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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 10 main sections:

1. Blood Component Collection and Production: Blood collection methods and devices (including apheresis); blood component preparation; inventory management; collection and storage of tissues; quality management and good manufacturing practice; automation and information technology; plasma fractionation techniques and plasma derivatives;
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3. Donors and Donations: Donor recruitment and retention; donor selection; donor health
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6. International Forum: Section in which topics related to any aspects of the transfusion chain (from technical to scientific, including socioeconomic and ethical facets) are discussed by invited participants and summarized by guest editors
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REVIEW

What influences decisions to donate plasma? A rapid review of the literature

Mary Berger[1] | Adam Easterbrook[1,2] | Kelly Holloway[3,4] | Dana Devine[2,3] | Nick Bansback[1,2]

[1]Centre for Health Evaluation and Outcome Sciences, Vancouver, British Columbia, Canada
[2]Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada
[3]Donation Policy & Studies, Canadian Blood Services, Toronto, Ontario, Canada
[4]Institute of Health Policy, Management & Evaluation, University of Toronto, Toronto, Ontario, Canada

Correspondence
Nick Bansback, Faculty of Medicine, University of British Columbia, 317-2194 Health Sciences Mall, Vancouver, BC V6T 1Z3, Canada.
Email: nick.bansback@ubc.ca

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Abstract

Background and Objectives: Plasma has become an essential ingredient for various medical treatments. Many blood collection agencies rely on voluntary non-remunerated donation when collecting plasma, but at present many do not collect sufficient plasma to meet domestic demands. This rapid review sought to explore the factors that have been found to influence people's decisions to donate plasma to inform future research.

Methods: Searches were conducted in PubMed, PsycINFO, Social Sciences Citation Index and CINAHL for peer-reviewed journal articles that discussed plasma donation and the factors associated with donor behaviour. Pertinent information from included articles was extracted and arranged in themes.

Results: In total, 33 articles were included in this review. Three main themes were identified by the authors. The first focused on site-level factors related to blood collection agencies' engagement with plasma donors and their influence on plasma donation experiences. The second theme considered how individual characteristics and experiences influence willingness to donate plasma. The third theme examined social and cultural-level factors, such as how social networks and community shape perceptions and experiences with donation.

Conclusion: Our findings suggest that the current understanding of plasma donation is focused mainly on converting whole blood donors and also centres on individual-level factors to donation. Further research must examine what factors attract non-whole blood donors to become plasma donors, focusing on broader social-level influences. This review will inform policies and interventions for blood collection agencies to increase plasma donors.

Keywords

donor motivation, donor retention, donors, plasma

Highlights

- This rapid review explores what influences people's decision to donate plasma. Most studies focus on existing whole blood donors, examining individual-level factors such as intention, altruism and fear surrounding the donation process.

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- There is little literature on non-whole blood donors or exploring social and cultural factors such as community influences and accessibility for diverse groups.
- Given that the findings show a lack of research on the influences of ethnicity, socioeconomic status and gender on plasma donation, specifically with non-donors, future studies should explore these factors in more depth.

INTRODUCTION

Plasma protein therapies are vital for patient recovery in clinical settings [1]. Plasma forms the treatment for patients with autoimmune disorders, immune deficiencies, rare blood disorders, various cancers, tetanus infections, nervous system disorders and so forth [1]. It can also be transfused directly into patients in hospitals or manufactured into specialized medicines called plasma protein products [2]. Due to the increasing use of these plasma protein products, the demand for plasma collection has increased globally [3].

Many blood collection agencies worldwide rely on voluntary, non-remunerated donations when collecting plasma. However, most fail to collect sufficient plasma to meet demand. Therefore, many countries purchase plasma from the international market, which often includes plasma from countries where people are paid to donate. While international purchasing allows countries to provide care, it raises concerns about the security of the supply. These concerns have been exacerbated during the COVID-19 pandemic, which has led to increasing costs of plasma products and disruptions to the supply chain [4].

It is important to understand the factors that influence people to either donate or not donate plasma to increase domestic plasma donation. Since whole blood donation is dominant in donation literature, recognizing the work done in plasma donation literature is a timely contribution. As discussed below, most plasma studies focus on converting whole blood donors to plasma. Addressing plasma donations' unique barriers and facilitators is vital to increase the plasma donor pool with new, inexperienced donors to ensure the whole blood donor pool remains stable. Thus, this review aims to summarize current plasma literature to provide insight for future research on improving plasma donor recruitment.

MATERIALS AND METHODS

A rapid review of plasma donation literature was done from February to April 2022. A rapid review follows a similar strategy to a systematic review but is more efficient due to limiting the search parameters and aspects of the synthesis. By applying a more streamlined approach to data collection and analysis, rapid reviews are best utilized in areas where information needs are immediate. This is relevant for plasma research, as the need for increasing supply is ever-growing; therefore, we chose a rapid review to quickly disseminate knowledge on how this demand can be met in a time of dire need. While there is no formal rapid review guideline, we used reviews that discussed the various approaches other authors have used [5–7], as well as supplemented the preliminary findings of the unpublished PRISMA-RR protocol [8].

Information sources

The search was completed by one reviewer (M.B.). The databases that we searched included PubMed, PsycINFO, CINAHL and Social Sciences Citation Index. We searched for peer-reviewed journal articles that discussed plasma donation and the factors associated with intention, motivation, facilitators and deterrents. The search term used was 'plasma don*' and was further specified using either [title/abstract] or depending on the database. The search began on 25 February 2022 and was completed on 19 April 2022. Test searches were performed before the official search using more specific terms alongside plasma don* (e.g., willingness, intent, barriers). However, the search results were too specific and did not provide a sufficient number of articles necessary for the review's aim. We searched Social Sciences Citation Index using the search terms and parameters; however, no relevant or new articles emerged. Therefore, we chose not to include any articles found in this database.

Inclusion criteria

We included journal articles that focused on factors of plasma donation that impact donors' willingness to, or not to, donate. These factors included motivation, intent, self-efficacy, barriers, facilitators, donor identity and willingness. In addition, eligible studies included those discussing factors that impacted the amount people donated, recruitment of new plasma donors, experiences of people who donated and perceptions surrounding plasma donation. More succinctly, we focused on the donor experience and how their backgrounds influence their perceptions and attitude towards donating plasma.

Exclusion criteria

Articles were excluded if not written in English or based outside North America, Europe or Australia. Articles outside of this geographic region examine healthcare systems that are structurally different from the Canadian system. Even with included countries varying in their reliance on remunerated donations, they historically have collected and distributed blood and plasma donations through a not-for-profit Blood Collection Agency (BCA) or an entity separate from the public health authority. Therefore, we examine countries with similarities in procurement, as the collection agencies often determine the type of people who donate [9]. We did not review any medical articles or letters to the editor. We chose not to include articles that described donor demographics (e.g., sex, age) alone as the review's focus is on

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how to increase donations, and descriptions of donor demographics are insufficient for this understanding. We also chose not to include op-ed articles or letters to the editor that discussed the ethical implications of paying donors (Figure 1).

Synthesis

Articles were organized in an Excel spreadsheet using descriptions of the studies, including methodology, sampling, study aims and findings. The authors kept notes on the emergent themes. The wider study team developed final themes by identifying each study's main goals and outcomes and grouping similar articles. In these groups, the authors highlighted how key findings fit together to create common trends across the articles, allowing for the development of broader themes. Moreover, grouping articles in such a way allowed the authors to find potential knowledge gaps explored in the discussion. Preliminary themes were developed by one author (M.B.), and then verified by another author (A.E.), with any disagreements resolved by a third author (N.B.).

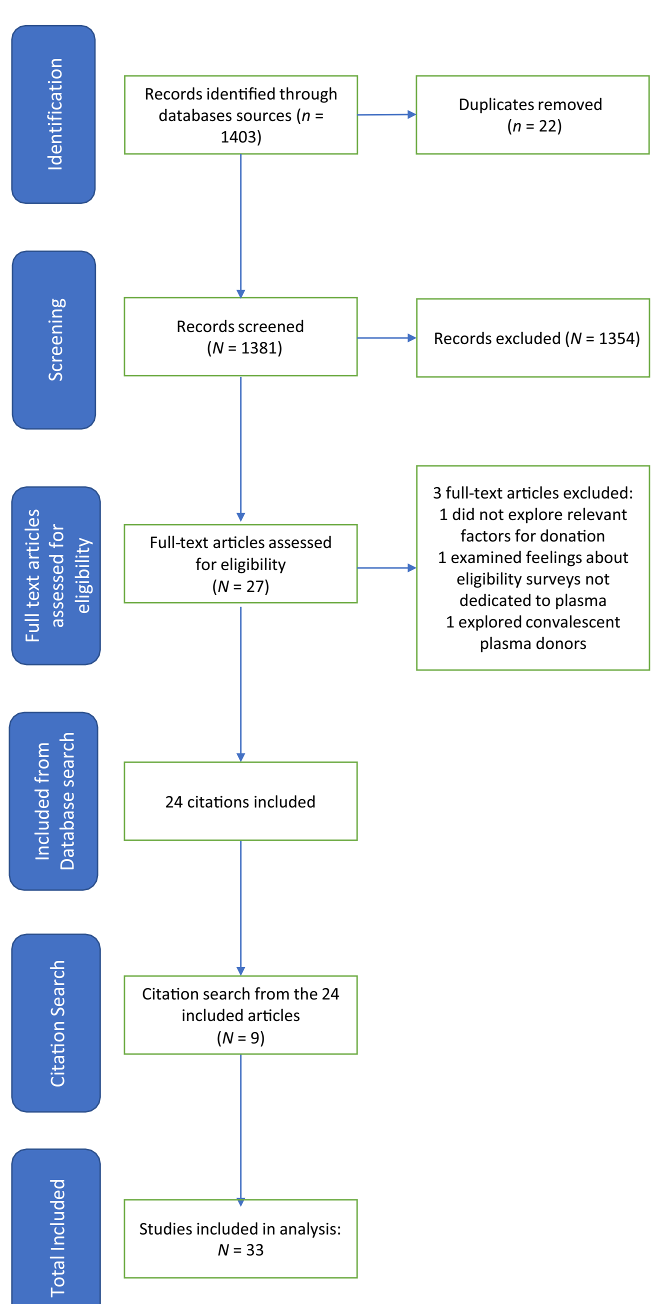

FIGURE 1 Flow chart of the selection process.

RESULTS

The electronic databases yielded 1403 articles. From these, 51 met our inclusion and exclusion criteria. After eliminating repeat articles, 24 unique studies met the inclusion/exclusion criteria to review fully. An additional nine articles were added from citation searches for a total of 33 articles (Table S1). The year range is from 1999 to 2022, as no articles appeared before 1999 in our search. Ten articles were qualitative and nine were quantitative. Additionally, three were mixed methods, and seven were experimental studies (e.g., randomized control trials). One article was a systematic review of whole blood donor willingness to convert to another substance of human origin [10]. Three were narrative reviews of motivations and deterrents towards plasma donation [11], motivations and compensation for plasma donation [12], and a summary of the current literature to provide strategies for BCAs [13].

We grouped our findings into three broad themes (Figure 2). The first theme focused on site factors, which include processes related to accessing plasma donation sites. The second theme was on individual-level factors, which focus on the motivations and deterrents of people who are approached to donate or already donate plasma. The third theme examined social/cultural-level factors, which explore the broader influences communities and social institutions have on individuals' perceptions of donating plasma.

Site and blood collection agency factors

Influence of BCA staff

To increase plasma donation, BCAs have explored both the recruitment of new donors and the conversion of whole blood donors to increase plasma donation [14–16]. The study by Thorpe et al. [16] aimed to increase the retention of first-time donors by comparing four strategies: calling donors, e-mailing donors, approaching donors to make advanced appointments and a simple 'thank you' message (control group). Compared with the control group, all intervention groups reported higher return rates and increased frequency of plasma donation. The most impactful strategy for getting donors to return included a call from the BCA asking about their experiences. This intervention demonstrated the importance of post-donation communication since

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FIGURE 2 An illustration of the themes that emerged from the review, beginning with the individual-level factors in the innermost circle, and moving out to the site-level factors, and finally the larger social- and cultural-level factors that influence donation.

retention and recruitment improved when staff took the time to explain plasma donation and how to book an appointment.

Similarly, other studies [15, 17] compared the efficacy of different strategies for converting whole blood donors to plasma donation and found that in-person conversations with centre staff were more effective than call-centre phone conversations. Moreover, the relationships donors create with staff strongly influence return donations. Bove et al. [22] interviewed plasma donors on what triggered them to donate and what made them continue. For many, their relationship with the staff prompted them to switch from whole blood to plasma and kept them returning.

Time to donate plasma

Plasma donation takes approximately 1 h due to the separation and the return of the red blood cells, which is four times the length of blood donation and is the primary barrier preventing people from donating [11, 13, 18–21]. Bagot et al. [20] interviewed donors who declined to convert to plasma from whole blood donation and found that across all groups, the amount of time it took to donate plasma was the most cited reason for not wanting to donate plasma. Thorpe et al. [19] found that most plasma donors who lapsed did so because of changes in life circumstances, such as changes to work schedules, shifting family obligations and the ebb and flow of everyday life, which causes plasma donation to get sidelined.

Individual-level factors

Incentives and compensation

There has been substantial research into financial compensation's impact on people's willingness to donate plasma [12]; however, findings remain mixed and contradictory. Two experimental studies found that incentives did not influence willingness to donate [23] and return rates [24]. Gyuris et al. [23] found that donor willingness did not differ between the group given a monetary reward and the group that had the social significance of plasma donation emphasized to them. However, those who were given the option to pay their monetary reward forward to a non-governmental organization reported higher willingness to donate. Chell et al. [24] provided participants with a voucher for a local cafe and found that while nearly 70% of the sample took the voucher, only 18% redeemed it. Moreover, the return rate

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between the control group that received no voucher and the experimental group with a voucher was not significantly different.

Two non-experimental studies explored the motivations of paid-plasma donors in the United States [25] and Hungary [26]. Anderson et al. [25] found that university students who were paid to donate their plasma were motivated by monetary incentives, not altruism. These students used the funds on activities they otherwise could not afford. Similarly, Trimmel et al. [26] surveyed paid plasma donors and voluntary whole blood donors in Hungary and found that paid plasma donors were motivated by monetary incentives and would not continue to donate if payment ceased. Moreover, paid donors had lower altruistic thinking levels than voluntary whole blood donors. More recently, France and France [27] surveyed university students on their preference to donate plasma or whole blood. They found that students' willingness to donate plasma rose from 3.9% to 47.5% after being informed that plasma donors are paid in the United States, where the study was based.

Anxiety and fear about donating plasma

Anxiety is often higher for new plasma donors due to navigating the apheresis machine and the unpleasant cold feeling experienced when blood is returned [28]. The medical environment in which plasma donation takes place appears to cause some unease and anxiety, especially for those new to the process.

A common factor for donor lapse is adverse events when donating, such as fainting. Vasovagal reactions (VVRs) are common for novice plasma donors [18, 28] and occur when individuals experience a sudden drop in blood pressure due to an extreme emotional response to a trigger, like seeing blood, and faint. Thijsen et al. [29] found that those plasma donors who experienced a VVR took significantly longer to return to donate and were more likely to experience another adverse event. Similarly, Thijsen et al. [30] explored the impact of a new policy in Australia, which allowed individuals to donate plasma without a previous whole blood donation. Compared to experienced donors, first-time plasma donors were the most likely to experience an adverse event and were not as likely to return.

Attempts have been made to address the anxiety and fears of new plasma donors. Gilchrist et al. [31] conducted an experimental study with two treatment groups for new donors. They gave the first group an informative brochure that normalized donor fear and provided stress reduction strategies—the second met with a trained research assistant who discussed concerns about with process with each donor. Both groups had fewer adverse events and higher positive support levels than the control.

Individual intention, motivation and self-efficacy

The most common framework for understanding individual intention and motivation towards plasma donation is the Theory of Planned Behaviour (TPB) [21, 32, 33]. The theory suggests that the best predictor of behaviour is intention. Three factors determine the strength of intention: perceived behavioural control, which is an individual's perception of how easily or not a behaviour can be performed; attitude, which is the evaluation of the behaviour as a positive or negative act; subjective norm, which is the perception of others approval towards the enacted behaviour. Godin and Germain [33] used TPB to evaluate which factors were the most impactful when determining the intention of plasma donors. They found that perceived behavioural control and attitude were the most significant determinants of intention, as well as age (i.e., older adults) and donor experience. Based on these findings, an individual's intention to donate plasma hinges on whether they believe they can do so successfully.

In response to these findings, studies have begun to supplement TPB with Bandura's concept of self-efficacy [34]. In this context, self-efficacy describes an individual's belief in their ability to carry out a behaviour well. Veldhuizen and van Dongen [32] used a modified TPB to identify the most significant predictor of conversion to plasma donation or the onset of a plasma donor career. Plasma donors had stronger positive attitudes than whole blood donors.

However, the most significant difference was the higher levels of self-efficacy reported in plasma donors. This result was mirrored in Bagot et al. [21]. This study incorporated additional factors such as moral norms and anticipated regret. Moral norms assess the individual's perceptions of whether the behaviour is right or wrong. Anticipated regret drives people to perform a behaviour to avoid feeling guilty if they fail to do so. Using these additional factors, Bagot et al. [21] found that moral norms were a significant predictor of intention to donate plasma; however, anticipated regret was only significant if the individual reported high moral norms.

Donor role

Bagot et al. [21] found that those blood donors with a strong tie to their 'blood donor identity' were less likely to convert to plasma donation. However, Thorpe et al. [35] found that converted plasma donors who felt whole blood donation was similar to plasma donation believed their donor identity remained stable. For many, their donor identity was also tied to the motivation to help as much as possible. Thus, when informed of the dire need for plasma, those who switched to plasma donation did not experience tension in the donor identity since it was seen as helpful and did not conflict with their blood donor identity [35].

Social and cultural-level factors

Donation frequency and supporting community

Several studies explored why people converted to plasma donation and what made them stay [22, 36–40]. The increased frequency at which donors could donate was a draw to plasma donation, as several

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researchers found that donors often wanted to donate as much as possible to address the need for plasma and benefit the broader community [22, 36, 38]. Bagot et al. [39] examined underlying beliefs that promote the intention to donate plasma and found that individuals who made a second plasma donation reported a strong desire to help others.

Family influences

A strong emotional connection to whole blood or plasma increased donation likelihood. Charbonneau et al. [40] found that many plasma donors had family members who had previously donated, knew of someone close to them who needed whole blood or plasma, or had someone close to them get ill or pass away. Thus, learning first-hand the impact of plasma donation on people, whether it was a favourable report of the experience from a loved one or witnessing its lifesaving capabilities, is a powerful motivator for many plasma donors.

Discriminatory or stigmatizing practices

Programmes have been developed to mitigate the exclusion of LGBTQ+ donors by Hema-Quebec in Canada. They proposed that men who have sex with men (MSM) could donate plasma, but their donation would be quarantined for 3 months and would require them to return to be tested for blood-borne infections such as HIV [41]. Several studies explored how members of the community, specifically MSM, felt about the plasma programme [41–43]. Using a TPB lens, Veillette-Bourbeau et al. [42] found that study participants reported a moderate level of perceived behavioural control, suggesting that the MSM programme was something they could see themselves doing. While some participants were keen to join the programme, a significant number of people still felt that the programme reinforced donation as an exclusionary practice that perpetrated an 'us vs. them' mentality Grace et al. [43]. Therefore, while the study by Veillette-Bourbeau et al. [42] found that MSM may feel they *could* donate, both Grace et al. [43] and Caruso et al. [41] found that the programme perpetuates the notion that the blood of MSM is 'impure' and reinforces the stigmatizing and discriminatory practices of BCAs. Participants in both studies did feel that the programmes were a step in the right direction and were hopeful that they would aid in the reduction of harmful prejudices held against them.

DISCUSSION

This rapid review of the plasma donor literature examined the various factors influencing plasma donor behaviour. A primary finding is that much of this research has focused on converting whole blood donors. Given the shortages in the supply of whole blood donors, exacerbated due to COVID-19 [44], this group will not sufficiently resolve plasma supply issues meaningfully. Historically, recruiting whole blood donors has been an effective way of collecting plasma, which was considered secondary to whole blood donation, until the 1990s when demand for plasma-derived products dramatically increased [45]. More research is needed on people not currently donating blood, including diverse populations who tend to be underrepresented amongst whole blood donors [46, 47].

There was a gap in the literature surrounding how knowledge of plasma donation and its uses influenced donor motivations. This was surprising, considering in whole blood donation literature, knowledge is discussed as an important factor [47–51]. Further research should be implemented to understand knowledge's influence on individuals when deciding whether they will donate plasma.

We suggest a more thorough discussion of altruism as a motivator within the context of blood plasma donation. In whole blood donation literature, attempts to broaden definitions of altruism have included influences like self-worth (warm glow) [52] and moral obligations (reluctant altruism) [53]. However, these expanded definitions are not found within plasma donation literature. Furthermore, because the discussion of altruism is lacking in blood plasma literature, it is examined using individual-level factors that do not capture the nuance of this socially driven behaviour. Based on this, we argue for having a more nuanced exploration of altruism in blood plasma literature, which may allow for more effective targeting strategies for specific groups of people instead of general marketing approaches.

Exploring macro-level social factors would greatly increase the understanding of the pushes and pulls people experience when deciding whether or not to donate plasma. Although some studies have examined how ethnicity and cultural background influence whole blood [46, 54, 55] and organ [56] donation, this literature remains scant and other influences, such as gender, socio-economic status, disability status and language, remain largely under-explored. Greater attention to larger social influences such as immigration status and socio-economic status should be explored to improve understanding of the pushes and pulls towards plasma donation.

It is important to note the strengths and limitations of this rapid review. The strengths of this article include a strict inclusion/exclusion criterion created before the initial search. Moreover, throughout the search and writing this review, the primary reviewer (M.B.) collaborated with other authors to negotiate and determine the best way to organize the data and reinforce the rigour of the review. The limitations are the nature of the rapid review and the method's efficiency, which means the review's scope is limited. For example, we omitted scholarship on COVID-19 convalescent plasma donation, as this donor base has different motivations that were irrelevant for future non-convalescent donation. However, this area would likely inform future plasma collection strategies and should be examined with non-convalescent donation [57]. As such, limiting the search terms and the databases may have resulted in publications being missed. Further, excluding grey literature, op-ed articles and publications in languages other than English may have created a potential publication bias.

Overall, the main message across the literature is that the need for plasma donors is dire, and the plasma supply must be increased to ensure people get the treatment they need. Future research should

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look at how to recruit new donors from a diverse population and the larger structural- and cultural-level factors that influence people's desire to donate plasma or not.

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CONFLICT OF INTEREST STATEMENT

The authors declared no potential conflicts of interest concerning the authorship, and/or publication of this article.

ORCID

Mary Berger https://orcid.org/0000-0001-9193-6063
Kelly Holloway https://orcid.org/0000-0003-3867-1072
Dana Devine https://orcid.org/0000-0002-9059-0344

REFERENCES

1. Mathew J, Sankar P, Varacallo M. Physiology, blood plasma. StatPearls. Treasure Island, FL: StatPearls Publishing; 2022.
2. Jolles S, Sewell WAC, Misbah SA. Clinical uses of intravenous immunoglobulin. Clin Exp Immunol. 2005;142:1–1.
3. Prevot J, Jolles S. Global immunoglobulin supply: steaming towards the iceberg? Curr Opin Allergy Clin Immunol. 2020;20:557–64.
4. Kenan N, Diabat A. The supply chain of blood products in the wake of the COVID-19 pandemic: appointment scheduling and other restrictions. Transp Res E Logist Transp Rev. 2022;159: 102576.
5. Garritty C, Gartlehner G, Nussbaumer-Streit B, King VJ, Hamel C, Affengruber L, et al. Cochrane Rapid Reviews Methods Group offers evidence-informed guidance to conduct rapid reviews. J Clin Epidemiol. 2021;130:13–22.
6. Ganann R, Ciliska D, Thomas H. Expediting systematic reviews: methods and implications of rapid reviews. Implement Sci. 2010; 5:56.
7. Tricco AC, Antony J, Zarin W, Strifler L, Ghassemi M, Ivory J, et al. A scoping review of rapid review methods. BMC Med. 2015;13:224.
8. Stevens A, Garritty C, Hersi M, Moher D. Developing PRISMA-RR, a reporting guideline for rapid reviews of primary studies (protocol). EQUATOR Network. 2018 [cited 2022 Mar 7]. Available from: https://www.equator-network.org/wp-content/uploads/2018/02/PRISMA-RR-protocol.pdf
9. Healy K. Embedded altruism: blood collection regimes and the European union's donor population. Am J Sociol. 2000;10:1633–57.
10. Hyde MK, Masser BM, Coundouris SP. A review of whole-blood donors' willingness, motives, barriers and interventions related to donating another substance of human origin. Transfus Med. 2022; 32:95–114.
11. Beurel A, Terrade F, Lebaudy JP, Danic B. Determinants of plasma donation: a review of the literature. Transfus Clin Biol. 2017;24: 106–9.
12. Farrugia A, Penrod J, Bult JM. Payment, compensation and replacement—the ethics and motivation of blood and plasma donation. Vox Sang. 2010;99:202–11.
13. Masser B, Bagot K. Plasmapheresis: recruitment, retention and flexible donors. ISBT Sci Ser. 2015;10:268–74.
14. Beurel A, Terrade F, Danic B, Lebaudy JP. Conversion to non-remunerated plasmapheresis donation: effects of labeling and donation environment. Eur Rev Appl Psychol. 2020;70:100554.
15. Godin G, Germain M. How to motivate whole blood donors to become plasma donors. J Blood Transfus. 2014;2014:752182.
16. Thorpe R, Masser BM, Nguyen L, Gemelli CN, Davison TE. Bringing new plasma donors back: testing the efficacy of telephone and e-mail communications with first-time donors. Transfusion. 2020;60: 1463–9.
17. Bagot KL, Bove LL, Masser BM, White KM. Asking for something different from our donors: factors influencing persuasion success. Transfusion. 2014;54:848–55.
18. Masser BM, Bove LL, White KM, Bagot KL. Negative experiences and donor return: an examination of the role of asking for something different. Transfusion. 2016;56:605–13.
19. Thorpe R, Nguyen L, Masser BM, Van Dyke N, Davison TE. Gone, but haven't forgotten: insights on plasmapheresis donation from lapsed donors. Vox Sang. 2021;116:281–7.
20. Bagot KL, Bove LL, Masser BM, Bednall TC, Buzza M. Perceived deterrents to being a plasmapheresis donor in a voluntary, non-remunerated environment. Transfusion. 2013;53:1108–19.
21. Bagot KL, Masser BM, White KM. Using an extended theory of planned behavior to predict a change in the type of blood product donated. Ann Behav Med. 2015;49:510–21.
22. Bove LL, Bednall T, Buzza M. Understanding the plasmapheresis donor in a voluntary, nonremunerated environment. Transfusion. 2011;51:2411–24.
23. Gyuris P, Gáspár BG, Birkás B, Csókási K, Kocsor F. Help is in your blood—incentive to "double altruism" resolves the plasma donation paradox. Front Psychol. 2021;12:653848.
24. Chell K, White C, Karki S, Davison TE. An Australian trial of the effectiveness of a discount reward to increase plasma donor retention and frequency of donation. ISBT Sci Ser. 2021;16:188–95.
25. Anderson L, Newell K, Kilcoyne J. "Selling blood": characteristics and motivations of student plasma donors. Sociol Spectr. 1999;19: 137–62.
26. Trimmel M, Lattacher H, Janda M. Voluntary whole-blood donors, and compensated platelet donors and plasma donors: motivation to donate, altruism and aggression. Transfus Apher Sci. 2005;33: 147–55.
27. France CR, France JL. Preference for whole blood versus plasma donation among university students. Vox Sang. 2020;115:624–7.
28. Thijsen A, King A, Waller D. Lost in translation: knowledge, attitudes and practices in donors experiencing a vasovagal reaction. Transfus Apher Sci. 2016;54:384–9.
29. Thijsen A, Masser B, Gemelli CN, Davison TE. Trends in return behaviour after an adverse event in Australian whole blood and plasma donors. Transfusion. 2019;59:3157–63.
30. Thijsen A, Davison TE, Speedy J, Hoad V, Masser B. Offering new and returned donors the option to give plasma: implications for donor retention and donor adverse events. Vox Sang. 2021;116: 273–80.
31. Gilchrist PT, Thijsen A, Masser BM, France CR, Davison TE. Improving the donation experience and reducing venipuncture pain by addressing fears among whole-blood and plasma donors. Transfusion. 2021;61:2107–15.
32. Veldhuizen I, van Dongen A. Motivational differences between whole blood and plasma donors already exist before their first donation experience. Transfusion. 2013;53:1678–86.
33. Godin G, Germain M. Predicting first lifetime plasma donation among whole blood donors. Transfusion. 2013;53:157S–161S.
34. Bandura A. Self-efficacy: toward a unifying theory of behavioral change. Psychol Rev. 1977;84:191–215.

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35. Thorpe R, Masser BM, Jensen K, Van Dyke N, Davison TE. The role of identity in how whole-blood donors reflect on and construct their future as a plasma donor. J Community Appl Soc Psychol. 2019;30:73–84.
36. Thorpe R, Masser BM, Nguyen L, Davison TE. Understanding donation frequency: insights from current plasma donors. Vox Sang. 2020;115:174–81.
37. Bagot KL, Masser BM, Starfelt LC, White KM. Building a flexible, voluntary donation panel: an exploration of donor willingness. Transfusion. 2016;56:186–94.
38. Bagot K, Masser B, White K. A novel approach to increasing inventory with the current panel: increasing donation frequency by asking for a different blood product. Transfusion. 2015;55:1294–302.
39. Bagot KL, Masser BM, White KM, Starfelt LC. Recruiting and retaining plasmapheresis donors: a critical belief analysis. Transfus Apher Sci. 2015;52:350–7.
40. Charbonneau J, Cloutier MS, Fainstein B. How do people become plasma and platelet donors in a VNR context. J Clin Apher. 2018;33:236–48.
41. Caruso J, Germain M, Godin G, Myhal G, Pronovost F, Morin M, et al. "One step closer": acceptability of a programme of plasma donation for fractionation from men who have sex with men. Vox Sang. 2019;114:675–86.
42. Veillette-Bourbeau L, Otis J, Lewin A, Godin G, Germain M, Daunais-Laurin G, et al. Determinants of the intention to participate in a programme of plasma donation for fractionation among men who have sex with men. Vox Sang. 2021;116:1051–9.
43. Grace D, Gaspar M, Klassen B, Lessard D, Anand P, Brennan DJ, et al. Stepping stones or second class donors? A qualitative analysis of gay, bisexual, and queer men's perspectives on plasma donation policy in Canada. BMC Public Health. 2021;21:444.
44. Draaisma M. Canadian Blood Services urges donors to keep appointments as blood supplies reach new low. CBC News. 2022 [cited 2022 Aug 6]. Available from: https://www.cbc.ca/news/canada/toronto/canadian-blood-servicessupplies-low-mask-mandate-1.6543815
45. Robert P. Global plasma demand in 2015. Pharm Policy Law. 2009;11:359–67.
46. Tran NYL, Charbonneau J, Valderrama-Benitez V. Blood donation practices, motivations and beliefs in Montreal's Black communities: the modern gift under a new light. Ethn Health. 2013;18:508–29.
47. Renzaho AMN, Polonsky MJ. The influence of acculturation, medical mistrust, and perceived discrimination on knowledge about blood donation and blood donation status: acculturation and blood donation. Transfusion. 2013;53:162S–171S.
48. Francis KL, Polonsky MJ, Jones SC, Renzaho AMN. The effects of a culturally-tailored campaign to increase blood donation knowledge, attitudes and intentions among African migrants in two Australian states: Victoria and South Australia. PLoS One. 2017;12:e0188765.
49. Fernández de la Iglesia J d C, Martínez-Santos AE, Rodríguez-González R, Cebreiro B, Fernández-Morante C, Casal-Otero L. Service-learning to improve attitudes towards blood donation among university students. Health Educ J. 2020;79:812–25.
50. Allerson JT. Assessment of selected university students' knowledge of blood donation and the relationship with intent to donate blood. Mankato: Minnesota State University; 2012.
51. Hupfer ME, Taylor DW, Letwin JA. Understanding Canadian student motivations and beliefs about giving blood. Transfusion. 2005;45:149–61.
52. Ferguson E, Taylor M, Keatley D, Flynn N, Lawrence C. Blood donors helping behaviour is driven by warm glow: more evidence for the blood donor benevolence hypothesis. Transfusion. 2012;52:2189–200.
53. Ferguson E, Lawrence C. Blood donation and altruism: the mechanisms of altruism approach. ISBT Sci Ser. 2016;11:148–57.
54. Muthivhi TN, Olmsted MG, Park H, Sha M, Raju V, Mokoena T, et al. Motivators and deterrents to blood donation among Black South Africans: a qualitative analysis of focus group data. Transfus Med. 2015;25:249–58.
55. Charbonneau J, Tran NYL. The paradoxical situation of blood donation in the Haitian-Quebec community. Can Ethn Stud. 2015;47:67–92.
56. Morgan M, Deedat S, Kenten C. 'Nudging' registration as an organ donor: implications of changes in choice contexts for socio-cultural groups. Curr Sociol. 2015;63:714–28.
57. Masser BM, Ferguson E, Thorpe R, Lawrence C, Davison TE, Hoad V, et al. Motivators of and barriers to becoming a COVID-19 convalescent plasma donor: a survey study. Transfus Med. 2021;31:176–85.

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

The added value of ferritin levels and genetic markers for the prediction of haemoglobin deferral

Marieke Vinkenoog[1,2] | Jarkko Toivonen[3] | Matthijs van Leeuwen[2] | Mart P. Janssen[1] | Mikko Arvas[3]

[1]Donor Medicine Research, Sanquin Research, Amsterdam, The Netherlands

[2]Leiden Institute of Advanced Computer Science, Leiden University, Leiden, The Netherlands

[3]Research and Development, Finnish Red Cross Blood Service, Helsinki, Finland

Correspondence
Mart P. Janssen, Plesmanlaan 125, 1066CX Amsterdam, The Netherlands.
Email: m.janssen@sanquin.nl

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Abstract

Background and Objectives: On-site haemoglobin deferral for blood donors is sometimes necessary for donor health but demotivating for donors and inefficient for the blood bank. Deferral rates could be reduced by accurately predicting donors' haemoglobin status before they visit the blood bank. Although such predictive models have been published, there is ample room for improvement in predictive performance. We aim to assess the added value of ferritin levels or genetic markers as predictor variables in haemoglobin deferral prediction models.

Materials and Methods: Support vector machines with and without this information (the full and reduced model, respectively) are compared in Finland and the Netherlands. Genetic markers are available in the Finnish data and ferritin levels in the Dutch data.

Results: Although there is a clear association between haemoglobin deferral and both ferritin levels and several genetic markers, predictive performance increases only marginally with their inclusion as predictors. The recall of deferrals increases from 68.6% to 69.9% with genetic markers and from 79.7% to 80.0% with ferritin levels included. Subgroup analyses show that the added value of these predictors is higher in specific subgroups, for example, for donors with minor alleles on single-nucleotide polymorphism 17:58358769, recall of deferral increases from 73.3% to 93.3%.

Conclusion: Including ferritin levels or genetic markers in haemoglobin deferral prediction models improves predictive performance. The increase in overall performance is small but may be substantial for specific subgroups. We recommend including this information as predictor variables when available, but not to collect it for this purpose only.

Keywords

donor deferral, ferritin, genetic markers, hemoglobin

Highlights

- Ferritin is routinely measured in some blood services, and genetic information for several iron-related single-nucleotide polymorphisms (SNPs) are collected by some others.

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- Inclusion of ferritin levels or genetic markers in haemoglobin deferral prediction models only marginally increases the predictability of blood donor deferrals.
- For donors with minor alleles on SNP 17:58358769, or donors with ferritin levels between 30 and 50 μg/L, the predictability of donor deferral increases considerably.

INTRODUCTION

Deferral of blood donors with low haemoglobin levels is necessary to prevent iron depletion. Currently, in Finland and the Netherlands, haemoglobin is measured before donation, which leads to on-site deferral if haemoglobin is below the donation threshold of 7.8 mmol/L (125 g/L) for women or 8.4 mmol/L (135 g/L) for men. On-site deferral is demotivating for donors and can be a reason to drop out of the donor pool permanently [1]. Haemoglobin deferral prediction models can help reduce the on-site deferral rate: for invitation-based donations, predictions can be included in the decision-making process of which donors to invite; for walk-in donations, the prediction could be communicated to the donor (e.g., shown on a donor dashboard or app that many blood banks offer), who can use this information to decide when to visit the blood bank.

Currently, haemoglobin deferral prediction models are not very accurate at predicting deferral on the specific day a donor may visit the blood bank. Although it is possible to correctly predict most deferrals as such (and therefore prevent them), this comes at the cost of incorrectly predicting some non-deferrals to be deferrals, which results in a large net loss of donations if these donors are then not invited to the blood bank based on this incorrect prediction. However, in a previous study we showed that predicting haemoglobin deferral at different time points, and inviting a donor once the predicted outcome is 'non-deferral', results in non-deferred donors to be invited earlier and deferred donors to be invited later, thereby eliminating the loss of successful donations [2]. This tells us that haemoglobin deferral prediction models are useful, and it is worth the effort of trying to improve the predictions.

Multiple studies [3–5] have shown previous haemoglobin levels to be the most important predictor of future haemoglobin deferral. Researchers from blood services in different countries have investigated many different potential predictors of haemoglobin deferral, to assess whether the inclusion of these predictors improves prediction performance. Most of these predictors were found to not substantially improve the models: information on menstruation, diet, ethnicity and smoking all only slightly improve model performance, even though they are known to be associated with iron stores [4]. One small-scale study on 261 donors did show that ferritin, soluble transferrin receptor and hepcidin were associated with subsequent anaemia [5].

In this study, we investigate the added value of including ferritin levels and genetic information in haemoglobin deferral prediction models. Ferritin is routinely measured at Sanquin, the Dutch national blood service, and therefore available for all donors. Genetic information for several iron-related single-nucleotide polymorphisms (SNPs) is collected for many donors by the Finnish Red Cross blood service. Because the information in both countries is collected without targeting specific donors, our results provide a realistic indication of how much predictions would be improved if the prediction model was to be used in practice. Our results will therefore be useful for blood services that would like to collect additional donor information to improve haemoglobin deferral predictions.

METHODS

Data

Data on blood donation attempts by whole-blood donors from (almost) five recent years were extracted from the eProgesa database (MAK-SYSTEM, Paris, France) in Finland and the Netherlands. Only data from donors who explicitly provided informed consent for the use of their data for scientific research were used. This consent is given by more than 99% of all Dutch donors. All Finnish blood donors studied provided informed consent for biobank research in accordance with the Finnish Biobank Act and the study was approved by the Blood Service Biobank (project 004_2019). In Finland, ~23% of active blood donors have given this consent since the founding of the Blood Service Biobank in 2017.

Finnish data reflect data entries from January 2016 through April 2020 and Dutch data from January 2017 through December 2021. For each visit, the following information was collected in both countries: donor sex, donor age, donation date and haemoglobin level. Additionally, ferritin level is measured at every new donor intake and upon every fifth donation in repeat donors in the Netherlands.

In Finland, only donors participating in the Blood Service Biobank are included, as only for these donors, genetic information related to iron metabolism is available [6]. Four SNPs were identified as significantly associated with higher prevalence of iron deficiency anaemia in an iron deficiency anaemia meta-analysis on Finnish and UK data. Polygenic risk scores were derived for three related endpoints: iron deficiency anaemia, ferritin and haemoglobin [7].

In total, complete information on the predictor variables (see Table 1) was available for 172,508 donation attempts by 42,255 donors in Finland and 456,384 donation attempts by 157,423 donors in the Netherlands.

The variable of interest is 'HbOK', a dichotomous variable that indicates whether the result of the donation attempt was deferral (i.e., haemoglobin [Hb] level below the eligibility threshold for donation) or non-deferral (i.e., Hb level equal to or above the threshold).

Donor deferral due to low haemoglobin is similar in Finland and the Netherlands. Haemoglobin is measured using a capillary skin-prick device before each donation, and eligibility thresholds for donation are 7.8 mmol/L for women and 8.4 mmol/L for men. However, in case

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TABLE 1 Predictor variables used in each country.

Variable used	Unit or values	Description	Country/Countries where data are available
Sex	{male, female}	Biological sex of the donor; separate models are trained for men and women	Both
Age	Years	Donor age at time of donation	Both
Month	{1–12}	Month of the year that the visit took place	Both
NumDon	Donations	Number of successful (collected volume >250 mL) whole-blood donations in the last 24 months	Both
DaysSinceFirstDon	Days	The number of days since the donor visited the blood bank for the first time	Both
HbPrev*i*	mmol/L	Haemoglobin level at *i*th previous visit, for *i* between 1 and 5	Both
DaysSinceHb*i*	Days	Time since related Hb measurement at *i*th previous visit, for *i* between 1 and 5	Both
FerritinPrev	µg/L	Most recent ferritin level measured in this donor	The Netherlands
SNP 1:169549811	{0, 1, 2}	Number of minor alleles in SNP rs6025	Finland
SNP 6:32617727	{0, 1, 2}	Number of minor alleles in SNP rs3129761	Finland
SNP 15:45095352	{0, 1, 2}	Number of minor alleles in SNP rs199138	Finland
SNP 17:58358769	{0, 1, 2}	Number of minor alleles in SNP rs199598395	Finland
PRS_anaemia	Standard deviations	Standardized polygenic risk score for anaemia	Finland
PRS_ferritin	Standard deviations	Standardized polygenic risk score for ferritin	Finland
PRS_haemoglobin	Standard deviations	Standardized polygenic risk score for haemoglobin	Finland

Abbreviation: SNP, single-nucleotide polymorphisms.

the measurement is below the eligibility threshold in Finland, haemoglobin is measured again (using the same device) in a venous sample, and this measurement is used for the deferral decision. In the Netherlands, two additional capillary haemoglobin measurements are taken when the first measurement outcome is below the eligibility threshold, and the donor is allowed to donate if any of the three measurement outcomes is above the eligibility threshold.

Analyses

For both countries, two models were fitted for each sex: one with all predictor variables available (the full model), and one with only those predictor variables that are available in both countries (the reduced model). By comparing the full model with the reduced model in both countries, the added value of the extra predictor variables (i.e., genetic information in Finland and ferritin information in the Netherlands) can be assessed.

The prediction models used were based on models developed for an earlier study considering Dutch data only [2]. All models are based on support vector machines (SVMs), supervised machine learning models that learn a separation between outcome classes from a *training set*, after which the model can be used to predict donor deferral for observations in an unseen *test set*. Here, the training set consists of blood bank visits in the first 4 years of data, whereas the test set consists of data collected in the final year.

Given a dataset and a set of predictor variables, a model consists of 10 SVM sub-models. The sub-models are named SVM-*sex*-*n*, where *sex* indicates donor sex (m for male, f for female donors) and *n* indicates the number of previous blood bank visits that are used for prediction. That is, each sub-model includes HbPrev*i* and DaysSinceHb*i* for *i* ranging from 1 to *n* as predictor variables. If *sex* is omitted in the sub-model name, it refers to the combination of two sex-specific sub-models. The number of blood bank visits (*n*) considered in this study varies from 1 to 5, and so five sub-models per sex are created. Donors can be included in the SVM-*sex*-*n* sub-model only if they have at least *n* previous visits; therefore, the sizes of the datasets used for both training and testing decrease from SVM-1 to SVM-5. Hyperparameters were optimized separately for each sub-model using stratified (on the outcome variable) fivefold cross-validation within the training set data only. Hyperparameters were optimized using grid search, using the balanced accuracy (defined as the weighted average of recall in both classes) as scoring method, which is suitable for datasets with imbalanced outcome sizes, as mistakes in the minority class are penalized more than those in the majority class.

During model training, the classification threshold is chosen again by optimizing the balanced accuracy. The predictive performance of the models is assessed using precision (also known as positive predictive value) and recall (also known as sensitivity) at this classification threshold. For non-deferral prediction, precision is defined as the proportion of true non-deferrals out of all predicted non-deferrals; recall is defined as the proportion of predicted non-deferrals out of all true non-deferrals. In this context, the complement of the precision is the hypothetical new deferral rate if the model would be used to choose which donors to invite, and the complement of the recall is the

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TABLE 2 Number of donation attempts available per model for both countries; number of deferrals and deferral rates are given in brackets.

Model	Women		Men	
	Finland	Netherlands	Finland	Netherlands
SVM-1	83,628 (3216; 3.85%)	236,994 (7724; 3.26%)	88,880 (1480; 1.67%)	219,390 (2411; 1.10%)
SVM-2	68,718 (2494; 3.63%)	166,640 (5875; 3.53%)	78,268 (1264; 1.61%)	179,465 (2114; 1.18%)
SVM-3	55,011 (1859; 3.38%)	123,171 (4370; 3.55%)	68,225 (1054; 1.54%)	150,396 (1889; 1.26%)
SVM-4	43,164 (1307; 3.03%)	93,868 (3149; 3.35%)	58,951 (896; 1.52%)	127,807 (1667; 1.40%)
SVM-5	33,179 (868; 2.62%)	72,165 (2112; 2.93%)	50,540 (749; 1.48%)	108,832 (1424; 1.31%)

Abbreviation: SVM, support vector machines.

proportion of successful donations that would be missed by the model because the donors are incorrectly predicted to have a low haemoglobin level. Precision and recall can be calculated for both outcome classes ('deferral' and 'non-deferral').

The precision–recall (PR) curve is a graph in which the recall and the precision of a prediction model at varying classification thresholds are shown. The area under this curve (AUPR), is a number between 0 and 1, where 1 would indicate a perfect classifier. By subtracting the deferral rate from the AUPR, we get an adjusted AUPR, which reflects the improvement by the model over a strategy that would always predict non-deferral. Without this correction, the improvement made by the model would be biased by the difference in deferral rate. AUPR represents the ability of the model to distinguish between two classes at differing classification thresholds. It is possible for model A to have a higher AUPR than model B even if precision and recall at the optimal classification threshold are the same in both models.

Model explanations

Because SVMs do not provide model coefficients that can be directly interpreted, we use Shapley Additive exPlanations (SHAP) values to investigate the importance of different predictor variables [8]. SHAP is a model agnostic explainer that shows the contribution of each predictor variable to the predicted outcome. This contribution is calculated for each individual observation separately (in a subsample of the test set) and is therefore very informative.

Subgroup analysis

To further investigate the value of including ferritin and genetic information in the models, we perform additional analyses in which donors are placed in groups defined by ferritin level or genotype. Deferral rate, model performance and the difference between reduced and full model performance are calculated and compared to assess whether there are subgroups of donors for whom including the extra variables results in better predictions.

Software

All analyses were performed in Python 3.10 using packages *numpy* and *pandas* for data processing, *scikit-learn* for model training and predictions, *shap* for calculating SHAP values and *matplotlib* for creating graphs. All code is available on GitHub and is indexed on Zenodo at https://doi.org/10.5281/zenodo.7780718.

RESULTS

Table 2 shows the number of donation attempts used for each model in both countries. Deferral counts and rates are given in brackets. Sample sizes are much larger in the Netherlands than in Finland. This is because the total number of blood donations is much higher in the Netherlands than in Finland, which is due to a larger population (17.4 million vs. 5.5 million in 2020), but also because genetic information is available in Finland in only a subgroup of donors, whereas ferritin measurements are available for all Dutch donors.

Deferral rates are very similar in both countries, around 3% for women and 1% for men. The biggest difference in deferral rates is found in men with at least one previous haemoglobin measurement, where the deferral rate is 0.57 percentage points higher in Finland. In most cases, deferral rates go down whenever more previous visits are included; this is most likely the result of self-selection, where donors with lower haemoglobin levels are less likely to return for subsequent donations than donors with higher haemoglobin levels. Surprisingly, for Dutch men this pattern seems to some extent to be reversed as their deferral rate goes up with an increasing number of donations.

Table S1 shows the marginal distribution of the predictor variables, combined for all sub-models. Donors in Finland are older than

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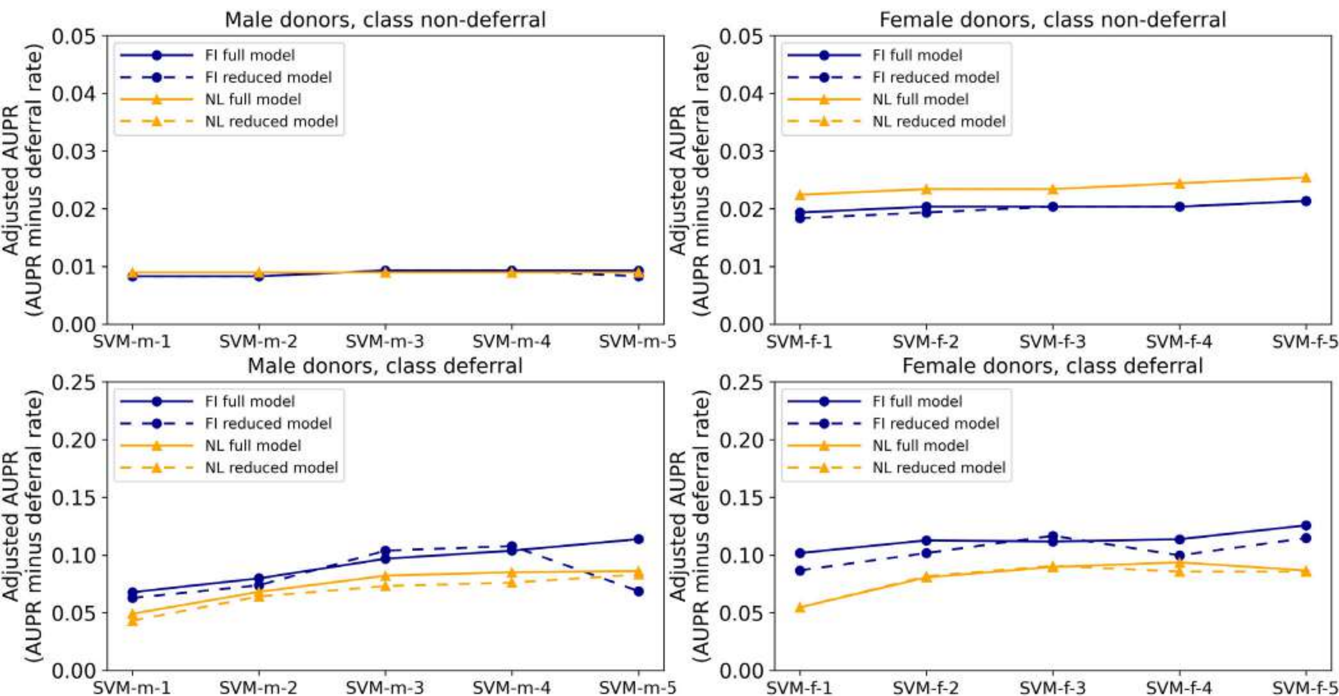

FIGURE 1 Adjusted area under precision–recall curve (AUPR) by sub-model for the Netherlands (NL) and Finland (FI) for both sets of predictor variables. SVM, support vector machines.

donors in the Netherlands (median age 46 vs. 30 years in women, 52 vs. 34 years in men) and the number of donations in the past 2 years ('NumDon') is also higher, with a difference in median donations of 2 for both sexes. This difference can be explained by the sample composition: the Finnish dataset consists of participants of the Blood Service Biobank, who have given consent for medical research and are typically regular, committed blood donors. Genetic information is available only for these donors.

Haemoglobin levels are slightly higher in Finland for both sexes for all variables HbPrev*i*, by 0.1–0.3 mmol/L. The time between subsequent donation attempts (variables DaysSinceHb*i*) is slightly shorter for Finnish women than for Dutch women, but almost identical for men. This difference can be partly explained by a difference in minimum donation interval between blood donations: for women, 91 days in Finland versus 122 days in the Netherlands; for men, 61 days in Finland versus 57 days in the Netherlands.

Predictive performance

Predictive performance can be assessed for individual sub-models, or for all sub-models combined, by using the most complex sub-model possible to predict each outcome. When more previous blood bank visits are taken into consideration, more predictor variables are used, and we expect the performance of the sub-model to increase. Figure 1 shows that this is the case for both the full and reduced model in both countries. The adjusted AUPR increases from SVM-1 to SVM-5 almost everywhere. An exception is the AUPR for class deferral in SVM-m-5, where the reduced model for Finnish donors shows an unexpected drop in the adjusted AUPR. For male donors, class non-deferral, the adjusted AUPR does not seem to change from SVM-m-1 to SVM-m-5.

Overall model performance and the difference in model performance between the full and reduced models are assessed by PR curves and adjusted AUPR values as described in Section 2. Figure 2 shows the PR curves for various models (SVM-1 through SVM-5, using the model with the most predictor variables possible for each donation attempt) by sex and true outcome class. In general, models are better at identifying non-deferrals (the most common outcome) than deferrals, even with scoring methods that weigh mistakes in both outcome classes proportionally. However, all curves are well above the baseline, indicating a structural improvement as compared to random guessing.

When comparing the reduced models with each other, one can observe that the performance is very similar in both countries. For women the AUPR is higher in Finland than in the Netherlands for the class deferral, but lower for the class non-deferral. This indicates that deferrals are more likely to be predicted correctly, but at the cost of more inaccuracies when predicting non-deferrals.

Moving from the reduced to the full model has virtually no effect on the AUPR for the class non-deferral: the AUPR of the full model is almost identical to that of the reduced model for both countries and sexes. For the class deferral, however, there is a difference: in Finland, AUPR increases by 58% (from 0.066 to 0.104) for men and by 8.5% (from 0.106 to 0.115) for women. In the Netherlands, AUPR remains the same for women (0.086 for both) but increases by 8.3% (from 0.072 to 0.078) for men.

Table 3 provides the confusion matrices of model predictions by the reduced and full models for both countries. In the Finnish data, going from the reduced to the full model causes 7 (1.9%) more

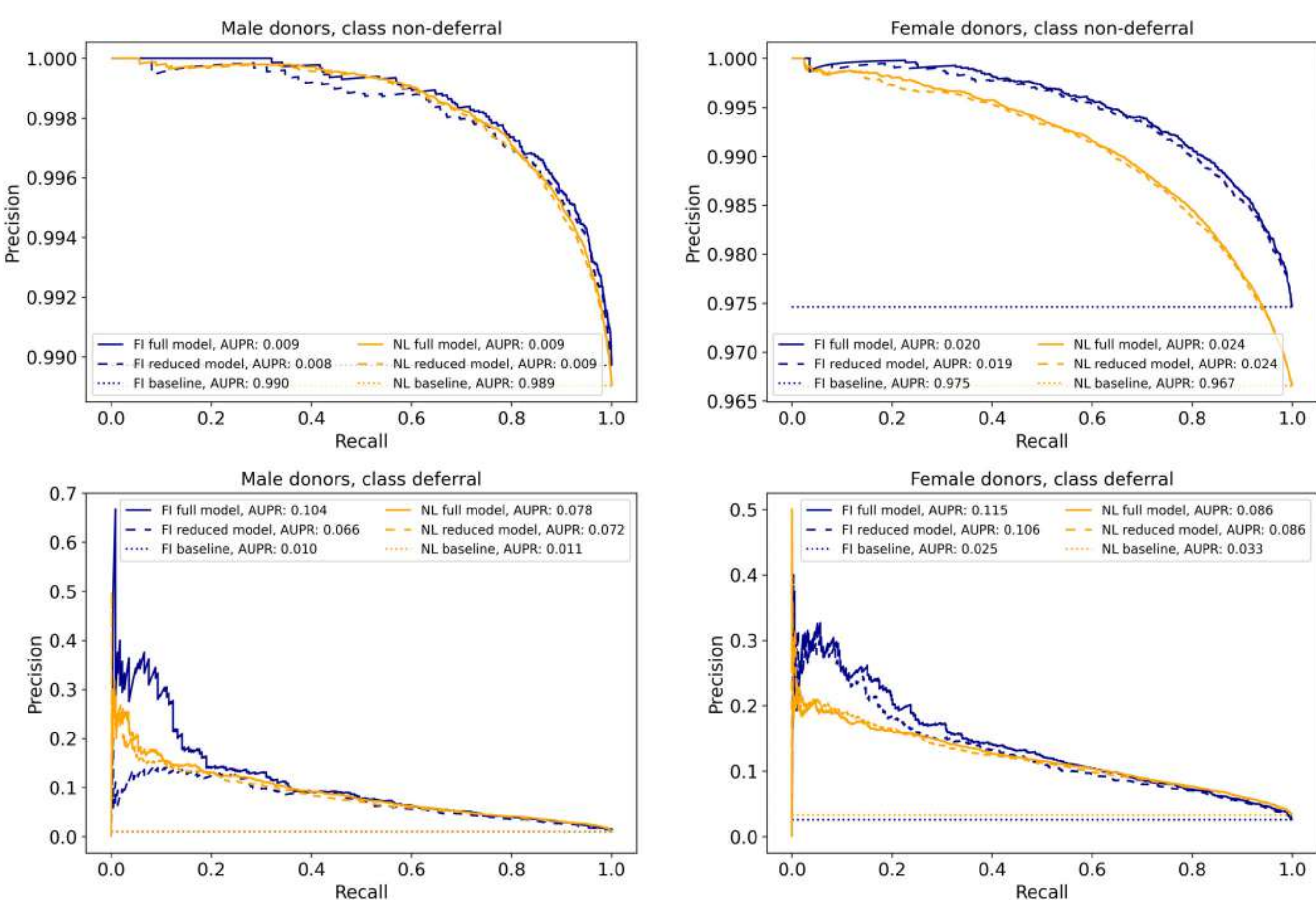

FIGURE 2 Precision–recall curves for the prediction models. For the Netherlands (NL) and Finland (FI), the curve is shown for the reduced and full prediction models. The baseline (proportion of observations belonging to this outcome class, i.e., for class deferral, the deferral rate) is shown as a dotted horizontal line. AUPR, adjusted area under precision–recall.

TABLE 3 Confusion matrices of predictions by the reduced and full models.

Finnish donors: Reduced-model			**Finnish donors: Full model**		
	Predicted deferral	**Predicted non-deferral**		**Predicted deferral**	**Predicted non-deferral**
True deferral	363	166	True deferral	370 (+7)	159 (−7)
True non-deferral	4573	18,713	True non-deferral	4662 (−59)	18,624 (+59)
Dutch donors: Reduced model			**Dutch donors: Full model**		
	Predicted deferral	**Predicted non-deferral**		**Predicted deferral**	**Predicted non-deferral**
True deferral	3762	957	True deferral	3775 (+13)	944 (−13)
True non-deferral	56,676	145,549	True non-deferral	55,203 (−1473)	147,022 (+1473)

Note: Numbers are summed over both sexes and over all sub-models SVM-1 through SVM-5. Observations that can be predicted with multiple sub-models are included in the most complex sub-model.
Abbreviation: SVM, support vector machines.

deferrals to be predicted correctly, while 59 (0.3%) more non-deferrals are predicted correctly. These improvements were all for female donors; at the chosen threshold values, no net changes in the confusion matrix were seen for male donors. In the Dutch data, 13 (0.3%) more deferrals, as well as 1473 (1.0%) more non-deferrals, are predicted correctly by the full model as compared with the reduced model.

Note that the large increase in AUPR for Finnish male donors, class deferral, is not reflected in the confusion matrices. The PR curve in Figure 2 shows that the AUPR increase is due to higher precision in the full model between a recall of 0 and 0.2. However, the optimal classification threshold that is used by the models corresponds to a recall of 0.7, at which point precision in the full model is exactly equal to precision in the reduced model.

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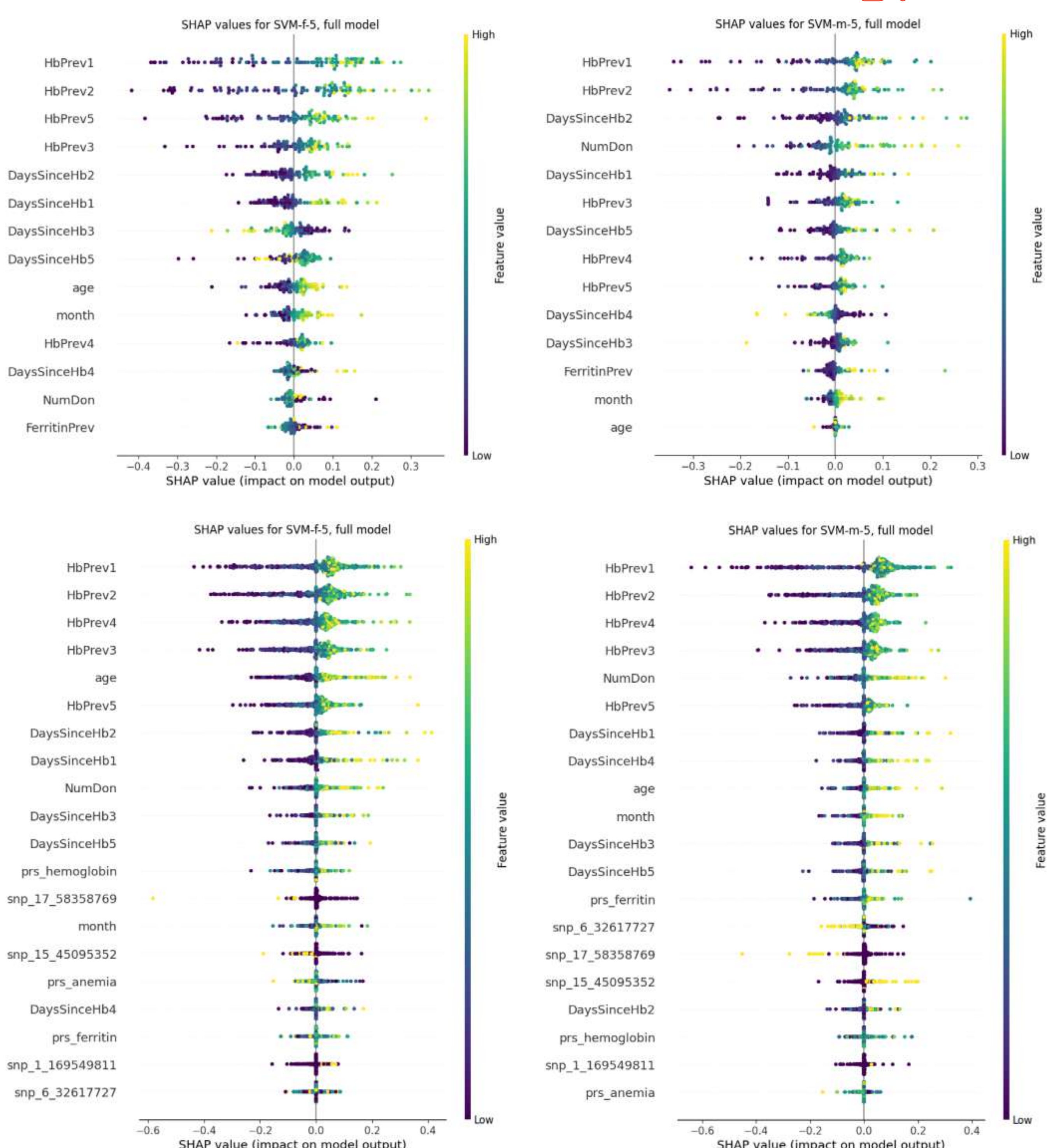

FIGURE 3 Shapley Additive exPlanations (SHAP) plots for the full models on Dutch (top row) and Finnish (bottom row) data, for women (left column) and men (right column) separately. SVM, support vector machines.

Variable importance

For all sub-models, SHAP values show the importance of the different predictor variables on the predicted outcome. Figure 3 shows SHAP plots of the sub-model SVM-5 of the full model, separately for both sexes and countries.

These plots show that in both countries and for both sexes, the most important predictor variable is HbPrev1, that is, the most recent haemoglobin measurement. The direction of the association between the impact on the model output and the feature value for all HbPrev*i* variables is sensible: a lower haemoglobin measurement is predictive of deferral. Age is a more important predictor variable for women than

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TABLE 4 Sample sizes, deferral rates and precision and recall of outcome class deferral for subsets of donors based on values for four SNPs (single-nucleotide polymorphisms).

SNP	Minor alleles	*N*	Deferral rate	Precision (class deferral)		Recall (class deferral)	
				Reduced model	Full model	Reduced model	Full model
SNP 1:169549811	0	22,810	0.022	0.073	0.073	0.686	0.702
SNP 6:32617727	1 or 2	1005	0.026	0.087	0.095	0.692	0.692
	0	7268	0.021	0.063	0.067	0.573	0.587
	1	11,908	0.022	0.072	0.074	0.704	0.742
	2	4639	0.026	0.092	0.081	0.790	0.756
SNP 15:45095352	0	20,831	0.022	0.073	0.073	0.676	0.691
	1 or 2	2984	0.022	0.080	0.080	0.758	0.773
SNP 17:58358769	0	23,427	0.021	0.071	0.071	0.683	0.687
	1 or 2	388	0.077	0.156	0.129	0.733	0.933
Total	-	23,815	0.022	0.074	0.074	0.686	0.701

for men in both countries, which is known from previous studies: young women have the highest probability of being deferred because of low haemoglobin, due to monthly iron loss with menstruation.

The additional genetic and ferritin variables for either country end up rather low in the variable importance ranking. The importance of all polygenic risk score and SNP variables in the Finnish models is very low. However, having the minor allele present in either SNP 6:32617727, SNP 15:45095354 or SNP 17:58358769 impacts the model output negatively. This effect is more pronounced in male than female donors.

Subgroup analysis in Finnish data

To further investigate the effect of the SNPs on deferral prediction, model performance was calculated for groups of donors with the same value for one SNP at a time. Donors with values 1 and 2 are grouped together, as the proportion of donors with value 2 is extremely low, except for the SNP on chromosome 6.

Table 4 shows that for the SNPs on chromosomes 1, 6 and 17, deferral rates are higher among donors with one or two minor alleles than in donors with only major alleles. As these SNPs are selected because of their association with iron deficiency or anaemia, this is to be expected. Additionally, precision and recall of class deferral are generally higher for donors with minor alleles than for those without, for both the reduced and full models. The SNP 17:58358769 shows this same trend, but the difference between donors with and without minor alleles is much larger. Precision in this subgroup is about twice as high as the overall precision in both the reduced and full model. The increase in recall between the full and reduced model (which changes from 0.733 to 0.933) is the highest of all subgroups.

An additional analysis on the distribution of haemoglobin measurement per donor showed that the higher deferral rate among donors with minor alleles on SNP 17:58358769 can be explained through a combination of a slightly lower average haemoglobin level and a slightly higher variance. This causes these donors to have a slightly higher deferral probability (median 32.6% for donors without minor alleles, median 36.6% for those with minor alleles). This difference was not observed for the other SNPs.

Subset analysis in Dutch data

Similar to the subset analysis in Finnish data, model performance was calculated for groups of donors with similar ferritin levels: <15, 15–30, 30–50, 50–100 and >100 µg/L. The first two groups are those that would be deferred for 12 or 6 months, respectively, in accordance with Sanquin's ferritin deferral policy.

Table 5 shows that precision and recall are highest for donors with ferritin levels between 30 and 50 µg/L. This is also the group of donors with the highest deferral rate: 3.2%, versus an overall deferral rate of 2.3%. The fact that this group has the highest deferral rate, and not donors with lower ferritin levels, can be explained by the fact that donors with ferritin levels below 30 µg/L were deferred for 6 months (12 months for ferritin levels below 15 µg/L) in accordance with Sanquin's ferritin deferral policy. This delay for the next donation provides the donors with sufficient time to replenish their iron stores and therefore reduces the deferral probability. Hence, donors with ferritin levels just above the ferritin-deferral threshold will have the highest haemoglobin-deferral rate, as they have neither the advantage of the donation break nor that of a very high ferritin level, which also protects against low haemoglobin levels.

DISCUSSION

Predicting deferral for low haemoglobin levels is a topic of interest to many blood banks, as accurate predictions could aid in decreasing deferral rates. This study investigates the added value of including

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TABLE 5 Sample sizes, deferral rates and precision and recall of outcome class deferral for various subsets of donors based on their ferritin level.

Ferritin level	*N*	Deferral rate	Precision (class deferral)		Recall (class deferral)	
			Hb only model	All variables	Hb only model	All variables
<15 μg/L	7172	0.022	0.054	0.054	0.700	0.681
15–30 μg/L	19,903	0.022	0.058	0.056	0.744	0.783
30–50 μg/L	62,140	0.032	0.082	0.079	0.815	0.833
50–100 μg/L	65,141	0.024	0.064	0.063	0.798	0.799
>100 μg/L	52,588	0.010	0.033	0.040	0.801	0.730
Total	206,944	0.023	0.062	0.064	0.797	0.800

Abbreviation: Hb, haemoglobin.

information on the donor's ferritin level or iron-related genetic information to improve haemoglobin deferral prediction. This is done by comparing prediction models with and without information on genetic markers and ferritin levels for the Finnish and Dutch blood bank, respectively. The reduced models (i.e., without the additional information) use the exact same predictor variables in both countries. The increase in AUPR is larger for adding genetic markers than it is for adding ferritin levels. Especially for the Finnish male donors, including genetic markers in the prediction model improves the ability of the model to distinguish between the two outcome classes, although at the optimal classification threshold precision and recall do not increase from the reduced model. The SHAP values of the predictions by the full models in both countries show that both genetic markers and ferritin levels have a much smaller impact on the prediction than the variables included in the reduced models, as confirmed by the modest increase in AUPR between the reduced and full models.

Overall, including either genetic or ferritin information has little effect on the predictions made by the models. Both increase the proportion of deferrals that are predicted correctly: 1.9% and 0.3% more deferrals are correctly identified in the Finnish and Dutch setting, respectively, when the full model is used rather than the reduced model. However, we found that in both countries, there is a subgroup of donors for which the full model performs substantially better than the reduced model. These are Finnish donors with minor alleles on SNP 17:58358769 and Dutch donors with ferritin levels between 30 and 50 μg/L. In both cases, these are subgroups of donors with a higher than average deferral rate. Performance for these subgroups is already higher than average in the reduced model, but when using the full model this difference increases even further.

Other studies have shown that previous haemoglobin measurements are the most influential predictors for haemoglobin deferral. Including lifestyle behaviour, smoking, ethnicity or menstruation in prediction models also improves performance, but only marginally [4]. A Finnish study showed that genetic information does not improve the predictive performance of haemoglobin levels (as opposed to haemoglobin deferral) [9]. This study confirms that the performance of prediction models increases slightly when either ferritin or genetic information is added. Still, considering the large number of donation visits blood banks receive yearly, even a small increase could potentially prevent hundreds of deferrals. It should be noted that the Finnish population is more genetically homogenous than in other countries and that they are also genetically distinct from other countries due to several historic population bottlenecks and geographical isolation [10]. According to the Genome Aggregation Database (gnomAD) [11], the SNP 17:58358769 minor allele frequency in the Finnish population is 0.0147 but only 0.0007 in the European (non-Finnish) population. It is not found in any other populations and was discovered by an iron deficiency GWAS in the FinnGen project [7]. This means that findings on Finnish genetic data may not be representative for other countries, but analyses in other populations may discover similar population-specific variations that may make the use of genetic data more beneficial.

The main limitation of this study is that the effect of including ferritin and genetic information is studied in two different countries, rather than in a single population. By comparing against the reduced model and reporting the relative increase in performance, we attempt to mitigate this limitation. The very similar adjusted AUPRs of the reduced models and the similarity in SHAP values of the models indicate that the countries are rather comparable. A second limitation is that all Dutch donors could be included in this study but only Finnish donors from the Blood Service Biobank, as genetic information is not available for other donors.

In general, we again confirm that accurately distinguishing deferrals from non-deferrals by predictive modelling is a complex task that comes at the cost of losing a substantial number of successful donations by incorrectly predicting them to be deferrals. A major reason for the low performance of our prediction models is the measurement variability, partly caused by the (pre-) analytical variability of the capillary haemoglobin measurements [12]. As long as we try to predict an outcome that is highly variable, the performance of any prediction model will remain unsatisfactory, regardless of the number of predictor variables included.

However, in the absence of a better measurement or decision strategy, it is worthwhile investigating which information would lead to better haemoglobin deferral predictions, as it still leads to a better understanding of the underlying process(es). Based on our results, we would recommend including ferritin and genetic information in prediction models in case these are readily available. Compared

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with the reduced model, including genetic information would have resulted in 7 fewer deferrals and 59 more donations in 1 year, at a cost of genotyping approximately 24,000 donors. Including ferritin levels results in 13 fewer deferrals and 1473 more donations in 1 year, and although measuring ferritin levels is less expensive than genotyping, this measurement must be repeated regularly whereas genotyping has to be performed only once for each donor. We would therefore not recommend collecting this information explicitly for the use in haemoglobin deferral prediction, as the marginal increase in performance is not likely to be worth the investment of both time and money.

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All authors contributed to the study design; M.V. and J.T. developed the software and analysed the data; M.V. aggregated the results and wrote the paper and all authors reviewed and edited the paper.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

As the data used for this study contains personal information of blood donors, the data will not be shared outside the organisations.

ORCID

Marieke Vinkenoog https://orcid.org/0000-0001-5653-8078
Jarkko Toivonen https://orcid.org/0000-0002-6843-5831
Matthijs van Leeuwen https://orcid.org/0000-0002-0510-3549
Mart P. Janssen https://orcid.org/0000-0002-1682-7817
Mikko Arvas https://orcid.org/0000-0002-6902-8488

REFERENCES

1. Spekman MLC, van Tilburg TG, Merz E-M. Do deferred donors continue their donations? A large-scale register study on whole blood donor return in the Netherlands. Transfusion. 2019;59:3657–65.
2. Vinkenoog M, van Leeuwen M, Janssen MP. Explainable haemoglobin deferral predictions using machine learning models: interpretation and consequences for the blood supply. Vox Sang. 2022;117:1262–70.
3. Vinkenoog M, Toivonen J, Brits T, de Clippel D, Compernolle V, Karki S, et al. An international comparison of haemoglobin deferral prediction models for blood banking. Vox Sang. 2023;118:430–9.
4. Baart AM, Timmer T, de Kort WLAM, van den Hurk K. Lifestyle behaviours, ethnicity and menstruation have little added value in prediction models for low haemoglobin deferral in whole blood donors. Transfus Med. 2020;30:16–22.
5. Pasricha S-R, McQuilten ZK, Keller AJ, Wood EM. Hemoglobin and iron indices in nonanemic premenopausal blood donors predict future deferral from whole blood donation. Transfusion. 2011;51:2709–13.
6. Kurki MI, Karjalainen J, Palta P, Sipilä TP, Kristiansson K, Donner KM, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. Nature. 2023;613:508–18.
7. Toivonen J, Castrén J, FinnGen AM. The value of genetic data from 665,460 individuals in predicting anemia and suitability to donate blood. Genet Epidemiol. 2022;46:477.
8. Lundberg SM, Lee S-I. A unified approach to interpreting model predictions. Adv Neural Inf Proces Syst. 2017;30.
9. Toivonen J, Koski Y, Turkulainen E, Prinsze F, Della Briotta Parolo P, Heinonen M, et al. Prediction and impact of personalized donation intervals. Vox Sang. 2022;117:504–12.
10. Översti S, Majander K, Salmela E, Salo K, Arppe L, Belskiy S, et al. Human mitochondrial DNA lineages in Iron-Age Fennoscandia suggest incipient admixture and eastern introduction of farming-related maternal ancestry. Sci Rep. 2019;9:16883.
11. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature. 2020;581:434–43.
12. Janssen MP. Why the majority of on-site repeat donor deferrals are completely unwarranted…. Transfusion. 2022;62:2068–75.

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

In vitro comparison of cold-stored whole blood and reconstituted whole blood

Sanna Susila[1,2] | Tuukka Helin[3] | Jouni Lauronen[2] | Lotta Joutsi-Korhonen[3] | Minna Ilmakunnas[2,4,5]

[1]Department of Anesthesiology and Intensive Care Medicine, Päijät-Häme Central Hospital, Lahti, Finland

[2]Finnish Red Cross Blood Service, Helsinki, Finland

[3]Department of Clinical Chemistry, HUS Diagnostic Center, Helsinki University Hospital and University of Helsinki, Helsinki, Finland

[4]Meilahti Hospital Blood Bank, Department of Clinical Chemistry, HUS Diagnostic Center, Helsinki University Hospital and University of Helsinki, Helsinki, Finland

[5]Department of Anesthesiology and Intensive Care Medicine, Helsinki University Hospital and University of Helsinki, Helsinki, Finland

Correspondence
Sanna Susila, Department of Anesthesiology and Intensive Care Medicine, Päijät-Häme Central Hospital, Keskussairaalankatu 7, 15850 Lahti, Finland.
Email: sanna.l.susila@gmail.com

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Abstract

Background and Objectives: Cold-stored whole blood (CSWB) is increasingly used in damage control resuscitation. Haemostatic function of CSWB seems superior to that of reconstituted whole blood, and it is sufficiently preserved for 14–21 days. To provide evidence for a yet insufficiently studied aspect of prehospital CSWB use, we compared in vitro haemostatic properties of CSWB and currently used in-hospital and prehospital blood component therapies.

Materials and Methods: Blood was obtained from 24 O RhD positive male donors. Three products were prepared: CSWB, in-hospital component therapy (red blood cells [RBCs], OctaplasLG and platelets 1:1:1) and prehospital component therapy (RBCs and lyophilized plasma 1:1). Samples were drawn on days 1 and 14 of CSWB or RBC cold storage. On day 14, platelet concentrates at their expiry (5 days) were used for 1:1:1 mixing. Conventional clotting assays, rotational thromboelastometry, thrombin formation and platelet function were assessed.

Results: Haemoglobin, platelet count, fibrinogen and coagulation factor levels remained closest to physiological in CSWB. Factor VIII activity decreased markedly by day 14 in CSWB. The decline in platelet function was prominent in CSWB. However, CSWB on day 14 yielded physiological EXTEM MCF, suggesting haemostatically sufficient platelet function. Despite haemodilution and lower coagulation factor levels, in-hospital component therapy was haemostatically adequate. Prehospital component therapy formed the weakest clots. Thrombin formation potential remained comparable and stable in all groups.

Conclusion: Current prehospital component therapy fails to offer the clotting potential that CSWB does. CSWB and current in-hospital 1:1:1 component therapy show similar haemostatic potential until 14 days of storage.

Keywords

haemostasis, lyophilized plasma, platelet function, reconstituted whole blood, whole blood, whole blood storage

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Highlights

- Cold-stored whole blood retains physiological coagulation factor levels during storage, except for factor VIII and von Willebrand factor activities.
- Platelet count and function declined during storage but remained haemostatically sufficient in cold-stored whole blood.
- Although coagulation factor levels were lower in reconstituted whole blood, it provides overall similar haemostatic potential to cold-stored whole blood.

INTRODUCTION

The most common preventable cause of death of trauma patients is massive haemorrhage [1]. Most deaths occur before reaching the hospital. A fifth of all trauma deaths could be prevented by better haemostatic control [1]. In developed countries, prehospital blood products are routinely used in damage control resuscitation (DCR). Blood products, typically red blood cells (RBCs) and fresh frozen plasma or lyophilized plasma (LP), may decrease mortality, although data are contradictory [2, 3].

Cold-stored whole blood (CSWB) has gained increasing interest in civilian DCR. CSWB, with easier logistics and thus potentially faster transfusions, can provide haemostatically active cold-stored platelets for prehospital transfusions. CSWB, compared to component therapy, contains less anticoagulant and additive solutions and may, therefore, reduce haemodilution in massive transfusion. CSWB may thus be superior to conventional component therapy in DCR. However, mortality and morbidity data are lacking as randomized controlled trials are still underway [4–6].

In previous studies [7–13], haemostatic function of CSWB has been superior to that of reconstituted whole blood (RWB) and sufficiently preserved up to 14–21 days. However, to the best of our knowledge, no studies have compared the haemostatic properties of CSWB to current prehospital and in-hospital component therapies for DCR. Here, prior to introducing CSWB to clinical use, we compared in vitro haemostatic properties of CSWB and the current component therapies used in massive haemorrhage.

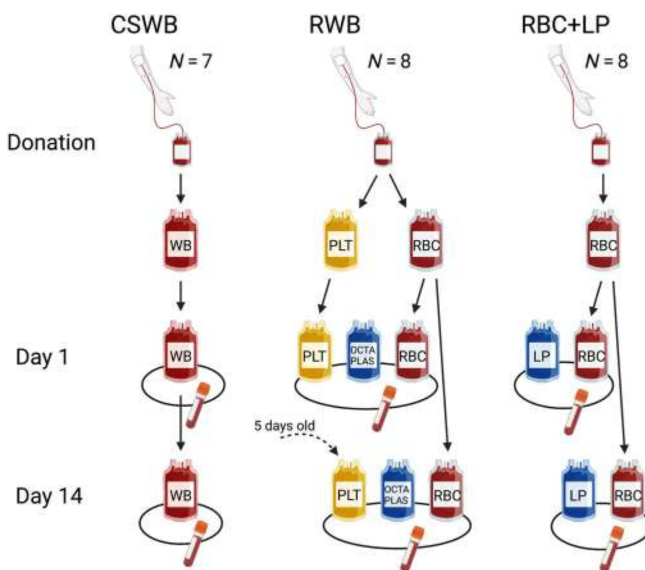

FIGURE 1 Preparation of blood products. Donated whole blood was leukoreduced 18–24 h after donation. Cold-stored whole blood (CSWB) and packed red blood cells (RBC) were stored at +5°C and PLT at +22°C. Unit volumes: CSWB 445 mL, RBC 260 mL, LP 200 mL, OctaplasLG® 200 mL and PLT 244 mL. Created with BioRender.com. LP, lyophilized plasma; PLT, buffy coat platelets; RWB, reconstituted whole blood, WB, whole blood.

MATERIALS AND METHODS

Twenty-four adult male O RhD positive regular donors were recruited through Finnish Red Cross Blood Service (FRCBS) electronic newsletter for blood donors. Standard FRCBS donor eligibility criteria were used, including haemoglobin level above 135 g/L. Use of non-steroidal anti-inflammatory drugs and herbal medications was prohibited 2 weeks prior to donation. Blood was collected following FRCBS standard procedures.

The study was approved by the ethics committee for Helsinki and Uusimaa Hospital District (HUS/699/2021). All participants signed an informed consent prior to donation.

Blood product preparation

We compared three different products (Figure 1), with eight donations allocated to each group: CSWB group, RWB mimicking hospital massive transfusion protocol with 1:1:1 blood product ratio (RWB group) and RBCs and LP with 1:1 ratio used in prehospital setting (RBC+LP group). To determine baseline parameters, control samples were drawn from eight random study participants before donation. All donated units were stored at +22°C prior to processing.

In the CSWB group, whole blood was collected with a Terumo Imuflex® WB-SP collection set (Terumo Europe N.V., Leuven, Belgium) containing 63 mL of citrate phosphate dextrose (CPD). Whole blood was leukoreduced 18–24 h after donation with a platelet-sparing filter. One CSWB donation coagulated in the collection bag due to erroneous placing of the bag on the mixer scale and was discarded. CSWB units were stored at +5°C after leukoreduction.

In the RWB and RBC+LP groups, whole blood was collected with Fresenius CompoFlow® Quadruple T&B collection set (Fresenius Kabi AG, Bad Homburg, Germany) containing 63 mL of CPD and 100 mL of SAGM. Whole blood was leukoreduced 18–24 h after donation with a platelet-sparing filter, then separated into RBC, plasma and buffy coat. RBC units were stored at +5°C. Plasma was discarded, as only solvent/detergent-treated pooled plasma is used in Finland.

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Buffy coats from four donors were pooled to form one platelet unit, stored in PAS-IIIM at +22°C.

In the RWB group, RBCs were mixed with blood group AB OctaplasLG® (Octapharma Nordic AB, Vantaa, Finland) and platelets in 1:1:1 ratio. On day 1 (d1), platelets were from the donations for this study. On day 14 (d14), platelets were O RhD positive buffy coat platelets from FRCBS surplus stock at unit expiry (5 days or within 12 h afterwards). In the RBC+LP group, RBCs were mixed with single-donor blood group AB LP (LyoPlas N-w®, DRK-Blutspendedienst West, Hagen, Germany) in 1:1 ratio.

Blood sampling and laboratory analyses

Control samples were drawn before blood donation to tubes with appropriate anticoagulants (BD Vacutainer® 3.2% Citrate and BD Vacutainer K2 EDTA, Becton Dickinson Finland, Vantaa, Finland; Vacuette Lithium Heparin, Greiner Bio-One GmbH, Kremsmünster, Austria). Samples from CSWB, RWB and RBC+LP groups were collected in test tubes without anticoagulants (Vacuette® Z No Additive, Greiner Bio-One GmbH, Kremsmünster, Austria).

Samples for laboratory analyses were obtained on d1 and d14 after donation. Samples were analysed within 3 h from collection. Citrated blood was centrifuged at 2500 g for 10 min before coagulation assays. For thrombin generation assay, citrated plasma was additionally centrifuged at 2500 g for 10 min, aliquoted and frozen at −80°C until analysis.

The blood count was analysed using Sysmex XN-9000® analyser (Sysmex Corporation, Kobe, Japan). Coagulation tests were done using routine analysers and methods; for fibrinogen, prothrombin time (PT), activated partial thromboplastin time (APTT), factor (F)V, FVIII, FIX, antithrombin, thrombin time (TT), D-dimer and von Willebrand factor glycoprotein Ib activity (VWF:Act) and antigen (VWF:Ag) with ACL TOP 500® (Instrumentation Laboratory, Werfen, Barcelona, Spain), for FII, FVII, FX, FXI, FXII, protein C, protein S free antigen with BCS XP® (Siemens Healthineers, Erlangen, Germany).

Platelet function analyser (PFA-200®, Siemens Healthineers, Erlangen, Germany) assay was performed from whole blood with collagen/epinephrine (PFA-EPI) and collagen/ADP (PFA-ADP) cuvettes in duplicate.

Multiple electrode aggregometry (MEA) for adenosine diphosphate (ADPtest) at 6.5 μM and thrombin receptor associated peptide-6 (TRAPtest) at 32 μM was done in duplicate from whole blood with Multiplate® analyser (Roche, Basel, Switzerland).

Thromboelastometry was done from whole blood using ROTEM® sigma analyser (Werfen, Barcelona, Spain), with cassettes containing extrinsic pathway (EXTEM), intrinsic pathway (INTEM) and fibrin formation (FIBTEM) tests. Clotting time (CT), clot formation time (CFT), amplitude at 5 min (A5), maximum clot firmness (MCF) and lysis index at 30 min (LI30) were reported.

Calibrated automated thrombogram® (CAT, Diagnostica Stago, Asnieres, France) was performed with 5 pM tissue factor, without thrombomodulin addition. Lag time, time to peak, thrombin peak and endogenous thrombin potential (ETP) were reported.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics, version 26. Non-parametric tests were used. As the control samples were not drawn from all donors, controls were treated as an independent group in statistical analyses. To quantify differences between the groups, ratios of group medians were calculated. Differences between the groups were tested with Kruskal–Wallis test. Time-dependent changes within groups were tested with Wilcoxon signed-rank test. Bonferroni correction was used to control the familywise error rate. A p-value of <0.05 was considered statistically significant.

RESULTS

Haemoglobin and haematocrit levels

Compared to controls, haemoglobin and haematocrit levels on d1 in the CSWB group were similar ($p = 0.094$), and in the RWB and RBC+LP groups significantly lower ($p < 0.001$) (Figure 2, Table S1). In the CSWB group, haemoglobin and haematocrit remained steady, and no clinically relevant changes occurred during storage. In the RWB and RBC+LP groups, haemoglobin concentrations and haematocrits were similar on d1 and d14 and were significantly higher in the CSWB group than in the RWB group.

Plasma coagulation factor levels

Compared to controls, coagulation factor levels on d1 in the CSWB group were similar ($p = 0.305$–0.903), except for lower FVIII activity ($p = 0.042$) (Figure 2, Table S2). All coagulation factor levels on d1 were significantly lower in the RWB ($p \leq 0.010$) and RBC+LP ($p \leq 0.001$) groups than in the controls.

In the CSWB group, coagulation factor levels decreased during storage, reflected in significantly decreased PT and increased APTT (Figure 2, Table S1). The storage effect was most prominent in FVIII levels (median 87 IU/dL on d1 and 43 IU/dL on d14, $p = 0.018$), which, in turn, prolonged APTT (Figure 2, Table S2). Fibrinogen level decreased during storage, but remained normal. Although FXII, FXI, FX, FV and FII levels decreased during storage, they remained within physiological range (Table S2). In the RWB and RBC+LP groups, the variation in coagulation factor levels between d1 and d14 was minimal. In the RBC+LP group, the FVIII level was significantly lower and thus APTT longer on d14 (Figure 2, Table S2). This was possibly due to variation in the single-donor LP composition.

Both on d1 and d14, coagulation factor levels were generally two-fold higher in the CSWB group than in the RWB and RBC+LP

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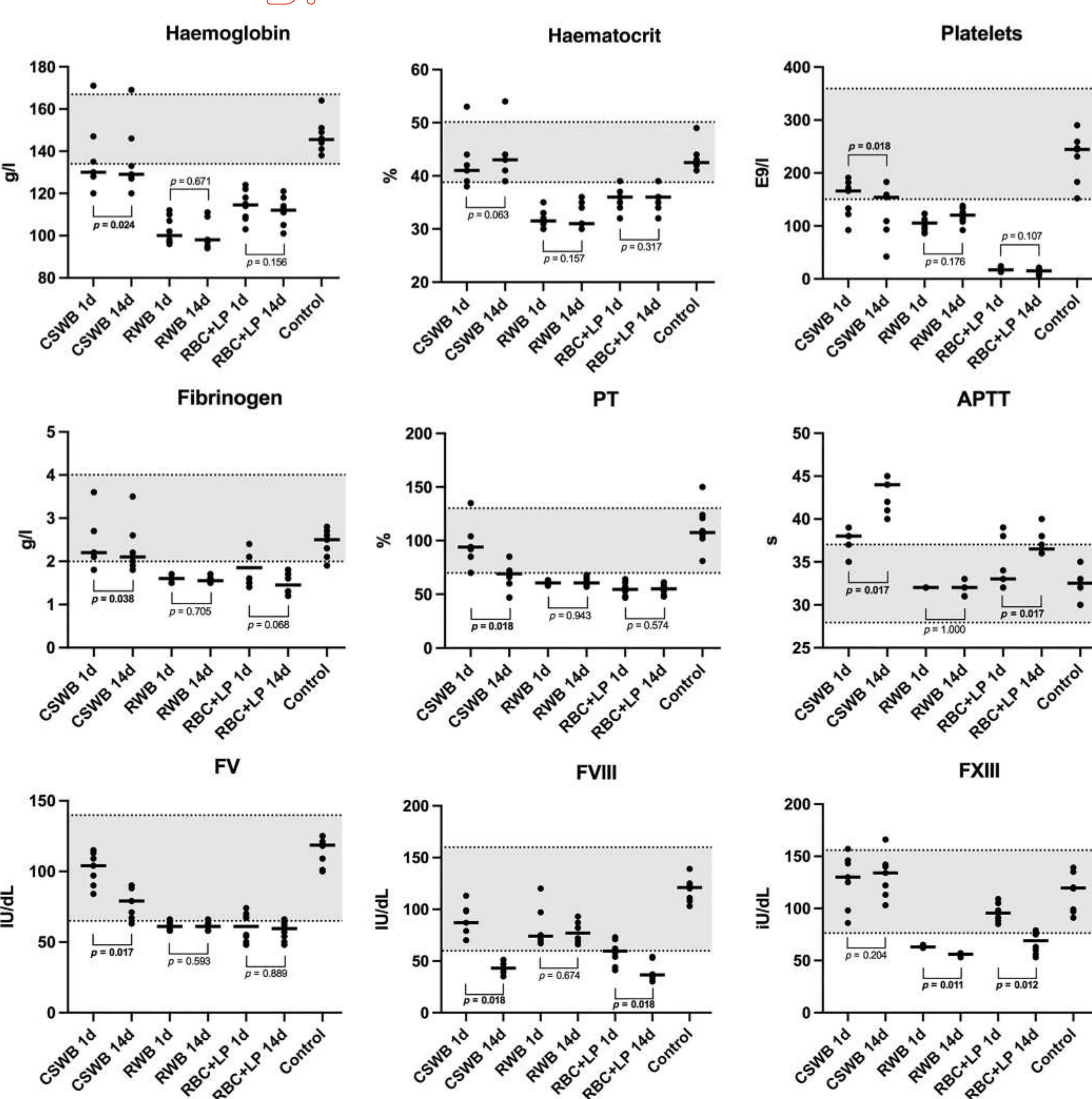

FIGURE 2 Blood count and coagulation assays. Individual measurements with median are shown. Laboratory reference range is shown in grey. Statistical comparison between groups is shown in Tables S1 and S2. CSWB, cold-stored whole blood; RBC+LP, packed red blood cells and lyophilized plasma; RWB, reconstituted whole blood.

groups (Figure 2, Table S2). In these groups, coagulation factor levels were approximately 50–70 IU/dL and thus below the physiological range. FVIII levels were significantly higher in the RWB group than in the RBC+LP group both on d1 ($p = 0.036$) and d14 ($p < 0.001$).

Thrombin generation

Compared to controls, CAT parameters on d1 in the CSWB group were similar, except for longer time to peak ($p = 0.010$) (Figure 3). In the RWB and RBC+LP groups, CAT parameters on d1 were comparable to controls, except for shorter lag time in the RBC+LP group ($p = 0.006$).

In the CSWB group, lag time increased during storage (Figure 3). Concomitantly, time to peak slightly decreased and peak height slightly increased. However, ETP in the CSWB group was similar to that in controls and did not change during storage, indicating overall normal thrombin generation in CSWB. In the RWB and RBC+LP groups, the variation in CAT parameters between d1 and d14 was minimal (Figure 3).

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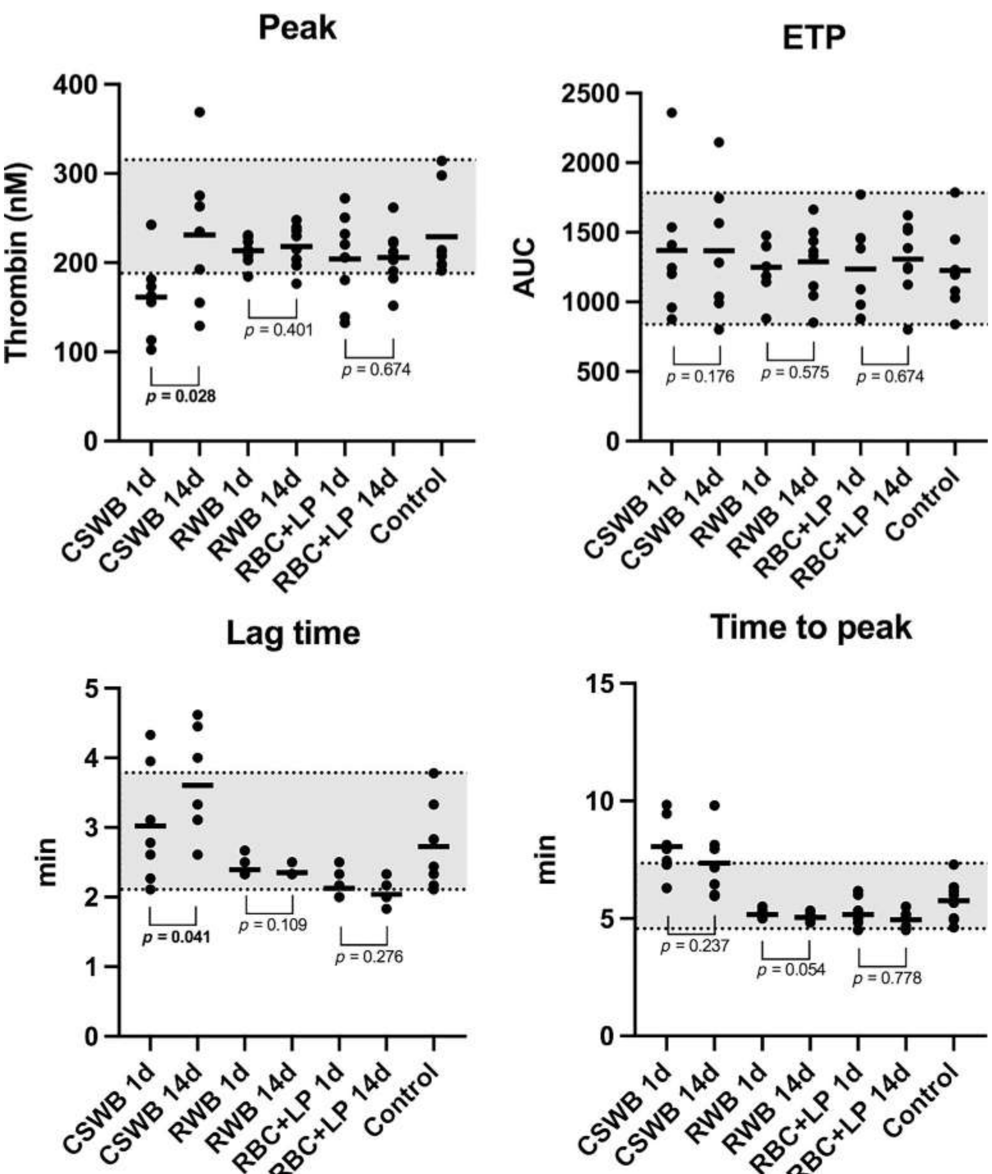

FIGURE 3 Thrombin generation assessed with Calibrated automated thrombogram. Individual measurements with median are shown. Control sample range is shown in grey. AUC, area under the curve; CSWB, cold-stored whole blood; ETP, endogenous thrombin potential; RBC +LP, packed red blood cells and lyophilized plasma; RWB, reconstituted whole blood.

Both on d1 and d14, time to peak was significantly longer in the CSWB group than in the RWB ($p = 0.007$ and $p = 0.004$, respectively) and RBC+LP ($p = 0.001$ and $p = 0.002$, respectively) groups (Figure 3). Likewise, on d14, lag time was significantly longer in the CSWB group than in the RWB ($p = 0.038$) and RBC+LP ($p < 0.001$) groups. However, ETP was similar in all groups on d1 and d14, indicating similar thrombin generation.

Viscoelastic properties

Compared to controls, EXTEM CT ($p < 0.001$) and INTEM CT ($p = 0.011$) on d1 were longer in the CSWB group (Figure 4). In the RWB and RBC+LP groups, viscoelastic properties were generally inferior compared to controls ($p \le 0.018$, except for EXTEM CT in the RWB group $p = 0.168$).

In the CSWB group, clotting times and EXTEM CFT increased during storage (Figure 4, Table 1). EXTEM A5 decreased significantly, but FIBTEM A5 and EXTEM MCF were comparable on d1 and d14. In the RWB group, EXTEM A5 and EXTEM MCF increased during storage. FIBTEM A5 was comparable on d1 and d14. In the RBC+LP group, clotting times increased and clot strength decreased during storage. These effects were likely due to variation in the single-donor LP composition.

Both on d1 and d14, EXTEM CT was longer in the CSWB than in the RWB group, and on d14 also, EXTEM CFT was longer (Figure 4, Table 1). EXTEM A5 and EXTEM MCF were the lowest in the RBC +LP group, explained by the fact that RBC+LP samples contained only some residual platelets.

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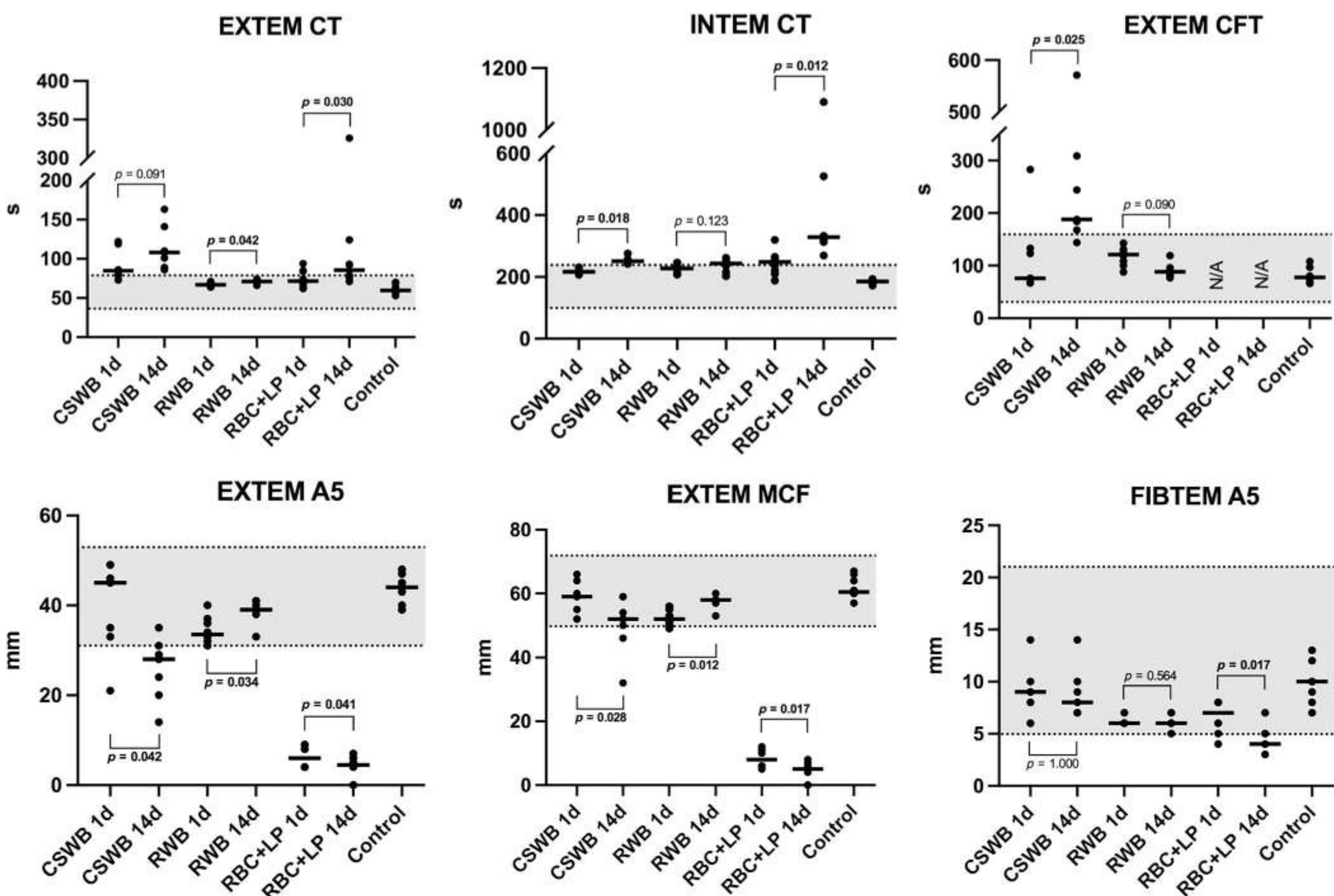

FIGURE 4 Viscoelastic properties assessed with rotational thromboelastometry (ROTEM). Individual measurements with median are shown. Laboratory reference range is shown in grey. Statistical comparison between groups is shown in Table 1. CFT, clot formation time; CSWB, cold-stored whole blood; CT, clotting time; RBC+LP, packed red blood cells and lyophilized plasma; RWB, reconstituted whole blood.

Platelet counts, function and VWF levels

Compared to controls, platelet count on d1 was lower in both the CSWB ($p = 0.011$) and RWB ($p < 0.001$) groups (Figure 2), whereas VWF:Ag and VWF:Act were similar (Table 2, Table S1). Compared to controls, platelet function on PFA and MEA on d1 was reduced both in the CSWB ($p \le 0.029$) and RWB ($p < 0.001$) groups. Interestingly, in controls, collagen/ADP closure time was slightly above laboratory reference.

Platelet count in the CSWB group decreased during storage but remained at an adequate haemostatic level (Figure 2, Table S1). Both on d1 and d14, platelet counts were slightly, yet not significantly, higher in the CSWB than in the RWB group (Figure 2, Table S1). In the RWB group, platelet counts achieved adequate haemostatic levels (>100 × 10E9/L) both on d1 and d14.

The platelet function declined significantly during storage in the CSWB group. In PFA, collagen/ADP closure time prolonged (Table 2). In MEA, the platelet function in both ADP and TRAP channels declined. In the RWB group, storage had no effect on the platelet function. On d1, the platelet function was comparable in CSWB and RWB groups. On d14, the platelet function was better preserved in the RWB than in the CSWB group.

VWF:Ag levels remained stable during storage in the CSWB group, whereas VWF:Act decreased significantly (Table 2). In the RWB group, both VWF:Ag and VWF:Act remained stable during storage. Both on d1 and d14, VWF:Ag levels were slightly, yet not significantly, higher in the RWB group than in the CSWB group. On d14, VWF:Act was higher in the RWB group.

DISCUSSION

CSWB is considered a superior prehospital blood product due to the availability of platelets and easier logistics. Most in vitro research has focused on how storage time influences CSWB haemostatic potential. To the best of our knowledge, only a few such studies compare CSWB and RWB, and none compare CSWB and the current common prehospital practice of RBC+LP. We, therefore, studied the haemostatic potential of CSWB, RWB and RBC+LP with up to 14 days of CSWB and RBC storage time.

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TABLE 1 Rotational thromboelastometry (ROTEM) results.

	Parameter	Laboratory reference range	Controls	CSWB	RWB	RBC+LP	Differences between groups: CSWB versus RWB	CSWB versus RBC+LP	RWB versus RBC+LP
Day 1	EXTEM CT (s)	38–79	60 (57–68)	85 (79–119)	67 (64–70)*	68 (63–84)*	1.27††	1.25	0.99
	EXTEM CFT (s)	34–159	78 (68–93)	76 (68–133)	121 (102–130)*	N/A	0.63	N/A	N/A
	EXTEM A5 (mm)	31–53	44 (41–47)	45 (33–49)*	34 (32–37)*	8 (4–9)*	1.34	5.63†††	4.19††
	EXTEM MCF (mm)	50–72	61 (60–66)	59 (52–64)*	52 (50–55)*	11 (6–11)*	1.13	5.62†††	4.95†
	EXTEM LI30 (%)	<15	100 (100–100)	100 (100–100)	100 (100–100)	100 (100–100)	1.00	1.00	1.00
	FIBTEM A5 (mm)	5–21	10 (8–12)	9 (6–10)	6 (6–6)	8 (5.5–8)*	1.67†	1.25	0.75
	INTEM CT (s)	100–240	185 (177–191)	217 (216–219)*	228 (211–241)	230 (210–260)*	0.95	0.94	0.99
Day 14	EXTEM CT (s)			108 (89–141)	71 (71–72)*	79 (75–92)*	1.51††	1.37	0.9†
	EXTEM CFT (s)			188 (168–309)	89 (82–95)*	N/A	2.12††	N/A	N/A
	EXTEM A5 (mm)			28 (20–31)*	39 (38–40)*	5 (4–6)*	0.72	5.60	7.8†††
	EXTEM MCF (mm)	See above	See above	52 (46–59)*	58 (57–58)*	6 (5–7)*	0.90	9.45†	10.55†††
	EXTEM LI30 (%)			100 (100–100)	100 (100–100)	100 (100–100)	1.00	1.00	1.00
	FIBTEM A5 (mm)			8 (7–10)	6 (5.3–6.8)	4.5 (4–5.5)*	1.33	1.78†††	1.33
	INTEM CT (s)			251 (248–256)*	244 (226–253)	320 (313–333)*	1.03	0.79†	0.76†††

Note: Median values and interquartile range are shown. Values outside laboratory reference are in grey. * $p < 0.05$ between days 1 and 14 within each group. Differences between groups are shown as a ratio of medians. $^{†}p < 0.05$, $^{††}p < 0.01$ and $^{†††}p < 0.001$ (Bonferroni corrected) between the groups at each time point.

Abbreviations: CFT, clot formation time; CSWB, cold-stored whole blood; CT, clotting time; MCF, maximum clot firmness; N/A, not applicable; RBC+LP, packed red blood cells and lyophilized plasma; RWB, reconstituted whole blood.

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TABLE 2 Platelet function assessed by platelet function analyser (PFA), multiple electrode aggregometry (MEA) and VWF assays.

	Parameter	Laboratory reference range	Controls	CSWB	RWB	CSWB versus RWB
Day 1	PFA ADP (s)	62–100	106 (89–110)	150 (126–165)*	>300 (>300–>300)	N/A[†††]
	PFA EPI (s)	82–150	118 (114–144)	>300 (270–>300)	>300 (>300–>300)	N/A
	Multiplate ADP (U)	40–110	67 (62–80)	19 (11–22)*	14 (12–20)	1.36
	Multiplate TRAP (U)	70–130	92 (88–99)	30 (16–43)*	23 (18–30)	1.33
	VWF:Ag (IU/dL)	50–190	110 (98–112)	99 (90–118)	119 (114–120)	0.83
	VWF:Act (IU/dL)	50–190	97 (84–104)	87 (75–99)*	87 (85–88)*	1.01
Day 14	PFA ADP (s)			>300 (>300–>300)*	>300 (>300–>300)	N/A
	PFA EPI (s)			>300 (>300–>300)	>300 (>300–>300)	N/A
	Multiplate ADP (U)	See above	See above	5 (4–11)*	11 (7–17)	0.48
	Multiplate TRAP (U)			10 (4–16)*	25 (21–28)	0.40[††]
	VWF:Ag (IU/dL)			99 (85–110)	118 (117–119)	0.84
	VWF:Act (IU/dL)			54 (42–58)*	93 (90–97)*	0.58[††]

Note: Median values and interquartile range are shown. Values outside laboratory reference are in grey. *($p < 0.05$) between days 1 and 14 within each group. Comparison between groups is shown as ratio of medians. [††] ($p < 0.01$) and [†††]($p < 0.001$) between the groups at each time point.
Abbreviations: ADP, adenosine diphosphate; CSWB, cold-stored whole blood; EPI, epinephrine; N/A, not applicable; RWB, reconstituted whole blood; TRAP, thrombin receptor activating peptide-6; VWF:Act, von Willebrand factor activity; VWF:Ag, von Willebrand factor antigen.

In line with previous data [14], we demonstrated that platelets are essential for well-maintained in vitro haemostatic function. CSWB platelet count depends primarily on storage time and leukoreduction, and in the latter case, whether a platelet-sparing filter was used [7–10, 12, 14–18]. In our study, median platelet count was slightly higher in CSWB (leukoreduced with a platelet-sparing filter) than in RWB despite the decrease in platelet count in CSWB over time. Due to the single-donor nature of CSWB, the variance in platelet count is wide and CSWB units may display divergent haemostatic function, which may be clinically relevant.

Platelet function was abnormal in both CSWB and RWB groups. As fibrinogen concentration and FIBTEM A5 remained stable throughout storage in CSWB, the observed increase in EXTEM CFT and decrease in EXTEM A5, both clearly outside the reference range on day 14, are likely due to impaired platelet function in CSWB. Indeed, in line with previous studies [9, 10, 16, 18], both ADP- and TRAP-induced platelet aggregation in CSWB decreased already during 14-day storage. As platelets in CSWB are exposed to cold storage, this impaired aggregation is seemingly in contradiction with increased platelet activation and enhanced haemostatic capacity of cold-stored platelet concentrates [19, 20], but in accordance with the finding of attenuated aggregation responses to ADP and TRAP in CSWB as compared to cold-stored platelet concentrates [10]. This implies that other CSWB components, namely, RBCs and plasma, may influence platelet functionality. We observed a significant decrease in VWF activity, which possibly contributed to decreased CFT and firmness (EXTEM A5) through impaired platelet-to-platelet adhesion. Nevertheless, the platelet function in CSWB seemed haemostatically sufficient, as MCF remained within the physiological range throughout storage. This also supports that, instead of measuring coagulation factor concentrations or platelet function separately, whole blood-based assays such as viscoelastic tests probably give the most accurate estimate of the total CSWB haemostatic capacity.

Although CSWB can provide platelets for prehospital DCR, platelet concentrates are commonly used for in-hospital DCR. We demonstrated that platelet count in RWB, in contrast to CSWB, was more uniform and achieved good haemostatic levels (median 121 $\times$ 10E9/L) even when RWB was prepared with platelets at the end of their shelf life. Interestingly, in RWB with stable fibrinogen content and older platelets, we detected enhanced CFT and MCF, suggesting platelet activation during traditional storage contributed to enhanced haemostasis [21].

An unwanted but unavoidable by-product of blood product storage is dilution due to anticoagulant and additive solutions. We demonstrated that CSWB, with retained physiological haematocrit, is less diluted than in-hospital (RWB) or prehospital (RBC+LP) component therapy. Accordingly, despite individual variation in CSWB units, coagulation factor levels were generally twice as high in CSWB than in RWB or RBC+LP. However, both in the RWB and RBC+LP groups, fibrinogen concentration, APTT and PT were within the currently recommended levels in traumatic bleeding [22]. FVIII is labile and, affirming previous studies [8, 12, 14, 15, 23], decreased considerably during CSWB storage. This, and the decrease in other intrinsic pathway coagulation factors, was reflected in prolonged APTT, INTEM CT and thrombin generation lag time. Despite this, overall thrombin generation in CSWB remained robust and similar to that of RWB and RBC+LP. Finally, FXIII and natural anticoagulants were well preserved in CSWB compared to RWB and RBC+LP, which may be beneficial in maintaining the stability of the formed clots and limiting hypercoagulation after traumatic bleeding.

Blood group affects haemostasis, a feature largely undiscussed in the CSWB literature. Surprisingly, few in vitro studies clearly state having used universal donor group O CSWB [8, 9, 11, 14]. Group O

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blood has lower VWF [24] and FVIII [25] levels and longer PFA closure times [26]. Indeed, this effect on the platelet function is evident in our control group, where ADP response in PFA was attenuated. The clinical haemostatic effects of transfusing group O, as opposed to group-specific, CSWB remain unclear.

There are several limitations to this study. First, the storage time was only 14 days. Although we show that CSWB retains haemostatic potential for at least 14 days, the effects of continued storage up to 21–28 days cannot be predicted. Studies with longer storage times have reported stable haemoglobin and fibrinogen concentrations but continued decrease in platelet count and function and clot firmness after 14 days [7–10, 12, 15–17]. Based on these studies, a CSWB shelf life of 14 [9] to 21 [7, 10] days has been suggested. The length of storage in this study was based on the planned 14-day storage time for the FRCBS CSWB product. Second, the sample size of eight donors per group is relatively small but likely sufficient to detect major differences between groups and time points. We considered it unethical to recruit a large number of donors for research purposes only. The small sample size is reflected in the marked variation in especially the CSWB platelet count. However, this accurately mimics clinical transfusion of non-pooled blood products, where the achieved response may vary significantly between units. A third, intrinsic limitation is that in vitro conditions do not imitate in vivo circumstances. Effects of acidosis, hypothermia, inflammation and endotheliopathy, all important in haemostasis and trauma-induced coagulopathy, were not addressed in this study. Also, post-transfusion platelet function remains unclear, as storage-induced platelet dysfunction may be restored after transfusion [21]. Similarly, this study cannot elucidate whether the patient's own platelets along with transfused RBC and LP are able to support haemostasis sufficiently.

Taken together, our study suggests that the coagulation properties of CSWB are superior to those of RWB or RBC+LP and supports its use in prehospital care where platelets are otherwise unavailable. Leukoreduced CSWB retains its haemostatic function over storage time of 14 days and is comparable with that of RWB. After initial DCR in prehospital care, in-hospital RWB seems a viable option when continued blood product use is warranted.

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S.S., J.L. and M.I. designed the study. J.L. recruited the blood donors. S.S. and M.I. collected the blood samples and data and wrote the paper. S.S. analysed the data. T.H., L.J.-K. and M.I. designed the laboratory assessments. J.L., T.H. and L.J.-K. reviewed the article.

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 from the corresponding author upon reasonable request.

ORCID

Sanna Susila https://orcid.org/0000-0001-5131-0656
Minna Ilmakunnas https://orcid.org/0000-0002-8469-4091

REFERENCES

1. Spinella PC, Cap AP. Prehospital hemostatic resuscitation to achieve zero preventable deaths after traumatic injury. Curr Opin Hematol. 2017;24:529–35.
2. Guyette FX, Sperry JL, Peitzman AB, Billiar TR, Daley BJ, Miller RS, et al. Prehospital blood product and crystalloid resuscitation in the severely injured patient: a secondary analysis of the prehospital air medical plasma trial. Ann Surg. 2021;273:358–64.
3. Crombie N, Doughty HA, Bishop JRB, Desai A, Dixon EF, Hancox JM, et al. Resuscitation with blood products in patients with trauma-related haemorrhagic shock receiving prehospital care (RePHILL): a multicentre, open-label, randomised, controlled, phase 3 trial. Lancet Haematol. 2022;9:e250–61.
4. Dishong D, Cap AP, Holcomb JB, Triulzi DJ, Yazer MH. The rebirth of the cool: a narrative review of the clinical outcomes of cold stored low titer group O whole blood recipients compared to conventional component recipients in trauma. Hematology. 2021;26:601–11.
5. Hazelton JP, Ssentongo AE, Oh JS, Ssentongo P, Seamon MJ, Byrne JP, et al. Use of cold-stored whole blood is associated with improved mortality in hemostatic resuscitation of major bleeding: a multicenter study. Ann Surg. 2022;276:579–88.
6. Spinella PC, Pidcoke HF, Strandenes G, Hervig T, Fisher A, Jenkins D, et al. Whole blood for hemostatic resuscitation of major bleeding. Transfusion. 2016;56:S190–202.
7. McRae HL, Kara F, Milito C, Cahill C, Refaai MA. Whole blood haemostatic function throughout a 28-day cold storage period: an in vitro study. Vox Sang. 2021;116:190–6.
8. Schubert P, Culibrk B, Devine DV, Chen Z, Wambolt R, Mctaggart K, et al. Cold-stored leukoreduced whole blood: extending the time between donation and filtration has minimal impact on in vitro quality. Transfusion. 2021;61:S131–43.
9. Assen S, Cardenas J, George M, Wang YW, Wade CE, Meyer D, et al. Hemostatic potential of cold-stored non-leukoreduced whole blood over time: an assessment of platelet function and thrombin generation for optimal shelf life. J Trauma Acute Care Surg. 2020;89:429–34.
10. Huish S, Green L, Kempster C, Smethurst P, Wiltshire M, Prajapati C, et al. A comparison of platelet function in cold-stored whole blood and platelet concentrates. Transfusion. 2021;61:3224–35.
11. Santangini MR, Leuckel SN, King KA, Cheves TA, Sweeney JD. In vitro comparison of CPD whole blood with conventional blood components. Transfus Apher Sci. 2023;62:1–6.
12. Wang S, Zhao G, Li N, Hou T, Shao X, Tang W, et al. An in vitro study of coagulation properties in refrigerated whole blood and reconstituted whole blood. Vox Sang. 2019;114:694–700.
13. Kornblith LZ, Howard BM, Cheung CK, Dayter Y, Pandey S, Busch MP, et al. The whole is greater than the sum of its parts: hemostatic profiles of whole blood variants. J Trauma Acute Care Surg. 2014;77:818–27.
14. Haddaway K, Bloch EM, Tobian AAR, Frank SM, Sikorski R, Cho BC, et al. Hemostatic properties of cold-stored whole blood leukoreduced using a platelet-sparing versus a non-platelet-sparing filter. Transfusion. 2019;59:1809–17.
15. Pidcoke HF, McFaul SJ, Ramasubramanian AK, Parida BK, Mora AG, Fedyk CG, et al. Primary hemostatic capacity of whole blood: a

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comprehensive analysis of pathogen reduction and refrigeration effects over time. Transfusion. 2013;53:137–49.
16. Meledeo MA, Peltier GC, McIntosh CS, Bynum JA, Cap AP. Optimizing whole blood storage: hemostatic function of 35-day stored product in CPD, CP2D, and CPDA-1 anticoagulants. Transfusion. 2019;59:1549–59.
17. Sivertsen J, Braathen H, Lunde THF, Kristoffersen EK, Hervig T, Strandenes G, et al. Cold-stored leukoreduced CPDA-1 whole blood: in vitro quality and hemostatic properties. Transfusion. 2020;60:1042–9.
18. Remy KE, Yazer MH, Saini A, Mehanovic-Varmaz A, Rogers SR, Cap AP, et al. Effects of platelet-sparing leukocyte reduction and agitation methods on in vitro measures of hemostatic function in cold-stored whole blood. J Trauma Acute Care Surg. 2018;84:S104–14.
19. Zhao H, Devine DV. The missing pieces to the cold-stored platelet puzzle. Int J Mol Sci. 2022;23:1100.
20. George CE, Saunders CV, Morrison A, Scorer T, Jones S, Dempsey NC. Cold stored platelets in the management of bleeding: is it about bioenergetics? Platelets. 2023;34:1-9.
21. Ng MSY, Tung JP, Fraser JF. Platelet storage lesions: what more do we know now? Transfus Med Rev. 2018;32:144–54.
22. Spahn DR, Bouillon B, Cerny V, Duranteau J, Filipescu D, Hunt BJ, et al. The European guideline on management of major bleeding and coagulopathy following trauma: fifth edition. Crit Care. 2019;23:98.
23. Feng L, Zhao Y, Zhao H, Shao Z. Effects of storage time and temperature on coagulation tests and factors in fresh plasma. Sci Rep. 2014;4:6–10.
24. Gill JC, Endres-Brooks J, Bauer PJ, Marks WJ, Montgomery RR. The effect of ABO blood group on the diagnosis of von Willebrand disease. Blood. 1987;69:1691–5.
25. Preston AE, Barr A. The plasma concentration of factor VIII in the normal population. Br J Haematol. 1964;10:238–45.
26. Cho YU, Chi HS, Jang S, Park CJ. Reconfirmation of preanalytical variables and establishment of reference intervals of platelet function analyzer-100 closure times in Korean adults. Korean J Lab Med. 2007;27:318–23.

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

Impact of donor ferritin testing on iron deficiency prevention and blood availability in France: A cohort simulation study

Pascale Richard[1] | Anne-Marie Fillet[1] | Lucile Malard[1] | Carole Leclerc[2] | Claire Chanut[3] | Geneviève Woimant[1] | Chantal Jacquot[2] | Henri Leleu[4] | Pascal Morel[1] | Alexandre Vimont[4]

[1]Medical Department, Etablissement Français du Sang, la Plaine Saint-Denis, France
[2]Blood Product Collection Department, Etablissement Français du Sang, la Plaine Saint-Denis, France
[3]Innovation and Projects Department, Etablissement Français du Sang, la Plaine Saint-Denis, France
[4]Public Health Expertise, Paris, France

Correspondence
Pascale Richard, Medical Department, Etablissement Français du Sang, 20 Avenue du Stade de France, 93218 la Plaine Saint-Denis, France.
Email: pascale.richard@efs.sante.fr

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Abstract

Background and Objectives: Implementing a ferritin testing policy for whole blood (WB) donors may prevent iron deficiency (ID, ferritin <26 ng/mL) and anaemia, but may induce donation losses. As part of a national prevention plan in France, we aimed to estimate its impact on ID, anaemias and WB donations among donors at high risk of ID.

Materials and Methods: A micro-simulation model was developed to evaluate different scenarios compared to the current situation without ferritin testing as a reference scenario. The following scenarios were simulated: a minimum scenario with a 6-month deferral for donors with absent iron store (AIS, ferritinemia <15 ng/ml), a main scenario with additional delayed invitations for donors with ferritinemia 15–25 ng/ml and a supplementation scenario with additional iron supplementation for 50% of the donors with AIS.

Results: In the main scenario, 52,699 WB donations per year were estimated to be lost after 1 year (−8%), falling to 27,687 (−4.7%) after 5 years. IDs and anaemias were reduced by 13.6% and 29.3%, respectively, after 1 year. The supplementation scenario increased the number of prevented IDs and anaemias to 24.1% and 35.4%, respectively, after 1 year, and halved the number of anaemias at 5 years. The latter scenario also had the least impact on the number of donations (−3.2% after 5 years).

Conclusion: A ferritin testing policy resulting in delayed donations for ID donors is effective in reducing IDs and anaemias, but significantly impacts the number of donations, thereby posing a self-sufficiency challenge.

Keywords

blood collection, blood donation testing, donor health

Highlights

- A ferritin testing policy resulting in delayed donations for iron-deficient donors is effective in reducing iron deficiencies (IDs) and anaemias.
- Iron supplementation in 50% of high-risk donors has a substantial positive impact.

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- Donations recovered from donors in whom ID and anaemia have been prevented do not compensate for the loss of donations resulting from extended inter-donation intervals, prolonged deferral periods and changes in donor behaviour.

INTRODUCTION

Approximately 3 million blood donations are carried out annually by Etablissement Français du sang (EFS) to treat 1 million patients. As whole blood (WB) donation leads to a 1 g/dl decrease of haemoglobin (Hb) levels [1], various measures are implemented in France to prevent anaemia in WB donors: (1) a mandatory 8-week inter-donation interval; (2) donations limited to four per year for women and six per year for men; (3) investigation into anaemia history and symptoms during the pre-donation interview; (4) pre-donation Hb testing in defined cases (initial donation or no donation in the previous 2 years, past donation Hb <12.5 g/dl for women or 13.5 g/dl for men, history or symptoms of anaemia) and (5) systematic post-donation Hb testing. Below a Hb threshold of 13 g/dl for males and 12 g/dl for females, defining anaemia, donors are deferred for 6 months. These thresholds are 0.5 g/dl below the European thresholds considering French West Indies donors whose Hb values are lower than those of the Caucasian populations [2].

Each donation of a WB unit results in an iron loss of 200–250 mg [1]. Iron stores are a key element in efficient erythropoiesis to compensate for red blood cell losses. Post-WB donation anaemia is due not only to direct Hb loss but primarily to iron loss.

The prevention of iron deficiency (ID), defined by ferritinemia <26 ng/ml [3], is expected to improve donor retention and donations.

The Canadian Blood Services and the Dutch national blood service Sanquin questioned blood product availability further to the implementation of a ferritin monitoring policy and subsequent donor deferral. The introduction of a ferritin monitoring policy to mitigate the effects of repeat donations on iron stores led to a significant decrease in donor availability and reduced donations in the Netherlands [4, 5]. A simulation model assessed the impact of ferritin testing on Canadian WB donors and concluded there would be a strong likelihood of a decline in WB donations [5].

ID in French WB donors was investigated in the FERRIDON research study. The prevalence was estimated to be 39.5% for women and 18.0% for men [6]. Four subgroups of donors at high risk of ID were determined (Table 1). Ferritin testing in these high-risk subgroups was implemented in early 2022 to further prevent ID in WB donors in France.

The aim of this study was to estimate in these subgroups the number of IDs and anaemias detected and the impact on the number of WB donations arising from these new measures with a specific focus on the long-term impact on WB availability to ensure national self-sufficiency.

MATERIALS AND METHODS

Overview

A patient-level micro-simulation model was designed using Visual Basic for Applications. The model was structured to run different scenarios focusing on WB donation, ID, absent iron store (AIS) and anaemia following the implementation of ferritin testing in the high-risk donor population. AIS was defined by ferritinemia <15 ng/ml. Donors with AIS were deferred for 6 months and advised to consult their general practioner (GP) for follow-up and possible iron supplementation. A moderate ID (15–25 ng/ml ferritinemia) led to delayed donation invitations, from 8 weeks to 3 months for male and to 4 months for female donors.

TABLE 1 Definition of groups of whole blood donors at high risk of iron deficiency (<26 ng/ml)

Group	Population	Definition of high-risk subgroups	Proportion among all donors (%)
G1	Repeat female donors	Women with last donation under 4.5 months ago, TCMH at last donation ≥30 pg and aged <31.5 years old	1.21
G2		Women with last donation under 4.5 months ago and TCMH at last donation <30 pg	9.65
G3	New female donors	Women aged <29.5 years old and with a pre-donation Hb level <13.7 g/dl	2.37
G4	Repeat male donors	Men with at least two donations within last year and: • Last donation under 3.5 months ago and TCMH at last donation <29.5 pg • Or last donation more than 3.5 months ago and TCMH at last donation <27.4 pg	7.23

Abbreviation: Hb, haemoglobin; TCMH, mean corpuscular hemogobin content.

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Several scenarios were compared, with and without ferritin testing, to determine the impact of changes in inter-donation interval and deferrals as a result of ferritin testing.

Simulation model

The micro-simulation model was an open cohort model comprising donors simulated individually and independently on a weekly basis. The model allowed new donors to enter the cohort and current donors to drop out of the cohort each week. Donors could exit the cohort if they did not return to donation before 24 months. Entry cohorts were assumed to encompass several subgroups of donors, such as repeated donors who transit from low-risk to high-risk, persistent AIS and drop-out donors returning to donation. Every new donor was assigned to a risk group and to different time-dependent states following the initial donation (at-risk for testing, ID, anaemia and drop out for the next donation). Pre-donation Hb testing was simulated, and donors with anaemia were deferred for 6 months. A fall in ferritin levels was assumed post-donation with a return to levels ≥26 ng/ml after several weeks, depending on risk factors for ID, such as gender, pre-donation ID and time to the next donation [7]; an ID occurred in the model when a donation was given before ferritin levels had returned to normal. Donors with anaemia were counted once, and the model did not count them for ID.

The model was structured with two loops: a weekly cohort loop, simulating new high-risk donors every week and updating results (Figure 1a), and an individual donor loop, simulating the course of donations and various time-dependent states (Figure 1b) for each donor. New high-risk donors were followed until their next donation, unless they dropped out and were included in the new weekly repeat donors for their additional donations thereafter. The model assessed total donations, total IDs at the time of donations and pre- and post-donation anaemias per week for each risk group over the model period for each scenario.

Once a donor was assigned to a risk group, time-dependent states, interval to the next donation and ferritin level recovery period were simulated. Donors no longer at risk for testing were kept in the cohort; the model accounted for their next donations so that they could switch to high-risk groups again at any time. Time-dependent states were simulated from the uniform distribution, donation intervals from gamma distributions and ferritin recovery periods from normal distributions (Table S1). An ID was assumed when the time to donation occurred before the ferritin recovery period. Two classes of ID were considered: <15 ng/ml and 15–25 ng/ml distributed according to the prevalence observed in the FERRIDON study. The ferritin recovery period was assumed to be longer for a level of ferritin <15 ng/ml compared to 15–25 ng/ml.

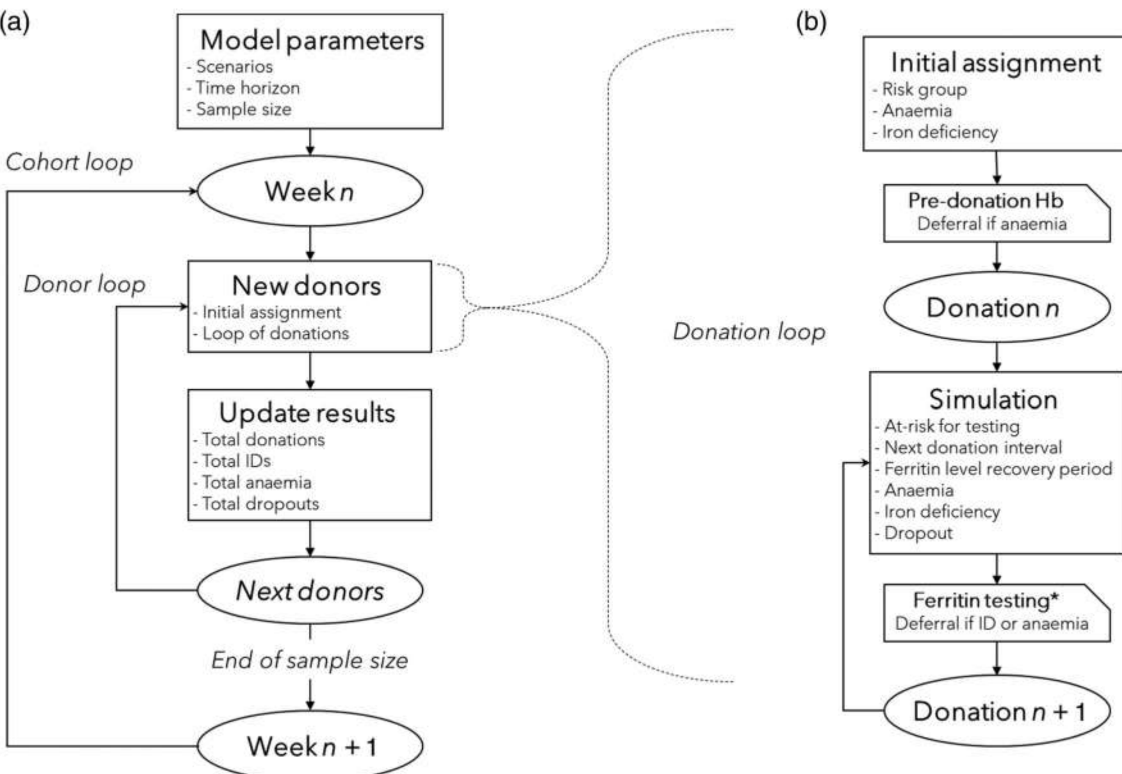

FIGURE 1 Structure of the patient-level micro-simulation model. *Only in scenarios with ferritin testing: <15 ng/ml: 6 months deferral. 15–25 ng/ml: informed by letter advising them to consult their GP and to postpone their next donation for at least 3 months for men and 4 months for women. Hb, haemoglobin; ID, iron deficiency

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TABLE 2 Clinical inputs from FERRIDON study

Variables	G1	G2	G3	G4
Prevalence of ID				
15–25 ng/ml	32.2%	26.0%	22.1%	25.5%
<15 ng/ml	38.9%	45.6%	24.0%	31.2%
Prevalence of anaemia at initial donation, by ID status				
15–25 ng/ml	6.6%	11.5%	5.7%	4.0%
<15 ng/ml	9.1%	16.5%	9.3%	6.8%
No ID	3.0%	2.0%	3.9%	0.5%
Prevalence of anaemia at subsequent donations, after ID				
15–25 ng/ml	0.0%	2.0%	7.1%	0.5%
<15 ng/ml	0.0%	2.0%	7.1%	0.5%
Drop out, by ID status				
No ID, no anaemia	35.0%	20.4%	56.6%	20.0%
15–25 ng/ml	35.0%	20.4%	56.6%	20.0%
<15 ng/ml	49.7%	32.1%	65.0%	27.8%
Anaemia	49.7%	32.1%	65.0%	27.8%

Abbreviation: ID, iron deficiency.

Model data

ID prevalence was derived from a national, cross-sectional, multicentre study (FERRIDON study) that estimated ID risk factors in French WB donors [6]. Four groups of donors at high risk of ID were determined based on the following independent risk factors: gender, age, donor status (new or repeat donor), mean corpuscular haemoglobin, Hb level, number of donations in the previous 12 months and interval since previous donation (Table 1). Model inputs from the FERRIDON study are presented in Table 2.

In the FERRIDON study, ID donors (<26 ng/ml) received a letter advising them to consult their GP and to postpone their next donation for at least 6 months. Their follow-up in the EFS database provided inter-donation interval data according to donor status, with and without ID and risk group. Gamma distributions were used and calibrated to reproduce the empirical distribution of donation intervals observed in the FERRIDON study for each risk group by ID status (Figure S1).

The time required for ferritin to revert to normal levels (>26 ng/ml) was taken from the HEIRS study published by Kiss et al. [7]. The study aimed to determine the effect of oral iron supplementation on the recovery of iron stores in donors with 'low ferritin' (≤26 ng/ml) and 'higher ferritin' (>26 ng/ml) levels. It was found that, following a 500-ml donation of WB, ferritin levels fell by approximately 30 and 8 ng/ml over a 30-day period in the high and low ferritin groups, respectively. Among blood donors with normal Hb levels, low-dose iron supplementation versus no supplementation reduced the ferritin recovery period to approximately 1 month in the low ferritin (≤26 ng/ml) or higher ferritin (>26 ng/ml) groups. Another study showed that without iron supplementation, iron stores were not replenished for approximately 84 and 168 days plus in the high and low ferritin groups, respectively [8]. Normal distributions were used to simulate recovery intervals for each risk group according to ID status (Table S1).

Internal validation

Model validation was conducted by calibrating the scenario without ferritin testing, which is the current situation in France, using annual data for each risk group (Figure S2). The model was calibrated to reproduce the overall annual number of donations from donors at high risk of ID, for whom a 20,000 reduction in annual donations was considered to reflect the donation trend. IDs and anaemias were calibrated for each risk group and were stable over time. Simulated model data were compared to historical data (Figure S2).

Statistical analysis

Four scenarios were simulated for each risk group:

1. *A reference scenario*: current situation without ferritin testing and a minimum inter-donation interval of 8 weeks.
2. *Minimum scenario*: implementation of ferritin testing for every donor of each risk group, involving a 6-month deferral period only for donors with ferritin levels <15 ng/ml. No specific intervention is planned for donors at 15–25 ng/ml.
3. *Main scenario*: minimum scenario, plus an additional intervention for donors at 15–25 ng/ml, involving a minimum return interval of 3 and 4 months for male and female donors, respectively.
4. *Supplementation scenario*: main scenario, plus an oral iron supplementation for 50% of donors with ferritin levels <15 ng/ml.

The model was simulated over a 5-year period. Results were calculated separately for each risk group and aggregated for the first, second and fifth year in terms of WB donations, IDs and anaemias. The dispersion was measured with the half-width used to estimate the confidence interval, which measures the error in the estimate of the true mean and is defined as the 95% confidence coefficient times the standard deviation over the square root of the sample size. A sampling rule of 1:10 donors was simulated to reflect monthly donations from high-risk donors.

Sensitivity analyses were conducted to assess uncertainty relating to various data sources for the following outcomes: rates of anaemia, ferritin recovery period, time to next donation and drop-out rate. Deterministic sensitivity analyses were performed for each type of input by applying ±25% of the reference input value to all risk groups (the error margin was assumed to be similar between the risk groups).

RESULTS

Reference scenario

For the first year, the model estimated around 660,000 donations in the high-risk population for the reference scenario, with 230,000 AISs (<15 ng/ml), 140,000 IDs of 15–25 ng/ml and 60,000 anaemias. Estimated changes over time for these high-risk groups are presented in Figure S2 and Table 3.

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TABLE 3 Estimated results in high-risk donors eligible for ferritin testing over a 5-year timeline

	Reference scenario	Minimum scenario	Main scenario	Iron supp. <15 ng/ml
WB donations				
First year	662,286	615,973	609,587	632,122
Second year	651,431	607,795	607,352	620,153
Fifth year	594,627	567,209	566,940	575,394
Difference versus reference scenario (loss of WB donations)				
First year change	-	−**46,313**	−**52,699**	−**30,164**
% Change	-	−7.0%	−8.0%	−4.6%
Second year change	-	−**43,636**	−**44,079**	−**31,278**
% Change	-	−6.7%	−6.8%	−4.8%
Fifth year change	-	−**27,418**	−**27,687**	−**19,232**
% Change	-	−4.6%	−4.7%	−3.2%
ID				
Total (<26 ng/ml)				
First year	369,847	328,114	319,532	280,612
Second year	316,828	265,229	258,428	235,498
Fifth year	296,411	250,459	243,078	222,006
<15 ng/ml				
First year	228,094	200,828	196,256	173,168
Second year	195,806	162,586	158,727	145,551
Fifth year	184,774	154,308	150,266	137,750
15–25 ng/ml				
First year	141,753	127,286	123,276	107,445
Second year	121,022	102,643	99,701	89,947
Fifth year	111,637	96,151	92,813	84,256
Difference versus reference scenario (prevented ID)				
Total				
First year change		−**41,733**	−**50,315**	−**89,235**
% Change		−11.3%	−13.6%	−24.1%
Second year change		−**51,599**	−**58,400**	−**81,330**
% Change		−16.3%	−18.4%	−25.7%
Fifth year change		−**45,952**	−**53,333**	−**74,406**
% Change		−15.5%	−18.0%	−25.1%
<15 ng/ml				
First year change		−**27,266**	−**31,838**	−**54,927**
% Change		−12.0%	−14.0%	−24.1%
Second year change		−**33,220**	−**37,079**	−**50,256**
% Change		−17.0%	−18.9%	−25.7%
Fifth year change		−**30,466**	−**34,508**	−**47,024**
% Change		−16.5%	−18.7%	−25.4%
15–25 ng/ml				
First year change		−**14,466**	−**18,476**	−**34,308**
% Change		−10.2%	−13.0%	−24.2%
Second year change		−**18,378**	−**21,321**	−**31,075**
% Change		−15.2%	−17.6%	−25.7%
Fifth year change		−**15,486**	−**18,825**	−**27,381**
% Change		−13.9%	−16.9%	−24.5%

(Continues)

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TABLE 3 (Continued)

	Reference scenario	Minimum scenario	Main scenario	Iron supp. <15 ng/ml
Anaemia				
First year	60,215	43,755	42,589	38,876
Second year	49,121	35,849	35,004	32,786
Fifth year	44,115	33,449	32,709	30,820
Difference versus reference scenario (cases of anaemia prevented)				
First year change	-	−**16,460**	−**17,626**	−**21,339**
% Change	-	−27.3%	−29.3%	−35.4%
Second year change	-	−**13,272**	−**14,117**	−**16,335**
% Change	-	−27.0%	−28.7%	−33.3%
Fifth year change	-	−**10,666**	−**11,406**	−**13,295**
% Change	-	−24.2%	−25.9%	−30.1%

Abbreviations: ID, iron deficiency; WB, whole blood.

Minimum scenario

The expected number of donations, IDs and anaemias for the minimum scenario and the estimated difference compared to the current scenario are presented in Table 3. Following the extended 6-month deferral for donors with ferritin <15 ng/ml, a loss of approximately 46,000 donations was estimated in the first year compared to 27,000 in the fifth year, corresponding to a reduction of 7.0% and 4.6%, respectively.

This scenario could prevent around 42,000 IDs in the first year (−11.3%) and 46,000 in the fifth year (−15.5%) (of which 65% AIS). An overall reduction of around 16,000 anaemias (−27.3%) was estimated for the first year and 11,000 (− 24.2%) for the fifth year.

Main scenario

When the delayed donation was applied for donors with ferritin levels ranging from 15 to 25 ng/ml, losses ranging from approximately 53,000 donations (−8.0%) in the first year to 28,000 (−4.7%) in the fifth year were estimated, with the highest impact being observed for G2, followed by G4 (Figure 2).

Around 50,000 IDs (−13.6%) could be prevented in the first year and 53,000 (−18.0%) in the fifth year. An overall reduction of around 18,000 anaemias (−29.3%) was estimated in the first year and 11,000 (−25.9%) in the fifth year.

Compared to the minimum scenario, the additional extended deferral period increased losses by one point (6500 donations) in the first year, with quite no differences in the fifth year. This scenario could prevent almost 10,000 additional IDs per annum over the 5-year period.

Supplementation scenario

In addition to the main scenario, this scenario assumed that 50% of donors with ferritin levels <15 ng/ml received iron supplements. A loss of around 30,000 donations (−4.6%) was estimated in the first year, falling to 19,000 (−3.2%) in the fifth year.

The supplementation could prevent 40,000 additional IDs and 4000 anaemias in the first year compared to the main scenario and 20,000 IDs and 2000 anaemias in the fifth year.

Sensitivity analyses

Deterministic sensitivity analyses are presented (Figure S3) and highlight the impact on WB donations during the first year of ferritin testing and the fifth year on a cumulative basis. In cases where the normal ferritin level recovery period was increased by 25%, additional losses of 35% and 39% were observed in the first and fifth years, respectively; conversely, when it fell by 25%, 20% and 32% of WB donations were saved. Decreasing inter-donation intervals and drop-out rates had a substantial impact, with additional losses ranging from 24% to 43% in the first year and 28% to 33% in the fifth year.

DISCUSSION

ID is a common issue in the general population. Of the 18–74-year-old French adults, 6.9% present AIS, mostly affecting women (11.9%) rather than men (1.5%) [9]. Donors at high risk of ID were investigated in France. AIS was found to be more prevalent in WB donors, with women being affected in 19.3% of cases and men in 6.4% of cases, this figure increasing to 45.6% in the high-risk group of repeat female donors [6].

This study aimed to assess the impact of a ferritin testing policy on WB donations, IDs and anaemias through patient-level simulation and data observed in the real-world setting.

Our simulation showed encouraging results: between 15% and 25% of IDs could be prevented in high-risk ID groups depending on the scenario. Regardless of the scenario, approximately one third of anaemia cases were prevented during the first year (Table 3).

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FIGURE 2 Changes in the loss of donations after implementation of ferritin testing (main scenario). (a) Changes in loss of donations for the population at high risk of iron deficiency (main scenario). (b) Changes in loss of donations for each risk group (main scenario)

Compared to the current situation, the number of anaemia cases in these groups fell to almost 50% after 5 years. These figures are underestimated since they only refer to ID and anaemia prevented among WB donors who return to donation, while data are censured for donors lost to follow-up.

Our study highlighted the fact that, after 5 years, donations recovered from donors in whom ID and anaemia have been prevented do not compensate for the loss of donations resulting from deferral and drop out. The maximum of WB donation loss occurred during the first year, decreasing thereafter over time, while the reduction in ID was stable over time (∼50,000 IDs per year). Our study also showed that the impact of iron supplementation in 50% of donors with very low ferritin levels (<15 ng/ml) had a substantial positive impact on donations (+20,000) and IDs (−40,000) in the first year.

In a Canadian study [5] using a different simulation model with a higher threshold of ferritinemia for deferral (<26 ng/ml), an extended 6-month deferral period, an increased drop-out rate and reduced donation frequency, the authors estimated a donation loss of 17% and a new donor requirement of 29%. These scenarios, based on a pilot study [10], could be pessimistic since return rates could improve with further retention and educational effort. In a scenario with no reduction in donation frequency, which may be overly optimistic, they estimated a donation loss of 9.5%.

In the Netherlands, donors undergo ferritin testing at entry and every five donations. Ferritin levels between 15 and 30 ng/ml are

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deferred for 6 months, and those below 15 ng/ml for 12 months [4]. This new ferritin monitoring policy in the Netherlands resulted in fewer donations and lower response rates to donation drives [11]. These results revealing a high percentage of lost donations have led EFS to defer only WB donors with AIS for 6 months and to delay invitations for moderate ID to avoid jeopardizing self-sufficiency in blood products.

One strength of our study lies in the fact that assumptions regarding donor behaviour were derived from the FERRIDON study, an observational study performed in 2019, including over 9000 WB donors representative of our donor population [6]. This should mitigate the impacts resulting from the unknown proportion of donors already using iron supplementation, having a low ferritin level unrelated to blood donations or from different behaviours depending on the site of donation.

Another strength of our study is that various scenarios have been considered and sensitivity analyses conducted. A delayed period to the next donation and an increase in drop-out rate were considered for donors based on ID level. Sensitivity analyses showed that a shorter inter-donation interval (−25%) and a lower drop-out rate (−25%) had a more adverse impact on WB donation from repeat donors (Figure S3). This was confirmed by the 8% reduction in the average number of annual donations for repeat donors for all risk groups (data not shown). A very recent Finish study corroborated these results, showing that donation activity accounted for most of the variation in ferritin levels [12].

Another key assumption was derived from the HEIRS trial, which studied the time to recovery of Hb and iron levels following a single donation [7]. Without iron supplementation, recovery of iron stores to pre-donation levels took longer than 24 weeks on average. In contrast, donors assigned to receive iron pills (38 mg) in this study displayed uniformly accelerated recovery of both Hb and iron levels. These results were applied in our model, and sensitivity analyses showed that when the ferritin recovery period was shortened by 25%, 32% of WB donations were saved over the 5 years.

In France, the monitoring policy recommends a GP visit for donors with ferritin levels <15 ng/ml. Iron supplementation will be given at the discretion of both patients and GPs. In the STRIDE study—a 2-year randomized controlled trial of 692 donors assigned to educational groups or interventional groups with iron supplementation—a small increase in de-enrolment at 60 days for interventional groups was observed. No difference was observed in donors receiving iron and placebo tablets, which suggests that some donors will not comply with regular dosing regimens [13]. Follow-up data of up to 24 months after the final STRIDE visit [14], however, showed increased donations and decreased Hb deferrals in donors receiving iron supplementation compared to controls. In addition, 57% of donors who received a letter advising of low ferritin levels with recommendations to take iron supplements or delay future donations initiated iron supplementation. This figure was five times higher compared to those who received letters with no specific recommendation. Another study suggested that informing donors of their iron status might be sufficient to stimulate donor initiation of iron supplementation [15] and could increase further with the dispensing of iron supplements [16] or after phoning the donors [17]. In line with these results, iron supplementation for 50% of AIS donors seems to be a realistic expectation that would have a significant impact, although 3.8% of the general French population was shown to take an iron supplementation [9].

In conclusion, our study shows that the prevention of anaemia and ID with a strategy of ferritin testing in donors at risk for ID significantly improves donor health: at least 11%–24% of ID and 27%–35% of anaemias will be prevented among high-risk WB donors. Because of extended inter-donation intervals, prolonged deferral periods and changes in donor behaviour, its implementation will, however, reduce the amount of WB collected from 5% to 8% in the high-risk subgroups after 1 year. The impact on WB donations could even be higher if donors were encouraged to convert to plasma donation, especially for those donating at fixed blood sites. This will need to be offset by combined efforts in terms of retention, education and recruitment of donors. Without public health policies for the prevention of ID in the general population, a significant proportion of donors, especially young women, will, however, continue to present with an ID unrelated to blood donations. Every blood establishment implementing a ferritin testing policy to improve donor health should be aware of the ensuing challenge of maintaining a sufficient blood supply.

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P.R. designed the research study, analysed the results and wrote the paper. A-M.F. wrote the draft of the paper. L.M. performed the statistical analysis, contributed to the design and wrote the paper. C.L., C.C., G.W., C.J. and P.M. reviewed the final draft of the paper. H.L. performed the statistical analysis. A.V. designed the research study, analysed the results and wrote the paper.

CONFLICT OF INTEREST

No conflict of interest to declare.

ORCID

Pascale Richard https://orcid.org/0000-0003-1864-3824

REFERENCES

1. Boulton F. Evidence-based criteria for the care and selection of blood donors, with some comments on the relationship to blood supply, and emphasis on the management of donation-induced iron depletion. Transfus Med. 2008;18:13–27.
2. Malard L, Richard P, Maire F, Djoudi R, Gross S, Fillet A-M. Factors associated with recovery of haemoglobin levels after whole-blood donation in the French West Indies in 2015. Transfus Med. 2019;29:72–5.
3. Mei Z, Addo OY, Jefferds ME, Sharma AJ, Flores-Ayala RC, Brittenham GM. Physiologically based serum ferritin thresholds for iron deficiency in children and non-pregnant women: a US National Health and Nutrition Examination Surveys (NHANES) serial cross-sectional study. Lancet Haematol. 2021;8:e572–82.

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4. Vinkenoog M, Hurk K, Kraaij M, Leeuwen M, Janssen MP. First results of a ferritin-based blood donor deferral policy in the Netherlands. Transfusion. 2020;60:1785–92.
5. Blake JT, O'Brien SF, Goldman M. The impact of donor ferritin testing on blood availability in Canada. Vox Sang. 2021;117:17–26.
6. Fillet A, Martinaud C, Malard L, Le Cam S, Hejl C, Chenus F, et al. Iron deficiency among French whole-blood donors: first assessment and identification of predictive factors. Vox Sang. 2021;116:42–52.
7. Kiss JE, Brambilla D, Glynn SA, Mast AE, Spencer BR, Stone M, et al. Oral iron supplementation after blood donation: a randomized clinical trial. JAMA. 2015;313:575–83.
8. Schotten N, Pasker-de Jong PCM, Moretti D, Zimmermann MB, Geurts-Moespot AJ, Swinkels DW, et al. The donation interval of 56 days requires extension to 180 days for whole blood donors to recover from changes in iron metabolism. Blood. 2016;128:2185–8.
9. Study of health on environnement, biosurveillance, physical activity and nutrition (Esteban), 2014–2016. Nutrition portion. Chapter biological dosages: vitamins and mineral. Saint-Maurice: Santé Publique France; 2019. 61p. Cited 2020 February 4. Available from: https://www.santepubliquefrance.fr/determinants-de-sante/nutrition-et-activite-physique/documents/rapport-synthese/etude-de-sante-sur-l-environement-la-biosurveillance-l-activite-physique-et-la-nutrition-esteban-2014-2016-.-volet-nutrition.-chapitre-dosages
10. Goldman M, Uzicanin S, Scalia V, O'Brien SF. Iron deficiency in Canadian blood donors. Transfusion. 2014;54:775–9.
11. Spekman MLC, Ramondt S, Sweegers MG. Whole blood donor behavior and availability after deferral: consequences of a new ferritin monitoring policy. Transfusion. 2021;61:1112–21.
12. Lobier M, Castrén J, Niittymäki P, Palokangas E, Partanen J, Arvas M. The effect of donation activity dwarfs the effect of lifestyle, diet and targeted iron supplementation on blood donor iron stores. PLoS One. 2019;14:e0220862.
13. Bialkowski W, Bryant BJ, Schlumpf KS, Wright DJ, Birch R, Kiss JE, et al. The strategies to reduce iron deficiency in blood donors randomized trial: design, enrolment and early retention. Vox Sang. 2015;108:178–85.
14. Cable RG, Birch RJ, Spencer BR, Wright DJ, Bialkowski W, Kiss JE, et al. The operational implications of donor behaviors following enrollment in STRIDE (Strategies to Reduce Iron Deficiency in blood donors). Transfusion. 2017;57:2440–8.
15. Mast AE, Bialkowski W, Bryant BJ, Wright DJ, Birch R, Kiss JE, et al. A randomized, blinded, placebo-controlled trial of education and iron supplementation for mitigation of iron deficiency in regular blood donors. Transfusion. 2016;56:1588–97.
16. Gorlin J, Katz L, Elsmore D, Kirbach K, Erickson Y, Hove A, et al. Prevalence of blood donor iron deficiency and feasibility ferritin-based iron replacement: a blood collection agency-based study. Vox Sang. 2016;111:206–8.
17. Magnussen K, Ladelund S. Handling low hemoglobin and iron deficiency in a blood donor population: 2 years' experience. Transfusion. 2015;55:2473–8.

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

Proof of concept for detection of staphylococcal enterotoxins in platelet concentrates as a novel safety mitigation strategy

Sylvia Ighem Chi[1,2] | Basit Yousuf[1,2] | Carina Paredes[1,2] | Jennifer Bearne[3] | Carl McDonald[3] | Sandra Ramirez-Arcos[1,2]

[1]Medical Affairs and Innovation, Canadian Blood Services, Ottawa, Ontario, Canada

[2]Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada

[3]National Health Service Blood and Transplant, London, UK

Correspondence
Sandra Ramirez-Arcos, Medical Affairs and Innovation, Canadian Blood Services, Ottawa, ON K1G 4J5, Canada.
Email: sandra.ramirez@blood.ca

Funding information
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Abstract

Background and Objectives: *Staphylococcus aureus* is a predominant contaminant of platelet concentrates (PCs) that can evade detection during screening with culture methods. Importantly, *S. aureus* produces staphylococcal enterotoxins (SEs) during PC storage, which are linked to slow growth and enhanced biofilm formation. This study investigated timing of SE production during PC storage and feasibility of SE detection as a PC safety strategy.

Materials and Methods: Genomic and transcriptomic data of transfusion-relevant *S. aureus* PS/BAC/169/17/W, PS/BAC/317/16/W, CI/BAC/25/13/W and CBS2016-05 were used to determine the presence and differential expression of exotoxin genes in PCs. Trypticase soy broth (TSB) and PCs were inoculated with 1.0E+06 cfu/mL of *S. aureus* PS/BAC/169/17/W and CBS2016-05. Expression of SEs at different growth phases was confirmed with Western blotting. PCs were inoculated with 30 cfu/unit of the same strains, and SE detection during PC storage was optimized with a sandwich dot-ELISA assay.

Results: *S. aureus* genomes contain multiple exotoxin genes including those encoding for SEs. Transcriptome data revealed significant upregulation (0.5–6.7-fold, $p < 0.05$) of SE genes in PCs versus TSB. Western blots demonstrated SE production at all growth phases. Notably, dot-ELISA detected clinically relevant concentrations of SEs (∼0.2 μg/mL) at 32 h of PC storage when *S. aureus* PS/BAC/169/17/W and CBS2016-05 counts were 1.8E+04 and 1.4E+04 cfu/mL, respectively.

Conclusion: Genomic analyses revealed that staphylococcal exotoxins are widely distributed and highly conserved among transfusion-relevant *S. aureus* isolates. Furthermore, SEs are significantly upregulated in PCs and detected at 30 h of PC storage. Therefore, bacterial toxin detection could supplement mitigation strategies to enhance PC safety.

Keywords

enterotoxins, platelet concentrates, staphylococcal exotoxins, *Staphylococcus aureus*

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Highlights

- Genomes of transfusion-relevant *Staphylococcus aureus* isolates encode for a variety of staphylococcal exotoxins, including superantigens such as staphylococcal enterotoxins (SEs), which are likely responsible for septic shock symptoms of patients transfused with contaminated platelet concentrates (PCs).
- Expression of SE genes is significantly upregulated in PCs contaminated with *S. aureus* in comparison to *S. aureus* grown in laboratory media.
- Detection of SEs in *S. aureus* contaminated PCs is feasible as early as after 30 h of PC incubation. Importantly, SE concentration at 32 h of PC incubation is approximately 0.2 μg/mL, which is considerably higher than 0.1 pg/mL, an SE concentration reported to cause septic shock symptoms in immunocomprised patients.

INTRODUCTION

Contamination of donated blood with bacteria remains a significant concern in transfusion medicine. Platelet concentrates (PCs), which are therapeutic for preventing or treating thrombocytopenia, are at higher risk of bacterial proliferation, compared to other transfusable blood components such as plasma and red blood cell concentrates. PCs are stored in glucose-rich, neutral pH solutions and gas-permeable plastic bags at 22 ± 2°C under agitation for up to 7 days [1]. These storage conditions are essential for platelet viability; however, they are also amicable for the proliferation of the majority of PC bacterial contaminants. To enhance the safety of donated blood, mitigation strategies have been implemented prior to and during blood collection, including donor selection, disinfection of donor's arm at the venipuncture area and diversion of the first aliquot of collected blood [2, 3]. Additionally, post-collection safety protocols are applied in different centres, which comprise PC screening for bacterial contamination or PC treatment with pathogen reduction (PR) technologies (PRTs) [1, 4]. Over the years, a remarkable success in decreasing bacterial contamination of PCs has been achieved worldwide, especially in high income countries [5].

Bacterial testing of PCs can be performed using culture-based approaches or rapid methods [6]. During the last two decades, automated culture-based systems have been widely implemented worldwide. PC culture diagnostic systems are credited with reducing septic events from PC transfusion by 69.7% [7]. Notwithstanding, there is no absolute guarantee of PC sterility since culture systems only validate the release of PC units on a negative-to-date basis, without considering the inoculated volume, sampling time, presence of slow growing pathogens or formation of surface-attached aggregates known as biofilms during PC storage [1, 8].

Gram-positive and gram-negative bacteria have been isolated from PCs with *Staphylococcus aureus* ranking as a major cause of transfusion related sepsis and fatalities [2, 9–14]. This species can evade detection during routine PC screening with automated culture systems due to slow growth and ability to form biofilms in PCs [13, 14].

Remarkably, *S. aureus* can secrete a compendium of exotoxins such as exfoliative toxins, staphylococcal superantigens (SAgs), and haemolysins among others [15]. SAgs are potent immunostimulatory proteins that include the toxic shock syndrome toxin-1 (TSST-1), staphylococcal enterotoxins (SEs), which have been involved in septic transfusion cases, and superantigen-like toxins (SSLs) [13, 14, 16]. Within all SEs, SE-type G (SEG) and SE-type H (SEH) are relevant for this study. While SEG was detected in a contaminated PC unit involved in a septic transfusion reaction, the *seh* gene was found in the genome of another *S. aureus* strain reported to be involved in a septic transfusion event [9, 13]. Furthermore, we have recently demonstrated that SEG and SEH production during PC storage increases the formation of biofilms and impairs growth of *S. aureus*, likely contributing to missed detection of this pathogen with culture methods [17]. In this study, we investigated time of SE production during PC storage, which could be used as a supplementary test to enhance transfusion patient safety.

MATERIALS AND METHODS

PC units

PC units were manufactured at the Canadian Blood Services netCAD Blood4Research Facility (netCAD, Vancouver, Canada) in agreement with standard procedures. PCs were shipped to the Canadian Blood Services Microbiology laboratory in Ottawa, Canada. Ethical approval for this study was granted by the Canadian Blood Services Research Ethical Board.

S. aureus isolates

Four *S. aureus* strains listed in Table 1 were used in this study, including one isolated in Canada [9] and three isolated in England [10–12]. Two of the English isolates (PS/BAC/169/17/W and PS/BAC/317/16/W) were detected during routine PC screening using the BACT/ALERT culture system at the National Health Service Blood and Transplant (NHSBT) (i.e., confirmed positives). The third English strain (CI/BAC/25/13/W) and the Canadian isolate (CBS2016-05) were missed during BACT/ALERT PC screening (i.e., false negative screening). While the PC unit contaminated with *S. aureus* CI/BAC/25/13/W

TABLE 1 Staphylococcal exotoxin genes encoded by the genomes of four *Staphylococcus aureus* strains.

S. aureus strain	BACT/ALERT PC screening result	Staphylococcal enterotoxins and superantigen-like genes
CBS2016-05	False negative[a]	*seg*, *seh*, *sei*, *sem*, *sen*, *seo*, *ses*, *selu*, *selw*, *selx*, *sely*, *exo*, *ssl1*, *ssl3*, *ssl4*, *ssl5*, *ssl7*, *ssl8*, *ssl9*, *ssl10*, *ssl11*, *ssl12*, *ssl13*, *ssl14*
CI/BAC/25/13/W		*seb*, *selp*, *selw*, *selx*, *selz*, *ssl1*, *ssl2*, *ssl3*, *ssl4*, *ssl5*, *ssl7*, *ssl8*, *ssl9*, *ssl10*, *ssl11*, *ssl12*, *ssl13*, *ssl14*
PS/BAC/169/17/W	Confirmed positive[b]	*seg*, *sei*, *sem*, *sen*, *seo*, *selu*, *selw*, *ssl1*, *ssl2*, *ssl3*, *ssl4*, *ssl5*, *ssl7*, *ssl9*, *ssl10*, *ssl11*, *ssl12*, *ssl13*, *ssl14*
PS/BAC/317/16/W		*selw*, *ssl1*, *ssl2*, *ssl3*, *ssl4*, *ssl5*, *ssl7*, *ssl9*, *ssl10*, *ssl11*, *ssl12*, *ssl13*, *ssl14*

Abbreviation: PC, platelet concentrate.

[a]False negative BACT/ALERT screening results refers to bacteria missed during routine screening of PCs and detected during investigation of transfusion reactions or near-misses [1].

[b]Confirmed positive BACT/ALERT screening results indicate the same bacterial species identified during initial and confirmatory PC screening [1].

was identified prior to transfusion by visual inspection due to clot formation ('near-miss') [12], the PC unit carrying CBS2016-05 was transfused causing a septic transfusion reaction [14].

Staphylococcal exotoxin gene identification

The genomes of all four *S. aureus* isolates were next-generation sequenced. Illumina paired end DNA libraries of the isolates were sequenced using the Miseq platform, assembled, annotated at the Health Canada genomics facility, and published at the NCBI database [9–12]. The presence of SE genes in the next-generation sequences of all studied strains was further confirmed by Sanger sequencing. Information from the genome sequences was used to design primers for specific PCR amplification of genes encoding for SEs and SSLs. OneTaq DNA polymerase kit (New England Biolabs, Ipswich, MA, USA) was used as follows: each reaction tube containing a mixture of 2 μL (10 ng) genomic DNA, 5.0 μL buffer (1×), 0.5 μL dNTPs (200 μM), 1.0 μL primer pair (0.4 μM), 0.125 μL polymerase (0.625 unit) and nuclease-free water summing to 25 μL reaction volume was run on a thermal cycler with initial denaturation (94°C); annealing and extension 30 cycles (94°C, 30 s: 45–68°C, 1 min; 68°C, 1 min); final extension (68°C, 5 min). Following the PCR run, the amplicons were resolved on 1% agarose gel (Sigma-Aldrich, Oakville, ON, Canada).

Differential expression of SE genes

RNAseq assays and comparative transcriptome analyses were performed using protocols recently reported [17]. Quantitative reverse transcription PCR (RT-qPCR) [18] verification of upregulated SE genes was performed in duplicate and repeated once. Approximately 1 μg total RNA was subjected to reverse transcription (RT) with QuantiTect Reverse Transcription kit protocol (Qiagen, Germantown, MD, USA) after treatment with TURBO DNAse enzyme (Invitrogen, Waltham, MA, USA). The RT reaction mixture, composed of the purified RNA, RT buffer, RT primer mix, and quantitect reverse transcriptase, was incubated at 42°C for 30 min. Two microliters (10 ng) of the cDNA was subsequently added to a qPCR mixture comprising 5 μL of 2× SYBR Green PCR master mix from QuantiNova SYBR Green PCR kit (Qiagen, Germantown, MD, USA), 1 μL of forward and reverse gene specific primers (0.7 μM) and 2 μL nuclease-free water. Each gene and condition were run in duplicate using equal amounts of cDNAs in trypticase soy broth (TSB) and PCs. For controls, *gyrA* (positive) and nuclease-free H_2O (negative) were used. CFX96 Touch™ Real-Time PCR Detection System (BioRad, Hercules, California, CA, USA) was used to run the experiments. Transcript copy numbers were determined using concentrations generated by mean Cq (quantification cycle) values of the samples obtained by standard curve analyses based on 10-fold dilutions of their respective PCR amplicons of known concentrations.

Western blotting detection of staphylococcal SE types G (SEG) and H (SEH) at different growth phases of *S. aureus*

TSB and PC cultures were inoculated with *S. aureus* CBS2016-05 and PS/BAC/169/17/W, representing strains that missed detection or were captured during BACT/ALERT screening, respectively, at a target concentration of approximately 1.0E+06 cfu/mL. TSB cultures were incubated at 37°C/18 h with agitation, and PC cultures were incubated under PC storage conditions (20–24°C under agitation for 120 h [5 days]). *S. aureus* spiked TSB or PC cultures obtained at mid-exponential, late-exponential, and stationary growth phases were analysed by Western blotting [19, 20]. Secreted proteins in TSB supernatants were precipitated for a minimum of 1 h at −20°C in 25% trichloroacetic acid [21], and the pellet was washed in 500 μL ice-cold acetone, dried and rehydrated in nuclease-free H_2O. PC supernatants were obtained by centrifugation of samples at 10,000 rpm for 15 min. Both TSB and PC protein samples were resolved on 12% SDS-PAGE, transferred onto polyvinylidene difluoride membrane on iBlot™ 2 Gel Transfer Device (Invitrogen, Waltham, MA, USA). Thereafter, the membranes were blocked with 5% bovine serum albumin (BSA) buffer for 1 h, followed by incubation with mouse polyclonal antibodies (1:5000) against enterotoxin SEG (GenTex.com, Irvine, CA, USA) or against enterotoxin SEH (Abcam, Waltham, MA, USA) and washed in Tris-buffered saline (TBS) supplemented with 0.1% Tween® 20 (TBST).

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Membranes were then incubated with horseradish peroxidase (HRP) conjugated goat anti-mouse IgG secondary antibody (1:10000) (Abcam, Waltham, MA, USA). The protein bands were visualized in the ChemiDoc imaging system (BioRAD, Hercules, California, CA, USA) after incubating for 5 min in detection solution, Pierce ECL Western Blotting Substrate (ThermoScientific, Waltham, MA, USA).

Semi-quantitative sandwich dot-ELISA assay for detection of SEs during PC storage

The limit of detection of SEs was investigated using a semi-quantitative sandwich dot-ELISA assay as described by Venkataramana et al. [22], with some modifications. PC cultures were inoculated with *S. aureus* CBS2016-05 and PS/BAC/169/17/W at a target concentration of approximately 30 cfu/PC and incubated under PC storage conditions. In brief, 10 μL aliquots of combined mouse polyclonal antibody anti SEG and SEH (1:1000) were dotted on 1.0 × 1.0 cm nitrocellulose membrane pieces that had been placed in a 12-well plate. To optimize SE detection, antibodies against SEG and SEH were combined. Blocking of unbound sites was achieved with addition of 5% BSA and incubation for 1 h at 45°C in TBS followed by washing with TBST. Subsequently, 500 μL of PC samples (initially inoculated with 30 cfu/PC), which were collected at 0, 24, 30, 32, 48, 72, 96 and 120 h of incubation, were added to different wells. Five hundred microliters of non-spiked PCs and 5 μL of a 20 μg/mL solution of purified enterotoxin SEG protein (0.1 μg) (Antibodies-online, Limerick, PA, USA) were included as negative and positive controls, respectively. The plate was incubated for 1 h

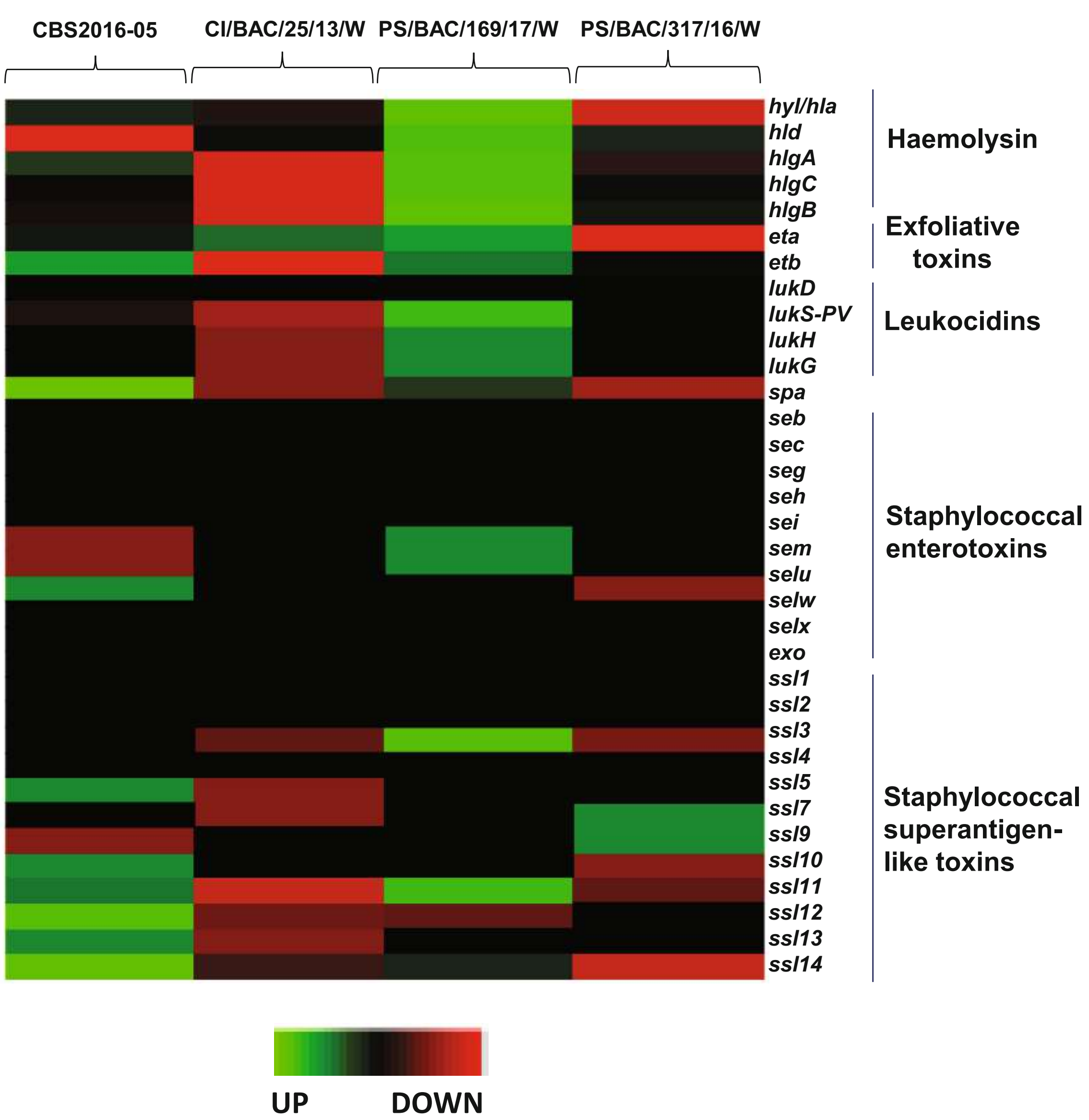

FIGURE 1 Heatmap of differentially expressed *S. aureus* exotoxin genes in PCs compared to TSB as revealed by RNAseq analyses. Experiments were performed in triplicate. The differential expressions were based on fold change values of ≥0.5 with TSB as the baseline for all strains. The statistical significance of RNAseq-based foldchange DE genes ($p < 0.05$) was determined using two-tailed t-tests on gene raw transcript counts performed in triplicate between the study conditions.

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at 37°C, washed and further incubated with 300 μL of 1:500 monoclonal SEs antibody cocktail under the same conditions. After washing, HRP conjugated goat anti-mouse IgG (Abcam, Waltham, MA, USA) was added onto the wells, incubated for 45 min at 37°C, washed and colour developed with TMB + 0.4% H_2O_2 (3,3′,5,5′-tetramethylbenzidine substrate plus hydrogen peroxide). This assay was performed twice.

Statistical analyses

Statistical tests were performed to determine the difference in gene expression between PCs and TSB inoculated with *S. aureus*. The mean quantification cycle numbers obtained in the RT-qPCR analyses were used to calculate the standard error mean deviation over the biological and technical replicates. Normalized count values of the RNAseq transcripts were used to calculate differential gene expression between the PC and TSB conditions for each bacterial strain. Data were provided with a *p* value and a FDR/q value that correct the *p* value for multiple testing using the Benjamini–Hochberg approach. This analysis was done in *R*. Normalized data were further used to determine the statistical significance of the RNAseq-based foldchange differentially expressed (DE) genes using two-tailed *t*-tests on gene raw transcript counts performed in triplicates between the study conditions. A *p* value <0.05 was considered significant.

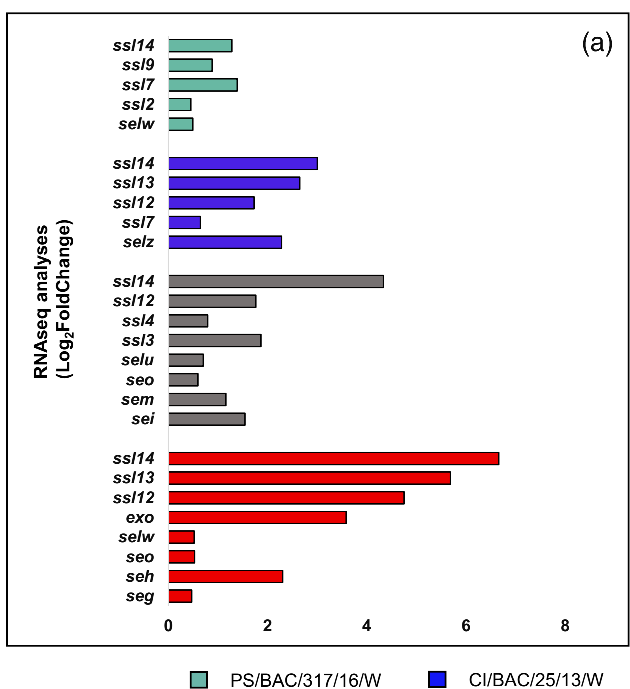
RESULTS

S. aureus genomes encode multiple exotoxin genes

Whole genome sequence analyses of the four *S. aureus* studied herein revealed an arsenal of genes coding for exotoxins in all strains. Genes of all staphylococcal exotoxin classes consisting of pore-forming toxins (haemolysins, leukocidins and phenol soluble modulins), cytotoxic enzymes (exfoliative toxins), and SAgs, including staphylococcal protein A (*spa*), SSLs, and SEs, were present in the genomes of the *S. aureus* isolates (Table 1). The presence of genes coding for SE and SSL superantigens was confirmed by PCR, as shown in Figure S1.

SSLs and SEs are upregulated in PCs compared to TSB

Comparative RNAseq analyses performed on the *S. aureus* strains grown in PCs and TSB revealed differential expression of exotoxin genes (Figure 1). Among the upregulated genes are haemolysins and leukocidins in strains CBS2016-05 and PS/BAC/169/17/W, exfoliative toxins in all strains but PS/BAC/317/16/W, and superantigens in all strains (0.5–6.7-fold change, $p < 0.05$). In general, SEs had increased transcripts in PCs compared to TSB (Figure 2a), with enterotoxin type H (*seh*), enterotoxin-like Z (*selz*) and an uncharacterized enterotoxin gene (*exo*) being significantly upregulated (2.3–3.6-fold



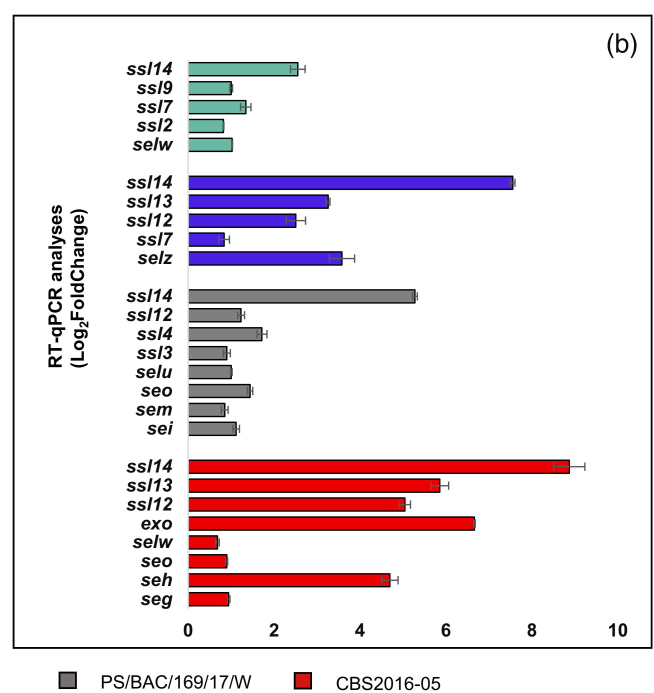


FIGURE 2 Upregulated staphylococcal SAg genes in PCs compared to TSB (baseline). (a) RNAseq data analyses. (b) Quantitative reverse transcription PCR (RT-qPCR) validation of the RNAseq results. Differential expression fold change of ≥0.5 was considered for all strains. For RT-qPCR, each gene and conditions were analysed in duplicate, and assays were repeated twice. *ssls* are superantigen-like exotoxin genes while *ses* are staphylococcal enterotoxin genes. The standard error mean (SEM = 0.01–0.37) (represented by the bars) of upregulated genes between PCs and TSB was determined based on the mean quantification cycle numbers obtained in the RT-qPCR.

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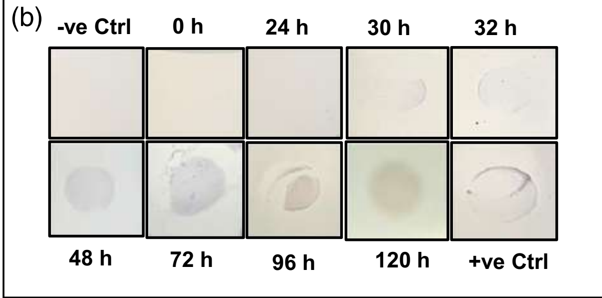
(a) -ve Ctrl 0 h 24 h 30 h 32 h
48 h 72 h 96 h 120 h +ve Ctrl



(c)

PC incubation time (h)	Bacterial counts (CFU/mL)	
	S. aureus CBS2016-05	*S. aureus* PS/BAC/169/17/W
0 h	<1.0E+00	<1.0E+00
24 h	1.5E+02	2.0E+02
30 h	1.1E+04	1.6E+04
32 h	1.4E+04	1.8E+04
48 h	1.8E+05	2.0E+05
72 h	3.7E+05	3.9E+05
96 h	1.6E+06	1.8E+06
120 h	2.5E+07	2.8E+07

FIGURE 3 Determination of the limit of detection of SEs in PCs by semi-quantitative sandwich dot-ELISA. Enterotoxins from *Staphylococcus aureus* CBS2016-05 (a) and PS/BAC/169/17/W (b) were detected on samples collected at different times during PC incubation (c). Negative control (−ve Ctrl) is non-spiked PCs and positive control (+ve Ctrl) corresponds to 0.1 μg of commercially obtained staphylococcal enterotoxin SEG. ($N = 2$).

change, $p < 0.05$). Remarkably, the greatest fold differences were observed for superantigen-like toxin genes with *ssl14* being significantly upregulated in *S. aureus* CBS2016-05 and marginally upregulated in the CI/BAC/25/13/W strain ($p = 0.04$ and $p = 0.05$, respectively) (Figure 2a). The DE candidate genes selected and validated by RT-qPCR (Figure 2b) corroborated the RNAseq results.

Detection of SEs during PC storage

Western blotting was used to detect the presence of secreted SEG and SEH in TSB and PC cultures inoculated with high bacterial counts (approximately 1.0E+06 cfu/mL) of *S. aureus*. The SEs were secreted in mid-exponential, post-exponential and stationary growth phases in TSB and PC cultures of CBS2016-05 and PS/BAC/169/17/W as shown in Figure S2.

After the initial assessment and confirmation of SE production in PCs by Western blotting, semi-quantitative sandwich dot-ELISA assays were performed to determine the analytical sensitivity of SEs in contaminated PC samples collected at different time points. The results of the immune-dot assays revealed time-dependent SE production. No SEs were detected at 0 or 24 h of PC incubation when *S. aureus* concentration was approximately 1.0E+02 cfu/mL (Figure 3). SE detection was apparent at 30 h of PC incubation when bacterial counts had reached approximately 1.0E+04 cfu/mL (Figure 3). Intensity of the dots increased with time and bacterial concentration indicating greater SE concentration at 120 h of PC storage when the bacterial concentration was approximately 1.0E+07 cfu/mL (Figure 3). Dots observed at 32 h of PC incubation and beyond were similar or darker than the dot of the positive control (0.1 μg of SEG) indicating that SE concentration in the PCs was ≥0.2 μg/mL (calculated based on 0.5 mL PCs blotted on the membranes).

DISCUSSION

Detection of PC contaminated with *S. aureus* is sometimes challenging with automated culture systems due to slow growth and ability to form biofilms by this species during PC storage [1, 13]. The safety risk of transfusing PCs contaminated with *S. aureus* is exacerbated by SE production during PC storage as exemplified by several reports of transfusion reactions with the detection of staphylococcal superantigens in the transfused units [13, 14, 16]. Remarkably, we have recently shown that SE production during PC storage enhances biofilm formation and decreases growth of *S. aureus* [17], which is likely linked to missed detection of this species with culture methods. We have previously demonstrated that transfusion-relevant *Serratia marcescens* strains form biofilms in PCs, resulting in decreased detection by the BACT/ALERT automated culture system [23].

The results of this study show that SEs exhibit biomarker characteristics for detection of *S. aureus* in PCs stored for 30 h. First, we showed that different SE genes are present in the genomes of *S. aureus* isolated from contaminated PC units. Findings by previous studies have also shown that nearly 80% of methicillin-susceptible

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S. aureus and >90% of methicillin-resistant *S. aureus* isolates secrete several superantigen toxins [24, 25]. The wide distribution and high sequence conservation of SEs in transfusion-relevant *S. aureus* isolates endorse their nomination as indicators of the presence of this bacterium in PCs. Second, we observed significant upregulation of SE gene expression in PCs compared to TSB cultures in all *S. aureus* isolates, corroborating the recommendation by the Food and Drug Administration (FDA) stating that ideal biomarkers must have increased expression in testing conditions [26]. Furthermore, the immunological detection of SEs in stored PC cultures complies with the FDA requirement for ease of detection of toxins in biological fluids as an important biomarker characteristic [26, 27].

Notably, we detected SEs from PC samples taken as early as 30 h of storage when bacterial counts were approximately 1.0E+04 cfu/mL, which is below the concentration that has been reported to be clinically significant (1.0E+05 cfu/mL) [28]. This result also indicates that SEs could be detected before culture results are obtained when PCs are screened using a large volume delayed testing algorithm, which requires PC sampling at ≥36 h post-blood collection [29, 30]. Recipients of PCs contaminated with *S. aureus* are at elevated risk of sepsis as SE concentrations ≥0.2 μg/mL were detected after 32 h of PC incubation, which are considerably higher than the 0.1 pg/mL concentration reported to cause toxin shocks in susceptible patients [31]. Hence, ideally, an immunoassay developed to detect SEs or other staphylococcal exotoxins in PCs should have high sensitivity and be able to detect lower levels of toxins earlier during PC storage. Others have optimized detection of SEs using dot-ELISA assays in the range of 32 pg/mL to 50 ng/mL [32, 33]. Our data provide a proof of principle of the importance of detecting SEs in PCs. Further complementary studies are needed to expand detection of enterotoxins by other *S. aureus* clinical isolates. Similarly, it would be interesting to optimize detection of toxins secreted by other bacteria such as superantigens produced by streptococci.

Exemplifying SEs secreted by *S. aureus* as potential biomarkers, we introduce the idea of supplementing bacterial detection with screening for bacterial toxins. Alternatively, toxin detection could also be a stand-alone point-of-care strategy to detect toxins in PCs that have been treated with PRT, as these methods have not been demonstrated to inactivate endotoxins or other pyrogens [34]. It is not known if whole blood derived PCs would contain bacterial toxins secreted prior to PC manufacturing that could resist PR treatment. Having a strategy to detect bacterial toxins in PCs will, therefore, further decrease the residual safety risk of PCs that are either screened with culture methods or pathogen reduced.

Investigating the presence of SEs is not common practice when septic transfusion reactions involving *S. aureus* are reported [35–37]. Therefore, the applicability of the proof of concept developed in this study is directly related to patient safety as it has been proven that staphylococcal superantigens such as TSST-1, SEG and SEU have been implicated in transfusion septic reactions [13, 14, 16]. It is recognized that the positive impact of implementing this additional safety measure cannot be quantified due to lack of data on the incidence of enterotoxin production when septic transfusion reactions are investigated. Patients receiving PCs contaminated with *S. aureus* develop septic shock symptoms likely due to the secretion of exotoxins in PCs and adding bacterial toxin detection will supplement current mitigation strategies to prevent transfusion of bacterially contaminated PC units. It is, however, acknowledged that implementing bacterial toxin detection should be feasible and efficient for blood banks.

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S.C.I. and S.R.-A. designed the study. J.B. and C.M. isolated and characterized English *S. aureus* strains. B.Y. and C.P. prepared RNA from PCs and TSB cultures and obtained RNAseq. S.C.I. performed RNAseq data analyses and experimental work related to immunological assays for detection of staphylococcal exotoxins. S.C.I. and S.R.-A. wrote the manuscript, which was reviewed by B.Y., C.P., J.B., and C.M.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The genome sequences that have been used to design primers are available; they have been published as Genome Sequence Announcements (references 9-12 of the manuscript). The transcriptome data is available at the NCBI site, BioProject: PRJNA915492 found at https://dataview.ncbi.nlm.nih.gov/object/PRJNA915492?reviewer=413fkhgmqdmj42p7u0ursh41tt.

ORCID

Carl McDonald https://orcid.org/0000-0003-3230-3287
Sandra Ramirez-Arcos https://orcid.org/0000-0003-0705-4987

REFERENCES

1. Ramirez-Arcos S, Evans S, McIntyre T, Pang C, Yi QL, DiFranco C, et al. Extension of platelet shelf life with an improved bacterial testing algorithm. Transfusion. 2020;60:2918–28.
2. Fatalities Reported to FDA Following Blood Collection and Transfusion Annual Summary for FY2018. [Cited 2023 March 6]. Available from: https://www.fda.gov/media/136907/download
3. Levy JH, Neal MD, Herman JH. Bacterial contamination of platelets for transfusion: strategies for prevention. Crit Care. 2018;22:271.
4. Pati I, Masiello F, Pupella S, Cruciani M, De Angelis V. Efficacy and safety of pathogen reduced platelets compared with standard apheresis platelets: a systematic review of RCTs. Pathogens. 2022;11:639.
5. Michael RJ. FDA guidance on bacterial contamination risk control strategies to enhance the safety and availability of platelets: advantages and limitation. Ann Blood. 2021;21:10.
6. Störmer M, Vollmer T. Diagnostic methods for platelet bacteria screening: current status and developments. Transfus Med Hemother. 2014;41:19–27.
7. Benjamin RJ, McDonald CP, ISBT Transfusion Transmitted Infectious Disease Bacterial Workgroup. The international experience of

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bacterial screen testing of platelet components with an automated microbial detection system: a need for consensus testing and reporting guidelines. Transfus Med Rev. 2014;28:61–71.
8. Freudenburg-de Graaf W, Spelmink S, Heijnen J, de Korte D. Transfusion transmitted bacterial infections (TTBI) involving contaminated platelet concentrates: residual risk despite intervention strategies. Ann Blood. 2022;7:26.
9. Yousuf B, Flint A, Weedmark K, Pagotto F, Ramirez-Arcos S. Genome sequence of *Staphylococcus aureus* strain CBS2016-05, isolated from contaminated platelet concentrates in Canada. Microbiol Resour Announc. 2021;26:10.
10. Yousuf B, Flint A, Weedmark K, McDonald C, Bearne J, Pagotto F, et al. Genome sequence of *Staphylococcus aureus* strain PS/BAC/317/16/W, Isolated from Contaminated Platelet Concentrates in England. Microbiol Resour Announc. 2021;2:10.
11. Paredes C, Chi SI, Flint A, Weedmark K, McDonald C, Bearne J, et al. Complete genome sequence of *Staphylococcus aureus* PS/BAC/169/17/W, isolated from a contaminated platelet concentrate in England. Microbiol Resour Announc. 2021;11:10.
12. Paredes C, Chi SI, Flint A, Weedmark K, McDonald C, Bearne J, et al. Complete genome sequence of *Staphylococcus aureus* CI/BAC/25/13/W, Isolated from Contaminated Platelet Concentrates in England. Microbiol Resour Announc. 2021;11:10.
13. Chi SI, Kumaran D, Zeller MP, Ramirez AS. Transfusion of a platelet pool contaminated with exotoxin-producing *Staphylococcus aureus*: a case report. Ann Blood. 2022;7:43.
14. Loza-Correa M, Kou Y, Taha M, Kalab M, Ronholm J, Schlievert PM, et al. Septic transfusion case caused by a platelet pool with visible clotting due to contamination with *Staphylococcus aureus*. Transfusion. 2017;57:1299–303.
15. Hu DL, Li S, Fang R, Ono HK. Update on molecular diversity and multipathogenicity of staphylococcal superantigen toxins. Anim Dis. 2021;1:7.
16. Perpoint T, Lina G, Poyart C, de Barbeyrac B, Traineau R, Jeanne M, et al. Two cases of fatal shock after transfusion of platelets contaminated by *Staphylococcus aureus*: role of superantigenic toxins. Clin Infect Dis. 2004;39:e106–9.
17. Chi SI, Ramirez-Arcos S. Staphylococcal enterotoxins enhance biofilm formation by *Staphylococcus aureus* in platelet concentrates. Microorganisms. 2022;11:89.
18. Wilson GJ, Tuffs SW, Wee BA, Seo KS, Park N, Connelley T, et al. Bovine *Staphylococcus aureus* superantigens stimulate the entire T cell repertoire of cattle. Infect Immun. 2018;86:e00505–18.
19. Grispoldi L, Popescu PA, Karama M, Gullo V, Poerio G, Borgogni E, et al. Study on the growth and enterotoxin production by *Staphylococcus aureus* in canned meat before retorting. Toxins. 2019;11:291.
20. Schwendimann L. Evaluation and prediction of staphylococcal enterotoxins. Human health and pathology. PhD Thesis. Université Paris-Est. 2021 https://theses.hal.science/tel-03666896/file/TH2021PESC0002.pdf
21. Vernozy-Rozand CM, Mazuy-Cruchaudet C, Bavari C, Richard Y. Improvement of a concentration protocol based on trichloroacetic acid for extracting staphylococcal enterotoxins in dairy products. Rev Méd Vet. 2004;155:533–7.
22. Venkataramana M, Rashmi R, Uppalapati SR, Chandranayaka S, Balakrishna K, Radhika M, et al. Development of sandwich dot-ELISA for specific detection of Ochratoxin A and its application on to contaminated cereal grains originating from India. Front Microbiol. 2015;6:511.
23. Greco-Stewart VS, Brown EE, Parr C, Kalab M, Jacobs MR, Yomtovian RA, et al. *Serratia marcescens* strains implicated in adverse transfusion reactions form biofilms in platelet concentrates and demonstrate reduced detection by automated culture. Vox Sang. 2012;102:212–20.
24. Fischer AJ, Kilgore SH, Singh SB, Allen PD, Hansen AR, Limoli DH, et al. High prevalence of *Staphylococcus aureus* enterotoxin gene cluster superantigens in cystic fibrosis clinical isolates. Genes (Basel). 2019;12:10.
25. Ferry T, Thomas D, Genestier AL, Bes M, Lina G, Vandenesch F, et al. Comparative prevalence of superantigen genes in *Staphylococcus aureus* isolates causing sepsis with and without septic shock. Clin Infect Dis. 2005;41:771–7.
26. Sahu P, Pinkalwar N, Dubey RD, Paroha S, Chatterjee S, Chatterjee T. Biomarkers: an emerging tool for diagnosis of a disease and drug development. Asian J Res Pharm Sci. 2011;1:9–16.
27. Jain KK. Biomarkers of infectious diseases. The handbook of biomarkers. New York, NY: Humana Press; 2017. p. 219–38.
28. Jacobs MR, Good CE, Lazarus HM, Yomtovian RA. Relationship between bacterial load, species virulence, and transfusion reaction with transfusion of bacterially contaminated platelets. Clin Infect Dis. 2008;46:1214–20.
29. Delage G, Bernier F. Bacterial culture of platelets with the large volume delayed sampling approach: a narrative review. Ann Blood. 2021;6:30.
30. Kamel H, Ramirez-Arcos S, McDonald C, ISBT Transfusion-Transmitted Infectious Disease Bacterial Working Party Bacterial Subgroup. The international experience of bacterial screen testing of platelet components with automated microbial detection systems: an update. Vox Sang. 2022;117:647–55.
31. Proft T, Fraser JD. Bacterial superantigens. Clin Exp Immunol. 2003;133:299–306.
32. Singh M, Agrawal RK, Singh BR, Mendiratta SK, Agarwal RK, Singh MK, et al. Development and evaluation of simple dot-blot assays for rapid detection of staphylococcal Enterotoxin-a in food. Indian J Microbiol. 2017;57:507–11.
33. Golafrouz H, Ahari H, Anvar SA, Shahbazzadeh D. Detection of *Staphylococcus aureus* enterotoxin a (SEA) using dot-ELISA in milk samples. J Med Microbiol Infect Dis. 2020;8:132–6.
34. Cloutier M, De Korte D. ISBT transfusion-transmitted infectious diseases working party, subgroup on bacteria. Residual risks of bacterial contamination for pathogen-reduced platelet components. Vox Sang. 2022;117:879–86.
35. Abela MA, Fenning S, Maguire KA, Morris KG. Bacterial contamination of platelet components not detected by BacT/ALERT®. Transfus Med. 2018;28:65–70.
36. Brailsford SR, Tossell J, Morrison R, McDonald CP, Pitt TL. Failure of bacterial screening to detect *Staphylococcus aureus*: the English experience of donor follow-up. Vox Sang. 2018;113:540-6.
37. Coutinho H, Galloway A, Ajdukiewicz K, Cleeve V. A case of *Staphylococcus aureus* septicaemia following platelet transfusion. J Clin Pathol. 2010;63:262–3.

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

A Danish national, multicentre evaluation of the new donor vigilance system among different staff groups

Christina Mikkelsen[1,2] | Helene Martina Paarup[3] | Mie Topholm Bruun[4] | Louise Ørnskov Pedersen[5] | Sys Hasslund[5] | Rune Larsen[6] | Bitten Aagaard[7] | Betina Samuelsen Sørensen[7]

[1]Department of Clinical Immunology, Copenhagen University Hospital, Copenhagen, Denmark

[2]Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health Science, Copenhagen University, Copenhagen, Denmark

[3]Danish Medicines Agency, Regulatory & Clinical Assessment, Copenhagen, Denmark

[4]Department of Clinical Immunology, Odense University Hospital, Odense, Denmark

[5]Department of Clinical Immunology, Aarhus University Hospital, Aarhus, Denmark

[6]Department of Clinical Immunology, Naestved Hospital, Naestved, Denmark

[7]Department of Clinical Immunology, Aalborg University Hospital, Aalborg, Denmark

Correspondence
Christina Mikkelsen, Department of Clinical Immunology, Copenhagen University Hospital, Blegdamsvej 9, Copenhagen 2100, Denmark.
Email: christina.mikkelsen@regionh.dk

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Abstract

Background and Objectives: Two years after implementing a new national donor vigilance system, the Danish Haemovigilance Committee conducted a nationwide survey to evaluate the implementation among different staff groups. We present the results here.

Materials and Methods: The study was designed as an anonymous online survey to evaluate the satisfaction with the new registration, understanding of the parameters used and the user-friendliness. The REDCap platform was used. The questionnaire consisted of 22 questions. Ordinal variables were answered using five-point Likert scale (1 = strongly disagree to 5 = strongly agree). The data were analysed using descriptive statistics. Successful implementation was defined as mean overall satisfaction ≥4 and mean understanding of the individual components (adverse reaction category, severity and imputability) in the registration ≥4.

Results: In all, 104 staff members (77.9% donation staff) participated. The mean (SD) overall satisfaction among all participants was 3.96 (0.94), highest among medical doctors (4.43 (0.78)) and lowest for administrative or other personnel (2.78 (1.09)). The mean scores for understanding the adverse reaction categories, severity and imputability were 3.92 (0.94), 3.92 (0.94) and 3.88 (1.00), respectively. Experience with a previous donor vigilance system was associated with lower scores. The most successful implementation programme included a medical doctor for introduction and a contact person.

Conclusion: The goal for successful implementation was not met. However, the overall attitude towards the new registration was positive and indicates that the system is suitable for different staff groups. Our results suggest that implementation could benefit from special attention to administrative staff and those accustomed to another donor vigilance system.

Keywords

donors, donor health, haemovigilance

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Highlights

- International standards for the categorization of adverse reactions and for their severity and imputability were found to be easy for donation staff to apply.
- Implementation of a new donor vigilance system is more challenging when a donor vigilance system is already in place.
- Implementation can be improved by an in-person introduction to the system and by having a defined contact person.

INTRODUCTION

Denmark is divided into five healthcare regions. In each, a regional blood establishment (BE) manages blood donation and registration of donation-related adverse reactions in blood donors.

In January 2020, the Danish Haemovigilance Committee implemented a new national donor vigilance system. The system was based on three parameters: (1) adverse reaction categories as defined by the ISBT; (2) severity as defined by the AABB; and (3) a modified version of imputability levels adapted to donor vigilance by the ISBT [1].

In the new donor vigilance system, registration of donation-related adverse reactions is performed on-site at the donation facilities in case of immediate reactions or later in case of delayed reactions.

The registration is done directly in the blood bank IT system and is a standardized code that includes a category number and a severity and imputability score. It is registered in the donor's donation chart, as a separate entry under the corresponding donation. Staff members can access previous registrations using donor id.

The staff member initially notified about the adverse reaction is responsible for ensuring immediate and correct registration. Therefore, donation staff and secretaries perform the majority of registrations. More complicated or severe reactions can be passed on to the attending physician for clinical assessment and subsequent registration of the adverse reaction. The Haemovigilance Committee provided national guidelines that included definitions of the three parameters used and examples of how to rate severity and imputability. This was included in regional standing operating procedures, which staff members had to sign off when read.

The regional BE annually provides the Haemovigilance Committee with data extraction using a predefined template. The committee then prepares the national report.

Currently, two IT systems are used in the Danish BE. One region uses Blodflödet and the remaining four, ProSang. In Blodflödet, three separate entries are made during the registration, one for each parameter. In ProSang, all three parameters are embedded in a single three-digit code.

The ISBT definitions and AABB severity tool have already been validated across different staff groups [2, 3], and users especially found imputability hard to assess. However, participants were predominantly senior staff members.

Following implementation of this new registration in Denmark, the Haemovigilance Committee repeatedly received questions concerning imputability ratings, in particular from staff at donation sites. Therefore, to evaluate the success of the implementation and to identify areas for improvement, a national survey was planned in order to study both the user attitude towards the system and the feasibility including user-friendliness of the system. The survey included all staff members working with blood donors in any of the Danish blood banks and blood collection sites, which enables us to investigate potential differences among staff groups, different age groups and to evaluate the potential differences between educational groups, age groups among staff and to evaluate the different methods of implementation on a national level.

MATERIALS AND METHODS

Survey design

The survey principles by Dillmann et al. [4] and Schleyer and Forrest [5] were followed, while the results were reported in accordance with Eysenbach [6]. The survey design was a structured format comprising a maximum of 22 questions, with adaptive questioning to reduce complexity and volume for the participants. For staff members in the Capital region, two follow-up questions were included due to a different IT system than in the other four regions. Single and multiple-choice questions were included with answer types assigned to nominal, ordinal and ratio scales. Ordinal variables were answered using five-point Likert scale (1 = strongly disagree to 5 = strongly agree). The fully translated questionnaire is included in the Supplementary Material. In the following, we present a brief overview.

The survey-landing page described the survey topics and length, goals and provided information about data handling according to the European General Data Protection Regulation (GDPR). The survey was voluntary, non-incentivized and fully anonymous. Survey information was not suitable to draw any conclusions to the participant, nor were technical identifiers (IP address, or other) stored. To start the survey, participants were required to give consent to their participation. The first survey section included demographical questions related to the respondent's age, gender, professional position, educational background, time of employment in a BE and healthcare region. In the second section, participants were asked a series of questions concerning the previous donor vigilance system and the implementation procedure of the new system. The third section addressed participant satisfaction with different aspects of the system including IT solutions and the three parameters. The fourth section addressed the

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system's user-friendliness and understanding both overall and for each of the three parameters. The fifth and final section asked participants whether they were aware of different ways to add comments or do follow up registration and whether they knew that data were routinely published.

Successful implementation was defined from the point of view of feasibility and user satisfaction as an overall satisfaction score of ≥4 and a score of ≥4 in understanding each of the three parameters.

Survey draft and validation

The Haemovigilance Committee drafted the questionnaire. Then, —one or two staff members from each region were invited to participate in a focus group meeting to inquire about: (1) the overall perception and understanding of the donor vigilance system; (2) the course of implementation; (3) expectations to results from the system; and (4) others. The participants in the focus group meeting unanimously commented that the survey should address the following: (1) Are users familiar with the option to provide additional comments if deemed relevant? (2) Do users omit registration due to lack of routine or time? (3) Do users know that results are used to improve donor safety and whether they know where to find more information about the results? (4) Do the users understand how to rate severity and imputability and are they satisfied with the guidelines? Item one through three were addressed in the survey's fifth section and item four in the fourth section as previously described in Section 2.1.

These four points were included in a revised version of the questionnaire. We then asked the focus group to fill out the questionnaires and provide their comments.

We received five responses. They commented on the use of abbreviations and highlighted that questions addressing technical issues in the IT system Blodflödet should be limited to participants from the relevant region. Based on these comments, we revised the questionnaire and asked a new group of staff members to test it and provide their comments. We received nine responses in total from three different regions, five include comments/suggestions and four respondents had no comments.

The respondents reported that the questionnaire took between 5 and 10 min to complete, which we considered acceptable. Furthermore, they asked that the Danish translation of imputability be used. Also, to add the possibility to reply if they themselves did not do the actual registration. Finally, for a couple of multiple-choice questions, they missed the opportunity to reply, "Do not know".

Recruitment

The target group included BE staff members working with the new donor vigilance system and included IT staff, secretaries, medical doctors, nurses, phlebotomists and all other staff members in contact with blood donors. The survey was administered between 01.04.2022 and

TABLE 1 Presentation of the study cohort

Study cohort	Number (percentage)
Sex	
Female	99 (95.2)
Male	5 (4.8)
Age	
20–29	8 (7.7)
30–39	18 (17.3)
40–49	31 (29.8)
50–59	23 (22.1)
>60	24 (23.1)
Education	
Medical laboratory technologist	42 (41.3)
Nurse	42 (40.4)
Medical doctor	7 (6.7)
Other[a]	12 (11.5)
Position in the blood establishment	
Donation staff	81 (77.9)
Medical doctor	7 (6.7)
Other	16 (15.4)
Time employed in the blood establishment	
<1 year	13 (12.5)
1–3 years	13 (12.5)
3–5 years	9 (8.7)
>5 years	15 (14.4)
>10 years	54 (51.9)
Region	
1	43 (41.3)
2	23 (22.1)
3	17 (16.3)
4	17 (16.3)
5	<5 (<4)
Response rate by region	
1	61%
2	33%
3	57%
4	22%
5	29%
National response rate	40%
IT system	
Blodflödet	43 (41.3)
ProSang	61 (58.7)
Previous donor vigilance system	
Yes	66 (63.5)
No	8 (7.7)
Do not know	30 (28.8)

[a]Includes administration, secretary, other health and non-health background.

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30.04.2022 (01.05.2022–31.05.2022 in the Zeeland region) via REDCap [7, 8]. REDCap is a freeware system approved for research projects in the Capital Region by the Danish Data Protection Agency (I-suite Nb. 05196).

Information about and a link to the survey was administered from the Danish Society of Clinical Immunology to the Organization of Transfusion Centers in Denmark (OTCD), who distributed the email to their staff members. Reminder emails were sent 2 weeks before the survey ended.

Data exclusion

The estimated target study population was 260 staff members. Only completed questionnaires were included. Of 140 participants, 36 were excluded due to missing data, defined as a questionnaire that had not been fully completed. In total, the completion rate was 74%, and 104 participants were included in the analysis.

Statistical analysis

The main outcome variables of the survey were overall user satisfaction, user-friendliness and understanding of the parameters. All outcome variables mentioned can be assigned to ordinal scales. Descriptive variables include nominal scales (gender, position, professional background, region, implementation and follow-up), ordinal scales (five-point Likert scale for user satisfaction, friendliness, understanding and attitude) and ratio scales (age and time of employment in BE). The percentage of respondents who chose each item was calculated. The descriptive data analysis was carried out using R studio. Results are presented as mean and standard deviation (SD). Groups with less than five individuals are either combined with other groups or presented as <5. Data were analysed in R studio 2022.02.03 Build 492.

RESULTS

Details of the study cohort are given in Table 1. The regional distribution of participants largely follows the size and activity of the BEs, with the highest number of participants coming from the two largest regions. Regions with a larger geographical coverage and fewer centralized donation sites have lower participation. The sex distribution is extremely skewed but is thought to reflect the gender composition of BE staff.

From Table 2 it can be seen that the overall satisfaction on a scale from 1 (very dissatisfied) to 5 (very satisfied) did not reach the goal of a score of 4 or higher. However, all were still above the neutral value of 3, thus in the positive part of the scale. Slightly surprising was the fact that 12%–24% of the included 104 participants were not able to

TABLE 2 Results from the survey presented as mean (SD)

	Number (percentage) of responses	Mean (SD)	Percentage of non-responders
Satisfaction with the new registration			
Overall satisfaction	83 (79.8)	3.96 (0.94)	20.2
Satisfaction with the IT solution	79 (76.0)	4.05 (0.90)	24
Satisfaction with the construction of the registration codes	91 (87.5)	3.76 (0.90)	12.5
Satisfaction with the three categories used (type of complication, severity and imputability)	90 (86.5)	3.79 (0.83)	13.5
User-friendliness and understanding of the new registration			
The new registration is easy to use	88 (84.6)	3.93 (1.05)	15.4
The new registration is not time-consuming	89 (85.6)	3.82 (1.09)	14.4
The adverse reaction categories are easy to use and understand	88 (84.6)	3.92 (0.94)	15.4
The severity categories are easy to use and understand	91 (87.5)	3.99 (0.91)	12.5
The imputability categories are easy to use and understand	91 (87.5)	3.88 (1.00)	12.5
Did you know an annual donor vigilance report is published			
Yes	54 (51.9)	–	
No	50 (48.1)	–	
Do you think the new registration will improve donation safety?			
Yes	17 (16.3)	–	
No	42 (40.4)	–	
Do not know	45 (43.3)	–	

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TABLE 3 Responses stratified according to demographics

	Overall satisfaction ($n = 83$)	Overall user-friendliness and understanding ($n = 88$)
Sex		
Female	3.95 (0.95)	3.89 (1.05)
Male	4.20 (0.84)	4.75 (0.50)
Age group		
20–29	4.00 (0.86)	4.13 (0.99)
30–39	4.33 (0.99)	3.88 (1.02)
40–49	3.81 (0.69)	3.85 (1.08)
50–59	3.74 (1.24)	4.05 (1.10)
>60	4.17 (0.86)	3.89 (1.08)
Time employed in BE		
<1 year	4.00 (0.71)	4.09 (0.83)
1–3 years	4.17 (0.94)	4.25 (0.75)
3–5 years	4.33 (0.82)	4.33 (1.21)
>5 years	3.69 (1.18)	3.86 (1.03)
>10 years	3.93 (0.94)	3.78 (1.15)
Position		
Donation staff	4.07 (0.82)	4.04 (0.96)
Medical doctor	4.43 (0.78)	4.50 (0.55)
Other	2.78 (1.09)	2.80 (1.23)
IT system		
Blodflödet	3.75 (0.98)	3.53 (1.08)
ProSang	4.10 (0.90)	4.19 (0.95)
Introduction of the new system		
New standard of operations		
Yes	3.96 (0.83)	3.93 (1.03)
No	3.97 (1.13)	3.94 (1.09)
Introduction by daily leader		
Yes	3.64 (1.15)	4.13 (0.99)
No	4.03 (0.89)	3.89 (1.06)
Introduction by medical doctor		
Yes	4.00 (0.78)	4.15 (0.80)
No	3.96 (0.98)	3.89 (1.09)
Introduction by a colleague		
Yes	4.08 (0.78)	3.82 (1.09)
No	3.92 (1.00)	3.98 (1.03)
No introduction		
Yes	2.50 (0.71)	2.33 (0.58)
No	4.00 (0.92)	3.99 (1.02)
Designated contact person		
Daily leader	4.00 (1.10)	4.06 (0.66)
Appointed medical doctor	4.20 (0.78)	4.43 (0.65)
Appointed colleague	3.90 (0.72)	3.68 (1.04)
Other	4.00 (1.26)	3.71 (1.25)
No	3.67 (1.05)	4.00 (1.32)
Do not know	4.09 (1.04)	3.67 (1.30)

Note: In total, 87 females and 5 males are included in the satisfaction results and 84 females and 4 males in the user-friendliness and understanding results. Results are presented as mean (SD).

give a score and instead replied "Do not know" (here named non-responders). When looking deeper into the groups of non-responders, we found that they had a higher percentage of participants replying that they did not do the registration themselves compared with the responders. For the satisfaction questions listed in Table 2, 14.3% of non-responders versus 6.0% of responders had replied that they themselves did not perform the registration. For user-friendliness, the numbers were 25.0% versus 3.4%, respectively. No difference in demographics between responders and non-responders was seen.

In the three specific questions addressing user-friendliness and understanding of the adverse reaction categories, severity and imputability, 10%, 5.5% and 11%, respectively, answered that they did not find the parameters easy to use or understand. Of these, 70%–75% further replied that this was because they did not understand the grading.

Despite the overall positive responses, only 16.3% believed that the registration would improve donation safety and only half of the participants knew that the Haemovigilance Committee publishes an annual report.

When looking closer into the responses for overall satisfaction and user-friendliness, we observed some interesting patterns (Table 3). First, mean scores were higher among men and medical doctors. For age and employment time in BB, the youngest and newest colleagues were in general more positive towards the new system. Most interesting was that non-donation/medical staff, that is, administrative personnel rated much lower than the other groups and below neutral. The new registration had the largest impact on daily routine for Blodflödet users and they had lower overall satisfaction as well as lower satisfaction with the technical aspect of the registration compared with ProSang users (3.83 (1.05) vs. 4.18 (0.78)).

The method of introduction to the system seemed to have some effect. While new standard of operations, newsletters or information on the intranet did not seem to have an effect, introduction by a daily leader, medical doctor or colleague did. Participants who responded that they had an appointed medical doctor or leader as their primary contact person also had improved satisfaction.

We proceeded to investigate regional variation and the effect of a previous donor vigilance system (Table 4). The higher the percentage of staff members who were acquainted with a previous registration in their region, the lower the scores for the two main parameters. This was not dependent on region, size, time of employment in the BE, IT system or participant age. However, as previously observed, administrative personnel had lower ratings (data not shown). This could indicate that even though familiar with a previous system, performing "bed side" registration in close collaboration with the donor improves the experience compared with those who have simply changed from one system to another without the clinical context.

To access the specific challenges in Blodflödet, where three separate entries must be made per registration, a follow-up question was included for Blodflödet users only. When asked to estimate how often they registered all three parameters, 51% replied "always" and 14% replied "always, if I find all three relevant", 7% registered more than 75% of the time, another 7% registered it half of the time, 12% did not register themselves and the remaining 9% replied "do not know".

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TABLE 4 Responses stratified by healthcare region and previous donor vigilance system.

	Region 1	Region 2	Region 3	Region 4	Region 5
Previous registration					
Yes	78.1	40	80	53.8	66.7
No	0	20	6.7	23.1	0
Do not know	21.9	40	13.3	23.1	33.3
Survey responses					
Overall satisfaction	3.75 (0.98)	4.30 (1.03)	3.87 (0.74)	4.15 (0.69)	3.67 (1.53)
Overall user-friendliness	3.53 (1.08)	4.50 (0.802)	3.93 (1.10)	4.15 (0.801)	3.50 (1.29)

Note: Previous registration is presented as percentage and responses by mean (SD).

DISCUSSION

The overall attitude towards the new donor vigilance system in Denmark is positive. Based on this survey, we did not meet our criteria for a successful implementation, although we were extremely close. However, if looking only at donation staff, our primary group of interest, we did succeed.

The donor vigilance system in Denmark builds on validated, international standards. However, the ISBT and AABB validations were limited to senior and academic staff. As the primary users in Denmark are donation staff, the Danish Haemovigilance Committee wished to evaluate their perception of the new system, both to identify areas with a need for revision or better guidelines, and also in the interest of colleagues elsewhere, who are in the process of implementing new guidelines for donor vigilance.

Our results show that even though the system was implemented during the COVID-19 pandemic, most staff members are positive towards the system and its individual components. We initially anticipated that imputability would receive low ratings for user-friendliness and understanding as this was the parameter that sparked the most debate and questions. Nevertheless, even though the ratings were marginally lower, they were still overall positive.

One of the main challenges of the implementation phase was to design a system that could work in two very different IT systems. In particular, the registration in Blodflödet was a concern, as the three parameters (category, severity and imputability, respectively) had to be registered with three separate entries, whereas the staff had previously been accustomed to a three-digit single entry. Given this, it is not surprising that Blodflödet users have the lowest satisfaction and find the new registration harder to use than ProSang users, who were subject to very few changes in their daily registration routine. As Denmark is preparing a national implementation of a shared ProSang-based IT system, no further actions will be taken by the Haemovigilance Committee on this matter.

The new system was differently implemented in all five regions. Based on our results, an in-person presentation improved the user's experience and understanding, whereas a written guideline did not seem to make a difference in how the users perceived and understood the registration.

One concern is the low number of staff members who know that donor vigilance data are compiled and published in an annual report. It could be suspected that this is also one of the reasons why more than 40% did not believe that the new system will improve donation safety. The Haemovigilance Committee is currently working on a strategy to improve the information provided to our staff as well as the possibilities to use the data in, for example, national campaigns to reduce donation-related adverse reactions.

The main strength of our survey is the large number of responses from donation staff. To our knowledge, this is the first time these international standards have been evaluated by this staff group. However, the study also has some important limitations. First, for comparison, a higher number of medical doctors and administrative staff should have been preferred. Second, in the design of the survey, the option to answer, "Do not know" instead of a score does mean that for some of the key questions, we have a high number of non-responders. However, given that not all staff groups use the new registration in the same way, that is, some do not register themselves, this was considered the best option. In addition, it also revealed that some staff members do not consider themselves informed well enough to answer, which also gives cause for reflection.

In total, 36 incomplete responses had to be excluded. As most staff members do not have designated office times, they had to fill out the questionnaire while working at the donation site with the risk of being interrupted. We therefore expect that the incomplete response reflects this, as the design of the survey did not give the possibility to save and resume at another time point.

In conclusion, even though the implementation of the Danish donor vigilance system did not meet our criteria for successful implementation, staff members were predominantly positive towards the new registration in terms of satisfaction, user-friendliness and understanding. Our results show that international standards for adverse reaction categories, severity and imputability are suitable for most staff groups.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ORCID

Christina Mikkelsen https://orcid.org/0000-0002-2945-6197
Rune Larsen https://orcid.org/0000-0002-7635-8012

REFERENCES

1. Mikkelsen C, Paarup HM, Topholm Bruun M, Ørnskov Pedersen L, Hasslund S, Larsen R, et al. The new donor vigilance system in Denmark reveals regional differences in adverse reactions supposedly caused by variation in the registration. Vox Sang. 2022;117:321–7.
2. Townsend M, Kamel H, van Buren N, Wiersum-Osselton J, Rosa-Bray M, Gottschall J, et al. Development and validation of donor adverse reaction severity grading tool: enhancing objective grade assignment to donor adverse events. Transfusion. 2020;60:1231–42.
3. Land KJ, Townsend M, Goldman M, Whitaker BI, Perez GE, Wiersum-Osselton JC. International validation of harmonized definitions for complications of blood donations. Transfusion. 2018;58:2589–95.
4. Dillman DA, Tortora RD, Bowker D. Principles for constructing web surveys. Joint Meet Am Stat Assoc. 1998;64:1–16.
5. Schleyer TKL, Forrest JL. Methods for the design and administration of web-based surveys. J Am Med Inform Assoc. 2000;7:416–25.
6. Eysenbach G. Improving the quality of web surveys: the Checklist for Reporting Results of Internet E-Surveys (CHERRIES). J Med Internet Res. 2004;6:e34.
7. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap consortium: building an international community of software platform partners. J Biomed Inform. 2019;95:103208.
8. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42:377–81.

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

Monitoring viral genomic sequences in transfusion-transmitted viruses

Daniel Candotti[1,2] | Steven J. Drews[3,4] | Helen M. Faddy[5,6] |
ISBT Transfusion-Transmitted Infectious Diseases Working Party, Subgroup on Viruses

[1]Department of Blood Transmitted Agents, National Institute of Blood Transfusion, Paris, France

[2]Department of Virology, Henri Mondor Hospital, Paris-Est University, Inserm U955-IMRB-Team 18, Créteil, France

[3]Department of Microbiology, Canadian Blood Services, Edmonton, Alberta, Canada

[4]Division of Diagnostic and Applied Microbiology, Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada

[5]School of Health, University of the Sunshine Coast, Petrie, Queensland, Australia

[6]Research and Development, Australian Red Cross Lifeblood, Kelvin Grove, Australia

Correspondence

Daniel Candotti, Department of Virology, Henri Mondor Hospital, Paris-Est University, Inserm U955-IMRB-Team 18, Créteil, France.
Email: daniel.candotti@inserm.fr

Helen M. Faddy, School of Health and Behavioural Sciences, University of the Sunshine Coast, The Sunshine Coast, Queensland, Australia.
Email: hfaddy@usc.edu.au

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Abstract

Background and Objectives: Monitoring genomic sequences of blood-borne viruses infecting blood donors enables blood operators to undertake molecular epidemiology, confirm transfusion transmission and assess and characterize molecular and serological screening assays. The purpose of the study was to determine how blood operators globally value viral diversity surveillance and to assess its impact.

Materials and Methods: An electronic questionnaire was developed and circulated to members of the International Society of Blood Transfusion-transmitted infectious diseases working party. Responses were compiled and complete data sets were analysed.

Results: Ninety-seven percent of respondents agreed that monitoring viral genomic sequences was important to blood operators and the transfusion community. However, only 47% of respondents are currently doing this monitoring. The main limitations reported were a lack of financial resources and expertise. Sequencing techniques, primarily next-generation sequencing and also Sanger sequencing, were considered most appropriate, with the preferred option for testing being regional or national reference centres. Respondents agreed that engagement with public health authorities needs to be enhanced.

Conclusion: Monitoring genomic sequences of blood-borne viruses is widely considered important by the transfusion community because of its direct applications for transfusion safety, and beyond for public health in general. Therefore, there is a need to strengthen collaboration between blood operators and public health authorities. While national and regional reference centres may be the most appropriate structure for such testing, international collaborations should not be overlooked. Overcoming financial barriers will be an important hurdle for many.

Keywords

diversity, public health, sequencing, transfusion safety, virus

Highlights

- Blood operators and the transfusion community favoured the implementation of monitoring genomic sequences of blood-borne viruses.

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- Lack of financial resources and experienced staff are the main limitations.
- Enhanced collaboration between blood operators and public health authorities is needed.

INTRODUCTION

Rapid advances and continuous improvements in serology assays and nucleic acid amplification testing (NAT) have reduced the risk of transfusion-transmitted infectious diseases (TTIDs). In addition, the introduction of molecular screening has stimulated the development and use of increasingly sophisticated molecular methods to confirm initial screening results. Concomitantly, the development of molecular biology has led to an unprecedented increase in the knowledge of the genetics of TTIDs including viruses, and, in particular, their diversity.

Differences in the nucleic acid sequences of a viral genome are driven by genetic variability leading to phenotypic changes. This is the result of various drivers, such as the natural history of viral infection, high replication rates and host–pathogen interactions [1]. This can occur through a range of mechanisms including the high intrinsic error rate of the viral reverse transcriptases or the RNA-dependent RNA polymerases, reassortment or template switching [2]. RNA viruses tend to display greater amounts of genetic diversity than DNA viruses, while diversity is higher in single-stranded compared with double-stranded viruses [2, 3]. Viruses with a smaller genome also tend to exhibit more genetic diversity than those with larger genomes [2, 4]. There is wide variability in the genetic diversity of blood-borne viruses, such as the single-stranded RNA viruses, hepatitis C virus (HCV) [5] and human immunodeficiency virus (HIV) [6], and the partially double-stranded DNA virus, hepatitis B virus (HBV) [7, 8]. Based on genetic diversity, viruses can be separated into genotypes and eventually sub-genotypes. HIV-1 is broken down into four groups (main [M], outlier [O], non-M [N] and P), with group M subdivided into nine subtypes [9], while HCV is classified into at least eight genotypes, each with several subtypes (over 80 in total) [10, 11], and HBV into nine genotypes with subtypes identified in all genotypes except E and G [12]. Inter-genotype genetic recombination, leading to new viral circulating recombinant forms (CRFs), has also been documented for HIV and HBV [13, 14].

Different methodological approaches are used to characterize and monitor genomic sequences in viruses infecting blood donors and eventually recipients. First, direct amplification of viral DNA or RNA by nested (RT)-PCR methods is usually needed to generate viral amplicons in sufficient quantity and length to obtain informative viral sequences. It might be necessary to increase the amount of viral nucleic acid template in the amplification reaction by increasing the volume of plasma in the nucleic acid extraction procedure or by concentrating viral particles in the sample prior to extraction [15–17]. Whole or partial genome sequencing is then performed by standard Sanger or next-generation sequencing (NGS) methods. Sanger sequencing is based on the random incorporation of dideoxynucleotides. NGS methods, however, allow for higher throughput, automated sequencing. Non-sequencing approaches, such as strain-specific nucleic acid tests or proteomics can also be used [18, 19]. Viral genetic characterization can be performed by blood transfusion centres either in-house, using technologies adapted to local resources and infrastructure, through regional or national reference centres, or, via international collaborative networks.

The analysis of viral genomic sequences in blood donors can have both direct and indirect impacts on transfusion safety. It may shed light on nucleic acid and amino acid variations that can reduce the performance of NAT or serological detection by altering primers/probes hybridization or antigenicity [20–22]. Genetic polymorphism may also affect the natural history of infection and negatively impact viraemia and antigenemia, which may challenge the analytical sensitivity of detection assays [23]. Identification of the molecular features responsible for detection failure has proven essential to improve not only molecular and serological blood screening tests but also both qualitative and quantitative viral diagnosis. Monitoring viral genomic sequences can also allow investigation of cross-reactive samples leading to false-positive results, as shown in donors vaccinated against the Japanese encephalitis virus and tested falsely reactive for West Nile virus (WNV) RNA [24]. Characterization of TTIDs is essential for assessing the transmissibility of (re)emerging viruses, and the effectiveness of new screening strategies on the residual risk to blood safety. However, in the absence of a recipient pre-transfusion sample free of viral markers, a high genetic similarity of the viral strains found in both donor and recipient is required to definitively differentiate between transfusion transmission, reactivation of persistent viral infection and iatrogenic infection, especially in highly endemic areas [25]. In addition, molecular epidemiology is essential for monitoring the constant genetic evolution of viruses that may result in changes in genotype geographical distribution and the emergence of inter-genotype CRFs and viral variants with potential differences in their replicative and infectious properties, pathogeny and sensitivity to antiviral treatments or vaccines [26–28]. Examining diversity in specific genes can provide insight into single or multiple nucleotide polymorphisms associated with phenotypic changes. For example, a single amino acid change to the WNV NY99 genotype resulted in the development of the WN02 genotype, which had a shorter extrinsic incubation period leading to rapid spread across the United States eventually displacing the original NY99 strain [26, 29].

Monitoring the prevalence of viral variants in blood donors appears to be important to assess the risk of transfusion transmission and future epidemiological changes in order to continuously evaluate and improve the performance of screening tests to ensure blood safety. Blood operators are in a unique position to enable investigations for variants of transfusion-transmitted viruses.

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However, the place of this type of molecular investigation in the blood transfusion field is still a matter of debate. An interactive session focused on monitoring transfusion-transmitted virus diversity was developed by the Virology subgroup of the International Society of Blood Transfusion TTID Working Party (ISBT TTID WP) during the 31st regional congress of the ISBT—ISBT in Focus!—in June 2021. Following on from widespread interest during and after this session, the present study was conceived to understand how blood operators globally value monitoring for viral diversity and assess the potential impact of monitoring for viral diversity in the transfusion and blood operator fields.

MATERIALS AND METHODS

A survey, which contained 19 questions, was developed to gather data on the value of monitoring for viral diversity and its possible impact on blood operators (see Data S1). The electronic link to the survey was first sent to members of the ISBT TTID WP on 27 October 2021, with a reminder sent on 4 February 2022. No new responses were accepted after 28 February 2022.

Response data were analysed, and basic graphs were generated using the GraphPad Prism v9.4.1 software.

RESULTS

Thirty-two questionnaires were received that contained sufficient data to be analysed. Twenty-nine respondents provided information about the World Health Organization (WHO) income classification of their country, with 13 (45%), 10 (35%) and 6 (21%) classified as high, middle, and low income, respectively. Of all respondents, 11 (34%) identified themselves as blood service/operator, 7 (22%) as transfusion medicine laboratory, 9 (28%) as both transfusion medicine and blood service/operator, 4 (13%) as other (including virology research laboratory, reference laboratory, industry and medical practitioner) and 1 (3%) did not provide the information. Among the 28 respondents whose activities are related to blood donation qualification, preparation and distribution of blood products as well as post-donation expertise, 46%, 14% and 29% were from national, regional and local organizations, respectively (Table 1). One (4%) was from a regional (blood screening)/national (reference laboratory) hybrid structure, and no information was provided for two (7%). National organizations were more frequent in high-income countries (80%) compared to middle- (33%) and low-income countries (50%). There was no difference between middle- and low-income countries with local organizations accounting for 44%–50% (data not shown). Four respondents had activities not linked to blood donations.

TABLE 1 Organizational status of 28 respondents with blood transfusion activities.[a]

Status	Blood services/ operators	Transfusion medicine laboratories	Blood services/ operators + transfusion medicine laboratories	Reference laboratory	Total (%)
National	6	5	1	1	13 (46.4%)
Regional	1	–	3	–	4 (14.3%)
Local	3	1	4	–	8 (28.6%)
Other[b]	1	–	–	–	1 (3.6%)
NA[c]	–	1	1	–	2 (7.1%)

[a]Excluding four respondents identified as virology research laboratory, industry, medical practitioner or without identification.
[b]Hybrid regional (blood screening)/national (reference laboratory) structure.
[c]Not available (information not provided by the respondent).

TABLE 2 Estimating the potential impact of viral diversity monitoring on blood service/operator policy and public health activities.

	Participants			WHO national income classification			
Impact estimation	Total ($n = 32$)	Monitoring viral diversity ($n = 15$)	Not monitoring viral diversity ($n = 17$)	Low ($n = 6$)	Medium ($n = 10$)	High ($n = 13$)	NA ($n = 3$)
Blood service/operator policy							
Yes	12 (37%)	7 (47%)	5 (29%)	5 (83%)	3 (30%)	3 (23%)	1 (33%)
Maybe	15 (47%)	5 (33%)	10 (59%)	1 (17%)	4 (40%)	9 (69%)	1 (33%)
No	5 (16%)	3 (20%)	2 (12%)	–	3 (30%)	1 (8%)	1 (33%)
Public health activities							
Yes	16 (50%)	7 (47%)	9 (53%)	6 (100%)	5 (50%)	3 (23%)	2 (67%)
Maybe	11 (34%)	6 (40%)	5 (29%)	–	4 (40%)	6 (46%)	1 (33%)
No	5 (16%)	2 (13%)	3 (18%)	–	1 (10%)	4 (31%)	–

Abbreviations: NA, information not provided; WHO, World Health Organization.

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Thirty-one participants (97%) indicated that monitoring for viral diversity in blood donors is important. Only one participant from a reference laboratory in a high-income country indicated that this was not important but acknowledged that monitoring viral diversity was still relevant to public health. Most participants (84%) estimated that monitoring viral diversity had or could have an impact on both blood service/operator policy and public health activities, particularly in low- and middle-income settings (Table 2). Irrespective of whether viral diversity monitoring was performed, 69% (22/32) of participants stated that national or local blood services/operators were involved in informing and/or developing the public health strategy, and 64% (18/28 reporting) considered this contribution important in their respective countries regardless of national income classification. However, 97% of responders still highlighted the need for improved engagement between blood operators and public health authorities.

Participants noted a range of benefits from monitoring viral diversity, including identification and monitoring of viral variants that may compromise the performance of molecular and serological screening tests, surveillance of circulating viral strains evading therapeutic and immunoprophylactic treatments, investigation of TTID, monitoring national and/or international molecular epidemiology, and improving knowledge of the natural history of infections (Figure 1). A similar importance was attributed to these different benefits, with the exception of the last. However, just under half of the participants (47%, $n = 15$) reported that they currently monitor viral diversity, with 40% (6/15) indicating that this monitoring was conducted in a national/regional reference centre, followed by 40% (6/15) in-house at the local level. One participant reported the viral diversity to be monitored both in-house and in a national/regional reference centre, and the remaining two in a consortium of blood services and a public

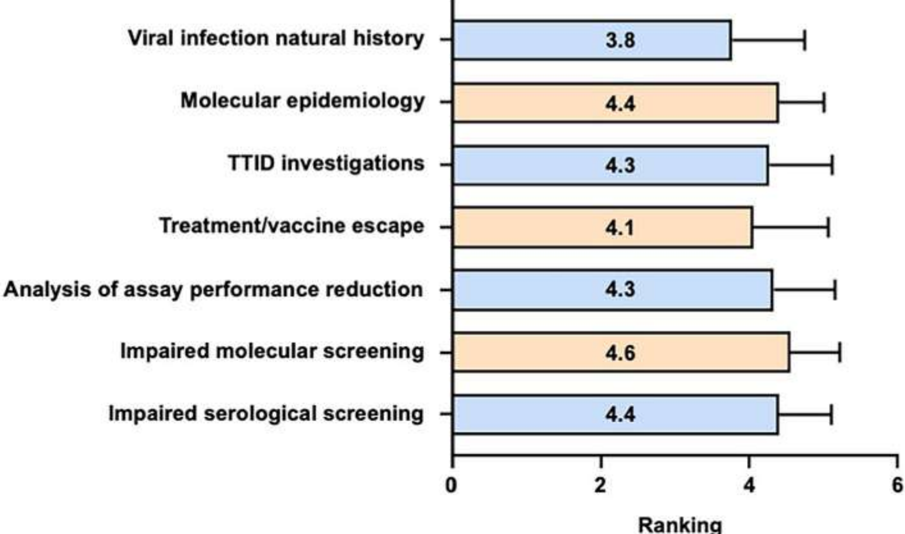

FIGURE 1 Perceived benefits of monitoring viral diversity. Participants ranked benefits from 1 (not relevant) to 5 (high importance). Columns represent means, with error bars showing standard deviations. TTID, transfusion-transmitted infectious disease.

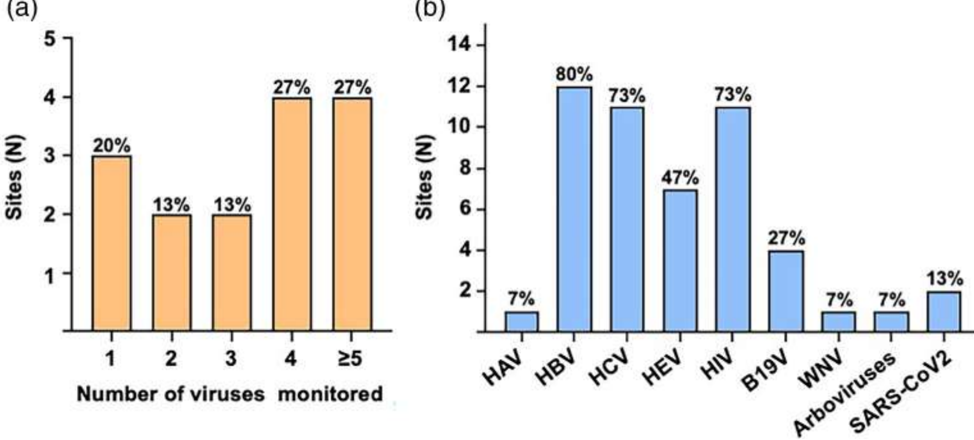

FIGURE 2 Number (a) and types of viruses (b) reported by 15 participants who monitor viral diversity.

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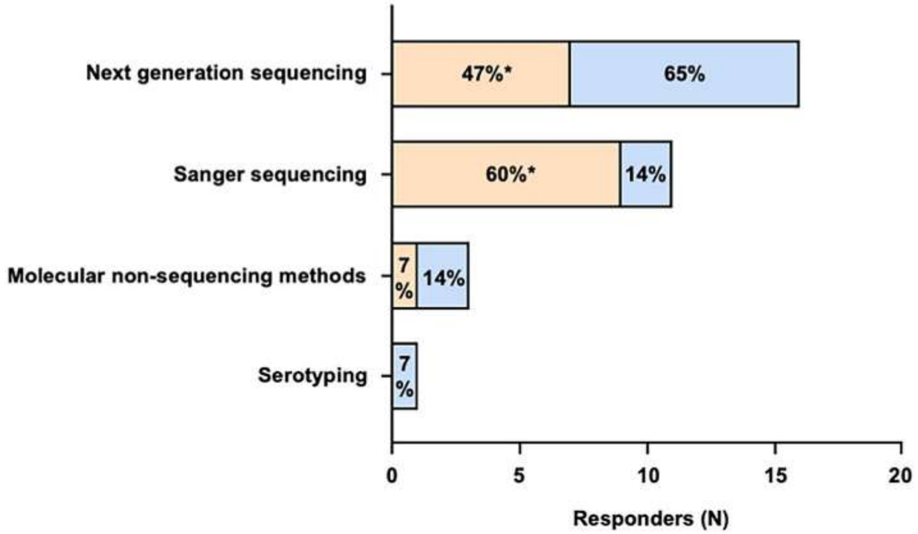

FIGURE 3 Methods to monitor viral diversity in blood donors currently used (grey bars) or wishing to be used (white bars) by 15 and 14 participants, respectively. *Two participants reported to use both sequencing (Sanger and NGS) and non-sequencing methods. NGS, next-generation sequencing.

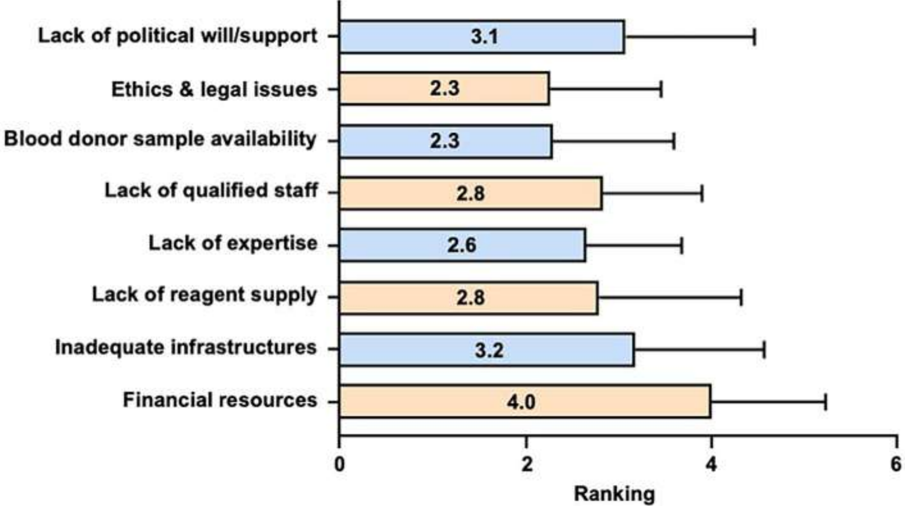

FIGURE 4 Limitations to implementing viral diversity monitoring. Participants ranked benefits from 1 (not relevant) to 5 (high importance). Columns represent means, with error bars showing standard deviations.

health institute. Of those 15 responders, monitoring 4 or more viruses was the most common approach (54%), followed by monitoring for a single virus (20%) (Figure 2a). HBV genetic diversity was monitored by the majority of participants (80%), followed by HCV and HIV (73%), then HEV (47%) (Figure 2b). Sanger sequencing and NGS were the methods used by 60% (9/15) and 47% (7/15) of respondents, respectively, including two respondents reporting the use of both methods (Figure 3). One participant reported using non-sequencing molecular methods (e.g., genotype-specific nucleic acid tests). NGS was used exclusively in national/regional reference centres or public health institutes, whereas Sanger sequencing was mainly used in-house locally. Although the use of NGS was limited to high- and middle-income countries, there was no significant difference overall compared with the use of Sanger sequencing that was also used in three low-income settings (data not shown). Of 14 respondents interested in implementing viral diversity monitoring, 9 (65%), 2 (14%), 2 (14%) and 1 (7%) preferred NGS, Sanger sequencing, non-sequencing molecular methods and serotyping, respectively (Figure 3).

Overall, the main limitations to implementing viral diversity monitoring were reported to be financial, followed by inadequate infrastructures, and lack of political support (Figure 4). When examining the responses in relation to the WHO national income classification, financial resources and inadequate infrastructures were the main limitations for those categorized as low- or middle-income (data not shown). While financial reasons were still the main limitation for high-income countries, lack of qualified staff and lack of expertise were also key limitations. Various operational models for monitoring viral diversity with viruses were proposed, with 54% of responses

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TABLE 3 Best-fit model of viral diversity monitoring based on replies from 28 participants, with blood transfusion activities,[a] stratified according to WHO national income classification.

Models	WHO national income classification			Total (n = 28)
	Low (n = 6)	Medium (n = 10)	High (n = 12)	
Regional/national reference centre	4 (67%)	4 (40%)	7 (58%)	15 (54%)
In-house local resources	2 (33%)	2 (20%)	1 (8%)	5 (18%)
International collaborative network	–	4 (40%)	2 (17%)	6 (21%)
Other[b]	–	–	2 (17%)	2 (7%)

Abbreviation: WHO, World Health Organization.
[a]Excluding four respondents identified as virology research laboratory, industry, medical practitioner or without identification.
[b]Including collaboration with local research groups or different models used to address different questions.

favouring the use of regional or national reference centres (Table 3). Although developing an international collaborative network was the next most frequent response (24%), no responder classified as low by the WHO national income classification favoured this option. The development of suitable in-house testing based on local resources was also on the radar of 17% of participants.

DISCUSSION

Blood safety is directly challenged by the continuous, and mostly unpredictable, emergence of new viruses (e.g., SARS-CoV-2) and variants of well-characterized viruses that are not limited to those viruses known to be highly variable (e.g., parvovirus B19). Indeed, viral variants have been repeatedly identified in blood donors in recent years [28, 30–32]. Therefore, monitoring viral genomic sequences appears clearly an important initiative for blood operators as agreed by 97% of participants to the present survey. This figure may be biased by the number of participants in the present survey (n = 32) who may represent primarily individuals already actively involved in viral genetic surveillance and may constitute a limitation of the study. However, only slightly less than half of the respondents (47%) indicated that they were currently monitoring viral genomic sequences. An imbalance between participants from countries with different resource levels could also introduce a bias. Although the majority (45%) of respondents were from countries classified as high income, countries classified as middle (35%) and low (21%) income were also represented.

The reasons for the limited monitoring of viral genomic sequences appear to be primarily the lack of financial resources, irrespective of income classification. Limited financial resources result in a lack of adequate infrastructure, and limited access to advanced technologies, but possibly also the lack of qualified staff. Viral genetic monitoring and characterization require a sufficient number of qualified, trained and competent staff. It might be particularly challenging for blood operators to establish training programmes and develop valorization measures to retain experienced staff [32]. Expertise in viral genetics was also reported as a key limiting factor, suggesting that efforts should be made to more actively involve clinicians and researchers with expertise in molecular virology, epidemiology and infectious diseases in the blood transfusion field. Ultimately, the development of methods for monitoring viral genomic sequences will depend heavily on political will to allocate the necessary resources, as participants indicated.

Different methods exist for monitoring viral genomic sequences. Genotype-specific (RT)-PCR amplification is a relatively inexpensive approach that does not require very advanced technology. However, the level of information provided remains limited. Sanger sequencing of PCR amplicons of full-length or partial viral genomes directly or after cloning remains a method of choice to characterize viral diversity. NGS is becoming an attractive alternative for 47% of participants. Compared to Sanger sequencing, NGS can identify a greater diversity of variants and provide the information to enable the broader comparison of genetic relationships in a population of viruses [33]. However, NGS requires costly sophisticated equipment, not only for sequencing itself but also for sample preparation and expensive maintenance. The development of portable third-generation sequencing, based on nanopore technology, is a promising alternative, which applies long-read single-molecule sequencing directly to amplified whole viral genomes, allowing also for the direct identification of recombination events within and between virus species [34]. This technology has been rapidly implemented successfully in complicated clinical situations to provide rapid viral genome consensus sequencing from field-collected samples in resource-limited settings [35]. However, genomic data analysis is complex and may require additional bioinformatics resources and staff training, regardless of the NGS methodology used [36]. Despite these limitations, NGS-based approaches may offer a greater level of data enrichment that may allow for more substantial analyses [18]. Ultimately, the choice of methodological approach will depend on the type of infrastructure available.

Various operational models have been proposed for monitoring viral genomic sequences, ranging from in-house to national and regional reference centres. The majority of participants favoured the use of regional or national reference centres, which present the advantage of re-grouping resources and expertise, and potentially allow for the implementation of the most advanced technologies. However, this option requires initially expensive infrastructure, adequate centralized management and a strong political will to provide financial resources over the long term. Small local facilities may be

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more affordable when combined with the use of less sophisticated but nonetheless valuable sequencing facilities, particularly in resource-limited settings that may face practical difficulties in transporting samples to centralized sequencing facilities. The establishment of international collaborations to pool resources and expertise can be an interesting approach. Collaborative networks such as those developed within the ISBT TTID WP have proven to be effective in sharing knowledge, providing practical training and securing national and international funding. However, only 21% of participants, mainly from middle-income settings, favoured this option. Although limited in number, no participant from a low-resource region chose this option. The reasons why are unclear but may be related to difficulties in sample transport and delays in getting information back, local regulations or concerns around being too dependent on other organizations. Although analysing donor viral sequences has many applications, there are limitations to relying only on such sequences, and future studies should utilize viral sequences from donors and the general population. Ultimately, the approach used will depend on individual settings or regional circumstances.

It is clear that blood operators and public health are inherently linked. The present survey showed that there is a dominant feeling among blood operators that collaboration between the two needs to be improved. Efforts should be made to break down any silos between the two. It would be interesting to hear from public health actors about this question. Nevertheless, blood operators often have national coverage, have access to specimens from a healthy subset of the population and have experience undertaking large-scale donor surveillance using the most sensitive methods available [37]. The role of blood operators as members of the one-health community became evident with the SARS-CoV-2 pandemic, which saw blood operators undertake large seroprevalence studies to inform public health policy [38]. Moreover, blood donor viral sequences have informed various public health initiatives [39, 40]. In front of the globalization of human and animal population travel, human activities with increased risk of zoonotic infections, and the environmental changes leading to the geographical expansion of viral vectors, blood operators and the transfusion medicine community should be prepared to actively engage in surveillance of emerging viral infections as a major actor in public health and in order to ensure optimal blood safety.

In conclusion, monitoring for genetic diversity of blood-borne viruses appears important given the many applications for blood safety and global public health. It is important for enhanced collaboration between blood operators and public health authorities to enable the greatest benefits to be afforded. While national and regional reference centres may provide the most suitable place for such monitoring, international collaborations should not be overlooked. Overcoming financial hurdles will be important. This review of practices inevitably leads to questions regarding the next steps that blood operators and the transfusion medicine community can play in the genetic characterization of blood-borne viruses. There are three core options including (1) conceptualization of specific multi-regional studies to characterize blood-borne viruses identified in blood donors; (2) development of networks to support the implementation of viral molecular characterization, especially in limited-resourced settings; and (3) creation of grant-writing teams to identify and compete for research grants focused on this theme.

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CONFLICT OF INTEREST STATEMENT

S.J.D. has been a paid consultant on transfusion-transmitted arboviruses and malaria for Roche. He has functioned as a content expert for respiratory viruses for Johnson & Johnson (Janssen) and has received funding in-kind from Abbott. D.C. has received research funding from Grifols Diagnostic Solutions Inc. H.F. has received research funding and/or honoraria from Grifols Diagnostic Solutions Inc. and Roche Diagnostic Solutions.

DATA AVAILABILITY STATEMENT

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

ORCID

Daniel Candotti https://orcid.org/0000-0001-7738-5373
Steven J. Drews https://orcid.org/0000-0003-2519-1109
Helen M. Faddy https://orcid.org/0000-0002-3446-8248

REFERENCES

1. Nasir A, Kim KM, Caetano-Anolles G. Viral evolution: primordial cellular origins and late adaptation to parasitism. Mob Genet Elements. 2012;2:247–52.
2. Sanjuan R, Domingo-Calap P. Genetic diversity and evolution of viral populations. Encyclopedia of Virology. 2021:53–61.
3. Duffy S, Shackelton LA, Holmes EC. Rates of evolutionary change in viruses: patterns and determinants. Nat Rev Genet. 2008;9:267–76.
4. Domingo E, Perales C. Viral quasispecies. PLoS Genet. 2019;15: e1008271.
5. Zhang Y, Gao Z, Wang S, Liu J, Paul N, He T, et al. Hepatitis C virus genotype/subtype distribution and evolution among Chinese blood donors: revealing recent viral expansion. PLoS One. 2020;15: e0235612.
6. Bes M, Piron M, Casamitjana N, Gregori J, Esteban JI, Ribera E, et al. Epidemiological trends of HIV-1 infection in blood donors from Catalonia, Spain (2005–2014). Transfusion. 2017;57:2164–73.
7. Grabarczyk P, Garmiri P, Liszewski G, Doucet D, Sulkowska E, Brojer E, et al. Molecular and serological characterization of hepatitis B virus genotype A and D infected blood donors in Poland. J Viral Hepat. 2010;17:444–52.
8. Phan NMH, Faddy HM, Flower RL, Dimech WJ, Spann KM, Roulis EV. Low genetic diversity of hepatitis B virus surface gene amongst Australian blood donors. Viruses. 2021;13:13.

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9. Sharp PM, Hahn BH. Origins of HIV and the AIDS pandemic. Cold Spring Harb Perspect Med. 2011;1:a006841.
10. Nakano T, Lau GM, Lau GM, Sugiyama M, Mizokami M. An updated analysis of hepatitis C virus genotypes and subtypes based on the complete coding region. Liver Int. 2012;32:339–45.
11. Hedskog C, Parhy B, Chang S, Zeuzem S, Moreno C, Shafran SD, et al. Identification of 19 novel hepatitis C virus subtypes-further expanding HCV classification. Open Forum Infect Dis. 2019;6:ofz076.
12. Kramvis A. Genotypes and genetic variability of hepatitis B virus. Intervirology. 2014;57:141–50.
13. Hemelaar J, Gouws E, Ghys PD, Osmanov S. Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004. AIDS. 2006;20:W13–23.
14. Araujo NM. Hepatitis B virus intergenotypic recombinants worldwide: an overview. Infect Genet Evol. 2015;36:500–10.
15. Deng X, Guo X, Li T, Laperche S, Zang L, Candotti D. Alternative hepatitis B virus DNA confirmatory algorithm identified occult hepatitis B virus infection in Chinese blood donors with non-discriminatory nucleic acid testing. Blood Transfus. 2022;20:8–17.
16. Zahn A, Allain JP. Hepatitis C virus and hepatitis B virus bind to heparin: purification of largely IgG-free virions from infected plasma by heparin chromatography. J Gen Virol. 2005;86:677–85.
17. Satoh K, Iwata-Takakura A, Yoshikawa A, Gotanda Y, Tanaka T, Yamaguchi T, et al. A new method of concentrating hepatitis B virus (HBV) DNA and HBV surface antigen: an application of the method to the detection of occult HBV infection. Vox Sang. 2008;95:174–80.
18. Houldcroft CJ, Beale MA, Breuer J. Clinical and biological insights from viral genome sequencing. Nat Rev Microbiol. 2017;15:183–92.
19. Gilchrist CA, Turner SD, Riley MF, Petri WA Jr, Hewlett EL. Whole-genome sequencing in outbreak analysis. Clin Microbiol Rev. 2015;28:541–63.
20. Edelmann A, Kalus U, Oltmann A, Stein A, Unbehaun A, Drosten C, et al. Improvement of an ultrasensitive human immunodeficiency virus type 1 real-time reverse transcriptase-polymerase chain reaction targeting the long terminal repeat region. Transfusion. 2010;50:685–92.
21. Foglieni B, Candotti D, Guarnori I, Raffaele L, Berzuini A, Spreafico M, et al. A cluster of human immunodeficiency virus Type 1 recombinant form escaping detection by commercial genomic amplification assays. Transfusion. 2011;51:719–30.
22. El Chaar M, Candotti D, Crowther RA, Allain JP. Impact of hepatitis B virus surface protein mutations on the diagnosis of occult hepatitis B virus infection. Hepatology. 2010;52:1600–10.
23. Caligiuri P, Cerruti R, Icardi G, Bruzzone B. Overview of hepatitis B virus mutations and their implications in the management of infection. World J Gastroenterol. 2016;22:145–54.
24. Drews SJ, Makowski K, Wood H, Dimitrova K, Yan MTS, Young D, et al. A case series of inactivated Japanese encephalitis virus vaccination associated with positive West Nile virus blood donor screening nucleic acid tests. Transfusion. 2020;60:1097–103.
25. Candotti D, Assennato SM, Laperche S, Allain JP, Levicnik-Stezinar S. Multiple HBV transfusion transmissions from undetected occult infections: revising the minimal infectious dose. Gut. 2019;68:313–21.
26. Moudy RM, Meola MA, Morin LL, Ebel GD, Kramer LD. A newly emergent genotype of West Nile virus is transmitted earlier and more efficiently by Culex mosquitoes. Am J Trop Med Hyg. 2007;77:365–70.
27. Abdou Chekaraou M, Brichler S, Mansour W, Le Gal F, Garba A, Deny P, et al. A novel hepatitis B virus (HBV) subgenotype D (D8) strain, resulting from recombination between genotypes D and E, is circulating in Niger along with HBV/E strains. J Gen Virol. 2010;91:1609–20.
28. Baylis SA, O'Flaherty N, Burke L, Hogema B, Corman VM. Identification of rabbit hepatitis E virus (HEV) and novel HEV clade in Irish Blood Donors. J Hepatol. 2022;77:870–2.
29. Pesko KN, Ebel GD. West Nile virus population genetics and evolution. Infect Genet Evol. 2012;12:181–90.
30. Uche EI, Chukwukaodinaka NE, Akinbami AA, Adeyemi OI, Hassan AO, Bamiro RA, et al. Common hepatitis B virus genotypes among blood donors in Lagos, Nigeria. Niger Postgrad Med J. 2022;29:228–35.
31. Ye X, Liu L, Chen L, Nie X, Huang L, Ye D, et al. High-frequency notable HBV mutations identified in blood donors with occult hepatitis B infection from Heyuan city of Southern China. Front Immunol. 2022;13:754383.
32. van Hulst M, Smit Sibinga CT, Postma MJ. Health economics of blood transfusion safety—focus on sub-Saharan Africa. Biologicals. 2010;38:53–8.
33. Drews SJ. Taxonomy and classification of viruses. Manual of clinical microbiology. 12th ed. Washington DC: ASM Press; 2023.
34. McNaughton AL, Roberts HE, Bonsall D, de Cesare M, Mokaya J, Lumley SF. Illumina and nanopore methods for whole genome sequencing of hepatitis B virus (HBV). Sci Rep. 2019;9:7081.
35. Quick J, Loman NJ, Duraffour S, Simpson JT, Severi E, Cowley L, et al. Real-time, portable genome sequencing for Ebola surveillance. Nature. 2016;530:228–32.
36. Maljkovic Berry I, Melendrez MC, Bishop-Lilly KA, Rutvisuttinunt W, Pollett S, Talundzic E, et al. Next generation sequencing and bioinformatics methodologies for infectious disease research and public health: approaches, applications, and considerations for development of laboratory capacity. J Infect Dis. 2020;221:S292–307.
37. O'Brien SF, Drews SJ, Lewin A, Osiowy C, Drebot MA, Renaud C. Canadian blood suppliers: an expanding role in public health surveillance? Can Commun Dis Rep. 2022;48:124–30.
38. O'Brien SF, Caffrey N, Yi QL, Pambrun C, Drews SJ. SARS-CoV-2 seroprevalence among Canadian blood donors: the advance of Omicron. Viruses. 2022;14:2336.
39. Tassachew Y, Belyhun Y, Abebe T, Mihret A, Teffera T, Ababi G, et al. Magnitude and genotype of hepatitis delta virus among chronic hepatitis B carriers with a spectrum of liver diseases in Ethiopia. Ann Hepatol. 2023;28:100770.
40. Barzon L, Montarsi F, Quaranta E, Monne I, Pacenti M, Michelutti A, et al. Early start of seasonal transmission and co-circulation of West Nile virus lineage 2 and a newly introduced lineage 1 strain, northern Italy, June 2022. Euro Surveill. 2022;27:2200548.

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

Can volume-reduced plasma products prevent transfusion-associated circulatory overload in a two-hit animal model?

Esther B. Bulle[1,2] | Robert B. Klanderman[1,2,3] | Marit B. de Wissel[2] | Joris J. T. H. Roelofs[4,5] | Denise P. Veelo[3] | Charissa E. van den Brom[1,2,6] | Rick Kapur[7] | Alexander P. J. Vlaar[1,2]

[1]Department of Intensive Care, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

[2]Laboratory of Experimental Intensive Care and Anesthesiology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

[3]Department of Anesthesiology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

[4]Department of Pathology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

[5]Amsterdam Cardiovascular Sciences, Microcirculation, University of Amsterdam, Amsterdam, The Netherlands

[6]Department of Anesthesiology, Amsterdam UMC, VU University, Amsterdam, The Netherlands

[7]Sanquin Research, Department of Experimental Immunohematology, Landsteiner Laboratory, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

Correspondence
Esther B. Bulle, Laboratory of Experimental Intensive Care and Anesthesiology, Amsterdam UMC, University of Amsterdam, Meibergdreef 9, 1100DD Amsterdam, The Netherlands.
Email: e.b.bulle@amsterdamumc.nl

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Abstract

Background and Objectives: Transfusion-associated circulatory overload (TACO) is a pulmonary transfusion complication and a leading cause of transfusion-related morbidity and mortality. Volume overload and rising hydrostatic pressure as a consequence of transfusion are seen as the central pathway leading to TACO. A possible preventative measure for TACO could be the use of low-volume blood products like volume-reduced lyophilized plasma. We hypothesize that volume-reduced lyophilized plasma decreases circulatory overload leading to a reduced pulmonary capillary pressure and can therefore be an effective strategy to prevent TACO.

Materials and Methods: A validated two-hit animal model in rats with heart failure was used. Animals were randomized to receive 4 units of either solvent-detergent pooled plasma (SDP) as control, standard volume lyophilized plasma (LP-S) or hyperoncotic volume-reduced lyophilized plasma (LP-VR). The primary outcome was the difference between pre-transfusion and post-transfusion left ventricular end-diastolic pressure (ΔLVEDP). Secondary outcomes included markers for acute lung injury.

Results: LVEDP increased in all randomization groups following transfusion. The greatest elevation was seen in the group receiving LP-VR (+11.9 mmHg [5.9–15.6]), but there were no significant differences when compared to groups receiving either LP-S (+6.3 mmHg [2.9–13.4], $p = 0.29$) or SDP (+7.7 mmHg [4.5–10.5], $p = 0.55$). There were no significant differences in markers for acute lung injury, such as pulmonary wet/dry weight ratios, lung histopathology scores or PaO_2/FiO_2 ratio between the three groups.

Conclusion: Transfusion with hyperoncotic volume-reduced plasma did not attenuate circulatory overload compared to standard volume plasma and was therefore not an effective preventative strategy for TACO in this rat model.

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Keywords
lyophilized plasma, pulmonary oedema, pulmonary transfusion reaction, transfusion-associated circulatory overload, volume-reduced

Highlights

- There was no significant difference in pulmonary capillary pressure between animals transfused with volume-reduced plasma and animals transfused with a regular volume plasma product.
- Volume-reduced plasma was not effective in attenuating transfusion-associated circulatory overload in this animal model.
- In contrast with what was expected, a volume-reduced hyperoncotic plasma product led to a non-significant trend towards an increase in pulmonary capillary pressure.

INTRODUCTION

Transfusion-associated circulatory overload (TACO) is a pulmonary transfusion complication [1]. TACO is still responsible for about one third of transfusion-related deaths in spite of adopted transfusion practices [2–4]. The proposed pathophysiological mechanism of TACO is that a first hit, like heart or renal failure, results in volume incompliance [5]. Subsequently, transfusion leads to a fluid overload in the vascular system, causing the pulmonary capillary pressure to rise and fluid to extravasate from the capillaries into the lungs. This ultimately leads to pulmonary oedema and respiratory distress. Although other factors like inflammatory processes, glycocalyx injury, colloid osmotic pressure or storage lesion also may play a role in the pathophysiology of TACO, volume overload and increased hydrostatic pressure are seen as central features [6, 7].

Current preventative measures suggest reducing volume overload by using restrictive transfusion thresholds, single-unit transfusions, slow infusion rates and diuretics [8]. Considering volume overload in TACO is a fundamental factor, use of volume-reduced transfusion products could aid in preventing TACO.

Plasma transfusion has been identified as a risk factor for TACO in previous studies [9, 10]. Plasma can be freeze-dried resulting in lyophilized plasma. Lyophilized plasma in a standard volume has been used in military and trauma situations, because of the logistical benefits [11, 12]. It can, however, also be reconstituted in a smaller volume prior to patient administration, resulting in a more concentrated, hyperoncotic volume-reduced product. Especially in patients with volume incompliance and vulnerable for developing TACO, reduced volume transfusion might be a strategy to prevent TACO. The aim of this study was therefore to evaluate if concentrated volume-reduced lyophilized plasma could reduce the increase in pulmonary capillary pressure during transfusion compared to a standard volume plasma product.

MATERIALS AND METHODS

General information

Animal experiments were approved by the Dutch national committee for animal experimentation (project licence number: AVD118002017814) and executed according to the Animal Research Reporting of In Vivo Experiments (ARRIVE) guidelines and local guidelines [13]. Animals were housed in an animal housing facility and were kept on a 12-h light–dark cycle. Animals were fed standard rat chow and water ad libitum. The groups described here are part of a multi-armed randomized controlled trial. The control group (solvent-detergent pooled plasma [SDP]) is the same as previously published by our group. Results from this study including a detailed methods section have been previously published [14].

Animal procedures

In brief, experiments were performed using adult male Wistar rats weighing over 300 g. Animals were anaesthetised using 5% isoflurane with 100% oxygen, followed by an intraperitoneal injection with a mix of racemic ketamine (9 mg/100 g body weight), dexmedetomidine (6.25 μg/100 g) and atropine (0.25 μg/100 g). Then, a continuous intravenous mix of racemic ketamine (50 mg/kg/h) and dexmedetomidine (15 μg/kg/h) was administered through a tail vein cannula.

Animals were ventilated following a tracheotomy using a ventilator (Babylog 3000, Dräger, Lübeck, Germany). The right carotid artery was cannulated and a rat pressure–volume catheter (SPR-838, Millar, Houston, TX, USA) was inserted into the left ventricle. The right jugular vein was cannulated for monitoring central venous pressure and administration of fluids and transfusion products. The left carotid artery was cannulated for monitoring mean arterial pressure (MAP) and blood sampling for arterial blood gas analysis (RapidLab 500, Siemens, Erlangen, Germany).

Isovolemic anaemia was established by the exchange of arterial blood for a colloid solution (Tetraspan 6%, B. Braun, Melsungen, Germany) with a target haematocrit of 30% (±2%). Heart failure was induced by ligating the left anterior descending artery (LAD). The myocardial infarct was confirmed using electrocardiogram monitoring and visual inspection of the blanched myocardium. Animals were allowed to stabilize for 30 min, after which a transfusion product was administered. All animals received intravenous norepinephrine (2–8 μg/h) starting before LAD ligation till the end of the experiment. Animals were followed for 1 h and 30 min after start of transfusion, after which they were exsanguinated.

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Experimental groups and donor products

In this study, within the multi-armed trial, animals were randomized to receive either (1) lyophilized plasma (LyoPlas N-W [blood type AB], Germany Red Cross Blood Service West, Hagen, Germany) either as standard volume lyophilized plasma (LP-S) or (2) volume-reduced lyophilized plasma (LP-VR) or (3) solvent-detergent pooled plasma (SDP) (Omniplasma [blood type AB], Octapharma, Laachen, Switzerland). SDP transfusion was used to control for a regular plasma transfusion and LP-S transfusion controlled for possible differences within the lyophilized product. The randomization was executed in blocks, containing two interventions for each randomization group. A single unit of lyophilized plasma was reconstituted in 100 ml of sterile water (half of the recommended volume). From this hyperoncotic solution, 50 ml was stored (LP-VR) while the other half was then diluted to the recommended volume (LP-S). All products originated from human donors. Animals were transfused with an equivalent to 4 units (4 ml of LP-S or SDP or 2 ml of LP-VR). Infusion speed was kept equal in all groups at 8 ml per hour, as previous studies have shown that differing transfusion speeds will confound results [15, 16]. The number of units transfused and the infusion speed have been previously tested and validated when this animal model was developed [17]. Treatment allocation was blinded before administration. This was not possible to maintain during transfusion and follow-up because the LP-VR group received less volume in a shorter amount of time.

Haemodynamic measurements

The primary outcome of this study was the difference between left ventricular end-diastolic pressure (LVEDP) measured before and after transfusion (ΔLVEDP). LVEDP is a surrogate marker for pulmonary capillary pressure [18]. In the LP-S and SDP group, ΔLVEDP was calculated by subtracting the pre-transfusion LVEDP value from the post-transfusion measurement 30 min after start of transfusion. For the LP-VR group, the post-transfusion measurement was 15 min after start of transfusion. Haemodynamic data were recorded using LabChart (version 6.1, AD instruments, Oxford, UK). Blood conductivity was measured using the volume-cuvette procedure. Parallel conductance was measured after hypertonic saline bolus injections. Calibration processes were performed according to previously published protocols [19].

Sample processing and pathology

Secondary outcomes were markers of pulmonary injury. After exsanguination, the right lung was harvested and the lobes were separated. The right upper lobe was fixed in 4% formalin, processed into haematoxylin and eosin–stained slides and scored by an experienced pathologist blinded for intervention groups. Scores ranged from 0 to 3 based on the presence and extensiveness of perivascular and intra-alveolar oedema. The right lower lobe was dried in a dehumidifying stove at 37°C for 7 days and a lung wet/dry weight ratio was calculated.

In order to quantify the myocardial infarct size, the heart was excised and perfused with Evans blue dye. Hearts were frozen, cut transversely into 2-mm slices and counterstained with triphenyltetrazolium chloride. Slices were fixed in 4% formalin and scanned. The percentage of infarcted myocardium was calculated using image analysis software (ImageJ 1.50i, National Institutes of Health, USA) [20].

Sample size calculation

The sample size for the complete multi-armed randomized controlled trial was calculated based on the expected effects of SDP and Ringer's lactate [14]. We calculated a sample size of nine animals in each group would have 80% power to detect a difference in means of ΔLVEDP 4.15 mmHg. Assuming that the common standard deviation is 2.8 using a two-group *t*-test with a 0.05 two-sided significance level, one animal was added (±10%) to each group to account for post-randomization mortality.

Statistical analysis

Animals that died or could not maintain a MAP >65 mmHg before the transfusion intervention were excluded prior to randomization and replaced. If animals dropped out after the measurement 30 min after the start of transfusion, all haemodynamic data from these animals were included for analysis. Secondary pulmonary outcomes were analysed from animals that completed the whole experiment. Non-parametric tests were used for all analyses because of non-normally distributed data and small group sizes ($n = 10$). The Kruskal–Wallis test was used when comparing three groups and the Mann–Whitney *U* test was used for two-group comparisons. The Friedman test was used to test differences between timepoints within groups. All results are presented in medians and interquartile ranges. A two-sided *p*-value <0.05 was considered as statistically significant. Data were analysed using R (Rstudio, 4.0.3) and figures were created using GraphPad Prism (v9.1.0).

RESULTS

Total fluid input was reduced in the LP-VR group

All baseline characteristics prior to randomization were similar between groups (Table 1). Ten animals were randomized and successfully transfused in each group. The LP-S and LP-VR group both contained one animal that died after randomization. Isovolemic dilution was successful in all groups with an overall median haematocrit of 31% (30, 32) directly after dilution. At the end of the experiment, the total fluid input was significantly lower in the LP-VR group (7.8 ml [7.3, 8.5]) compared to the groups receiving LP-S or SDP (9.9 ml [9.4, 10.7] and 9.5 ml [9.2, 9.8], respectively, $p < 0.001$) due to the reduced transfusion volume.

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TABLE 1 Pre-randomization descriptive characteristics

Characteristics	LP-S, $n = 10$ (median, IQR)	LP-VR, $n = 10$ (median, IQR)	SDP, $n = 10$ (median, IQR)
Weight (g)	373 (362, 387)	355 (343, 365)	357 (331, 382)
Vent duration pre-transfusion (h)	2 h 05 min (1 h 45 min, 2 h 14 min)	1 h 50 min (1h 45 min, 1 h 59 min)	1 h 50 min (1 h 45 min, 1 h 59 min)
Fluid input pre-transfusion (ml)[a]	3.1 (3.0, 3.9)	3.0 (2.8, 3.7)	3.2 (2.9, 3.5)
Cardiac infarct size (%)	21 (18, 23)	22 (19, 24)	20 (19, 22)
pH	7.35 (7.33, 7.38)	7.37 (7.35, 7.39)	7.39 (7.35, 7.41)
Lactate (mmol/L)	3.2 (2.8, 3.8)	3.0 (2.6, 3.5)	2.7 (2.0, 3.1)
Hct (%)	36 (33, 39)	38 (36, 39)	36 (35, 39)

Abbreviations: Hct, haematocrit; IQR, interquartile range; LP-S, standard volume lyophilized plasma; LP-VR, volume-reduced lyophilized plasma; SDP, solvent-detergent pooled plasma.

[a]Fluid input was defined as all intravenously administrated fluids, but did not include volume used to achieve isovolemic anaemia, which was only used as volume replacement.

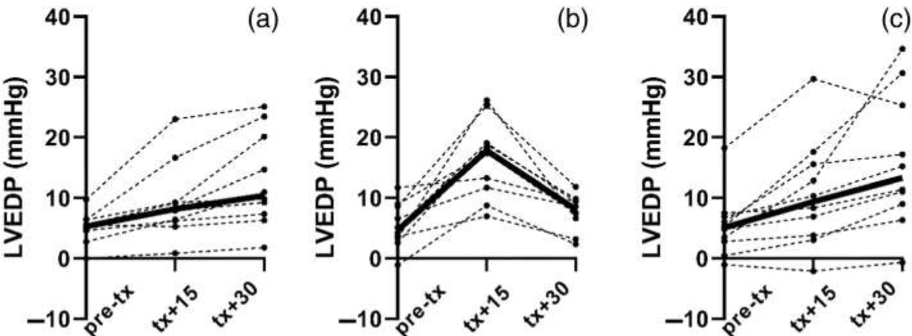

FIGURE 1 Left ventricular end-diastolic pressure (LVEDP) during transfusion. LVEDP values for all individual animals during transfusion (dotted lines) and median values per group (bold lines). (a) Standard volume lyophilized plasma ($n = 10$), (b) volume-reduced lyophilized plasma ($n = 10$) and (c) solvent-detergent pooled plasma ($n = 10$).

Transfusion with LP-VR did not decrease ΔLVEDP

LVEDP did not differ between groups pre-transfusion (median 5.1 mmHg [3.4, 6.6]) and increased significantly in all groups during transfusion with a maximum at the end of the transfusion, $p < 0.001$, $p = 0.002$ and $p < 0.001$ in groups receiving LP-S, LP-VR or SDP, respectively (transfusion of LP-VR ending after 15 min vs. 30 min in the SDP and LP-S groups). Post-transfusion LVEDP did not differ significantly between groups when all groups were compared ($p = 0.47$, Figure 1). ΔLVEDP was similar after 4 units of LP-S or SDP transfusion, 6.3 mmHg (2.9, 13.4) and 7.7 mmHg (4.5, 10.5), respectively ($p = 0.60$). There was a larger increase in ΔLVEDP after two units of LP-VR transfusion, 11.9 mmHg (5.9, 15.6). However, this was not significantly different from either the LP-S or the SDP group ($p = 0.29$ or 0.55, respectively). (Figure 2).

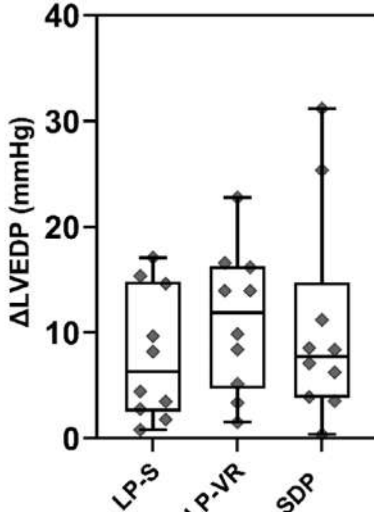

FIGURE 2 Left ventricular end-diastolic pressure (ΔLVEDP). Individual values for ΔLVEDP, boxplots display medians, interquartile ranges and min/max values. Peri-transfusion ΔLVEDP values were calculated as the post-transfusion LVEDP subtracted by the pre-transfusion LVEDP. The post-transfusion measurement for the standard volume lyophilized plasma (LP-S, $n = 10$) and solvent-detergent pooled plasma (SDP, $n = 10$) group was 30 min after start of transfusion, and for the volume-reduced lyophilized plasma (LP-VR, $n = 10$) group, it was 15 min after start of transfusion.

Post-transfusion haemodynamics were not significantly different between groups

Baseline haemodynamic variables did not differ between groups (Table 2). During transfusion, MAP increased significantly within all groups ($p = 0.002$, $p = 0.002$ and $p < 0.001$ in groups receiving LP-S,

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TABLE 2 Peri-transfusion haemodynamics

Pre-transfusion	LP-S, $n = 10$ (median, IQR)	LP-VR, $n = 10$ (median, IQR)	SDP, $n = 10$ (median, IQR)
Heart rate (bpm)	305 (280, 330)	280 (265, 317)	297 (277, 334)
MAP (mmHg)	75 (69, 87)	73 (69, 80)	77 (71, 81)
CVP (mmHg)	1.4 (0.8, 2.0)	1.4 (1.0, 2.0)	1.8 (1.2, 3.1)
LVEDP (mmHg)	5.3 (4.7, 6.2)	4.7 (3.5, 8.1)	5.1 (3.0, 6.7)
Cardiac output (ml/min)	17 (14, 24)	19 (15, 22)	20 (16, 30)
15 min after start of transfusion			
Heart rate (bpm)	311 (280, 340)	285 (277, 302)	304 (290, 352)
MAP (mmHg)	105 (81, 118)	109 (95, 127)	121 (102, 133)
CVP (mmHg)	NA	3.0 (2.5–4.0)	NA
LVEDP (mmHg)	8.2 (6.3, 9.3)	17.8 (12.1, 19.0)	9.4 (4.6, 14.9)
Cardiac output (ml/min)	20 (16, 26)	24 (19, 30)	27 (23, 33)
30 min after start of transfusion			
Heart rate (bpm)	333 (284, 339)		346 (311, 375)
MAP (mmHg)	132 (90, 142)		126 (112, 147)
CVP (mmHg)	1.5 (1.0, 2.4)		1.5 (1.1, 2.0)
LVEDP (mmHg)	10.4 (7.8, 18.8)		13.3 (9.5, 23.3)
Cardiac output (ml/min)	19 (16, 24)		25 (16, 31)

Abbreviations: bpm, beats per minute; CVP, central venous pressure; IQR, interquartile range; LP-S, standard volume lyophilized plasma; LP-VR, volume-reduced lyophilized plasma; LVEDP, left ventricular end-diastolic pressure; MAP, mean arterial pressure; NA, not applicable; SDP, solvent-detergent pooled plasma.

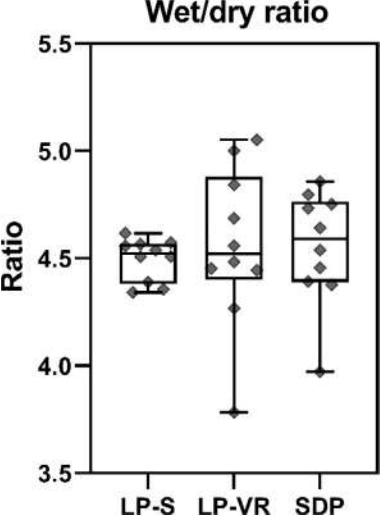

FIGURE 3 Pulmonary wet/dry weight ratio. Individual values for lung wet/dry weight ratio, boxplots display medians, interquartile ranges and min/max values. LP-S, standard volume lyophilized plasma ($n = 9$); LP-VR, volume-reduced lyophilized plasma ($n = 9$); SDP, solvent-detergent pooled plasma ($n = 10$).

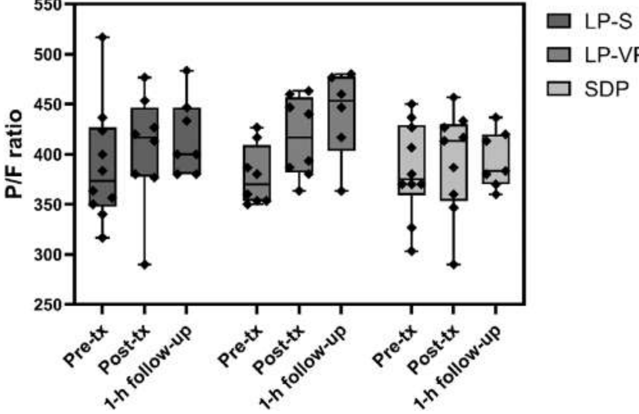

FIGURE 4 PaO_2/FiO_2 ratio (P/F ratio). Individual values for PaO_2/FiO_2 ratio during transfusion and follow-up; black lines display medians and interquartile ranges. LP-S, standard volume lyophilized plasma ($n = 9$); LP-VR, volume-reduced lyophilized plasma ($n = 9$); SDP, solvent-detergent pooled plasma ($n = 10$).

LP-VR or SDP, respectively). Heart rate increased significantly after SDP ($p < 0.001$), but increases in groups receiving LP-S or LP-VR were non-significant ($p = 0.150$ and 0.058, respectively). Hypertension and tachycardia are cardiovascular symptoms in line with TACO's diagnostic criteria. There was no difference in either heart rate or MAP between groups.

Reducing transfusion volume did not reduce pulmonary oedema, histopathological pulmonary injury or PaO_2/FiO_2 ratio

The median pulmonary wet/dry weight ratio was 4.52 (4.42, 4.56) in the LP-S group, 4.52 (4.45, 4.80) in the LP-VR group and 4.59 (4.41, 4.75) in the SDP group ($p = 0.685$) (Figure 3). The median pathology scores for pulmonary injury were 2.0 (2.0, 2.0), 1.0 (1.0, 2.0) and 1.0 (1.0, 1.75) for the group receiving LP-S, LP-VR and SDP, respectively

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($p = 0.072$). PaO_2/FiO_2 ratios for the baseline, pre-transfusion, post-transfusion and 1-h follow-up did not differ between groups. They are displayed in Figure 4. At termination, the PaO_2/FiO_2 ratio was 400 mmHg (390, 440) in the LP-S group, 453 mmHg (424, 473) in the LP-VR group and 383 mmHg (375, 417) in the SDP group ($p = 0.155$).

DISCUSSION

Volume overload is seen as a central pathway in the development of TACO; therefore, we hypothesized that concentrated volume-reduced lyophilized plasma would reduce pulmonary capillary pressure and could help preventing TACO. However, our results do not show a reduction in pulmonary capillary pressure after transfusion with LP-VR compared to regular volume plasma products like LP-S or SDP. Also, there was no effect from volume-reduced plasma on pulmonary injury.

Previously, our group developed a two-hit animal model to study the underlying pathophysiology of TACO [17]. In the present experiment, this model was used to study the effect of a volume-reduced plasma product on the development of TACO. Our model was executed successfully as hydrostatic pulmonary capillary pressure (ΔLVEDP) was previously shown to increase significantly in the transfusion groups compared to crystalloid fluids [14]. Also, ΔLVEDP was greater than 4 mmHg in all groups receiving plasma products, which was the predefined threshold for clinical relevance [15]. Most importantly, the volume reduction of the LP-VR product led to a significantly reduced total fluid input in that group. There were no significant differences found between groups receiving LP-S or SDP, indicating that the lyophilized plasma product by itself does not affect TACO development in this animal model.

Interestingly, no differences were found in pulmonary capillary pressure between the three different transfusion products. Although the LP-VR group had a significantly lower volume administered compared to the other groups, this did not lead to a decrease in ΔLVEDP. These results suggest that TACO is more complex than originally thought and that volume overload alone is not the most important factor in rising pulmonary capillary pressure after plasma transfusion in this model. LP-VR is hyperoncotic and therefore able to increase the intravascular colloid osmotic pressure, preventing extravasation of fluids and possibly recruiting volume from the extravascular compartment [21, 22]. While volume recruitment was not an unexpected effect of hyperoncotic LP-VR, the equivalent increase in pulmonary capillary pressure following a low-volume product was. Previous in vitro experiments suggested a minimal volume recruitment, however, previous animal experiments support the observation that colloid osmotic pressure might be involved in rising pulmonary capillary pressure [14, 21]. However, it is unlikely that colloid osmotic pressure alone is responsible for the development of TACO, since we have shown previously a rise in pulmonary capillary pressure after transfusion of red blood cell products and these have a low colloid osmotic pressure [17, 21]. In these cell-containing products, storage lesion could lead to NO scavenging substances, which causes vasoconstriction and rising pulmonary capillary pressure [23]. Other mechanisms that might be involved in TACO development are inflammation and damage of the glycocalyx as a result of the transfusion, however, this needs to be investigated further [6, 7, 24].

Important criteria for TACO are either respiratory compromise or pulmonary oedema [1]. In the present study, we did not find significant differences between groups in any of the markers for acute lung injury, that we measured. An observation that was also seen in previous experiments with this model, where there was no significant increase of pulmonary injury after transfusion [14, 17]. Because there is no additional pulmonary injury, LP-VR cannot reduce it. This absence of transfusion-related pulmonary injury could possibly be explained by the fact that LVEDP does not increase enough to start extravasation of fluids and cause hydrostatic pulmonary oedema. A study in dogs showed that a minimum left atrial pressure of 24 mmHg was required to develop pulmonary oedema [25]. This threshold decreased when the plasma protein concentration was reduced. However, in our experiment we administered plasma proteins, and in the LP-VR group, this was a concentrated hyperoncotic plasma product. This could have possibly raised the pressure threshold for fluid extravasation and prevented pulmonary oedema formation in the LP-VR group, despite a higher LVEDP.

There were some limitations to this study. First, this experiment was part of a multi-armed randomized controlled trial and was not specifically powered to review the effects of low-volume lyophilized plasma. However, since results show a trend towards a harmful effect from the volume-reduced plasma product on pulmonary capillary pressure, it seems highly unlikely that a beneficial effect would have been found in a larger sample size. Second, this experiment was performed with animals that were young and healthy aside from an induced acute myocardial infarction. This situation is not completely translatable from the clinical situation, where TACO patients are more often older with different comorbidities, like renal failure or other chronic cardiovascular problems [3, 5, 9]. Moreover, the fact that these animals did not have an impaired kidney function could contribute to their ability to process fluids and reduce the benefits of a low-volume transfusion product. Also, we transfused human plasma products in rats. This had the advantage that we could study different plasma products that are not available from rat donors like SDP. On the other hand, the use of cross species blood products could lead to a less translatable model. However, human plasma products have been successfully transfused in animal experiments using rats or mice in several other studies [26, 27]. Lastly, as mentioned before, this experimental model lacks pulmonary oedema or respiratory distress, which is the primary clinical symptom of TACO. This was also seen in previous experiments with red blood cells and other plasma products [14, 17]. The model is very suitable for researching changes in pulmonary capillary pressure after transfusion, but the absence of lung injury and oedema limits the possibility to research pathways leading to TACO-like inflammation or storage lesion of blood products [23, 24, 28].

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Our study showed no effect on pulmonary capillary pressure from a volume-reduced lyophilized plasma product. We could not research the effect on pulmonary oedema, due to an absence of pulmonary oedema caused by plasma transfusion. Future studies should focus on examining the effect of volume-reduced plasma in a model with pulmonary oedema but should also explore other possible preventative strategies for TACO after plasma transfusion. For example, alternative products for plasma transfusion like prothrombin complex concentrates have been shown to be superior to plasma to restore coagulation [29]. This product and other alternative products could be tested to review their effect on pulmonary capillary pressure and their capability to be an effective preventative strategy for TACO. In conclusion, we wanted to investigate if volume-reduced lyophilized plasma could reduce volume overload and attenuate TACO. In this randomized experimental study in a two-hit rat model, pulmonary capillary pressure increased in all groups during transfusion. A hyperoncotic volume-reduced lyophilized plasma product did not have a beneficial effect but resulted in a trend of larger increase in pulmonary capillary pressure. These data suggest that a volume-reduced plasma product did not attenuate pulmonary capillary pressure required to develop circulatory overload and was therefore not an effective preventative strategy for TACO in this animal model. More research is required to obtain insights into the TACO pathophysiology, in order to identify targetable pathways for preventing TACO.

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CONFLICT OF INTEREST

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ORCID

Esther B. Bulle https://orcid.org/0000-0002-1578-2813
Robert B. Klanderman https://orcid.org/0000-0001-5820-4530

REFERENCES

1. Schipperus MR, Wiersum-Osselton JC, Group I-I-ATDR. Updated definitions for respiratory complications of blood transfusion. Transfusion. 2019;59:2482–3.
2. Clifford L, Jia Q, Yadav H, Subramanian A, Wilson GA, Murphy SP, et al. Characterizing the epidemiology of perioperative transfusion-associated circulatory overload. Anesthesiology. 2015;122:21–9.
3. Lieberman L, Maskens C, Cserti-Gazdewich C, Hansen M, Lin Y, Pendergrast J, et al. A retrospective review of patient factors, transfusion practices, and outcomes in patients with transfusion-associated circulatory overload. Transfus Med Rev. 2013;27:206–18.
4. Narayan S, Poles ea D. on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2021 Annual SHOT Report. 2022. [cited 2022 Dec 5]. Available from: https://www.shotuk.org/shot-reports
5. Bosboom JJ, Klanderman RB, Zijp M, Hollmann MW, Veelo DP, Binnekade JM, et al. Incidence, risk factors, and outcome of transfusion-associated circulatory overload in a mixed intensive care unit population: a nested case-control study. Transfusion. 2018;58:498–506.
6. Semple JW, Rebetz J, Kapur R. Transfusion-associated circulatory overload and transfusion-related acute lung injury. Blood. 2019;133:1840–53.
7. Bulle EB, Klanderman RB, Pendergrast J, Cserti-Gazdewich C, Callum J, Vlaar APJ. The recipe for TACO: a narrative review on the pathophysiology and potential mitigation strategies of transfusion-associated circulatory overload. Blood Rev. 2021;52:100891.
8. Frazier SK, Higgins J, Bugajski A, Jones AR, Brown MR. Adverse reactions to transfusion of blood products and best practices for prevention. Crit Care Nurs Clin North Am. 2017;29:271–90.
9. Clifford L, Jia Q, Subramanian A, Yadav H, Schroeder DR, Kor DJ. Risk factors and clinical outcomes associated with perioperative transfusion-associated circulatory overload. Anesthesiology. 2017;126:409–18.
10. Roubinian NH, Hendrickson JE, Triulzi DJ, Gottschall JL, Michalkiewicz M, Chowdhury D, et al. Contemporary risk factors and outcomes of transfusion-associated circulatory overload. Crit Care Med. 2018;46:577–85.
11. Garrigue D, Godier A, Glacet A, Labreuche J, Kipnis E, Paris C, et al. French lyophilized plasma versus fresh frozen plasma for the initial management of trauma-induced coagulopathy: a randomized open-label trial. J Thromb Haemost. 2018;16:481–9.
12. Mok G, Hoang R, Khan MW, Pannell D, Peng H, Tien H, et al. Freeze-dried plasma for major trauma - systematic review and meta-analysis. J Trauma Acute Care Surg. 2021;90:589–602.
13. Percie du Sert N, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, et al. Reporting animal research: explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol. 2020;18:e3000411.
14. Bulle EB, Klanderman RB, de Wissel MB, Roelofs JJTH, Veelo DP, van den Brom CE, et al. The effect of plasma transfusion in an experimental two-hit animal model of transfusion-associated circulatory overload with heart failure. Blood Transfus. 2022.
15. Nand N, Gupta SP, Gupta MS. Hemodynamic evaluation of blood transfusion in chronic severe anemia with special reference to speed of transfusion. Jpn Heart J. 1985;26:759–65.
16. Klanderman RB, Wijnberge M, Bosboom JJ, Roelofs J, de Korte D, van Bruggen R, et al. Differential effects of speed and volume on transfusion-associated circulatory overload: a randomized study in rats. Vox Sang. 2022;117:371–8.
17. Klanderman RB, Bosboom JJ, Maas AAW, Roelofs J, de Korte D, van Bruggen R, et al. Volume incompliance and transfusion are essential for transfusion-associated circulatory overload: a novel animal model. Transfusion. 2019;59:3617–27.
18. Verel D, Stentiford NH. Comparison of left atrial pressure and wedge pulmonary capillary pressure. Pressure gradients between left atrium and left ventricle. Br Heart J. 1970;32:99–102.

19. Pacher P, Nagayama T, Mukhopadhyay P, Batkai S, Kass DA. Measurement of cardiac function using pressure-volume conductance catheter technique in mice and rats. Nat Protoc. 2008;3:1422–34.
20. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012;9:671–5.
21. Klanderman RB, Bosboom JJ, Korsten H, Zeiler T, Musson REA, Veelo DP, et al. Colloid osmotic pressure of contemporary and novel transfusion products. Vox Sang. 2020;115:664–75.
22. Woodcock TE, Woodcock TM. Revised Starling equation and the glycocalyx model of transvascular fluid exchange: an improved paradigm for prescribing intravenous fluid therapy. Br J Anaesth. 2012;108:384–94.
23. Berra L, Pinciroli R, Stowell CP, Wang L, Yu B, Fernandez BO, et al. Autologous transfusion of stored red blood cells increases pulmonary artery pressure. Am J Respir Crit Care Med. 2014;190:800–7.
24. Bosboom JJ, Klanderman RB, Migdady Y, Bolhuis B, Veelo DP, Geerts BF, et al. Transfusion-associated circulatory overload: a clinical perspective. Transfus Med Rev. 2019;33:69–77.
25. Guyton AC, Lindsey AW. Effect of elevated left atrial pressure and decreased plasma protein concentration on the development of pulmonary edema. Circ Res. 1959;7:649–57.
26. Chipman AM, Wu F, Pati S, Burdette AJ, Glaser JJ, Kozar RA. Fresh frozen plasma attenuates lung injury in a novel model of prolonged hypotensive resuscitation. J Trauma Acute Care Surg. 2020;89:118–25.
27. Letourneau PA, McManus M, Sowards K, Wang W, Wang YW, Matijevic N, et al. Aged plasma transfusion increases mortality in a rat model of uncontrolled hemorrhage. J Trauma. 2011;71:1115–24.
28. Blumberg N, Heal JM, Gettings KF, Phipps RP, Masel D, Refaai MA, et al. An association between decreased cardiopulmonary complications (transfusion-related acute lung injury and transfusion-associated circulatory overload) and implementation of universal leukoreduction of blood transfusions. Transfusion. 2010;50:2738–44.
29. Chai-Adisaksopha C, Hillis C, Siegal DM, Movilla R, Heddle N, Iorio A, et al. Prothrombin complex concentrates versus fresh frozen plasma for warfarin reversal. A systematic review and meta-analysis. Thromb Haemost. 2016;116:879–90.

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COMMENTARY

Haemovigilance: Giving it our best SHOT!

INTRODUCTION

In 2022, the United Kingdom's Serious Hazards of Transfusion (SHOT) programme celebrated its 25th anniversary [1]. SHOT is not the first haemovigilance system internationally (the first national programme was established in Japan in 1993), but it is one of the best known and most influential. In this Commentary, we summarize some of SHOT's achievements and consider what we can all learn from their experience and findings that will help us into the future.

Different definitions of haemovigilance exist, but all capture its broad scope (incorporating blood donor, product and transfusion recipient issues), its centrality to quality management and the importance of the systematic collection, analysis and reporting of robust data to improve blood systems, clinical practice and donor and patient outcomes [2, 3].

Haemovigilance itself has evolved from an initial focus on transfusion-transmitted infections (particularly HIV and hepatitis), which was the impetus for establishment of haemovigilance programmes in many parts of the world, to a much broader approach, as health systems have evolved over recent decades, and in response to lessons learned from haemovigilance itself. Haemovigilance links closely with national blood policy development, with patient blood management and with changes in blood product manufacturing. It also serves as a foundation for research efforts by identifying areas of unmet need and as a mechanism to measure the effects of changes as they are introduced [4].

Many gaps and challenges still exist. For example, evidence-based or consensus definitions for some important complications of donation or transfusion are still lacking or do not align well with the clinical picture—such as for some post-transfusion cardiopulmonary reactions. Frequently, data are incomplete or denominators unavailable, limiting analysis and comparison over time and between systems. Most haemovigilance systems struggle with availability of sufficient resources (people, tools and systems) to do their work effectively. Recommendations are often not taken up into policy or implemented into practice.

This article reflects the views of the author (BIW) and should not be construed to represent FDA''s views or policies.

In this context, it is worth briefly examining some of SHOT's achievements, and what we can all learn, from SHOT's experience. Firstly, let us consider some key elements of governance and structure.

INDEPENDENCE AND PROFESSIONAL LEADERSHIP

SHOT's professional independence enables it to conduct its activities and provide its reports freely and impartially. There is a demonstrated commitment to openness, transparency and reporting of findings, while not identifying or blaming individuals or organizations. Participation is high (see below) and is now professionally mandated. SHOT is affiliated to the UK Royal College of Pathologists (RCPath) and has established links with the UK Health Security Agency Epidemiology Unit (for transfusion-transmitted infection reporting and analysis) and the Medicines and Healthcare products Regulatory Agency (regarding product-focused safety issues). SHOT is managed by a small multidisciplinary team and supported by a wider expert group (see below). There are clear operational and reporting lines, and the programme has recruited (and retained) staff with relevant experience, including from both hospital and blood centre backgrounds and with data management expertise. Sustained funding from the four UK blood services (National Health Service Blood and Transplant [NHSBT, England], Northern Ireland Blood Transfusion Service, Scottish National Blood Transfusion Service and the Welsh Blood Service) has been secured. Some administrative functions are handled by NHSBT, permitting the SHOT team to focus on core activities.

SHARING EXPERTISE AND VIEWS AROUND A BIG TABLE

One of SHOT's strengths is the close collaboration with, and broad input from, across the professional spectrum (biomedical scientists, nurses, clinicians who prescribe transfusions, representatives of specialist colleges and societies) along with the UK blood services, clinical and laboratory transfusion experts, regulators and health safety experts. Very importantly, lay members, representing the

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voices of the broader community, have a seat at this table as members of the SHOT Steering Group. Founding members and former directors contribute corporate memory and ongoing advice in honorary roles. SHOT's Working Expert Group provides specialist input to targeted analyses, such as events affecting specific patient groups (e.g., paediatrics or patients with haemoglobin disorders) or settings (such as the emergency department) or specific blood products (such as the use of RhD immunoglobulin in pregnancy). They also serve as liaison with their specialties to provide input and disseminate messages from SHOT.

Next, let us consider some of SHOT's activities and outputs.

HIGH LEVELS OF PARTICIPATION

From 169 reports in 1996, more than 4000 cases were reported in 2021 from across the United Kingdom, both from the public (all NHS Trusts/Health Boards submitted at least one report) and private (non-NHS) sectors. Although participation has been very high for years, and is now professionally mandated, this is the first time that complete 100% national participation has been documented [1]. This is important because it gives confidence that SHOT's findings are truly representative of a national picture and that participation is valued and recognized as contributing to practice improvement.

A FOCUS ON THE BIG PICTURE, WHILE NOT NEGLECTING THE DETAILS

As is clear from its name, SHOT analyses reports of serious hazards of transfusion. There are different schools of thought on the ideal scope of haemovigilance reporting, with some systems including all cases of all severity, aiming to ensure a comprehensive picture of all adverse reactions and incidents. This can certainly be helpful in understanding the breadth and scale of potential clinical and procedural complications, as well as ensuring that cases that otherwise might have been missed are not. Many of the contributing factors are similar too, of course, whether serious or minor in consequence; however, this approach also creates a huge workload of cases for investigation that can distract from other, more clinically relevant events, which are SHOT's priorities. 'Near-miss' events are also an opportunity to learn, as many of the same factors contribute to these cases as those that result in patient harm; near misses are reportable to SHOT and account for a substantial proportion of cases.

UNDERSTANDING AND LEARNING FROM HUMAN ERRORS

Transfusion is a complex process with many steps and interdependencies [1, 5]. SHOT has documented and analysed how human errors and inadequate systems can contribute to both near-miss events and actual incidents, with consequences ranging from no harm to fatal outcomes for patients, and major impact (psychologically and professionally) on staff and other participants. The importance of a safety culture, and a learning culture, to identify and address hazards, is emphasized.

PROMOTING A HOLISTIC APPROACH TO TRANSFUSION SAFETY

SHOT promotes a combined Safety-I and Safety-II approach and recently introduced SHOT-ACE: Acknowledging Continuing Excellence in Transfusion. Recognizing errors and identifying improvement actions to prevent recurrence is the primary focus when incidents are investigated, typical of a Safety-I approach. Safety-II, a more proactive approach, seeks to understand the ability of healthcare staff to adapt to problems and pressures, and considers organizational resilience. It focuses on productivity and ensuring the best possible outcomes. Combining Safety-I and Safety-II approaches helps provide a more holistic understanding of the underlying reasons for errors and procedural violations. Reporting and studying success augment learning, enhance patient outcomes and experience through quality improvement work, and positively impact workplace resilience and culture.

SHARING THE FINDINGS—AND THE LESSONS—FROM HAEMOVIGILANCE

The Annual SHOT Report is essential reading for those interested in haemovigilance and the 'gold standard' for haemovigilance reports internationally [1]. The effort necessary to compile, analyse, draft, edit and present the annual report—245 pages in 2021—cannot be underestimated, but neither can the value of this rigorous and up-to-date document, written for a broad readership and with concrete recommendations for action to stakeholders. A series of chapters presents analyses of incidents from the past year and relevant cumulative data. De-identified clinical vignettes engage the reader and are highly useful for teaching purposes. Donor haemovigilance data are provided by UK blood service representatives. Sections focus on high-risk areas for attention or topics of interest, and recommendations are framed in a clear, positive and practical way, indicating parties responsible for action.

Annual SHOT symposia are open to all interested parties and supported by multiple professional organizations. Some have been collaborations with the International Haemovigilance Network, and for these, International Society of Blood Transfusion (ISBT) has provided ISBT Academy support to enable participants from low- and middle-income countries to attend, resulting in even greater international engagement. The symposia have an educational focus and include reviews of SHOT data and key themes from the annual reports, along with guest speakers and discussions. A communications expert participates in the meeting and distils important points into visual and written messages for wide distribution.

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SHOT regularly contributes to educational and professional activities, including through regional transfusion committee meetings, RCPath and other collaborative events, and peer-reviewed publications. SHOT's comprehensive website and social media presence help raise awareness of activities and findings. SHOT contributed to the ISBT-World Health Organization project to curate haemovigilance tools and resources and make these readily available to facilitate strengthening haemovigilance activities worldwide. These will be continuously updated and expanded to provide a comprehensive library of resources [2].

So, in summary, important messages from SHOT's 25-year experience are about being inclusive, collaborative and open to sharing resources and findings, with a focus on learning and practice improvement. It is also clear that haemovigilance takes time: SHOT is still making many of the same recommendations it has been making since the initial report in 1996, and many problems that prompted the establishment of haemovigilance programmes are still with us. However, much progress has been made in both understanding and improving transfusion safety, and SHOT has been a major contributor to this effort, across the United Kingdom and around the world. Congratulations to SHOT on this important anniversary—and to everyone working in haemovigilance internationally. This work is vital and must continue.

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Erica M. Wood[1,2]
Barbee I. Whitaker[3]
Mary Townsend[4]

[1]*School of Public Health and Preventive Medicine, Monash University, Melbourne, Australia*
[2]*Department of Clinical Haematology, Monash Health, Melbourne, Australia*
[3]*Office of Biostatistics and Pharmacovigilance, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland, USA*
[4]*Vitalant, Scottsdale, Arizona, USA*

Correspondence
Erica M. Wood, Monash University, 553 St Kilda Road, Melbourne, Victoria 3004, Australia.
Email: erica.wood@monash.edu

ORCID

Erica M. Wood https://orcid.org/0000-0001-7527-2340
Barbee I. Whitaker https://orcid.org/0000-0002-6311-6268
Mary Townsend https://orcid.org/0000-0001-7759-0676

REFERENCES

1. Narayan S, editor Poles D on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. et al. The 2021 Annual SHOT Report. 2022.
2. International Society of Blood Transfusion [cited 16 Jan 2023]. Available from: https://www.isbtweb.org/resources/hv-tools.html.
3. De Jonge LL, Wiersum-Osselton JC, Bokhorst AG, Schipperus MR, Zwaginga JJ. Haemovigilance: current practices and future developments. Ann Blood. 2022;7:23.
4. Wood EM, Ang AL, Bisht A, Bolton-Maggs P, Bokhorst A, Flesland O, et al. International haemovigilance: what have we learned and what do we need to do next? Transfus Med. 2019;29:221–30.
5. Burns KE, Haysom HE, Higgins AM, Waters N, Tahiri R, Rushford K, et al. A time-driven, activity-based costing methodology for determining the costs of red blood cell transfusion in patients with beta thalassaemia major. Transfus Med. 2019;29:33–40.

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ORIGINAL ARTICLE

Transfusion medicine research in Africa: Insights from investigators in the field

Tina S. Ipe[1,2] | Quentin Eichbaum[3] | Magdy El-Ekiaby[4] | Shirley Owusu-Ofori[5] | Marion Vermeulen[6] | Tonderai Mapako[7] | Claude Tayou Tagny[8] | Bamory Dembele[9] | Evan M. Bloch[10] | Linda S. Barnes[11,12] | for the AABB Global Transfusion Forum contributed equally

[1]Medical Division, Our Blood Institute, Oklahoma City, Oklahoma, USA

[2]Department of Pathology and Laboratory Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA

[3]Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, Tennessee, USA

[4]Blood Transfusion and Hemophilia Center, Shabrawishi Hospital, Cairo, Egypt

[5]Central Zonal Blood Center, National Blood Service Ghana, Kumasi, Ghana

[6]The South African National Blood Service, Roodepoort, South Africa

[7]Business Development Department, National Blood Service Zimbabwe, Harare, Zimbabwe

[8]Department of Hematology and Blood Transfusion, University of Yaounde, Yaounde, Cameroon

[9]National Blood Transfusion Center Laboratory and, Pharmaceutical and Biological Sciences Training and Research Unit, University Félix Houphouet-Boigny, Abidjan, Côte d'Ivoire

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

[11]School of Public Health, University of Illinois, Chicago, Illinois, USA

[12]Linda S. Barnes Consulting, Seattle, Washington, USA

Correspondence
Tina S. Ipe, Oklahoma Blood Institute, 1001 N. Lincoln Blvd. Oklahoma City, OK 73104, USA.
Email: tina.ipe@obi.org

Abstract

Background and Objectives: Research in low-resource settings is inherently challenging. We sought to assess the factors that have impeded or facilitated transfusion medicine (TM) research in various African settings.

Materials and Methods: A qualitative case study was conducted of selected investigators in Africa; selection was based on productivity-spanning publication, leadership and research in TM. We designed a questionnaire to explore the factors impeding or facilitating TM research to understand the impact on the investigators' careers. Written responses were independently coded and double-checked for precision. Qualitative analysis was conducted, whereby responses were grouped thematically and clustered by relationship. The initial findings were discussed with respondents to validate and refine the interpretations. The recorded transcript was analysed and incorporated into the final analysis.

Results: Six investigators participated in the study. Their responses yielded 471 coded comments: 389 from the questionnaires and 82 from the ensuing discussion. The most frequently cited factors described included knowledge and intellectual abilities ($n = 104$), personal effectiveness ($n = 99$), research and governance structure ($n = 97$), and engagement, influence and impact ($n = 75$). Four relationship clusters emerged from the facilitators ($n = 42$), barriers ($n = 28$), and common approaches ($n = 26$) to research, informing summary themes of adaptation, collaboration, perseverance, and resiliency.

Conclusion: Individual attributes were found to be central to a successful TM research career in African settings. However, given other public health priorities and constraints, interpersonal relationships, organizational structures and the broader research context were important to TM researchers. Overcoming complexities demands adaptation, collaboration, perseverance and resiliency.

Evan M. Bloch and Linda S. Barnes contributed equally as senior authors.

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Linda S. Barnes, 2247 Prescott Ave SW,
Seattle, WA 98126, USA.
Email: lbarne23@gmail.com

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Africa, qualitative research, transfusion medicine

Highlights

- Transfusion medicine researchers, both clinical and non-clinical, in Africa do not publish as frequently as their counterparts in other continents.
- This study showed personal effectiveness, knowledge and intellectual capability as attributes of successful researchers in Africa.
- Creating a pipeline of researchers in Africa is vital to building research capacity, not only in this continent but also in other low- and middle-income countries.

INTRODUCTION

Healthcare research is essential in low- and middle-income countries (LMICs) where the burden of disease is high but the resources are limited, thus underscoring the need for locally relevant, innovative, evidence-based and sustainable solutions to effect meaningful change [1, 2]. Research in low-resource settings is inherently challenging for local investigators [3]. Such is the case in large parts of Africa, where the challenges span funding, infrastructure, education and training [4, 5]. The challenges are amplified in niche disciplines, such as transfusion medicine (TM), that garner little attention.

TM researchers in Africa are grossly under-represented in the published literature. On average, 38 papers were published annually by researchers in sub-Saharan Africa (SSA) between 2008 and 2015, almost half (41%) of which focused on transfusion-transmitted infections [6]. Only a third (34%) of the publications were in transfusion-specialist journals, and an overwhelming majority of publications stemmed from collaborations outside of SSA. Consequently, policy recommendations originating in high-income countries such as those in Europe and North America are advanced where regional research might otherwise be used to guide practices that would be more appropriate and successful in a local context [7]. Examples include optimal blood donor engagement and testing strategies that increase the benefits of blood availability balanced against the risk of transfusion-transmitted infections in austere environments.

The tepid publication record may reflect a more systemic problem. Specifically, a framework for achieving success as a TM researcher in various African countries is lacking. Such a framework or roadmap could facilitate research careers for aspiring trainees and junior investigators to navigate the obstacles and leverage learnings from well-published TM researchers within the African context. The Association for the Advancement of Blood and Biotherapies (AABB) Global Transfusion Forum (GTF) Research Subcommittee undertook a qualitative study of this issue. A sample of TM experts from different regions in Africa were invited to share their perspectives to understand better the opportunities and challenges faced by African researchers. We sought to offer guidance to trainees and junior investigators, drawing on others' first-hand regional knowledge and experience of the TM research environment in Africa.

STUDY DESIGN AND METHODS

We employed a methodology known as interpretative phenomenology, whereby the lived experience of the research participant is incorporated into the research study in a participatory way [8]. Using a qualitative approach and two-phase case study design, the AABB GTF Research Subcommittee developed a questionnaire (Data S1) to describe the attributes, facilitators and challenges that either favoured or impeded the success of the participating investigators [8]. This allowed for the ascertainment of self-described factors that favour or impede the success of TM researchers. The questionnaire comprised 12 questions covering topics such as knowledge and intellectual ability, personal effectiveness, research governance and organization, engagement, influence and impact [3]. Responses were entered as free text.

The AABB GTF Research Subcommittee selected the invited respondents (i.e., TM research investigators). Selection of participants was based on an individual's publication record, research funding, engagement in leadership positions in the field of TM (e.g., Africa Society for Blood Transfusion) and reputation in TM. By design, the study was limited to a few notable investigators, with the intent to represent each of the geographic regions of Africa (i.e., Central, East, North, Southern and West Africa). The investigators were invited via email to participate. The questionnaire was distributed electronically to those who agreed to participate in the study. The responses were received by email from February to December 2020.

Data analysis

The analysis was led by a researcher experienced in qualitative methods. The respondents' free-text responses were received as Microsoft Office 365 Word (Microsoft, Seattle, WA, USA) files and analysed using MAXQDA 2020 (VERBI Software, Berlin, Germany), a software program that is designed for qualitative and mixed-methods research. We chose to use a priori constructs as concepts compiled from an existing framework to facilitate interpretation given the contextual nature of the study while allowing for emergent concepts. The Vitae Research Development Framework (RDF) [9] was used to characterize the concepts that emerged from the responses. The RDF was

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created by researchers in the United Kingdom who identified factors associated with successful research careers, developed from empirical data collected through interviews. The RDF recognizes four central factors and subordinate considerations (i.e., behavioural characteristics and environmental factors) that are important for success in research (Table 1). The four prominent factors are (a) knowledge and intellectual abilities, (b) personal effectiveness, (c) research governance and organization and (d) engagement, influence and impact. In addition, there are 12 sub-constructs; definitions are provided in the Codebook (Data S2). These factors informed an a priori coding scheme and the associated definitions that were incorporated into the Codebook.

Two independent coders coded at a paragraph level, initially assigning parent codes, followed by sub-codes; the responses were reviewed independently to improve inter-rater reliability. Construct and sub-construct definitions were refined to enhance consistent understanding of the application. Where appropriate, segments were multi-coded to fully capture the meaning of the content, including intersections. Emergent codes were added throughout the coding process, informed by concurrent in-document memos.

The frequency of codes within and across the responses informed patterns. Themes were developed by summarizing the coded content within and across the questionnaire responses. These themes were clustered by proximity to understand the relationships with facilitative features and barriers described by the respondents. This approach illustrates the relative emphasis of each of the features and their relatedness (i.e., intersections). Following the initial analysis of the questionnaire responses, the preliminary findings were reviewed and discussed with two of the respondents in a recorded webinar. This member-checking approach captured additional interpretive suggestions and contextual nuance and incorporated additional insights. The recording from the discussion was transcribed, coded and analysed using the same codebook, repeating the same thematic approach to enrich and enhance the findings. Results from both sets of data (i.e., the questionnaires and the member-checking discussion) were interpreted separately and together.

Human subjects

The study was approved by the institutional review boards (IRBs) at the University of Arkansas for Medical Sciences and Johns Hopkins University School of Medicine before initiation.

RESULTS

Of the 10 invited researchers, 6 (60%) agreed to participate and responded to the questionnaire. All respondents described being actively engaged in TM research. The respondents were from the following countries: Cameroon, Cote d'Ivoire, Egypt, Ghana, South Africa and Zimbabwe. Three respondents were physicians (MBchB/MBBch/MD) having between 10 and 30 years of experience in TM research. Two respondents held the PhD degree with more than 15 years of experience in TM-related fields. One respondent is a PhD candidate with more than 20 years of experience in senior technical/management roles including TM research. All reported receiving grant funding and holding a considerable record of peer-reviewed literature in TM (median 35.5; range 6–57).

Questionnaire responses

A total of 389 codes were applied to written segments, covering 94% (range 89%–96%) of the content of the questionnaires. The two coders reached a significant inter-coder agreement (91%) through iterative comparisons and improvement of definitions. Across all questionnaire responses (Figure 1), the most frequently referenced factors by the number of coded segments, in parentheses, were personal effectiveness ($n = 48$; 12.3%) followed by knowledge and intellectual abilities ($n = 46$; 11.8%) and research governance and organization ($n = 46$; 11.8%). More facilitators ($n = 37$; 9.5%) were described than barriers ($n = 24$; 6.2%). The least commonly mentioned considerations included personal qualities ($n = 8$; 2.1%), engagement and impact ($n = 7$; 1.8%), professional conduct ($n = 3$; 0.8%), and communication and dissemination ($n = 2$; 0.5%).

TABLE 1 A priori constructs, sub-constructs, and definitions applied to code questionnaires and the subsequent member-checking discussion based on the Vitae Researcher Development Framework (Vitae, © 2010 Careers Research and Advisory Centre [CRAC] Limited) [5].

Construct (concept)	Sub-construct (factors)	Definition
Personal effectiveness	Personal qualities Self-management Professional and career development	Personal qualities and approach to be an effective researcher
Knowledge and intellectual abilities	Knowledge base Cognitive abilities Creativity	Knowledge, intellectual abilities and techniques to do the research
Research governance and organization	Professional conduct Research management Finance, funding and resources	Knowledge of the standards and requirements, and professionalism to do the research
Engagement, influence and impact	Working with others Communication and dissemination Engagement and impact	Knowledge and skills to work with others and ensure wider impact of research

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FIGURE 1 Code frequency by construct across the accumulated questionnaires received from African transfusion medicine researchers.

Member-checking discussion

A total of 82 segments were coded from the discussion with respondents. The most frequently cited factor was knowledge and intellectual abilities ($n = 10$; 12.2%), followed by personal effectiveness ($n = 8$; 9.8%) and research governance organization ($n = 8$; 9.8%). Creativity ($n = 7$; 8.5%) and engagement, influence and impact ($n = 7$; 8.5%) were also rated high. We observed that personal qualities ($n = 2$; 2.4%) and professional conduct ($n = 1$; 1.2%) were less frequently mentioned.

Combined data

Across the collective questionnaire responses and member-checking discussion, we observed code intersections occurring when a response reflected more than one construct, leading to multi-coding. These intersections and co-occurrences were also examined. The most frequent intersections occurred between personal effectiveness and knowledge and intellectual abilities ($n = 31$ co-occurrences). We also observed that facilitators co-occurred with research governance and organization ($n = 23$), personal effectiveness ($n = 20$) and knowledge and intellectual abilities ($n = 20$). However, we noted that research and governance were most frequently identified as the highest ranking barriers ($n = 18$), followed by engagement, influence and impact ($n = 13$). Using the code map intersection, we found four relationships, herein called clusters, by map position between pairs of codes (Figure 2).

The clusters were further examined to identify the common developmental approaches described by the researchers as they advanced in their TM careers. Characterized as a Developing Researcher Framework, Figure 3 captures key attributes that arose from the study to facilitate advancement and overcome barriers to achieve a successful career as a TM researcher. Derived from notable quotes in the words of the researchers, these approaches are summarized as an adaptation of the research agenda to make it practicable in low-resource settings: international collaborations with other TM researchers and resiliency to overcome barriers through joint efforts to achieve the intended impact. We mapped the relationships of the themes to a hierarchy from foundational features to the self-actualization of the successful TM researcher. A set of recommendations compiled from the researchers summarizes the advice given to those pursuing a TM research career in the African context (Table 2).

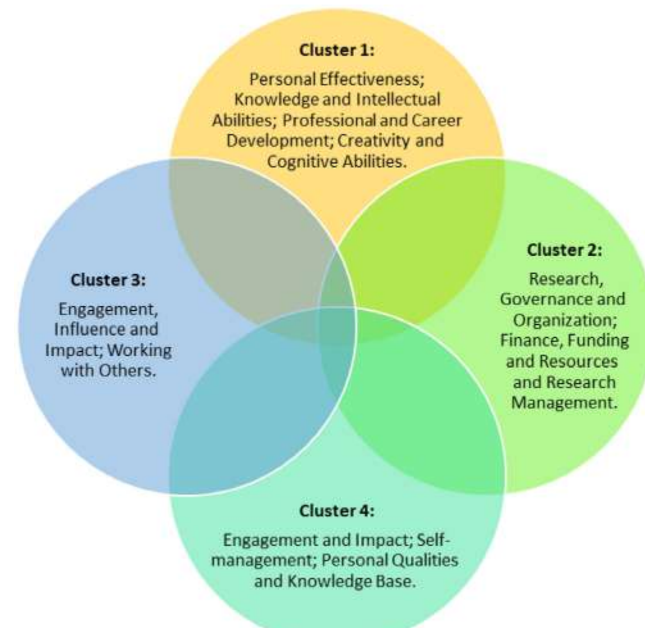

FIGURE 2 Four clusters that were developed based on the intersection of concepts after combining responses from the questionnaires and the member-checking discussion.

DISCUSSION

Our study findings highlight themes from the self-described perspectives of TM researchers who have had successful TM research careers in parts of Africa. Key findings emphasize personal effectiveness combined with knowledge and intellectual abilities. Research governance

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FIGURE 3 Developing Research Framework, synthesized from the four clusters illustrating the how foundational individual attributes build towards interpersonal and contextualized approaches, leveraging work with others and available financial resources, through personal effectiveness, knowledge and abilities and engagement, influence and impact. Common themes for overcoming hurdles included adaptation, collaboration, perseverance and resiliency.

TABLE 2 Consolidated recommendations for future transfusion medicine (TM) researchers, compiled from the questionnaire responses and discussion.

- Define your research interest in TM.
  - Always start your research by asking a question.
  - Perform a thorough literature review.
  - Define your aim of research.
  - Study options for funding your research.
  - Get trained on how to write a proper manuscript to publish the results of your research.
- Consider research as a career. There will be initial hurdles and disappointments, but there are high rewards to be gained if efforts are sustained quite well.
  - Identify mentors in your line of research interest.
  - Pursue publication of your research work.
  - Advance your education and embark on operational research.
  - Develop relevant skills for research. Aim to embark on ethical conduct of research always.
  - Utilize available free educational opportunities.
  - Endeavour to attend to scientific congresses as an active participant (present some scientific work).
  - Join allied associations such as Africa Society for Blood Transfusion (AfSBT), International Society of Blood Transfusion (ISBT), Association for the Advancement of Blood and Biotherapies (AABB), etc.
- Explore research networking opportunities in Africa Society for Blood Transfusion, International Society for Blood Transfusion and the Association for the Advancement of Blood and Biotherapies to enhance research interests and collaborations with other TM researchers.

and organization, including funding, had a significant impact on individuals' careers, favourable or otherwise. While not unique to an African setting, adaptation, collaboration, perseverance and resiliency notably contributed to positive outcomes. There was a perceived need for local, innovative, evidence-based and sustainable solutions, which have already been identified as deficient in the context of TM research in Africa and other low-resource settings [4–6]. Additionally, the respondents' lesser emphasis on communication and dissemination was striking, potentially explaining under-representation in scientific publication [2, 7].

A qualitative approach was better equipped to understand the developmental trajectories of a sampling of research investigators in Africa. By drawing on the researchers' perspectives, a qualitative approach affords a depth of context and insight, which is frequently lacking in quantitative methods. Our study found intersecting patterns in the questionnaire responses and discussion content supporting a strong relationship of a researcher's knowledge and abilities combined with personal effectiveness; these were key factors in facilitating the researchers' successful career development. Personal effectiveness, which encompassed unique personal qualities, self-management and professional and career development, was the dominant quality attributed to successful TM researchers in Africa. In other words, the personal initiative to conduct and sustain their research goals was vital to the researcher's success. To develop their professional TM research career, respondents shared that self-management was essential to creating a robust knowledge base and cognitive abilities as a researcher. Several respondents described the lack of training infrastructure in their countries to learn research skills as an impediment. Attendance in conferences, review courses or post-graduate education (e.g., in the United States and Europe) was essential to obtain and maintain valuable skills in research methodology and manuscript writing. There are successful examples, albeit few, where courses have

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been devised to impart foundational skills in either clinical TM or related research [3, 10–13].

Self-discipline was key to generating manuscripts and publications, where research tasks were often undertaken outside work hours. The researchers were relentless in their research pursuits, some describing self-funding of their research. Creativity was apparent, with one respondent sharing how they acted on the opportunity to become a 'research officer', which enabled them to further develop their research expertise as part of a formal professional role. However, another respondent succinctly stated, 'You will need to have perseverance and manage your time to be able to be successful in this area. You need to want to do it'.

The respondents observed that their personal attributes and mastery alone were insufficient to ensure a successful research career in TM. These researchers described being effective because they forged collaborations with industry sponsors, in-country and international TM clinicians and other researchers. They relied on mentors to help them network and establish relationships with other TM researchers. They also encouraged their research staff to advance their research through participating in local, national and international TM research. Notably, the most impactful research topics were locally and/or regionally relevant, often informed by challenges specific to the population and settings where they worked [14–19].

Even when motivated and collaborating on important works, the researchers described being sometimes constrained by their respective regions' research, governance and organizational structures. For example, their local environments were not conducive to research because of poor access to technology such as the Internet, lack of adequately trained research staff and the lack of internal (state, governmental or institutional) funding. The lack of administrative and technology support sometimes impaired their ability to coordinate research projects or even meet sponsors' deadlines for those projects. This hampered their ability to engage with or influence the national and international TM community. However, the respondents described adaptive ways to move beyond these challenges through collaboration and engagement with professional societies.

Surprisingly, the respondents did not refer to the regulatory review process as a notable obstacle to research. This was not stated explicitly; rather, the omission was conspicuous. Communication and the ability to disseminate research findings were not mentioned explicitly as research and career obstacles. Of note, none of the researchers spoke about the professional conduct of their colleagues or their own as being either a facilitator or barrier to conducting research.

During the member-checking discussion, the investigators emphasized self-management as a critical characteristic of a successful individual in LMICs. However, the foundational features of the Developing Research Framework (Figure 3) built a pathway towards self-actualization built upon the innovative approaches described by accomplished TM researchers [20]. These approaches included the adaptation of research specific to the local context, collaborations with local and international researchers, perseverance marked by courage and persistence to communicate unpopular findings and resiliency to overcome barriers to research. This approach allowed them to address locally pertinent research questions having a more significant bearing on policymakers, administrators, clinicians and public health collaborations. Examples of local research capacity building in Africa include the Francophone Africa Transfusion Medicine Research Training network, T-REC, and the NIH REDS-III and Fogarty South Africa progammes [13]. These research programmes have enrolled trainees at all levels, from short-term course participants in epidemiology to Masters and PhD candidates. It has increased the number of TM research publications originating from Africa, with at least 60 new manuscripts in the past several years. These programmes noted shortages of mentors and grant-writing skills as challenges faced by trainees. While these contextually rich insights are poorly described in the peer-reviewed literature, they may support advancing TM researchers in the African context and beyond.

This study has several limitations. First, the small sample size (i.e., small number of participants and skewed geographic representation) warrants highlighting. Although our sampling plan was broad, not all African regions were represented. However, this was deemed sufficient for a qualitative analysis given the narrow scope of the study and thematic saturation. We acknowledge that our definitions of 'success' and 'productivity' in research, as defined by publications, contributions to professional societies and grant funding, may not be the only measures of a successful career or impactful contribution. We did not solicit opinions of those who attempted a TM research career but did not meet our definitions. While such information may serve as an important comparison, this was not the focus of our study. We appreciate that the respondents' experience varied by individual and their context, representing a limitation in transferability to other settings. We applied reflexivity and member-checking to validate the observed patterns and found the thematic similarities striking. Although this study focused on experiences in African settings, its findings may be relevant to TM researchers with similar backgrounds and other comparable LMIC contexts.

The findings of this study point to the remarkable ability of a subset of African TM investigators to navigate a range of impediments and obstacles in conducting research in Africa. These accomplished TM researchers exercised personal effectiveness combined with knowledge and abilities. Their successes were further enhanced through adaptation, collaboration, perseverance and resiliency. A pipeline of future researchers is critical to increase the capacity of research in LMICs, enticing aspiring trainees. These findings warrant further exploration, mainly to understand how to teach, mentor and expand TM research specific to Africa.

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T.S.I. designed the research study, submitted the IRB, conducted the survey, acquired and analysed the data, wrote the first draft of the manuscript, wrote additional drafts of the manuscripts and reviewed and edited the manuscript; Q.E., M.-E., S.O.-O., M.V., T.M., C.T.T. and B.D. reviewed and edited the manuscript; E.M.B. designed the

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research study, conducted the survey and reviewed and edited the manuscript; L.S.B. enriched the qualitative design, analysed the data, wrote additional drafts of the manuscript and reviewed and edited the manuscript.

CONFLICT OF INTEREST STATEMENT

E.M.B. is a member of the US Food and Drug Administration (FDA) Blood Products Advisory Committee. Any views or opinions expressed in this article are Dr Bloch's and are based on his own scientific expertise and professional judgement; they do not necessarily represent the views of the Blood Products Advisory Committee or the formal position of the FDA and also do not bind or otherwise obligate or commit either the Advisory Committee or the FDA to the views expressed. T.S.I. is a consultant for Terumo Blood and Cell Technologies and Alexion, Inc. L.S.B. is a consultant to the Association for the Advancement of Blood and Biotherapies.

ORCID

Tina S. Ipe https://orcid.org/0000-0003-3554-5521
Quentin Eichbaum https://orcid.org/0000-0003-3480-7892
Magdy El-Ekiaby https://orcid.org/0000-0002-3881-1605
Shirley Owusu-Ofori https://orcid.org/0000-0002-5611-685X
Marion Vermeulen https://orcid.org/0000-0003-4383-4526
Tonderai Mapako https://orcid.org/0000-0003-2873-4420
Claude Tayou Tagny https://orcid.org/0000-0002-2179-3105
Bamory Dembele https://orcid.org/0000-0002-6165-9090
Evan M. Bloch https://orcid.org/0000-0001-8181-9517
Linda S. Barnes https://orcid.org/0000-0002-5090-0328

REFERENCES

1. Alemayehu C, Mitchell G, Nikles J. Barriers for conducting clinical trials in developing countries- a systematic review. Int J Equity Health. 2018;17:37.
2. Custer B, Zou S, Glynn SA, Makani J, Tayou Tagny C, El Ekiaby M, et al. Addressing gaps in international blood availability and transfusion safety in low- and middle-income countries: a NHLBI workshop. Transfusion. 2018;58:1307–17.
3. Murphy EL, McFarland W, Lefrère J-J. Teaching transfusion medicine research methods in the developing world. Transfusion. 2009;49:1532–4.
4. Weimer A, Tagny CT, Tapko JB, Gouws C, Tobian AAR, Ness PM, et al. Blood transfusion safety in sub-Saharan Africa: a literature review of changes and challenges in the 21st century. Transfusion. 2019;59:412–27.
5. Ifland L, Bloch EM, Pitman JP. Funding blood safety in the 21st century. Transfusion. 2018;58:105–12.
6. Fisher A, Wallis S, Hassall O, Martin R, Bates I. Collaborations on blood transfusion research in sub-Saharan Africa: who, what and where. Vox Sang. 2020;115:221–32.
7. Bates I. Final Report Summary - T-REC (Building research capacity of blood transfusion services in Africa) [monograph on the internet]. 2015 [cited 2022 Aug 10]. Available from: https://cordis.europa.eu/project/id/266194/reporting.
8. Yin RK. Case study research and applications: design and methods. 6th ed. United States: SAGE Publications; 2017.
9. Vitae. Vitae RDF [monograph on the internet]. 2021 [cited 2022 Aug 10]. Available from: https://www.vitae.ac.uk/images/researchers-professional-development/TransparentRDFgraphic.png/view.
10. Sibinga CT. Filling a gap in transfusion medicine education and research. Transfus Med Rev. 2009;23:284–91.
11. Guarner J, Amukele T, Mehari M, Gemechu T, Woldeamanuel Y, Winkler AM, et al. Building capacity in laboratory medicine in Africa by increasing physician involvement: a laboratory medicine course for clinicians. Am J Clin Pathol. 2015;143:405–11.
12. Louw VJ, Nel MM, Hay JF. Postgraduate education in transfusion medicine in the absence of formal residency training: assessment of factors needed to develop and sustain a postgraduate diploma program. Transfus Apher Sci. 2013;49:681–6.
13. Mapako T, Tagny CT, Laperche S, Bates I, Murphy EL. Building capacities in research for blood services in Africa. Transfus Clin Biol. 2021;28:171–4.
14. Tagny CT, Diarra A, Yahaya R, Hakizimana M, Nguessan A, Mbensa G, et al. Characteristics of blood donors and donated blood in sub-Saharan Francophone Africa. Transfusion. 2009;49:1592–9.
15. Vermeulen M, Swanevelder R, Chowdhury D, Ingram C, Reddy R, Bloch EM, et al. Use of blood donor screening to monitor prevalence of HIV and hepatitis B and C viruses, South Africa. Emerg Infect Dis. 2017;23:1560–3.
16. Mapako T, Mvere DA, Chitiyo ME, Rusakaniko S, Postma MJ, van Hulst M. Human immunodeficiency virus prevalence, incidence, and residual transmission risk in first-time and repeat blood donations in Zimbabwe: implications on blood safety. Transfusion. 2013;53:2413–21.
17. Owusu-Ofori S, Temple J, Sarkodie F, Anokwa M, Candotti D, Allain J-P. Predonation screening of blood donors with rapid tests: implementation and efficacy of a novel approach to blood safety in resource-poor settings. Transfusion. 2005;45:133–40.
18. Ala F, Allain JP, Bates I, Boukef K, Boulton F, Brandful J, et al. External financial aid to blood transfusion services in sub-Saharan Africa: a need for reflection. PLoS Med. 2012;9:e1001309.
19. Kouao MD, Dembele B, N'Goran LK, Konate S, Bloch E, Murphy EL, et al. Reasons for blood donation deferral in sub-Saharan Africa: experience in Ivory Coast. Transfusion. 2012;52:1602–6.
20. Kaufman S. What does it mean to be self-actualized in the 21st century? Sci Am. 2018. https://blogs.scientificamerican.com/beautiful-minds/what-does-it-mean-to-be-self-actualized-in-the-21st-century/. Accessed December 12, 2021.

SUPPORTING INFORMATION

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

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SHORT REPORT

Simulated effects of ferritin screening on C-reactive protein levels in recruited blood donors

Esa Turkulainen | Jarkko Ihalainen | Mikko Arvas

Research and Development, Finnish Red Cross Blood Service, Helsinki, Finland

Correspondence
Esa Turkulainen, Biomedicum
1, Haartmaninkatu 8, 00290 Helsinki, Finland.
Email: esa.turkulainen@veripalvelu.fi

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Abstract

Background and Objectives: Ferritin is commonly measured to evaluate iron stores in the body. Some countries have added or considered adding ferritin lower bounds to donor eligibility criteria. Ferritin is also elevated by inflammation. The main goal of this study is to estimate how different ferritin cut-offs would affect the proportion of donors with a C-reactive protein (CRP) level over 3 mg/L, which is the decision limit of the highest chronic cardiovascular risk.

Materials and Methods: To simulate recruitment of new blood donors, we selected participants from two Finnish general population cohorts, namely FINRISK 1997 ($n = 5369$) and Health 2000 ($n = 3278$), that would likely fulfil the selection criteria of blood donation. We then calculated the proportion of individuals with high-sensitivity CRP values above 3 mg/L, over a range of ferritin values.

Results: We found that for several ferritin cut-offs the proportion of potential donors with CRP > 3 mg/L would rise by a statistically significant amount. The trend was significant and similar for all subgroups but weaker for non-menstruating women as well as men.

Conclusion: Our results show that screening a population of potential blood donors with ferritin cut-offs raises the number of people with CRP > 3 mg/L within the blood donor population.

Keywords

CRP, donor health, donor management, ferritin, ferritin screening

Highlights

- We demonstrate the use of well-characterized Finnish general population cohorts (FINRISK 1997 and Health 2000) for simulating the selection of new donors with a ferritin screening policy in addition to regular eligibility criteria.
- In our simulations, filtering the cohort data with rising cut-off ferritin levels results in a proportional increase of C-reactive protein (CRP) within the chronic inflammation range.
- The association between increasing CRP and ferritin levels was most prominent in menstruating females. This finding emphasizes the importance of thorough pre-donation interview and assessment in this group of whole blood donors.

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INTRODUCTION

Ferritin is an intracellular protein that stores iron, protecting cells from its toxicity. It is found throughout the body and its synthesis and degradation align with cellular iron requirements. The iron-free form of ferritin, apoferritin, consists of H and L-subunits coded by two distinct, but similar genes. Ferritin is also secreted in blood by non-classical pathways mostly by macrophages [1], where it is composed primarily of iron poor L-subunits. Whether blood ferritin has an active regulatory role or just reflects cellular damage is debated, but it is not thought to have a role in iron uptake or transport [2].

A Cochrane library review recently concluded that there is low-certainty evidence that plasma or serum ferritin below 30 μg/L 'is reasonably sensitive and a very specific test for iron deficiency in people presenting for medical care' [3], while WHO recommends <15 μg/L to detect iron deficiency in apparently healthy adults. In contrast, in regular blood donors where blood ferritin levels are typically found to be below 50 μg/L and self-estimated health is on average good, no associations between self-estimated health and blood ferritin have been found so far [4, 5]. Some countries have considered adding ferritin lower bounds into donor eligibility criteria; for example, in the Netherlands this practice is already implemented. Although this can help in the management of donor iron stores, it can also introduce new challenges to donor health management and recruitment.

Ferritin levels are elevated in various diseases, from microbial infections to chronic metabolic diseases. This has been suggested to have a role in the modulation of immune responses and iron sequestration [1].

In general, the relationships between diseases and iron status appear to be complex. However, a meta-analysis concluded that ferritin levels are positively associated with metabolic syndrome [6].

C-reactive protein (CRP) is secreted by the liver as part of the acute-phase response to infection and inflammation. CRP has been used as a marker for bacterial infection, with 10 mg/L as a typical decision limit. However, CRP can be temporarily elevated by various environmental irritants and inflammatory responses, making the importance of minor CRP elevation difficult to interpret. Despite its unspecificity, high-sensitivity CRP (hs-CRP) at the levels beyond the acute-phase reaction (≤3 mg/L) has become a clinically useful marker for risk of cardiometabolic disease. A systematic review of 23 publications estimated that the relative risk of incident coronary heart disease was from 1.37 to 1.83 (95% confidence interval [CI]) higher in persons with CRP > 3 mg/L versus persons with CRP < 1 mg/L. The recent CANTOS trial on inflammation reduction using canakinumab provides strong evidence that inflammation, and in particular CRP, is causal for cardiovascular disease. Cardiovascular disease patients whose CRP was lowered by canakinumab to less than 2 mg/L had a hazard ratio of 0.66–0.85 (95% CI) of major adverse cardiovascular events, while those whose CRP was not lowered below 2 mg/L enjoyed no such benefit from the treatment [7].

Danish and Finnish regular blood donors have been found to have similar levels of minor CRP elevation and markedly lower levels than Finnish general population [8, 9]. This is likely due to the healthy donor effect: healthier individuals are selected to be donors and they can maintain the habit for years or even decades. Regardless, even in the healthy blood donor sub-population, low-grade inflammation (defined as a plasma CRP level between 3 and 10 mg/L) has been shown to be associated negatively with physical health-related quality of life [10].

As ferritin is both an iron and an inflammation marker, we hypothesized that adding ferritin lower bounds to blood donor selection policy might increase the level of inflammation in a blood donor population. As a surrogate for inflammation, we used CRP. With the help of Finnish representative general population cohorts, we here attempt to quantify the influence of ferritin cut-off levels to donor recruitment and donor health.

MATERIALS AND METHODS

We used data from two different Finnish health survey cohorts: FINRISK 1997 (FR97) and Health 2000 (H2000). Both FR97 and H2000 studies were approved by the Ethics Committee of the National Public Health Institute and carried out according to the recommendations of the Declaration of Helsinki. Permission to use the data for this project was granted by THL biobank (project THLBB2020_19).

Exact laboratory methods have been described previously for serum ferritin in FR97 and H2000 [11] and for serum hs-CRP in FR97 [11] and H2000 [12]. From these cohorts, we included individuals who had both a ferritin measurement and a measurement of hs-CRP. To improve the fit between the population cohorts and potential blood donors, we excluded individuals based on several donor eligibility criteria.

We assigned individuals into subgroups of men, menstruating women and non-menstruating women using their recorded sex and reported status of menstrual bleeding. In the H2000 cohort, women over the age of 55 were not asked about their menstruation status. We categorized these individuals as non-menstruating. The number of individuals in each subgroup for both cohorts is summarized in Table 1, along with the medians and interquartile ranges of CRP and ferritin.

To test whether ferritin filtering has the potential to increase the share of donors with an increase in inflammatory reaction in the otherwise eligible donor population, we examined the proportion of individuals over the commonly used threshold of 3 mg/L of serum hs-CRP for low-grade inflammation within sub-populations filtered using ferritin lower bounds ranging from 0 to 50 μg/L with a step size of 1 μg/L. We assign 95% CIs for the bootstrapped estimates (sample size 10,000) using normal approximation. We use these CIs to determine whether the proportion of possibly inflamed potential donors differs between the current eligibility criteria (no ferritin filtering) and an added ferritin lower bound requirement of 15, 30 and 50 μg/L in a statistically significant manner. The full R code of the analysis is available on Github (https://github.com/FRCBS/CRP_enrichment).

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TABLE 1 The number of individuals, along with medians (mds) and interquartile ranges (IQRs) for ferritin and C-reactive protein (CRP) levels across all subgroups within both cohorts.

	Subgroup	N	Ferritin (μg/L) md | (IQR)	CRP (mg/L) md | (IQR)
FR97	Menstruating women	1912	23.91 | (12.38, 42.53)	0.77 | (0.39, 1.86)
	Non-menstruating women	876	55.78 | (31.27, 92.76)	1.27 | (0.61, 2.65)
	Men	2581	112.05 | (65.87, 181.79)	0.89 | (0.46, 1.89)
H2000	Menstruating women	938	27.9 | (15.12, 48.98)	0.62 | (0.27, 1.82)
	Non-menstruating women	651	55.9 | (32.8, 95.45)	1.04 | (0.38, 2.42)
	Men	1689	124.9 | (76.6, 194.2)	0.77 | (0.35, 1.75)

Abbreviations: FR97, FINRISK 1997; Health 2000, H2000.

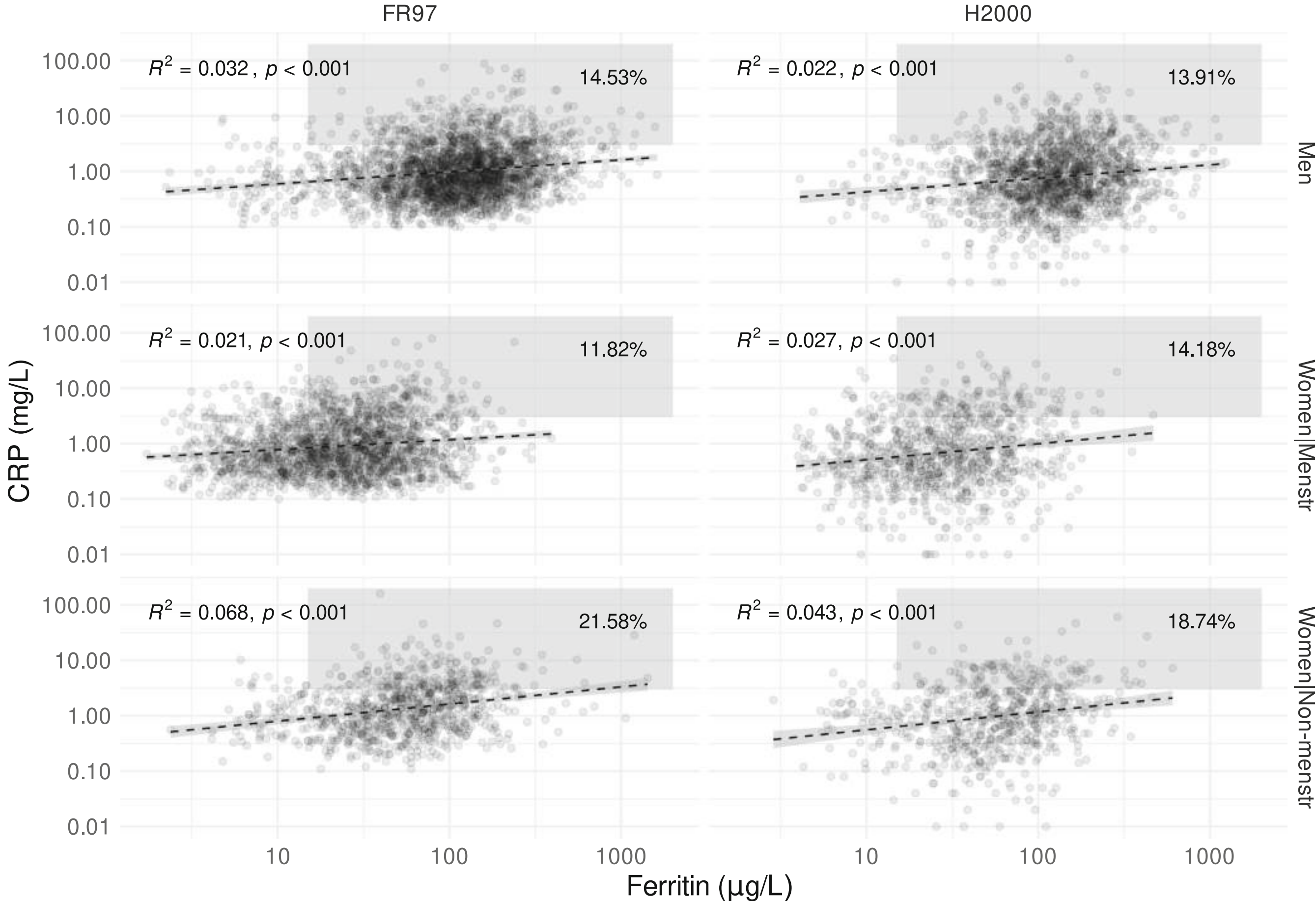

FIGURE 1 Scatter plots of C-reactive protein (CRP) and ferritin values in both cohorts and all subgroups. The grey boxes indicate the area where donors are both over 15 μg/L of ferritin and 3 mg/L of CRP. The proportion of people in the sample covered by the box is indicated with a percentage. FR97, FINRISK 1997; Health 2000, H2000.

RESULTS

The general relationship between CRP and ferritin within subgroups in the cohorts is shown as a scatterplot in Figure 1. The linear fits indicate a weak positive correlation between the variables for all subgroups ($R^2 = 0.021$–0.068, $p < 0.001$). Figure 2 presents the change in the proportion of people with CRP > 3 mg/L at different ferritin filtering levels. For example, if a ferritin cut-off of 30 were to be applied for menstruating women, the proportion of individuals >3 mg/L CRP among potential blood donors would rise by 2.3–6.3 percentage points based on the FR97 cohort. We find that for menstruating women in both cohorts, the proportion of people with CRP > 3 mg/L is significantly higher at ferritin filter levels of 15, 30 and 50 μg/L than without filtering. This holds for non-menstruating women

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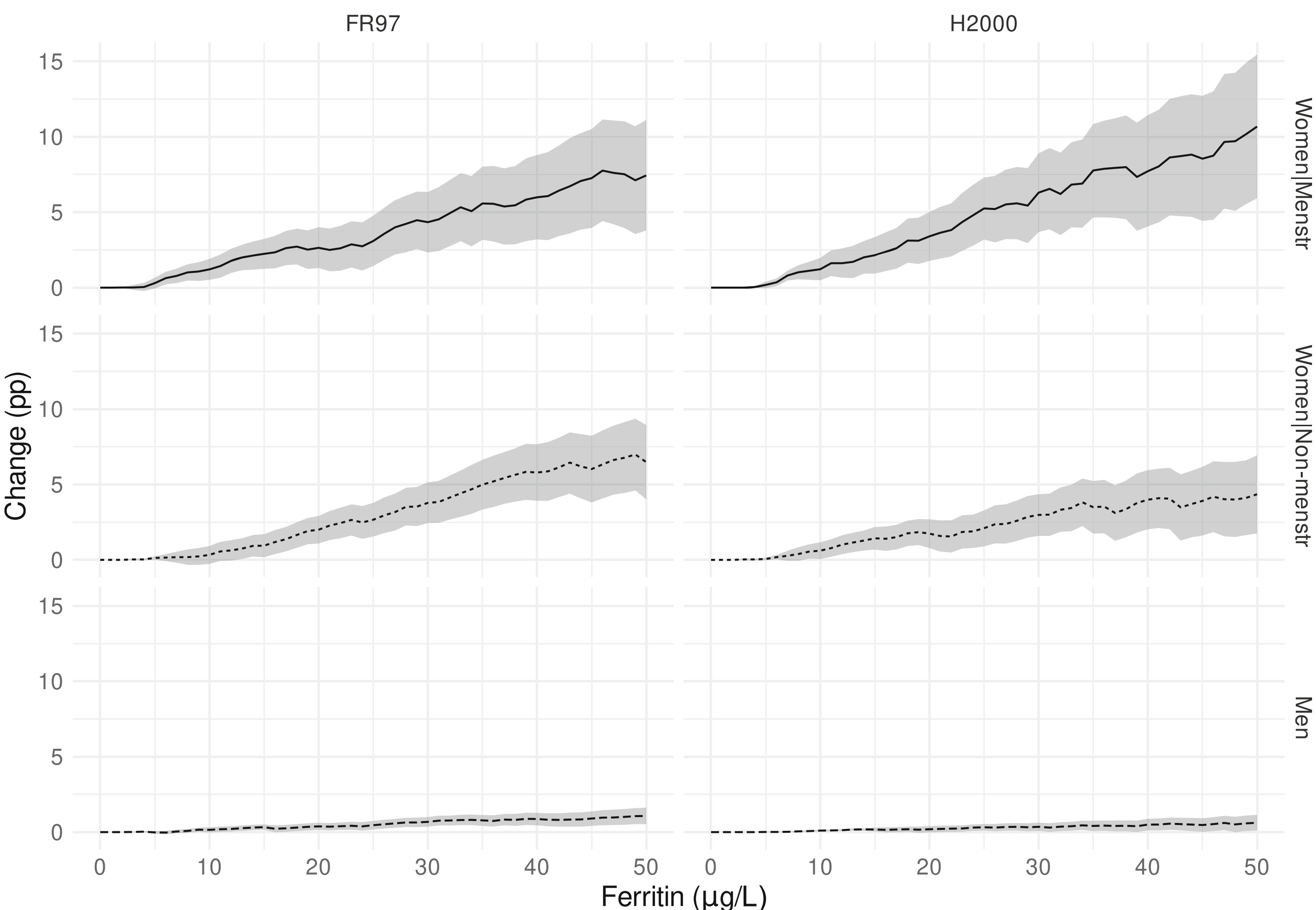

FIGURE 2 Line plots of the change in the proportion of people over 3 mg/L C-reactive protein at different ferritin filtering levels, with 95% confidence intervals. Change is presented in percentage points (pp). FR97, FINRISK 1997; Health 2000, H2000.

also. These differences are all significant in men, but the respective increases in proportions are much smaller.

DISCUSSION

As expected, a weak positive correlation between ferritin and CRP was found in the Finnish representative general population cohorts analysed (Figure 1). This is in line with the hypothesis that ferritin has a role in the modulation of immune responses and iron sequestration during microbial infection. For blood donation, it is important to note that the correlation of CRP and ferritin extends to the lower CRP range of ≤3 mg/L. Hence, even minor chronic inflammation will affect ferritin measurements.

Our simulation suggests that adding a ferritin threshold to the existing eligibility criteria when selecting blood donors from the general population will increase the percentage of donors with low-grade inflammation. These individuals might be less suited to tolerate the physiological stress of blood donation and to maintain long donation careers. Based on current research, high iron status could also be associated with higher disease risks, further aggravating this effect. Countries such as the Netherlands, where additional ferritin screening policies are in place, are well set to study this hypothesis and whether a ferritin measurement should be accompanied with a hs-CRP measurement to avoid these issues. Our results also provide further information on the effects on different ferritin cut-off levels, thus facilitating the selection of the most appropriate decision limit.

The effect of ferritin cut-off on CRP is highest in menstruating women. This suggests that a significant proportion of donors in this group may have ferritin levels in the expected range not because of adequate iron balance but because of an inflammatory response. Furthermore, ferritin filter levels of 15 and 30 µg/L exclude around 20% and 60% of otherwise eligible donors within this subgroup, respectively (Supplementary material). These considerations should be taken into account in designing the donor eligibility schemes.

Our approach suffers from the incomplete information available from the participants of the two cohort studies. Some of the people in question may have had conditions that would have been revealed in the pre-donation interview. Additionally, there were too few haemoglobin measurements in the FR97 cohort to include it in the eligibility criteria, while in reality haemoglobin is a very important criterion. However, as the results were similar in both cohorts, we believe that these challenges do not significantly undermine the statistical findings of our study.

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Of Finnish regular premenopausal female blood donors, 18%–23% were found to have ferritin <15 μg/L in 2015–2017 [4]. The Finnish Red Cross Blood Service reacted to this finding by recommending the young female donors to donate only once a year. They were also informed in educational materials about the risk of iron deficiency by blood donation. The iron replacement policy for them was also revised by doubling the course of daily 50 mg iron tablets from 2 to 4 weeks. Such practices could provide an alternative approach to guarantee iron stores of blood donors to the costly combination of ferritin and CRP measurements.

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E.T. wrote the analysis code, analysed the data and compiled the results; E.T., M.A. and J.I. all wrote and reviewed the manuscript; J.I. instructed the analysis and writing; M.A. and J.I. conducted the literature review and M.A. supervised the research.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data used for the research was obtained from the THL Biobank in Finland. Researchers can apply for access by following the application process outlined at https://thl.fi/en/web/thl-biobank/for-researchers/application-process. All research code is available for use under GNU General Public License at https://github.com/FRCBS/CRP_enrichment.

ORCID

Esa Turkulainen https://orcid.org/0000-0001-8844-9344
Jarkko Ihalainen https://orcid.org/0000-0003-3331-0307
Mikko Arvas https://orcid.org/0000-0002-6902-8488

REFERENCES

1. Moreira AC, Mesquita G, Gomes MS. Ferritin: an inflammatory player keeping iron at the core of pathogen-host interactions. Microorganisms. 2020;8:1–20.
2. Cullis JO, Fitzsimons EJ, Griffiths WJH, Tsochatzis E, Thomas DW. Investigation and management of a raised serum ferritin. Br J Haematol. 2018;181:331–40.
3. Garcia-Casal MN, Peña-Rosas JP, Urrechaga E, Escanero JF, Huo J, Martinez RX, et al. Performance and comparability of laboratory methods for measuring ferritin concentrations in human serum or plasma: a systematic review and meta-analysis. PLoS One. 2018;13:1–24.
4. Lobier M, Castrén J, Niittymäki P, Palokangas E, Partanen J, Arvas M. The effect of donation activity dwarfs the effect of lifestyle, diet and targeted iron supplementation on blood donor iron stores. PLoS One. 2019;14:e0220862.
5. Rigas AS, Pedersen OB, Sørensen CJ, Sørensen E, Kotzé SR, Petersen MS, et al. No association between iron status and self-reported health-related quality of life in 16,375 Danish blood donors: results from the Danish Blood Donor Study. Transfusion. 2015;55:1752–6.
6. Abril-Ulloa V, Flores-Mateo G, Solà-Alberich R, Manuel-Y-Keenoy B, Arija V. Ferritin levels and risk of metabolic syndrome: meta-analysis of observational studies. BMC Public Health. 2014;14:1–8.
7. Liberale L, Montecucco F, Schwarz L, Lüscher TF, Camici GG. Inflammation and cardiovascular diseases: lessons from seminal clinical trials. Cardiovasc Res. 2021;117:411–22.
8. Lobier M, Niittymäki P, Nikiforow N, Palokangas E, Larjo A, Mattila P, et al. FinDonor 10 000 study: a cohort to identify iron depletion and factors affecting it in Finnish blood donors. Vox Sang. 2020;115:36–46.
9. Sørensen CJ, Pedersen OB, Petersen MS, Sørensen E, Kotzé SR, Thørner LW, et al. Combined oral contraception and obesity are strong predictors of low-grade inflammation in healthy individuals: results from the Danish Blood Donor Study (DBDS). PLoS One. 2014;9:e88196.
10. Dinh KM, Kaspersen KA, Mikkelsen S, Pedersen OB, Petersen MS, Thørner LW, et al. Low-grade inflammation is negatively associated with physical Health-Related Quality of Life in healthy individuals: results from The Danish Blood Donor Study (DBDS). PLoS One. 2019;14:e0214468.
11. Salomaa V, Havulinna A, Saarela O, Zeller T, Jousilahti P, Jula A, et al. Thirty-one novel biomarkers as predictors for clinically incident diabetes. PLoS One. 2010;5:e10100.
12. Konstari S, Sares-Jäske L, Heliövaara M, Rissanen H, Knekt P, Arokoski J, et al. Dietary magnesium intake, serum high sensitivity C-reactive protein and the risk of incident knee osteoarthritis leading to hospitalization—a cohort study of 4,953 Finns. PLoS One. 2019;14:e0214064.

SUPPORTING INFORMATION

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

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LETTER TO THE EDITOR

Tetanus antibodies in normal human immunoglobulin preparations

We would like to draw attention to the issue that anti-tetanus immunoglobulin (TIG), that is, human immunoglobulin (IG) with known antibody content against the neurotoxin, is essential medicine (WHO) for post-exposure prophylaxis (PEP) and therapy of tetanus [1–3]; yet, TIG is not available in all countries [1] or has been difficult to obtain in recent years [4]. In the absence of TIG, PEP with human IG for intramuscular immunoglobulin (IMIG) or subcutaneous immunoglobulin (SCIG) use is an alternative [4]. For tetanus treatment, intravenous immunoglobulin (IVIG) at a dose of 200–400 mg/kg is recommended in the United States [1], whereas in the United Kingdom, a fixed dosage with selected IVIG preparations was defined [4]. As IG is not routinely tested for tetanus antibody concentrations, little information is available on lot-to-lot variability and dosage required to administer the recommended 250–500 IU of tetanus antibody for PEP or 3000–10,000 IU for therapy [1–4]. In this context, we received requests on tetanus antibody levels in 10% immunoglobulin for infusion (IGI) from practitioners recently, which reflects the need for further information.

We, therefore, investigated tetanus antibody concentrations of current 10% IGI preparations manufactured in 2021 from plasma collected in the United States and European Union, by apheresis (source plasma) as well as from blood donations (recovered plasma), which can be administered as IVIG (Gammagard Liquid/KIOVIG) as well as SCIG (HyQvia), using the Tetanus IgG enzyme-linked immunosorbent assay (ELISA) (Virotech Diagnostics GmbH, Rüsselsheim, Germany) and methodology validated according to ICH principles, reporting results against the second WHO International Standard for Anti-Tetanus Immunoglobulin Human (NIBSC code: 13/240).

We determined a mean ± standard deviation (SD) anti-tetanus potency of 29.2 ± 2.0 IU/mL for recent IGI, 10% lots (Table 1), where lots fractionated from US plasma had significantly ($p < 0.0001$) higher tetanus toxin antibody content (30.8 ± 1.2 IU/mL) than lots fractionated from EU plasma (27.5 ± 1.2 IU/mL). The lowest antibody concentration determined in any lot was 26.0 IU/mL (Table 1). There was no relevant difference in tetanus toxin antibody content for IGI lots fractionated from source or recovered plasma. These data were brought into context with tetanus toxin antibody concentrations reported for other IGI preparations by the National Institute for Biological Standards and Control (NIBSC) [4], where a selection for more recent test date (i.e., tested in 2016–2019) was made and values were normalized to 10% protein content. When tetanus toxin antibody concentrations of 39 lots of 13 different commercial IGI products of six different manufacturers [4] were normalized for protein content, a mean potency of 30.5 ± 2.5 IU/mL was calculated (Table 1), very similar to the antibody concentration determined in the current IGI, 10% preparations.

Our investigation showed that normal human IG preparations contain tetanus toxin antibodies at remarkably similar concentrations. This is true for lots of the same manufacturer, as well as for different commercial preparations of several manufacturers (Table 1) [4]. A difference in tetanus potency was seen for the 10% IGI preparations analysed here depending on the origin of plasma that was used for fractionation, that is, higher potency was seen in US plasma-derived IGI than in EU plasma-derived IGI. The geographic difference in tetanus potency was already reported previously and had been related to differences in tetanus vaccination policy and vaccine potency [5]. As the slightly lower tetanus toxin antibody content in EU plasma-derived IGI still provides for dosages well within PEP and therapy recommendations (see below), this potential lot-to-lot difference does not appear clinically relevant. A serum tetanus toxin antibody concentration of ≥0.1 IU/mL is indicative of immune protection.

TABLE 1 Summary of tetanus anti-toxin potency in recent immunoglobulin for infusion (IGI) preparations, normalized for 10% protein content.

Year of test	Number of different IGI products (number of manufacturers)	Number of lots analysed	Protein content (%)	ELISA IU/mL (mean ± SD) [min]	Reference
2021	2 (1)	36	10	29.2 ± 2.0 [26.0]	n.a.
2016–2019[a]	13 (6)	39	5, 10, 16, 16.5	30.5 ± 2.5 [24.0]	Lejtenyi and Mazer [5]

Abbreviation: n.a., not applicable.
[a]Protein content of IGI preparations prior to normalization to 10%.

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The 10% IGI preparations tested here are suitable for subcutaneous as well as intravenous application, making them amendable for tetanus PEP as well as tetanus therapy. With respect to PEP of tetanus, 10–20 mL of 10% IGI should suffice to administer the recommended 250–500 IU of tetanus antibody, assuming the lowest tetanus potency that was determined, that is, 26.0 IU/mL (Table 1). This corresponds well with the recommendation to manage tetanus-prone wounds with 4.5–9 mL of 20% SCIG [4], a product that is manufactured by the same pathway as the 10% IGI lots tested here, with a final adjustment of protein content. For tetanus therapy with 10% IVIG, a person with 80 kg of body weight would receive 4160 IU of tetanus antibodies, assuming treatment with the lowest recommended dosage of 200 mg/kg [1] and the lowest tetanus potency that was determined for a lot here (Table 1), that is, a dosage well within the recommended 3000–10,000 IU for therapy [1–4].

In conclusion, we provide comprehensive evidence that current 10% IGI preparations consistently contain tetanus antibodies at levels that allow for tetanus PEP as well as tetanus therapy, with negligible lot-to-lot variability. Although TIG does have the benefit of smaller volumes being required for treatment, the information of consistent anti-tetanus potency in 10% IGI aids to alleviate concern about the uncertain availability of specific TIGs and opens further options for tetanus PEP and treatment.

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M.R.F and T.R.K conceived the study; A.S. supervised the study; M.R.F and C.L. analysed the data and wrote the manuscript. All the authors revised the manuscript and contributed significantly to the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors are employees of Takeda Manufacturing Austria AG, Vienna, Austria. M.R.F and T.R.K have Takeda stock interests.

FUNDING INFORMATION

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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.

Maria R. Farcet
Cornelia Lackner
Andreas Schirmer
Thomas R. Kreil

Global Pathogen Safety, Takeda Manufacturing Austria AG, Vienna, Austria

Correspondence

Thomas R. Kreil, Global Pathogen Safety, Takeda Manufacturing Austria AG, Takeda, Benatzkygasse 2-6, 1221 Vienna, Austria.
Email: thomas.kreil@takeda.com

ORCID

Maria R. Farcet https://orcid.org/0000-0002-2335-8609
Thomas R. Kreil https://orcid.org/0000-0001-9970-0987

REFERENCES

1. American Academy of Pediatrics. Tetanus. In: Kimberlin DW, Brady MT, Jackson MA, Long SS, editors. Red book: 2018 report of the Committee on nfectious Diseases. 31st ed. Itasca, IL: American Academy of Pediatrics; 2018. p. 793–8.
2. European Medicines Agency. Committee for Medical Products for Human use, Core SPC for Human Tetanus Immunoglobulin for intramuscular use. CPMP/BPWG/3730/02. 2006 [cited 2023 Jul 7]. Available from: https://www.ema.europa.eu/en/core-summary-product-characteristics-human-tetanus-immunoglobulin-intramuscular-use
3. Robert Koch Institute. Tetanus RKI-Ratgeber. 2018 [cited 2023 Jul 7]. Available from: https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Tetanus.html;jsessionid=390A4F781973A18F0D42699C65BA4BA4.internet072#doc2398266bodyText10
4. Public Health England. Tetanus. Guidance on the management of suspected tetanus cases and on the assessment and management of tetanus-prone wounds. 2019 [cited 2023 Jul 7]. Available from: https://www.gov.uk/government/publications/tetanus-advice-for-health-professionals
5. Lejtenyi D, Mazer B. Consistency of protective antibody levels across lots of intravenous immunoglobulin preparations. J Allergy Clin Immunol. 2008;121:254–5.

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