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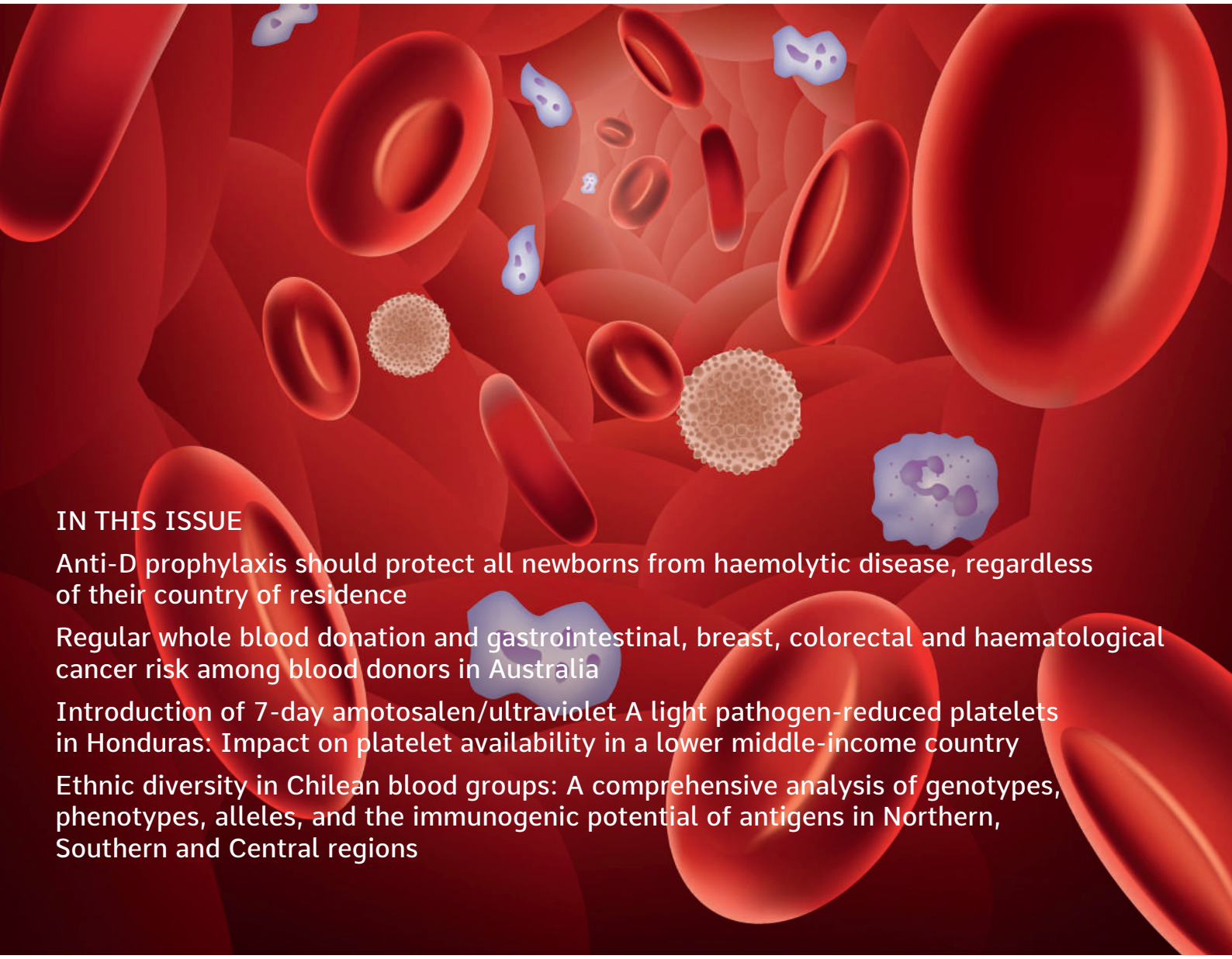

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Aims and Scope

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

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Vox Sanguinis

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COMMENTARY

Vox Sanguinis ISBT International Society of Blood Transfusion

Anti-D prophylaxis should protect all newborns from haemolytic disease, regardless of their country of residence

According to a World Health Organization (WHO) study, the observed failure rate of substandard and falsified medicines (SFM) in low- and middle-income countries is estimated to be over 10%, with an estimated spend of US$30.5 billion [1]. The problem embraces drugs in many areas of healthcare. We report on monoclonal anti-Ds, used for many years in low- and middle-income countries, for prophylaxis of RhD haemolytic disease of the foetus and newborn (HDFN), which have never been shown to meet the standards of anti-D immunoglobulin (Ig). The consequences of using these drugs in postnatal women might be very serious, even fatal, for their offspring.

The prevention of HDFN by administration of anti-D Ig to D-negative women has been a triumph of medicine. In Europe, North America and Australia, before prophylaxis, 16%–17% of D-negative women delivering ABO-compatible, D-positive infants developed anti-D after delivery of a second D-positive infant [2]. Several clinical trials, over 6 years, on male volunteers and then on thousands of D-negative primiparae delivering ABO compatible, D-positive infants, many of them tested after delivery of a second D-positive infant, showed that postnatal anti-D Ig could suppress RhD immunization and hence be used clinically in women [2]. Anti-D Ig acts, in part, by clearing foetal red blood cells from the maternal circulation. By 1971, anti-D Ig was introduced into clinical use in the United Kingdom, where mortality from HDFN fell from 46/100,000 births in 1953 [3] to 1.6/100,000 in 1989 [4]. However, in several low- and middle-income countries, due to limited availability and expense of prophylaxis, this success has not occurred, where it was estimated that about 150,000 D-negative pregnancies are affected per annum, resulting in numerous stillbirths and severely affected infants [5].

Hyperimmune anti-D plasma for the manufacture of anti-D Ig is obtained mainly by plasmapheresis of paid hyperimmunized subjects in the United States. It would be ideal if a monoclonal anti-D, in limitless supply, could replace this polyclonal product. Since 1980, numerous monoclonal anti-Ds were produced either from human EBV-transformed B cells, mouse-human heterohybridomas or recombinant anti-Ds from Chinese Hamster Ovary (CHO) or rat myeloma cells. In human volunteers, monoclonal anti-Ds from mouse or hamster cell lines caused defective red cell clearance and did not prevent RhD immunization [6]. This was attributed to non-human glycosylation (composition of sugars) of the anti-Ds, which, in part, changed the patterns of interaction with Fc receptors on mononuclear phagocytic cells. Glycosylation of antibodies is specific for each species of cell line used for their production [7].

Bharat Serums and Vaccines Ltd., has marketed, for over 20 years, monoclonal and recombinant anti-Ds, licensed locally in India. By 2018, Rhoclone had been administered to over 4 million mothers in India, Asia and Africa. Since then, sales have vastly expanded. However, despite its wide use, little is known about the different forms of Rhoclone. At first, it derived from a heterohybridoma from Russia, found to be unsuitable for clinical use because it failed to show efficacy in trials on volunteers [8]. Recently, Bharat has marketed Trinbelimab, a recombinant CHO cell-derived anti-D. Only four small clinical non-inferiority trials, of questionable statistical significance, have been published on Bharat's monoclonals (human B cells [9], heterohybridoma [10, 11] and CHO [12]); about 100 women in each trial, most with an unknown number of previous pregnancies or of ABO-compatibility with the newborn (only primigravidae delivering ABO compatible, D-positive infants and tested after delivery of a second D-positive infant, should have been studied), received monoclonal or recombinant anti-D. None made anti-D at 180 days (about 25% were lost to follow up). A phase IV safety study was published recently on Trinbelimab [13]. No data on studies in vitro or in D-negative volunteers can be found in the public domain. We have tested two lots of Rhoclone in ADCC (antibody-dependent cellular cytotoxicity assay), a key in vitro predictor of the efficacy of anti-D in vivo. Unlike polyclonal anti-D Ig, Rhoclone lacked haemolytic activity, indicating that it is highly unlikely to provide protection from D-immunization.

Bharat's anti-Ds would not have passed the stringent licensing criteria required by the Federal Drugs Administration of the USA, the European Medicines Agency or the : UK Medicines and Healthcare Regulatory Agency, since their three forms of anti-D have not undergone the scrupulous scrutiny the polyclonal anti-Ds have. These are new drugs that each need to be characterized, tested and tried properly, first in D-negative male volunteers subjected to repeated D-positive red cell injections and then in at least 1000 D-negative primiparae after delivery of their second D-positive infant [2]. It is of great concern that the manufacturer of these drugs is selling them in large quantities without any solid proof of efficacy. Despite Rhoclone being used in millions of D-negative women, no data are available on the rate of alloimmunization of such women after delivery of their first ABO-compatible, or of a second, D-positive infant—a crucial determinant of the immunosuppressive effect of the antibody. We have asked the company for post-marketing data on efficacy but have not received a reply.

Thus, the lack of convincing evidence that Rhoclone has a beneficial prophylactic effect, and the absence of post-marketing data, should raise great concern to health authorities worldwide. It is

worrying to read that, in Ethiopia, where polyclonal and Bharat's anti-Ds are used, 17% of RhD-negative women are still immunized, suggesting the absence of a prophylactic effect [14]. The same article provides even more striking data: 67.9% of alloimmunized women participating in the testing had received anti-D after their previous pregnancy.

Fortunately, the real efficacy of Rhoclone will finally be assessed. Recently, the AFRICARhE project has been launched, with the ultimate goal of eradicating HDFN in Africa [15]. One of the first objectives of this project is to retrospectively evaluate the efficacy of the anti-Ds from Bharat Serums and Vaccines Ltd. in a post-marketing Phase IV efficacy study.

Resource poor countries deserve an international regulatory body that will ensure that the drugs available to them in the market are as effective as those available in high income countries. There is hope now, with the WHO Global Surveillance and Monitoring System (GSMS), for identifying substandard and falsified medical products. In the absence of an international regulatory body, a first step could be the reporting, by user countries, of these types of non-fully validated anti-Ds to the GSMS, thus protecting public health and enabling informed decisions by clinicians worldwide.

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The authors declare no conflicts of interest. The authors contributed in equal measure to the writing of this Commentary.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

The prototypical UK blood donor, homophily and blood donation: Blood donors are like you, not me

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Abstract

Background and Objectives: Homophily represents the extent to which people feel others are like them and encourages the uptake of activities they feel people like them do. Currently, there are no data on blood donor homophily with respect to (i) people's representation of the average prototypical UK blood donor and (ii) the degree of homophily with this prototype for current donors, non-donors, groups blood services wish to encourage (ethnic minorities), those who are now eligible following policy changes (e.g., men-who-have-sex-with-men: MSM) and recipients. We aim to fill these gaps in knowledge.

Materials and Methods: We surveyed the UK general population MSM, long-term blood recipients, current donors, non-donors and ethnic minorities ($n = 785$) to assess perceptions of the prototypical donor in terms of ethnicity, age, gender, social class, educational level and political ideology. Homophily was indexed with respect to age, gender and ethnicity.

Results: The prototypical UK blood donor is perceived as White, middle-aged, middle-class, college-level educated and left-wing. Current donors and MSM are more homophilous with this prototype, whereas recipients and ethnic minorities

have the lowest homophily. Higher levels of homophily are associated with an increased likelihood of committing to donate.

Conclusion: The prototype of the UK donor defined this as a White activity. This, in part, may explain why ethnic minorities are less likely to be donors. As well as traditional recruitment strategies, blood services need to consider broader structural changes such as the ethnic diversity of staff and co-designing donor spaces with local communities.

Keywords

demography, equality, ethnicity, homophily, prototype, social class

Highlights

- The prototypical UK blood donor is White, middle-aged, middle-class, college-level educated and left-wing.
- Degree of homophily (the closeness of a person's perception of the prototypical UK blood donor to their own demography) predicts decisions to donate.
- Current donors and men-who-have-sex-with-men are more homophilous with the blood donor prototype; ethnic minorities have the lowest homophily, with White people having the highest.

INTRODUCTION

People are more likely to join groups, participate in sports, contribute to community initiatives/activities and seek healthcare if they feel that people who partake in those activities are similar to them [1–3]. This is called homophily [1, 3]. Homophily has important implications for donor services aiming to enhance diversity and equality in their donor panels by recruiting and retaining donors across more comprehensive ranges of ethnicity, sexuality and age [4, 5], Specifically, people who do not perceive themselves to be like—*homophilous* with—current donors are less likely to donate. This may, in part, explain why Black people and younger people are less represented in donor panels [4, 5]. Furthermore, following recent changes to United Kingdom (UK) donor policy men-who-have-sex-with-men (MSM) are eligible to donate [6]. Thus, it is useful to know if MSM perceive themselves as homophilous to blood donors, as this is likely to encourage more MSM to donate. Therefore, knowledge of donor homophily for these groups is important for blood services to aid the development of inclusive strategies. Finally, the perspective of those with sickle cell and thalassaemia is critical. As long-term recipients of blood treatment, efficacy is enhanced with well-matched blood from ethnic minorities. Thus, these recipients may have concerns about their treatment if they do not see donors as homophilous for ethnicity.

Therefore, we explore how people in the UK define the prototypical blood donor across key demographic characteristics (e.g., age, sex, ethnicity), from the perspective of different stakeholders (blood donors, MSM, recipients of blood, people from ethnic minorities) and how donor homophily predicts active decisions to become a blood donor [3].

BLOOD DONATION, HOMOPHILY, PROTOTYPE THEORY AND DONOR IDENTITY

Greater diversity of donors is beneficial both psychologically (e.g., increased well-being) [7] and clinically (e.g., improved treatment of sickle cell disease: SCD) [4]. However, few new young people [5, 8] and members of ethnic minorities [4] donate blood. As an example, recruiting and retaining more Black donors will enhance the efficacy of treating Sickle Cell by better blood matching [4]. Furthermore, recent changes to UK donor selection policy, based on individualized sexual behaviour, mean that MSM can donate [6]. Again, MSM are more likely to decide to donate the greater their perceived homophily with current donors.

We argue that homophily is an additional structural barrier to donation. Indeed, many barriers to blood donation have been documented [9]; including psychological (e.g., fear of needles) and structural (e.g., convenience, location) factors that influence everyone [9], and some that are more likely to influence people from the Black community (e.g., distrust, fear of negative health effects, differential deferral) [10–12]. However, one major structural barrier, not previously explored with respect to sexuality, ethnicity and age, relates to how far potential donors perceive themselves as similar to the prototypical donor—*homophily* [1, 13]. Theoretical models are described below, highlighting why this is a potentially important driver/barrier to blood donor behaviour.

The prototype-willingness model offers a dual-process account of behaviour driven by a reactive emotional/heuristic and a planned decision-making route [13]. The emotional/heuristic route encompasses the idea of behavioural prototypes: particular behaviours

(e.g., blood donation) are associated with specific prototypes and the greater the degree of perceived similarity a person feels to the prototype, the more likely they are to perform that behaviour [13, 14].

Linked to prototypicality, is the concept of homophily. Homophily states that people are more likely to join groups/communities or prototypes to which they feel similar, in terms of both psychological *and* demographic characteristics [1, 3]. Conversely, people avoid behaviours/groups where homophily is low [2]. Arguably, if people perceive the typical donor as a member of a group with which they do not identify, they are less likely to donate blood.

Donor identity, which is a key driver of donor return behaviour [15, 16], arises not only as a function of donating per se [17] but also by identifying with similar other donors (prototype and homophily) [18]. This in-group identity will reinforce the donor's self-identity as a donor, encouraging return behaviour, which will ultimately perpetuate the current status quo and donor prototype [18]. Thus, there is a self-reinforcing system whereby homophily enhances donor self-identity, which in turn enhances return behaviour of homophilous people, which then further reinforces self-identity.

Finally, there is a growing realization that 'space' is partly defined in terms of demography, including ethnicity, age, gender, social class and politics and that these characteristics influence who will be likely to enter these spaces [14, 19]. For example, if blood donors are perceived as being White, then ethnic minorities will be less willing to enter spaces where blood donation occurs.

WHO ARE THE DONORS?

So, what are the current characteristics of voluntary blood donors? Regarding demography, in the UK, blood donors tend to be White in their late 30s to mid-40s, with females slightly outweighing males [5]. Data from other countries indicate that blood donors are of higher socioeconomic and educational status and are educated to at least 18 years [20]. While there are no data on blood donors' political views, organ donors, who are also more likely to be blood donors, typically express a more politically left viewpoint [21]. If the prototype reflects these objective characteristics, people should view donors as equally likely to be male or female, of higher social status, educated, politically left, white and in their early 40s.

Therefore, in this article, we explore the perceived prototypical blood donor, calculate homophily scores for people from different cultural, social and health backgrounds to quantify their similarity to the prototypical donor and investigate whether those homophily scores predict decisions to donate.

METHOD

Sampling

Participants were recruited via (i) Prolific (https://www.prolific.com/about/) (18–23 November 2021), (ii) the UK Sickle Cell Society (23–29 November 2021) and (iii) UK Thalassaemia Society (22–29 November 2021). A two-stage sampling process was adopted for the Prolific sample. An initial gender-balanced UK adult sample was recruited, and the second was a UK adult sample of non-heterosexual, non-asexual identifying MSM. The samples were collected consecutively, and additional screening was performed to ensure no repeat recruitment. MSM were oversampled to explore awareness and beliefs about the (For the Assessment of Individualised Risk) FAIR project (not the focus of this paper). All respondents were paid £1.00 for participation, consistent with Prolific guidelines. The UK Sickle Cell Society sent the link to all their relevant social media channels and their registered members' email list and posted it on their dedicated blood donation awareness pages, 'Give Blood, Spread Love, England.' (https://www.instagram.com/givebloodspreadlove/). For the UK Thalassaemia Society, the link was distributed on all their relevant social media channels (Twitter, Facebook), their registered members' email list (there are 1600, including people with thalassemia, parents and doctors) and 4000 on their social media accounts. Responses were collected from 22 to 29 November 2021.

The survey

The survey was programmed in Qualtrics (https://www.qualtrics.com/uk/). The key variables used in this paper are described below (see Supplementary File S1 for the full survey focus and sampling).

Demographics

Demographic information on age, gender, sexual orientation, ethnicity, religion and UK location was collected. Participants were coded as LGBTQ+ if they reported a sexual orientation other than heterosexual/straight *and/or* non-binary gender identity. Participants were coded as MSM if they identified as bisexual, gay, queer, pansexual or bi-curious and were male.

Donor history

All respondents were asked whether they had ever donated blood in the UK (Yes/No/ I'm not sure/ Prefer not to say) and were coded as *blood donors* if they responded 'Yes'. Blood donors were subsequently asked (i) when they last donated blood (within the last 2 months/ 2 to 12 months ago/12 months to 2 years ago/Longer than 2 years/I cannot remember/Prefer not to say). *Non-donors* are those who have never donated, *lapsed donors* have donated but not within the last 2 years, and *current donors* have donated in the last 2 years. This is a validated and reliable estimate of past donor behaviour [11, 22].

Prototypical donor

To assess what participants perceived a typical donor to be like in terms of demography, we asked, 'In your mind, what does the

'typical' blood donor look like across the following demographic categories?' They then selected one category for *age* (18–29, 30–44, 45+), using these categories because the proportion of donors aged 45 and over has increased in recent years (from 48.7% in 2018/19 to 51.1% in 2022/2023, NHSBT 2024). They also select one category for each of the following: *gender* (male, female); *ethnicity* (Asian, Black, Mixed, Other, White); *education level* (no-qualification, General Certificate of Secondary Education (GCSE) or equivalent, A levels or equivalent, degree or equivalent); *social class* (working class, middle class, upper class) and *political affiliation* (left-wing, right-wing).

Homophily Index

To index homophily, we designate $\pi =$ prototype demographic categorization and $\sigma =$ person actual self-ascribed demographic categorization. Then, in a specific dimension, if $\pi - \sigma = 0$, homophily, $\eta = 1$, else 0. Then, overall homophily, $H = \Sigma\ (\eta)$. We calculated homophily scores using the demographic data available for both the respondents and their prototype judgements: age, gender and ethnicity. Thus, we have three dimension-specific homophily scores each with a value 0 (=non-similarity) or 1 (=the perceived donor categorization and participant categorization are the same). The total homophily scores, H, range from 0 to 3, where 0 indicates that the respondent shares neither age group, gender, nor ethnicity with a prototype donor, and 3 indicates that the respondent shares all three. We applied unit weighting to each demographic characteristic when assessing the overall homophily score. While some demographic characteristics may have greater salience, there are no previous data in this domain to estimate or justify a specific weighting. Therefore, we chose unit weighting in this case.

Active commitment to donate blood

Evidence shows that an active commitment to donate is an extremely strong predictor of subsequent donations (Ferguson et al., 2023). As such, it is useful to identify predictors of making an active commitment to donate. To assess this, we stated:

> In the UK, men can donate blood every 12 weeks, and women every 16 weeks. If you were to become a blood donor, would you expect to donate blood once or multiple times?

Participants then selected one of the following: Once, Multiple times, I'm not sure or prefer not to say. Selecting once or multiple times indicated an active decision to donate and selecting I'm not sure indicated hesitancy and indecision. This is a reliable index of future behaviour [23, 24].

Ethics

This survey study was approved by the School of Psychology, the University of Nottingham., Ethics Review Board (F1308) on the 15th of November 2021.

Power estimates

A small effect size is observed for cognitive and emotional factors on emotions and donor behavioural propensity [23–25]. Thus, to achieve 0.80 power, with an α of 0.05, requires 332 participants.

RESULTS

Sample

In total, 804 participants were recruited; four respondents did not provide full informed consent, 11 dropped out after receiving the participant information sheet and 4 dropped out immediately after providing informed consent, giving a final sample of 785 observations. A Combined Patient Group (CPG) comprised participants who reported living with either thalassaemia or sickle cell.

Table 1 provides the sample characteristics (Supplementary File S2 and Table S1 provides a sample breakdown and representativeness analysis). Excluding the oversampling of MSM and the patient sample, the sample was younger (median 34) than the UK population in 2010 (median 40) and included more White people (89% vs 82%), but was broadly representative by location and gender. This pattern was the same for the full sample, except the oversampling of MSM increased the proportion of men in the sample.

The Prototypical UK Blood Donor

Table 2 categorizes the prototypical donors as seen for the total sample, as well as by MSM, patients, current donors and ethnicity. Overall, the prototypical UK donor is perceived to be 30–44 years old, White, educated to A level (high school) or degree level, middle class and left-wing. There is no clear perception that donors are more likely to be male or female.

Homophily

Figure 1 shows the homophily scores by sample characteristics (see Supplementary File S3 and Table S2 for exact figures for Figure 1). We see that this is 2 out of 3 for the overall sample. Current donors have the highest overall homophily score of 2.15 out of 3, significantly higher than non-donors but similar to lapsed donors. This is driven by the ethnicity homophily score, in which current and lapsed donors

TABLE 1 Summary descriptive statistics.

Demographics	Freq	Mean/%	SD	Min	Max	*n*
Age		35.77	12.77	18	81	779
Gender						
Male	518	66%		0	1	780
Female	251	32%		0	1	780
Non-binary	11	1.5%		0	1	780
Prefer not to say	3	0.5%				
Sexual orientation						
LGBTQ+	328	42%		0	1	775
Straight	447	58%		0	1	775
MSM						
MSM	268	35%		0	1	767
Non-MSM	499	65%		0	1	767
Ethnicity						
Asian	63	8%		0	1	772
Black	23	3%		0	1	772
Mixed	24	3%		0	1	772
Other	13	2%		0	1	772
White	649	84%		0	1	772
Location						
England	660	85%		0	1	781
Scotland	75	10%		0	1	781
Wales	32	4%		0	1	781
Northern Ireland	14	2%		0	1	781
Blood donation						
Non-donors	546	70%		0	1	776
Lapsed donors	131	17%		0	1	776
Current donor	99	13%		0	1	776
Recipients of donated blood						
Recipient of donated blood/blood products	77	10%		0	1	768
Sickle cell	4	1%		0	1	785
Thalassaemia	36	5%		0	1	785
Ineligible to donate	138	18%		0	1	785
Friend/family member with sickle cell disease	27	4%		0	1	724
Friend/family member with thalassaemia	39	5%		0	1	710
Friend/family member who is a blood recipient	284	46%		0	1	616

Abbreviations: Freq, frequency; max, maximum; min, minimum; MSM, men-who-have-sex-with-men.

have a higher average ethnicity homophily score and are thus more likely to perceive the prototypical donor's ethnicity as the same as their own ethnicity. Patients had the lowest homophily score of 1.22, which is significantly lower than non-patients and, again, this is primarily related to ethnicity homophily. Patients view themselves as less similar in ethnicity to their perception of the prototypical donor. MSM had a higher homophily score (2.04) than non-MSM, driven by the gender homophily. Thus, MSM see their gender (men) as similar to their perception of the gender of the prototypical donor. Women have a higher homophily score than men, which is also driven by the gender homophily scores, with women perceiving themselves as more similar to the prototypical donor in terms of gender. Homophily also varied by ethnicity, with Asian, Black, mixed and other ethnicities all having lower homophily scores than White participants.

Predicting donation decisions

Seventy-eight people said they would donate once, 293 many times, 72 were unsure and two preferred not to say. We explored, using a multi-nominal regression model, the extent to which the overall homophily score predicts the active decision to make one or more

TABLE 2 Prototypical donors as seen by sub-groups.

	Responders sub-groups									
	All	**MSM**	**Patients**	**Current donors**	**White**	**Non-White**	**Asian**	**Black**	**Mixed**	**Other**
Prototype categories										
Sex										
Female	**404 (51.5)**	127 (47.4)	15 (37.5)	**55 (55.6)**	**343 (52.9)**	55 (45.2)	28 (48.3)	**14 (60.9)**	10 (41.7)	3 (23.1)
Male	381 (48.3)	**141 (52.6)**	**25 (62.5)**	44 (44.4)	306 (47.1)	**67 (54.8)**	**34 (51.7)**	9 (39.1)	**14 (58.3)**	**10 (76.9)**
p value	0.438	0.427	0.154	0.315	0.158	0.319	0.526	0.405	0.541	0.092
Age (years)										
18–29	235 (30.4)	86 (32.3)	8 (22.9)	29 (29.3)	177 (27.5)	**56 (47.5)**	**30 (50.8)**	**13 (56.5)**	9 (37.5)	4 (33.3)
30–44	**419 (54.3)**	**138 (51.9)**	**20 (57.1)**	**52 (52.5)**	**360 (56.0)**	51 (43.2)	25 (42.4)	8 (34.8)	**10 (41.7)**	**8 (66.6)**
45+	118 (15.3)	42 (15.8)	7 (20.0)	18 (18.2)	106 (16.5)	11 (9.2)	4 (6.8)	2 (8.7)	5 (20.8)	0 (0)
p value	**<0.001**	**<0.001**	**0.011**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.019**	0.417	0.248
Ethnicity										
Asian	12 (1.6)	0 (0)	1 (3.0)	1 (1.0)	0 (0.0)	12 (10.3)	12 (20.7)	0 (0.0)	0 (0.0)	0 (0.0)
Black	7 (0.9)	0 (0)	2 (6.1)	0 (0)	0 (0)	7 (6.0)	0 (0.0)	7 (31.8)	0 (0.0)	0 (0.0)
Mixed	49 (6.4)	16 (6.0)	5 (15.2)	3 (3.0)	32 (5.0)	14 (12.1)	5 (8.6)	1 (4.5)	8 (33.3)	0 (0.0)
Other	18 (2.3)	7 (2.6)	3 (9.1)	3 (3.0)	12 (1.9)	5 (4.3)	2 (3.4)	1 (4.5)	0 (0.0)	2 (16.7)
White	**681 (88.8)**	**243 (91.4)**	**22 (66.7)**	**92 (93)**	**596 (93.1)**	**78 (67.2)**	**39 (67.2)**	**13 (59.1)**	**16 (66.6)**	**10 (83.3)**
p value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.102	**0.021**
Education										
No qualifications	11 (1.5)	3 (1.1)	2 (7.1)	1 (1.0)	10 (1.6)	1 (0.9)	0 (0.0)	1 (4.8)	0 (0.0)	0 (0.0)
GCSE or equivalent	120 (16.0)	45 (17.1)	2 (7.1)	17 (17.5)	108 (17.1)	11 (10.1)	5 (9.1)	2 (9.5)	3 (13.0)	1 (10.0)
A level or equivalent	**315 (42.1)**	**114 (43.3)**	8 (28.6)	**43 (44.3)**	**274 (43.8)**	38 (34.9)	22 (40.0)	6 (28.6)	6 (26.1)	4 (40.0)
Degree or equivalent	303 (40.5)	101 (38.4)	**16 (57.1)**	36 (37.1)	240 (38.0)	**59 (54.1)**	**28 (50.9)**	**12 (57.1)**	**14 (60.9)**	**5 (50.0)**
p value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.003**	**0.015**	0.273
Social class										
Working class	214 (27.9)	66 (24.8)	6 (18.2)	23 (23.2)	177 (27.7)	35 (30.2)	15 (25.9)	7 (31.8)	11 (45.8)	2 (16.7)
Middle class	**541 (70.5)**	**198 (74.4)**	**27 (81.8)**	**73 (73.7)**	**465 (71.3)**	**76 (65.5)**	**40 (60.0)**	**14 (63.6)**	**12 (50.0)**	**10 (83.3)**
Upper class	12 (1.6)	2 (0.8)	0 (0.0)	3 (3.0)	7 (1.1)	5 (4.3)	3 (5.2)	1 (4.5)	1 (4.2)	0 (0.0)
p value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.003**	**0.010**	**0.021**
Political ideology										
Left-wing	**868 (84.7)**	**224 (85.2)**	**21 (72.4)**	**86 (88.7)**	**543 (85.6)**	**88 (80.0)**	**43 (76.8)**	**16 (76.2)**	**19 (82.6)**	**10 (100)**
Right-wing	115 (15.3)	39 (14.8)	8 (27.6)	11 (11.3)	91 (14.4)	22 (20.0)	13 (23.2)	5 (23.8)	4 (17.4)	0 (0.0)
p value	**<0.001**	**<0.001**	**0.024**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.027**	**0.003**	**0.002**

Note: A binomial test was used for dichotomous variables and chi-square for multi-category variables within the demographic target category. The figures in blod indicate the largst number in that category.

Abbreviation: MSM, men-who-have-sex-with-men. GCSE, General Certificate of Secondary Education

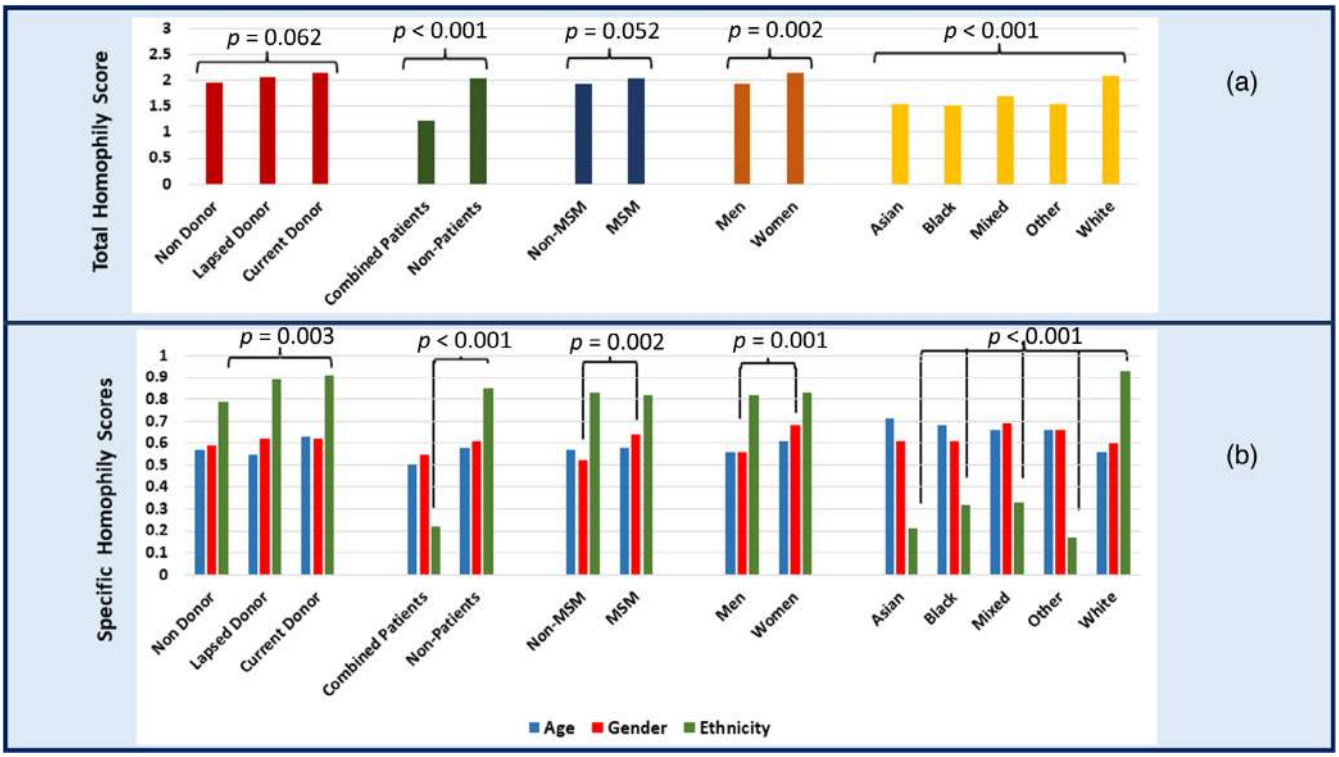

FIGURE 1 Homophily scores general (panel a) and specific (panel b) by sub-groups. p = exact p value.

TABLE 3 Multinominal regression for active donation decisions on overall homophily scores in eligible non-donors ($n = 420$).

	Coefficient (SE)	z	p value	95% CI	
				Lower	Upper
Uncertain					
Donate once					
Homophily score					
1	0.2336 (0.8316)	0.28	0.779	−1.3962	1.8634
2	0.4547 (0.8168)	0.56	0.578	−1.1462	2.0557
3	0.7646 (0.8206)	0.93	0.351	−0.8437	2.3729
Constant	−0.2877 (0.7638)	−0.38	0.706	−1.7846	1.2092
Donate many times					
Homophily score					
1	1.0371 (0.7194)	1.44	0.149	−2.4471	0.0373
2	1.4394 (0.7099)	2.03	0.043	0.0478	2.8301
3	1.4190 (0.7185)	1.97	0.048	0.0107	2.8274
Constant	0.2231 (0.6708)	0.33	0.739	−1.0916	1.5379

Note: Eligible non-donors do not include recipients of blood. This is a multinomial regression model with 'Uncertain about donation' as the reference category and, within the homophily scores, zero is the reference category.
Abbreviations: CI, confidence interval.

donations compared to uncertainty about donating. The results show that a homophily score of two or three predicts an active decision to make more than one donation, compared with feeling uncertain about donating (Table 3: This effect is robust to the inclusion of demographic and prototype information as controls; see Supplementary File S4).

DISCUSSION

The prototypical UK blood donor is seen as 30–44 years old, White, educated to A level (high-school) or degree level, middle class and politically left-wing. We explored the degree of homophily with this prototype across a set of key stakeholders, including donors and non-donors, to better understand the role of homophily with respect to donor retention (donors) and recruitment (non-donors). Recruiting people from ethnic minorities is a major focus of many blood collection agencies; as such, we explored homophily from the perspective of a number of ethnic minorities (Asian, Black and Mixed). Recent policy changes in the UK (the FAIR project) and across the world with respect to individualized risk assessment of sexual behaviour mean that previous deferral policies for MSM no longer apply [6]. Therefore, we explored if MSM perceive the prototypical donor as like them. In general, greater homophily should be associated with a greater willingness to become or remain a donor. Finally, we explored the patient perspective from the vantage point of long-term recipients of blood for those with sickle cell or thalassaemia. These recipients require multiple transfusions, and the efficacy of transfusions increases with well-matched blood from ethnic minority donors. As sickle cell and thalassaemia are more prevalent in Black and Asian communities, lower homophily with the prototypical donor may lead to recipients' concerns about the efficacy of their current and future treatment.

Current donors perceive themselves as being most similar to the prototype donor, followed by MSM, with blood recipients being the least similar. People from ethnic minorities also have low homophily scores. As greater homophily increases the probability of making an active decision to be a repeat donor, the UK prototypical donor accurately reflects, and is likely driven by, the aggregate demographic profile of UK blood donors [26]. Perceptions of prototypical donors are associated with the decision to donate via the homophily score, with smaller perceived differences between a person's prototype and their own personal demography increasing their likelihood of donating.

While the perception that the prototypical UK blood donor is 30–44 years old, White, college-educated, middle class and left-wing reflects the demography of UK blood donors [26], this is not simply a reflection of the UK's wider demography, as there are demographic profiles for different philanthropic acts. For example, volunteers and those who donate money to charity tend to be older (65+ years), with an even distribution across ethnicity [27–29].

Within the UK, White people constitute the largest ethnic group and, as such, many social, institutional and communal spaces become defined as White spaces. Nonetheless, there are spaces defined as Black and Asian, including clubs and cafes [29–32]. However, based on the prototypical donor, blood donation centres, like many UK institutions, are not. With that in mind, the perception of the prototypical donor may deter people from ethnic minorities and younger people. These are two groups blood services want to recruit [2, 5, 8]. One clear implication for blood services is that designing campaigns and strategies to change donor demography (Route A Interventions in Figure 2) [33] addresses only half the picture. Success with Route A interventions will, over time, change the aggregate donor demography and, ultimately, what the prototypical donor is perceived to be. However, interventions must also be considered to address how people perceive blood donation/donors (Route B Interventions in Figure 2). Fortunately, some evidence suggests prototypes can be malleable [34].

We initially consider what innovations are suggested by route A. Many campaigns and strategies have been implemented to recruit and retain more donors from ethnic minorities and younger age groups, and some have been successful [33]. As these have been reviewed and discussed at length, we focus on novel implications arising from knowing the UK prototypical donor.

Donors are seen as older, so blood donation is less likely to be perceived as relevant for young people [35], who are also less likely to have received a blood transfusion [36] or to know people who need a transfusion [37]. This implies the need to make the notion of blood donation salient for younger people. One way is to implement 'cognitive time travel' and have younger people consider their future selves and link to other future concerns important to younger generations, such as climate change [37].

The perception that the prototypical UK blood donor is middle class may be a previously unrecognized barrier to donors from working- and upper-class people. Social class, especially within the UK, is a strong social force with respect to group formation, social identity and behaviour [38]. A drive for wider social class inclusion will likely impact greater ethnicity and educational inclusions, as these characteristics are geographically clustered and related [39]. Blood drives and campaigns generally focused across wider geographical and social areas may be worth considering.

A novel and interesting finding is the perception that the prototypical UK blood donor is left-wing. Left-wing ideology, compared with right-wing ideology, is associated with increased compassion for others [40], which taps into wider associations of compassion, altruism and helping those in need [25]. Unfortunately, we do not know the current political ideology of UK blood donors. Without knowing this, it is difficult to propose effective strategies. However, having politicians from all ideologies jointly endorse blood donation as a compassionate act may encourage wider diversity of donors.

Prior research has identified a wide set of barriers to blood donation including psychological concerns (anxiety, phobia of needles and blood), structural issues (inconvenience, location and time), as well as issues specific to minorities, such as prejudice and differential deferral [9–12]. We show that homophily should be added as a structural and specific barrier.

Below, we explore how this barrier may be addressed, focusing on the types of intervention suggested by route B. As blood donors are both perceived as White and the majority *are* White, the perception of blood donation as a White activity in a White space will act as a barrier to ethnic minorities becoming blood donors [1].

Potential solutions could involve locating blood centres in geographical areas where the density of ethnic minorities is high. This could be enhanced further by increasing the diversity of donor centre staff. Ideally, blood donor centres should be co-designed with

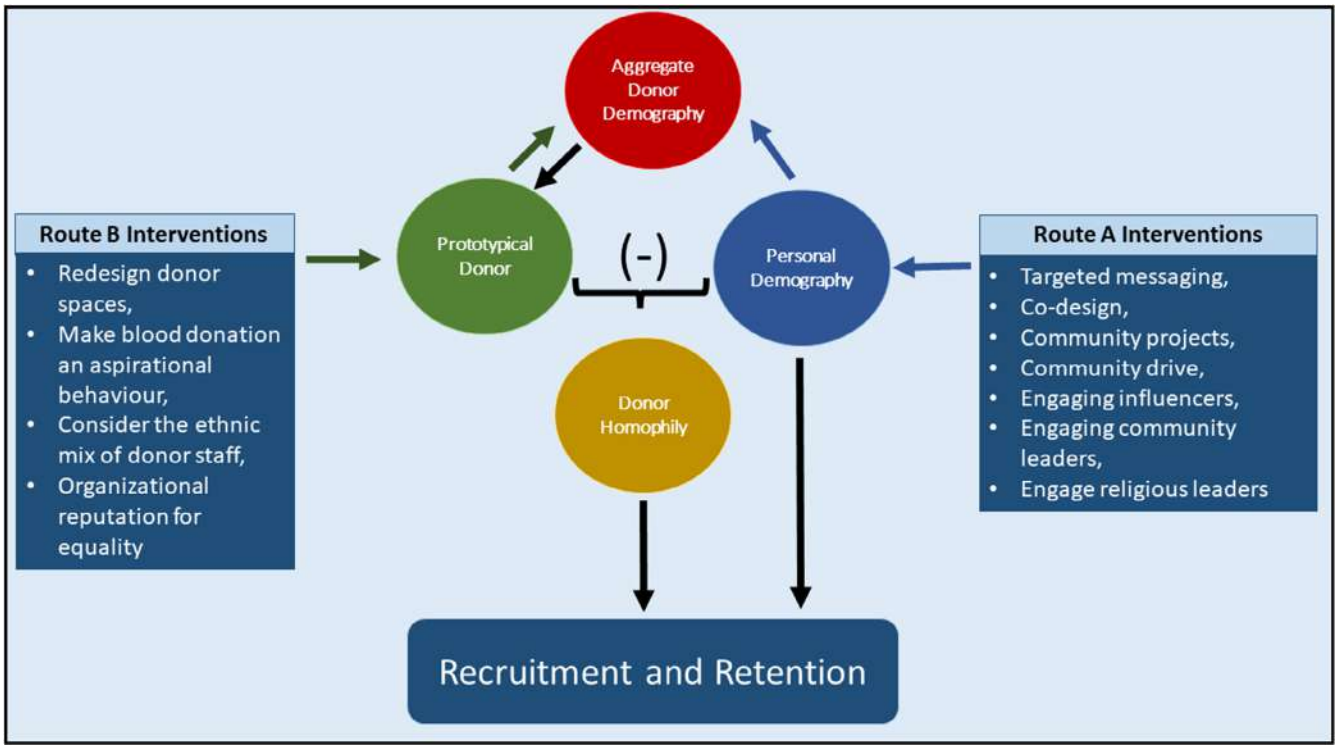

FIGURE 2 Theoretical and practical schema. The schema shows that homophily, defined as the difference between the perceived prototypical donor and the person's own demographic characteristics, drives donation decisions. This role for homophily highlights the dynamic relation between the prototypical donor and actual donor demography within a country. That is, actual demography predicts the prototype, but changing the perceived prototype (Route B) alters homophily and recruitment, altering the actual donor demography within a country and, thus, the prototype. Hence, there is a dynamic reinforcing link between the prototype and actual donor demography. This dynamic link can also be influenced by directly attracting a wider demography to donor panels (Route A).

members of the local ethnic minority communities to make these spaces more culturally relevant, welcoming and familiar. NHSBT's work with the new co-designed Brixton Blood Centre in London is an excellent example.

The donor centre location is also important in terms of how political ideology influences blood donor behaviour. What is important concerning political ideology and blood donor behaviour is not the *absolute* ideology (left-wing, right-wing) but rather partisanship, with individuals less likely to donate blood when their political ideology is very different from the representative political ideology of their area [41]. Specifically, those who perceive themselves as political outliers are less likely to donate blood. Therefore, political ideology is an important consideration for blood services. Again, this is another reason for blood donation centres to consider *where* their donor centres are placed and the importance of co-designing with the local demography and developing community-based partnerships and funding schemes.

Donor services need to change the perception of blood donation as an exclusively middle-aged activity, especially if they wish to recruit younger donors [42]. One possible strategy is to normalize and represent blood donation as a positive, socially normative activity through social media (e.g., Instagram, TikTok, BeReal or Snapchat posts). Blood donation could be presented as an aspirational and community-building activity for young people and made relevant to them.

This is a primarily descriptive study, and we make no claims of causality. We look at the prototype as an antecedent to recruitment but acknowledge that there are many complexities to donor recruitment. However, the implications of these results underscore the importance of the blood donor prototype and homophily, which should now be considered in future work.

The study has some limitations. The sample was not representative by ethnicity and age; however, the consistency of the findings by age, gender and ethnicity supports the contention that this did not affect the results. We also acknowledge that the age categories were not uniform, which may have contributed to the prototypical age effect being middle age; future research would benefit from incorporating a more comprehensive range of evenly distributed age bands. We assessed *active decisions to commit to donate blood* as this is a key predictor of actual donation [24]. As such, we did not assess directly if people were completely *un*willing to donate, and this should be explored in future studies. Finally, causality needs to be explored and the use of instrumental variable models, propensity score matching and Directed Acyclic Graphs (DAG)s can all be considered [43, 44].

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providing detailed feedback and revising the paper; all authors approved the final version.

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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.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Regular whole blood donation and gastrointestinal, breast, colorectal and haematological cancer risk among blood donors in Australia

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Abstract

Background and Objectives: Several studies have suggested that blood donors have lower risk of gastrointestinal and breast cancers, whereas some have indicated an increased risk of haematological cancers. We examined these associations by appropriately adjusting the 'healthy donor effect' (HDE).

Materials and Methods: We examined the risk of gastrointestinal/colorectal, breast and haematological cancers in regular high-frequency whole blood (WB) donors using the Sax Institute's 45 and Up Study data linked with blood donation and other health-related data. We calculated 5-year cancer risks, risk differences and risk ratios. To mitigate HDE, we used 5-year qualification period to select the exposure groups, and applied statistical adjustments using inverse probability weighting, along with other advanced doubly robust g-methods.

Results: We identified 2867 (42.4%) as regular high-frequency and 3888 (57.6%) as low-frequency donors. The inverse probability weighted 5-year risk difference between high and low-frequency donors for gastrointestinal/colorectal cancer was 0.2% (95% CI, −0.1% to 0.5%) with a risk ratio of 1.25 (0.83–1.68). For breast cancer, the risk difference was −0.2% (−0.9% to 0.4%), with a risk ratio of 0.87 (0.48–1.26). Regarding haematological cancers, the risk difference was 0.0% (−0.3% to 0.5%) with a risk ratio of 0.97 (0.55–1.40). Our doubly robust estimators targeted minimum loss-based estimator (TMLE) and sequentially doubly robust (SDR) estimator, yielded similar results, but none of the findings were statistically significant.

Conclusion: After applying methods to mitigate the HDE, we did not find any statistically significant differences in the risk of gastrointestinal/colorectal, breast and haematological cancers between regular high-frequency and low-frequency WB donors.

Keywords
blood donor, cancer, HDE, healthy donor effect, malignancy, whole blood

Highlights
- We used the 'qualification period' method along with advanced statistical methods, such as inverse probability weighting, and doubly robust g-methods with ensemble machine learning algorithms, to mitigate the impact of the 'healthy donor effect'.
- We found that regular high-frequency whole blood donation does not significantly alter the cancer risk.
- Studies with relevant data on ongoing health of donors are required to produce unbiased results when examining the effect of blood donation on long-term health outcomes.

INTRODUCTION

Studies have suggested that the level of iron in the human body may affect the occurrence of cancers [1–7]. Due to loss of iron from the body after each whole blood (WB) donation, it has been hypothesised that frequent WB blood donors may have different risk of cancers compared to less-frequent donors or non-donors [6]. Studies have also indicated that temporary immune system alterations such as lowering of the level and activity of natural killer cells and enhanced cell proliferation after each blood donation, could affect the risk of haematological cancers [8, 9]. In relation to the level of iron in the body, it has been observed that in iron overload diseases like hereditary hemochromatosis there is increased risk of hepatocellular carcinoma, particularly in patients with liver cirrhosis [10], and potentially other type of cancers such as colorectal cancer [5, 11].

Studies conducted in blood donors have reported contraindicatory findings in relation to the risk of cancers. Several studies have reported that the risk of cancers is lower or not different in donors compared to general population or less-frequent donors [6, 12–15]. However, some have also reported a higher incidence of overall cancers or some particular cancers among blood donors compared to the general population [6, 13, 14, 16].

The results from many of the above studies may have been impacted by a bias called the 'healthy donor effect (HDE)'. This bias arises when healthier people self-select to donate blood. Further health screening by blood collection agencies to ensure that donors are eligible to give blood compounds this effect. Comparison of this relatively healthier group without adequate adjustments for health differences from the non-donor population (or with low-frequency donors) usually suggests that blood donors have a lower risk of almost any health outcome measured [17].

In this study, we examined the possible association between regular high-frequency WB donation and the risk of gastrointestinal/colorectal, breast and haematological cancers among blood donors in Australia. To mitigate the HDE, we utilized a 5-year qualification period method, similar to the 'qualification period' method described by Peffer et al. and applied several statistical adjustments in the analyses [18]. The 'qualification period' refers to the time period during which the donor must be actively donating blood and must fulfil other qualifying criteria. This method identifies active donors (enabling the within donor comparison) within a defined time period and also separates the exposure period and follow-up period, which further reduces the reverse causation bias as the exposure and outcome cannot influence each other.

METHODS

Data sources and linkage

In this study, we used the Sax Institute's 45 and Up Study data, linked to other electronic health datasets—the Australian Red Cross Lifeblood Donor data, Registry of Birth, Deaths and Marriages-Deaths Registrations (RBDM), New South Wales Cancer Registry (NSWCR) and Medicare Benefit Schedule (MBS) data.

The Sax Institute's 45 and Up Study enrolled 267,357 individuals aged 45 years or above in New South Wales, Australia, between 2005 and 2009 [19]. The study recruited prospective participants through random selection from the Services Australia Medicare enrolment database, which includes all Australian and New Zealand citizens and Australian permanent residents, resulting in a participation rate of 19.2% [20]. People aged 80 years and above and people living in rural and remote areas were oversampled [19]. Participants completed an initial questionnaire that covered a wide range of topics, including socio-demographic information, health status, lifestyle choice and behaviours. Additionally, they provided consent for their data to be linked with various administrative datasets, allowing for long-term follow-up analysis.

Australian Red Cross Lifeblood is the sole agency responsible for collecting, processing and distributing blood and blood products in

Australia. It also keeps track of donor data in a central system called the National Blood Management System (NBMS). Before 2007, the methods used by Lifeblood to store donor data varied. However, after a national merger in 2007 of what was to that time separate, state-based sets of donor data, all donor information was consolidated within the NBMS. However, for New South Wales (NSW) complete records for blood donations were available from 1 June 2002. Therefore, for the purpose of data linkage, the dataset used included blood donation information spanning from 1 June 2002, to 31 December 2018.

The NSWCR keeps track of individuals diagnosed with cancer in NSW. Since 1972, the NSWCR has maintained comprehensive records that include demographic information, incidence data and death details for individuals who have been diagnosed with cancer. In our study, we used this dataset to ascertain the occurrence and date of cancer diagnosis. The data were complete up to December 2015.

The details of other datasets and the linkage process is presented in the Supporting Information: Data S1 and also described elsewhere [21].

Study population, qualification period

We employed a 5-year qualification period to select the participants and determine exposure status inspired by the method used by Peffer and colleagues [22] (Figure 1). The qualification period refers to the time in which the donor is needed to actively give blood while satisfying other requirements for eligibility to donate. In our analysis, this qualification period includes the time period 3 years before the enrolment into the 45 and Up Study data and 2 years thereafter. For our analysis, donors must have made at least one WB donation on the first and fifth years of the qualification period and be alive and cancer-free for the full 5-year period. The qualification period method can also be described as 'exposure window' method, as described and used by Edgren et al.; however, the qualification period method implemented in this study includes specific qualification criteria that ensure donors are active donors during the time period of exposure assessment as well as are free of the study outcome being measured [6]. We excluded donors who performed any plasma or platelet donation during the 5-year window to keep only WB donors for the analysis. Donors who had cancers before the start of qualification period were also excluded.

Exposure variable

We considered several exposure scenarios to measure the frequency and regularity of blood donations made by participants during each year of qualification period (i) at least one WB donation during each year of qualification period versus others, (ii) at least two WB donation during each year of qualification period versus others and (iii) at least three WB donation during each year of qualification period versus others.

Ascertainment of WB donation

Utilizing linked Lifeblood donation history data, instances of a WB donations were determined. If a person successfully donated a unit of WB, the individual was regarded as a WB donor.

Ascertainment of cancer

The primary outcomes of this study were gastrointestinal, colorectal, breast and haematological cancers. All the cancer information was ascertained from the linked NSWCR dataset. By using the international classification of disease 10th revision (ICD10) codes, an individual was confirmed to have experienced either gastrointestinal or colorectal cancer if the cancer diagnosis codes were C15 (oesophageal) or C16 (stomach) or C17 (small intestinal) or C22 (liver) or C23-C24 (gallbladder) or C25 (pancreatic) or C18 (colon) or C19-C21 (rectal). Moreover, an individual was confirmed to have experienced breast cancer if the diagnosis code was C50 (Breast). Furthermore, an individual was confirmed to have experienced haematological malignancy if the diagnosis codes were C920 (acute myeloid leukaemia) or C910 (acute lymphoblastic leukaemia) or C81 (Hodgkin lymphoma) or C8890 (multiple myeloma) or C82 (non-Hodgkin lymphoma) or C919 (other lymphoid leukaemia) or C929 (other myeloid leukaemia) or C94 (other specified leukaemia) (Table 1). We only considered the first diagnosed cancer for this analysis if a person had multiple malignancies over the follow-up period.

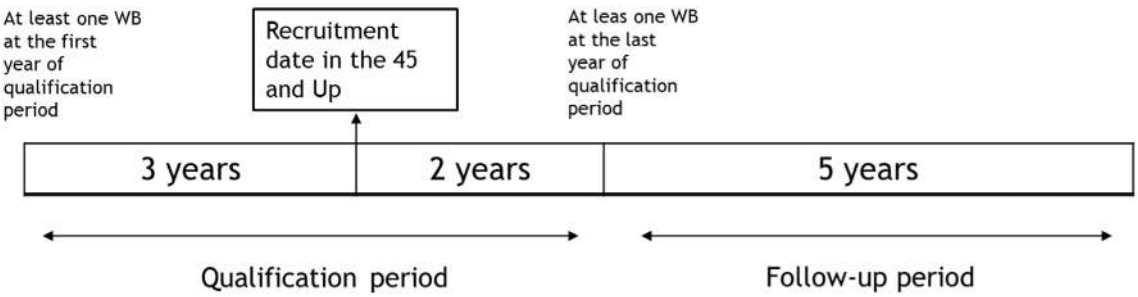

FIGURE 1 The 5-year qualification period and follow-up period.

TABLE 1 International classification of diseases 10th revision (ICD10) codes used to ascertain cancer cases.

Cancer group	ICD10 codes
Gastrointestinal or colorectal cancer	C15 (oesophageal) C16 (stomach) C17 (small intestinal) C22 (liver) C23-C24 (gallbladder) C25 (pancreatic) C18 (colon) C19–C21 (rectal)
Brest cancer	C50 (Breast)
Haematological malignancies	C920 (acute myeloid leukaemia) C910 (acute lymphoblastic leukaemia) C81 (Hodgkin lymphoma) C8890 (multiple myeloma) C82 (non-Hodgkin lymphoma) C919 (other lymphoid leukaemia) C929 (other myeloid leukaemia) C94 (other specified leukaemia)

Follow-up period

The follow-up period commenced from the last day of the qualification period and ended at the conclusion of either 5 years from the start of the follow-up, the death date or the cancer diagnosis date, whichever occurred first. This end date of follow-up was chosen to enable us to study the 5-year risk of cancer. For sensitivity analyses, we also considered an administrative end date of the follow-up, so that the study started from the last day of the qualification period and ended on 30 December 2015 (corresponding to available cancer registry data), death date or cancer diagnosis death, whichever occurred first.

Potential confounding factors

A number of demographic/socioeconomic, health status and blood donation-related variables were considered as potential confounding factors. The demographic/socioeconomic variables were age, sex (male, female), geographical location (metro, regional/remote), education (no formal education, school to diploma, university) and gross annual household income (<20 k, 20–39 k, 40–69 k, 70 k+ Australian dollars). The health status-related variables were body mass index (body mass index [BMI]—underweight, normal, overweight, obese), self-reported general health (excellent, very good, good, fair/poor), smoking status (never, former, regular), daily alcohol intake (≤1/day, >1/day), weekly physical activity (<1, ≥1), daily fruit or raw vegetable consumption (0–2, 3–4, 5+), intake of multivitamins and minerals (no, yes), consumption of red meat (<5/week, ≥5/week), consumption of processed meat (<3/week, ≥3/week), number of general practice (GP) visits in the last 1 year, number of specialist consultations and pathology test referrals in the last 1 year, family history of cancers (no, yes) and any cancer screening (no, yes). Blood donation-related variables were average blood pressure levels during the qualification period, average haemoglobin level during the qualification period and blood group. The detailed derivation of the variables is given in Table S1.

Ethics approval

The 45 and Up Study received approval from the Human Research Ethics Committee (HREC) at the University of NSW. Additionally, this specific study was approved by the NSW Population Health Services Research Ethics Committee and Lifeblood Ethics Committee.

Statistical methods

We calculated 5-year cancer risk, risk difference and risk ratio (RR) by inverse probability weighting (IPW) of a marginal structural model for gastrointestinal and colorectal cancers together and for breast and haematological cancers separately. We fitted a pooled logistic regression model by adding a constant plus linear and quadratic terms of time and also linear and quadratic product terms of donation status and time. The baseline covariates were adjusted by calculating the inverse probability weights and then using the weights in the outcome regression model. The IPW was truncated at the 99th percentile to remove any extreme weights from outliers. Finally, we used non-parametric bootstrapping with 500 samples to calculate all the 95% CIs. Inverse probability weighted Kaplan–Meier survival curves were also plotted for the cancer outcomes with three different exposure definitions.

We also utilized two alternative g-methods, namely the targeted minimum loss-based estimator (TMLE) and the sequentially doubly robust (SDR) estimators, to compute 5-year cancer risk, risk difference and RRs [23, 24]. These estimators, including IPW, rely on two mathematical models: the treatment model and the outcome model, both of which are functions of the confounding variables. The IPW is a singly robust estimator, as its accuracy depends on correctly specifying the treatment model. On the other hand, TMLE and SDR are doubly robust estimators, meaning that their estimates remain unbiased even if one of the treatment or outcome models is misspecified.

Additionally, the inverse probability weighted marginal structural models can produce a biased estimate if affected by violations of the positivity assumption. In contrast, doubly robust estimators often produce less biased results than IPW estimators, even if the positivity assumption is extremely violated [25, 26]. Moreover, these doubly robust estimators have the advantage of being able to utilize machine learning algorithms to fit the treatment and outcome models, allowing them to capture complex associations that may not be possible with simple regression-based approaches [24, 27]. As blood donation behaviour is assumed to be time-varying in nature, we also estimated time-varying TMLE and SDR estimators in one of the sensitivity analyses. We used the R package 'SuperLearner' version 2.0–29 and 'lmtp' version 1.4.0 to implement this analysis [28].

A few variables had missing values (maximum of approximately 16%). Although we assumed that the data were missing at random, we still did multiple imputations to calculate missing values, as

TABLE 2 Characteristics of the study participants.

Characteristics	At least 2 whole blood donations in each year of the qualification period	
	No (low frequency)	Yes (regular high frequency)
Participants, *n* (%)	3888 (57.6)	2867 (42.4)
Sex, *n* (%)		
Male	1717 (44.2)	1585 (55.3)
Female	2171 (55.8)	1282 (44.7)
Age at baseline, mean (SD)	57.72 (6.68)	60.3 (6.9)
Haemoglobin, g/dL, mean (SD)	140.99 (10.36)	143.38 (9.86)
Systolic blood pressure, mean (SD)	127.39 (12.05)	128.66 (11.17)
Diastolic blood pressure, mean (SD)	76.95 (6.84)	77.26 (6.25)
Total no. of WB donation in qualification period, mean(SD)	9.78 (3.56)	16.85 (2.65)
Blood group, *n* (%)		
Non-O	1976 (50.8)	1401 (48.9)
O	1912 (49.2)	1466 (51.1)
Body mass index, kg/m^2, *n* (%)		
Underweight	10 (0.3)	8 (0.3)
Normal	1306 (33.6)	897 (31.3)
Overweight	1527 (39.3)	1231 (42.9)
Obese	793 (20.4)	577 (20.1)
Missing	252 (6.5)	154 (5.4)
Body mass index, kg/m^2, mean (SD)	26.92 (4.35)	27.08 (4.21)
Smoking status, *n* (%)		
Never	2435 (62.6)	1884 (64.3)
Former	1282 (33.0)	921 (32.1)
Regular	157 (4.0)	90 (3.1)
Missing	14 (0.4)	12 (0.4)
Self-rated health, *n* (%)		
Excellent	1040 (26.8)	850 (29.7)
Very good	1791 (46.1)	1361 (47.5)
Good	854 (22.0)	564 (19.7)
Fair/poor	130 (3.3)	57 (2.0)
Missing	73 (1.9)	35 (1.2)
Alcohol consumption/day, *n* (%)		
None	877 (22.6)	600 (20.9)
≤1/day	1521 (39.1)	1100 (38.4)
>1/day	1461 (37.6)	1148 (40.0)
Missing	29 (0.8)	19 (0.7)
Vigorous physical activity in the last week, *n* (%)		
<1	1415 (36.4)	964 (33.6)
1–3	1331 (34.2)	967 (33.7)
4+	660 (17.0)	603 (21.1)
Missing	482 (12.4)	333 (11.6)
Education level, *n* (%)		
No formal education	215 (5.5)	175 (6.1)
School to Diploma	2432 (62.6)	1927 (67.2)
University	1213 (31.2)	747 (26.1)
Missing	28 (0.7)	18 (0.6)

TABLE 2 (Continued)

Characteristics	At least 2 whole blood donations in each year of the qualification period	
	No (low frequency)	Yes (regular high frequency)
Annual household income, *n* (%)		
<20 k	313 (8.1)	257 (9.0)
20–39 k	521 (13.4)	503 (17.5)
40–69 k	954 (25.5)	762 (26.6)
70 k+	1484 (38.2)	901 (31.4)
Missing	616 (15.8)	444 (15.5)
Location, *n* (%)		
Major city	1909 (49.1)	1161 (40.5)
Regional/Remote	1888 (48.6)	1646 (57.4)
Missing	91 (2.3)	60 (2.1)
Daily fruits/vegetable consumed, *n* (%)		
0–2	229 (5.9)	160 (5.6)
3–4	928 (23.9)	688 (24.0)
5+	2259 (58.1)	1685 (58.8)
Missing	472 (12.1)	334 (11.7)
Taking any vitamin or mineral supplement, *n* (%)		
No	2975 (76.5)	2236 (78.0)
Yes	912 (23.5)	631 (22.0)
Missing	<5 (<0.0)	<5 (<0.0)
Consumption of red meat, *n* (%)		
<5/week	2954 (76.0)	2134 (74.4)
≥5/week	865 (22.3)	697 (24.3)
Missing	68 (1.8)	36 (1.3)
Consumption of processed meat, *n* (%)		
<3/week	2869 (73.8)	2097 (73.1)
≥3/week	577 (14.8)	459 (16.0)
Missing	442 (11.4)	311 (10.9)
Family history of cancer, *n* (%)		
No	2058 (52.9)	1517 (52.9)
Yes	1830 (47.1)	1350 (47.1)
Cancer screening, *n* (%)		
No	421 (10.8)	301 (10.5)
Yes	3428 (88.2)	2545 (88.8)
Missing	39 (1.0)	21 (0.7)
No. of GP visits in the past 1 year, mean (SD)	4.68 (4.15)	4.15 (3.41)
No. of referrals in the past 1 year, mean (SD)	2.84 (2.69)	2.51 (2.35)
Outcomes		
Gastrointestinal/colorectal, *n* (%)	25 (0.6)	27 (0.9)
Breast[a], *n* (%)	40 (1.8)	21 (1.6)
Haematological, *n* (%)	23 (0.6)	20 (0.7)

Abbreviation: GP, general practice.
[a]Breast cancer cases are calculated only from female donors.

removing participants with missing values would lower the number of cases for analysis. The imputation was a fully conditional specification that used classification and regression trees and was implemented by the R package 'mice' version 3.16.0 (used method = 'cart' in the mice function) [29].

We used R version 4.2.2 to conduct all the statistical analyses.

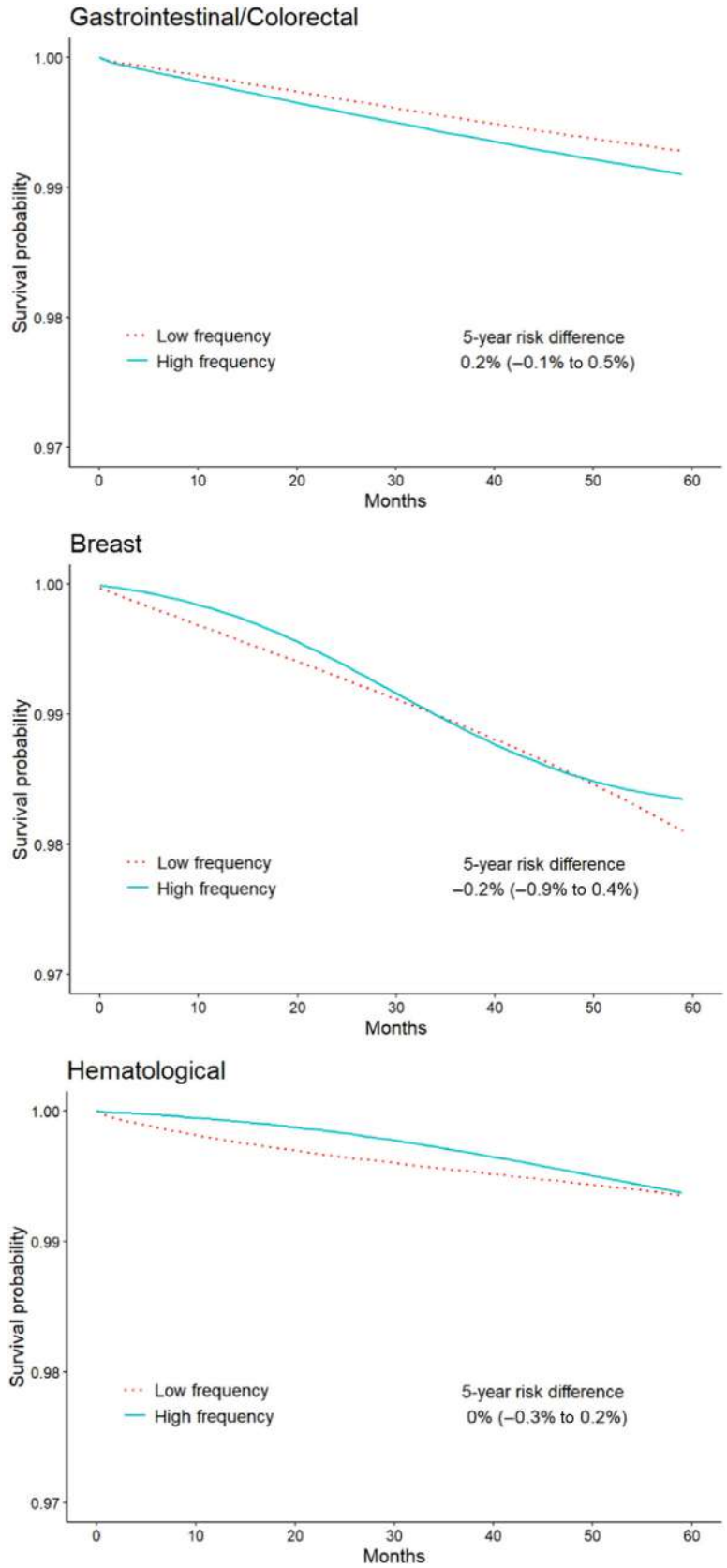

FIGURE 2 Weighted survival curves for a 5-year follow-up period for gastrointestinal/colorectal, breast and haematological cancers.

RESULTS

Table 2 shows the distribution of various characteristics of 6755 WB donors, of whom 2667 (42.4%) donated at least two WB units in each year of the qualification period (regular high-frequency donors), whereas 3888 (57.6%) donated less than two WB donations in any of the qualification year. Regular high-frequency donors were mostly male (55.3%) and also slightly older (average age 60.3 years) than low-frequency donors. Among the donors, 25/3888 (0.6%) from the low-frequency blood donor group were diagnosed with gastrointestinal/colorectal cancer during 5 years of follow-up, whereas 27/2867 (0.9%) were diagnosed with gastrointestinal/colorectal cancer in the high-frequency donor group. Among 3453 female donors, 40 (1.8%) breast cancer cases were identified from the low-frequency donor group and 21 (1.6%) from the high-frequency donor group during the 5-year follow-up period. For haematological cancer, we found 23 (0.6%) incident cases from the low-frequency donor group and 20 (0.7%) from the high-frequency donor group during the 5-year follow-up period. The detailed information about the variables in Table 2 can be found in the Table S1. Figure 2 shows no significant risk differences between low and high-frequency donors in the inverse probability weighted Kaplan Meyer survival curves for gastrointestinal/colorectal, breast and haematological cancers over a 5-year follow-up..

Table 3 presents the estimated 5-year cancer risk for gastrointestinal/colorectal, breast and haematological cancer, their risks, risk differences and RRs calculated by IPW, TMLE and SDR estimators. The IPW risk of gastrointestinal/colorectal cancer was 0.9% (95% confidence interval [CI], 0.6%–1.2%) for high-frequency donors and 0.7% (95% CI, 0.5%–0.9%) for low-frequency donors which resulted in the risk difference of 0.2% (95% CI, −0.1% to 0.5%) and RR of 1.25 (95% CI, 0.83–1.68). We found almost identical results from TMLE; the risk for high-frequency donors was 0.9% (95% CI, 0.7%–1.1%) and the risk for low-frequency donors was 0.7% (95% CI, 0.5–0.9), which resulted in risk difference of 0.2% (95% CI, −0.1% to 0.5%) and RR of 1.25 (95% CI, 0.86–1.81). The SDR estimator produced almost similar results (Table 3) to IPW and TMLE. The IPW risk of breast cancer was 1.6% (1.1%, 2.2%) for high-frequency donors and 1.9% (95% CI, 1.5%–2.3%) for low-frequency donors, which resulted in the risk difference of −0.2% (−0.9% to 0.4%) and the RR of 0.87 (0.48–1.26). Moreover, the IPW risk of haematological cancer was 0.6% (95% CI, 0.4%–0.8%) for high-frequency donors and 0.6% (95% CI, 0.5%–0.8%) for low-frequency donors, which produced a risk difference of 0.0% (95% CI, −0.3% to 0.2%) and RR of 0.97 (95% CI, 0.55–1.40). The TMLE produced almost similar results; risk of 0.6% (95% CI, 0.5%–0.8%) for high-frequency donors, risk of 0.6% (95% CI, 0.5%–0.8%) for low-frequency donors and risk difference of 0.0% (95% CI, −0.3% to 0.2%) and RR of 0.96 (0.66–1.40). The SDR estimator produced similar results to IPW and TMLE, except the RR was slightly higher than both estimators (RR, 1.01 [95% CI, 0.71–1.43]). None of the results for both gastrointestinal/colorectal and haematological cancer were statistically significant, indicating no increased/decreased risk of gastrointestinal/colorectal and haematological cancers among blood donors.

Sensitivity analysis

We found similar results to our main analysis when we ended the follow-up on 31 December 2015 instead of a fixed 5-year follow-up for each participant. The IPW RR for this analysis was 1.27 (95% CI, 0.74–1.80) for gastrointestinal/colorectal cancer, 0.99 (95% CI, 0.59–1.39) for breast cancer and 0.92 (95% CI, 0.53–1.30) for haematological cancer. For different exposure definitions, we also found similar

TABLE 3 Estimated 5-year cancer risk, risk difference and risk ratios for high- and low-frequency donors.

Outcomes	Models	Risk, % (95% CI)		Risk difference, % (95% CI)	Risk ratio (95% CI)
		Low frequency	High frequency		
Gastrointestinal/colorectal[a]	IPW	0.7 (0.5 to 0.9)	0.9 (0.6 to 1.2)	0.2 (−0.1 to 0.5)	1.25 (0.83 to 1.68)
	TMLE	0.7 (0.5 to 0.9)	0.9 (0.7 to 1.1)	0.2 (−0.1 to 0.5)	1.25 (0.86 to 1.81)
	SDR	0.8 (0.6 to 0.9)	1.0 (0.7 to 1.2)	0.2 (−0.1 to 0.5)	1.27 (0.89 to 1.80)
Breast[b]	IPW	1.9 (1.5 to 2.3)	1.6 (1.1 to 2.2)	−0.2 (−0.9 to 0.4)	0.87 (0.48 to 1.26)
	TMLE	1.9 (1.5 to 2.3)	1.7 (1.4 to 2.0)	−0.2 (−0.7 to 0.3)	0.89 (0.67 to 1.19)
	SDR	2.0 (1.6 to 2.4)	1.7 (1.4 to 2.0)	−0.3 (−0.8 to 0.3)	0.86 (0.65 to 1.14)
Haematological[a]	IPW	0.6 (0.5 to 0.8)	0.6 (0.4 to 0.8)	0.0 (−0.3 to 0.2)	0.97 (0.55 to 1.40)
	TMLE	0.6 (0.5 to 0.8)	0.6 (0.5 to 0.8)	0.0 (−0.3 to 0.2)	0.96 (0.66 to 1.40)
	SDR	0.7 (0.5 to 0.9)	0.7 (0.6 to 0.9)	0.0 (−0.2 to 0.3)	1.01 (0.71 to 1.43)

Abbreviations: CI, confidence interval; IPW, inverse probability weighting; SDR, sequentially doubly robust; TMLE, targeted minimum loss-based estimator.
[a]Adjusted for sex, age, haemoglobin, systolic blood pressure, diastolic blood pressure, blood group, body mass index (BMI), smoking status, self-rated health, alcohol consumption, education, annual income, physical activity, daily consumption of fruits and vegetables, vitamin/mineral intake, red meat consumption, processed meat consumption, family history of cancer, cancer screening, location, no. of general practice (GP) visits in the past 1 year, no. of referrals in the past 1 year.
[b]Adjusted for all the variables in a except for sex.

results, except for breast cancer risk, where RR (0.5 [95% CI, 0.03–0.96]) was significantly lower for high-frequency donors when considered at least three donations per every qualification year vs other donation categories. For all other sensitivity analyses, we did not find any statistically significant association. The detail results and description of the sensitivity analyses can be found in Tables S2–S4.

DISCUSSION

In this study, we examined the association between regular high-frequency WB donation and the risk of gastrointestinal/colorectal, breast and haematological malignancies among Australian blood donors. We did not find a statistically significant relationship between regular high-frequency WB donations and risk of developing the various cancer outcomes studied.

We used the 5-year qualification period technique to ascertain the exposure (high-frequency donor) and control (low-frequency donor) groups, which is comparable to the qualification period method used by Peffer et al. [18]. It is likely that the HDE has a substantial impact on the studies that only used the lifetime number of donations to determine exposure status. Thus, Peffer et al., in their study, only included active donors and separated the exposure period from the follow-up period in their analysis, which can significantly reduce the HDE [18]. Similar to Peffer et al. our 5-year qualification period method likely has a comparable effect on lowering the HDE. In addition, we had access to several other health-related variables to adjust for the effect of HDE in our analysis. Although Peffer et al. have used a three-category exposure variable based on the tertiles of donations made during the 10-year qualification period and we have categorized the exposure variable that was based on the frequency and consistency of the donation pattern, these differences are likely to have only a minor impact while comparing the studies.

Several studies have examined the incidence of cancer among blood donors. Many of these studies have reported a lower risk of cancer occurrence and mortality among blood donors [13, 14, 30]. In a Scandinavian study, researchers utilized a nested case–control design to investigate the impact of iron depletion through blood donation on Swedish and Danish donors [6]. The study found a trend towards a reduced risk of liver, lung, colon, stomach and oesophageal cancers in males with a latency period of 3 to 7 years, comparing the lowest to highest estimated iron loss from donations. Nevertheless, the authors acknowledged their inability to account for several important confounding factors, such as smoking, alcohol consumption, nutrition, physical activity, anthropometric measures and occupational exposures, which might have influenced the observed results [6]. Another study from the United States reported there is no difference in the risk of colorectal cancer in regular male blood donors compared to non-donors [12]. Although they did not use any established method to reduce the impact of HDE, our findings of gastrointestinal and colon cancer are consistent with their findings.

Although none of our findings were statistically significant, our point estimates for gastrointestinal/colorectal cancer in the main analysis were slightly higher than the null value (IPW RR, 1.25 [95% CI, 0.83–1.68]). Increased cancer risk in high-frequency donors has been reported in prior studies, but none of them could conclusively report the association as causal [6, 15]. In one of our sensitivity analysis, we defined the high-frequency exposure group with at least one and three donations each year of the qualification period which ruled out the possibility of an increased risk that could not be detected by our sample. In addition to that, time-varying TMLE and SDR estimators also found almost zero risk differences among high and low-frequency donors. Moreover, because of blood donors' continuous screening during their donation career and comparatively higher health consciousness, it is not uncommon to have more cancer detection among frequent blood donors compared to casual donors [15, 16].

Our study has several strengths. First, the use of a qualification period method decreased the HDE by comparing cancer outcomes among active donor populations with a continuous donation career and presumably less variance in health status. Second, our data linkage allowed us to adjust for a variety of potential confounding variables, something that was lacking in the majority of previous studies. In addition, we utilized doubly robust statistical models, such as TMLE and SDR, which incorporated machine learning algorithms to determine the risk estimates. As the findings of our IPW model and our doubly robust models are nearly identical, our treatment and outcome models are less likely to have been misspecified.

Our study also has limitations. The majority of participants were older adults. As a result, the findings of this study may not be generalized to all blood donors. However, the representativeness of the 45 and Up Study (~19% response rate) is unlikely to be of importance as our study examined the relative risks [20, 31]. Due to the fact that our donation records are only available on or after June 2002, we were unable to analyse the duration since the first donation or the cumulative impact of the entire donation history. Moreover, compared to some previous studies, our sample size is somewhat small, and our follow-up period is also shorter (a maximum of 5 years), resulting in a smaller number of events. This may cause lower statistical power to detect clinically important small effect sizes. Because of the smaller number of events, we also did not conduct a sex-stratified analysis, which may be relevant for iron-induced outcomes. However, given the majority of the female participant in the study are older and likely reached menopause, the differences by sex should be minimal.

In conclusion, we did not find any convincing evidence of an altered risk of gastrointestinal/colorectal, breast and haematological malignancy among high-frequency WB donors donating regularly. Further exploration is needed with a longer follow-up time to better understand the relationship between these cancer outcomes and regular high-frequency WB donation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Available from the Sax Institute upon request but subject to approvals.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Questions on travel and sexual behaviours negatively impact ethnic minority donor recruitment: Effect of negative word-of-mouth and avoidance

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Abstract

Background and Objectives: Donor selection questions differentially impacting ethnic minorities can discourage donation directly or via negative word-of-mouth. We explore the differential impact of two blood safety questions relating to (i) sexual contacts linked to areas where human immunodeficiency virus (HIV) rates are high and (ii) travelling to areas where malaria is endemic. Epidemiological data are used to assess infection risk and the need for these questions.

Materials and Methods: We report two studies. Study 1 is a behavioural study on negative word-of-mouth and avoiding donation among ethnic minorities ($n = 981$ people from National Health Service Blood and Transplant (NHSBT) and the general population: 761 were current donors). Study 2 is an epidemiology study (utilizing NHSBT/UK Health Security Agency (UKHSA) surveillance data on HIV-positive donations across the UK blood services between1996 and 2019) to assess whether the sexual risk question contributes to reducing HIV risk and whether travel deferral was more prevalent among ethnic minorities (2015–2019). Studies 1 and 2 provide complementary evidence on the behavioural impact to support policy implications.

Results: A high proportion of people from ethnic minorities were discouraged from donating and expressed negative word-of-mouth. This was mediated by perceived racial discrimination within the UK National Health Service. The number of donors with HIV who the sexual contact question could have deferred was low, with between 8% and 9.3% of people from ethnic minorities deferred on travel compared with 1.7% of White people.

Conclusion: Blood services need to consider ways to minimize negative word-of-mouth, remove questions that are no longer justified on evidence and provide justification for those that remain.

Keywords
blood donation, donor behaviour, ethnicity, HIV, sexual behaviour, travel

Highlights

- Donor selection questions on travel and sexual contact linked to human immunodeficiency virus (HIV)-endemic areas negatively impact ethnic minorities in terms of increased negative word-of-mouth and reduced willingness to donate.
- A total of 34% of Black non-donors decided not to donate because of the sexual contact question, with 17% of Black non-donors telling others not to. The travel question resulted in 17% of Black non-donors deciding not to donate and 11.3% telling others not to. These effects were mediated through increased perceived racial discrimination within the National Health Service.
- Surveillance data show that the number of donors with HIV attributed to sex with a higher risk partner from an endemic area is low. Travel questions disproportionately impact ethnic minorities, with 8%–9.3% of people advised not to attend to donate compared with 1.7% of White donors.

INTRODUCTION

While greater diversity within donor panels is clinically beneficial, disproportionately fewer people from ethnic minorities donate within countries in the global north [1]. One contributory factor we explore is the negative impact of deferral arising from blood donor selection questions differentially impacting ethnic minorities [2]. We explore this negative impact in terms of reduced propensity to donate (avoidance) [2, 3] and negative word-of-mouth ($_N$WoM) [4, 5].

Consequence of deferral: Personal avoidance and $_N$WoM

Deferral (i.e., being temporarily or permanently not allowed to donate blood) reduces the likelihood of a person donating again [3] and may have a wider social impact through $_N$WoM in terms of telling others not to donate [4–6]. Information through $_N$WoM is a major concern because it (i) is more believable than positive WoM ($_P$WoM) (e.g., information that would encourage and support blood donation) [4, 5, 7], (ii) spreads widely and quickly [8] and (iii) is hard to counteract [9, 10]. This is especially true if deferral is perceived as discriminatory and unjust [10]. While the negative impact of $_N$WoM on productivity is well documented in the business community [4, 5], less is known about $_N$WoM in the voluntary sector [11] and blood donation in particular [6, 12–17].

For blood donation, $_P$WoM encourages (i) recruitment ([6, 13, 14] but see [12]), (ii) positive donor attitudes [15] and (iii) subsequent $_P$WoM [16]. While $_N$WoM based on deferral has been reported [17], the impact of $_N$WoM on blood donation has not been explored. This article addresses this gap in the literature. We explored $_N$WoM arising from blood donor selection questions that differentially impact ethnic minority communities in the United Kingdom (UK) in 2019, predicting that $_N$WoM will be higher among ethnic minorities.

In terms of better understanding the mechanisms driving $_N$WoM for ethnic minorities, we propose that perceived racial discrimination within the UK National Health Service (NHS) and social isolation mediate the link between ethnicity and $_N$WoM. People from ethnic minorities report greater perceived racial discrimination within health services in the United Kingdom and worldwide [18-19], and greater social isolation is linked to feelings of marginalization [20, 21]. Thus, enhanced racial discrimination and isolation should foster greater $_N$WoM by confirming these opinions and experiences [20].

Awareness: Safety, family and need

We examine three aspects of awareness that should reduce the negative impact of questions: safety, family and need. Awareness that these questions are asked to ensure blood *safety* should mitigate negative impacts by providing a potential justification for their inclusion [22]. Similarly, knowing *family* members who donate should also reduce negative impacts by helping to normalize donation [23, 24]. Finally, awareness of the need for well-matched blood should also act as a mitigator by reinforcing the importance of the need for a diverse donor pool [19].

Sex and travel questions in the United Kingdom

We explore the effect of $_N$WoM and avoidance in two donor selection questions in the UK, which are more likely to impact people from ethnic minorities, using behavioural (Study 1) and epidemiological (Study 2) data collected from UK donors. The first question asked if, in the last 3 months, the potential donor has had sex with anyone who may ever have had sex in parts of the world where human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) is

common, including most countries in Africa: Termed the higher risk partner from sub-Saharan Africa question (HRP-SSA). If potential donors answered 'Yes' to the HRP-SSA question, they were asked not to donate, although, in England, the deferral could be removed if they had a regular partner willing to give a sample for testing. The second question asked if the potential donor had travelled recently. If they have returned from a tropical area affected by chikungunya, dengue, yellow fever or zika, this resulted in a 1-month deferral, and returning from the malarious area (e.g., parts of Africa) in a 4-month deferral followed by additional testing. While these processes, in place for many years, are intended to enhance blood safety, behaviourally, they differentially affect people from ethnic minorities who are more likely to have travelled to Africa, Asia and South America. Indeed, previous findings show that White donors had the lowest proportions of deferral at 2%, with 15% of donors of Indian ethnicity, 10% of Pakistani ethnicity donors and 8% of Black African donors advised not to attend [25].

Behavioural and epidemiological evidence for policy impacts

Any behavioural impacts, as evidenced by avoidance and $_N$WoM, would suggest that these questions should be removed or changed. However, the behavioural evidence only tells half the story, and to support policy change, it is also necessary to show no direct impact on donor safety. Therefore, triangulation with epidemiological data is essential. Thus, we explore the effects of the HRP-SSA question on the incidence of HIV in donors up to 2019 and discuss how many people from ethnic minorities are deferred on the travel question.

Historical context

The questions examined in this article were in place in 2019 on the donor health check (DHC) at the time. At the time the screening questionnaire in England, Northern Ireland and Scotland were pencil and paper, and in Wales, electronic, but they ask the same questions with slight differences in wording. The work reported in this article contributed to the subsequent removal (HRP-SSA) or led to an update of pre-donation information concerning the importance of the travel questions as part of the For the Assessment of Individualised Risk (FAIR) 2 initiative with NHS Blood and Transplant (NHSBT) (https://www.blood.co.uk/news-and-campaigns/news-and-statements/fair-steering-group; NHSBT is a special health authority that is part of the NHS. It is responsible for blood donation services in England) and, as a consequence, has enhanced inclusivity and equity. We report these data to show how triangulation across behaviour and epidemiology data provides robust evidence for policy change. Also, the behavioural data we report here (Study 1) extends previous reports by exploring the mediating role of perceived racial discrimination and social isolation and the social network of donors.

Aims and hypotheses

We tested the following behavioural hypotheses (Study 1). The HRP-SSA and travel questions will result in greater reported avoidance and $_N$WoM in ethnic minorities (H1). People from ethnic minorities will perceive greater racial discrimination within the NHS and greater social isolation (H2). The link between ethnic minorities with avoidance and $_N$WoM will be mediated by perceived racial discrimination within the NHS (H3a) and social isolation (H3b). Knowing family members who have donated blood (H4a), being aware that the questions are asked to enhance safety (H4b) and being aware of the need for well-matched blood (H4c) will ameliorate any adverse effects of the HRP-SSA and travel questions. Using epidemiological and donor management data (Study 2), we (i) examined the number of UK donors with HIV who later reported a potential HRP-SSA partner and (ii) tested the hypothesis that people from ethnic minorities were more likely to be deferred by the travel question (H5).

STUDY 1: BEHAVIOURAL EFFECTS ON $_N$WOM AND AVOIDANCE

Methods

Design and sampling procedure

Six thousand (3500 from ethnic minorities and 2500 from White backgrounds) current donors who had donated within the last 2 years were *randomly* selected from the NHSBT database (ethnicity data were 99% complete in 2019). Non-donors were recruited through a market research company (Code 3: www.code3research.co.uk: 8600 were randomly selected with 4300 from ethnic minorities and 4300 White people). Initial surveys and reminders were sent out between 14 June 2019 and the 2 August 2019 (see [11] and Supplementary File S1).

Coding ethnicity

Self-described ethnicity was coded using the UK Office of National Statistics (ONS) criteria (Supplementary File S2).

Materials

Supplementary File S3 provides the survey description, and Supplementary File S4 contains details of the measures.

Racial discrimination within the NHS

This was assessed with three items (e.g., 'Racial discrimination in a doctor's surgery is common': Supplementary File S4 for all items) (from [26]), summed to give a single scale with higher scores equating

to greater discrimination ($\alpha = 0.84$, $M = 6.75$, SD = 2.58, mode = 6, range = 3–15).

Social inclusion

We assess this with two items (e.g., 'Overall, how strongly do you feel about the extent to which you are included in broader society in the UK': Supplementary File S4 for all items) [27, 28], totalled with higher scores equating to greater social inclusion ($\alpha = 0.73$, $M = 6.87$, SD = 1.82, mode = 8, range = 2–10).

Both the perceived racial discrimination and social isolation questions were scored on a five-point Likert-type scale (1 = Strongly Disagree, 2 = Disagree, 3 = Neither Disagree nor Agree, 4 = Agree and 5 = Strongly Agree).

Family/community connections with blood donation

We asked, 'Do you know any people from the following groups who have donated blood?' (i) your family, (ii) your friends, (iii) your work colleagues and (iv) your neighbourhoods (Yes = 1, No/Don't Know = 0).

Awareness of need for ethnic minority blood

We asked, 'Were you aware that blood from ethnic minority groups is needed to treat diseases like Sickle Cell and Thalassemia? (Yes = 0, No = 1).'

Evaluation tasks for the 2019 HRP-SSA and travel questions

All participants were presented with the following stem:

> Before donating blood **everyone** must read an information booklet and complete a form which asks questions about lifestyle, health, and travel. In one question, those presenting to donate blood are asked …

Participants were then presented with the following specific wording for the following:

1. the HRP-SSA question,

> … if in the last three months, they have '*had sex with anyone who may ever have had sex in parts of the world where AIDS/HIV is very common (this includes most countries in Africa)?*' If they answer Yes, they are asked not to donate unless their partner is able to give a sample for testing.

2. the Travel question,

> … if they have travelled outside the UK in the last 12 months or since their last donation. Specifically, if donors have returned from an area where there is malaria, including many parts of Africa, Asia, and South America in the last 4 months they are asked not to donate.

After reading the HRP-SSA questions, participants answered questions on awareness and the two primary outcomes of avoidance and $_N$WoM. This was repeated for the Travel question.

Awareness of safety

Participants were asked: 'This question needs to be asked to keep blood safe for patients' (*Safety*) and 'The reason for asking this needs to be explained to the donor' (*Need*).

Primary outcome measures

Participants were also asked: (i) 'This question would put me off wanting to donate blood' (*Avoidance*) and (ii) 'question makes me want to tell others not to donate' ($_N$WoM).

Awareness and outcome measures were assessed on a five-point Likert-type scale (1 = Strongly Disagree, 2 = Disagree, 3 = Neither Disagree nor Agree, 4 = Agree and 5 = Strongly Agree).

Analytical strategy

Perceived racial discrimination and social inclusion were normalized using the formulae in Supplementary File S5. As the outcomes are correlated, seemingly unrelated regression (SUR) models accounted for this overlap in the residual error. Models were specified in SPSS-28 and Stata-18, with all *p*-values two-tailed. Power calculations showed that the sample size provides 80% power (Supplementary File S5).

Results

The final sample consisted of 981 participants, of which 182 were Asian, 141 Black, 158 mixed ethnicity, 24 other, 456 White and 20 missing. In total, 761 were current donors, 633 were female, 339 were male and 9 were missing, and the mean age was 44.65 years (SD = 14.57) (Supplementary File S2 for full details). There were 719 responses from NHSBT (12% response rate), 254 from code 3 (3% response rate) and 8 from the community sample. Donors were less likely to be Black and women (Table S2 for details).

Hypothesis 1. HRP-SSA and travel questions result in greater avoidance and $_N$WoM for ethnic minorities.

To explore H1, we grouped responses for the HRP-SSA and Travel questions into three combined categories: (i) 'strongly disagree/disagree', (ii) 'neither' and (iii) 'strongly agree/agree'. Figure 1 shows the percentage of responses in the 'strongly agree/agree' category by ethnicity for the whole sample, current donors and non-current-donors (Supplementary File S6 for percentages for all categories by ethnicity and donor experience). For the HRP-SSA and Travel questions, Black people were significantly more likely to 'strongly agree/agree' for 'avoidance', reaching 34% of Black non-donors. Regarding $_N$WoM, Black people were significantly more likely to endorse 'strongly agree/agree' for $_N$WoM, with this being 17.4% for Black non-donors. These findings support H1.

Table 1 shows that the predictions from H1 are robust in controlling for demographic factors, safety, need, awareness, discrimination, social inclusion, and family/community connections to blood donation. That is, after reading the HRP-SSA question, Black people were more likely to say they would avoid donation compared to White people. Similarly, after reading the travel question (Table 1), Black people were more likely to say they would avoid donating compared to White people.

The results in Table 1 also show that following the HRP-SSA question, avoidance was also higher if people (i) felt that the inclusion of the HRP-SSA question needed explaining, (ii) were less aware of the need for blood from ethnic minorities, and (iii) knew a family member who has donated blood. Avoidance was lower following the HRP-SSA question if people (i) believed the question was included to ensure safety and (ii) were current donors. Concerning $_N$WOM, people from Asian, Black, and Mixed ethnicities are more likely to say that they will tell others not to donate compared to White people. $_N$WOM was higher if people (i) were older, (ii) felt more socially isolated, and (iii) knew a family member who had donated blood. $_N$WOM was lower for current donors.

Table 2 also shows that following the travel question, avoidance was higher if people (i) felt that the inclusion of the Travel question needed explaining and (ii) perceived greater racial discrimination within the NHS. Avoidance was lower if people (i) believed the question was included to ensure blood safety and (ii) were current donors. For $_N$WOM, Asian and Black people were more likely to say that they would tell others not to donate compared to White people. $_N$WOM was also higher if people (i) were older and (ii) knew a family member had donated blood. $_N$WOM was lower for current blood donors, and if people felt the question was needed to ensure blood safety.

Hypothesis 2. Greater discrimination within the NHS and social isolation will be observed in ethnic minorities.

Supporting H2, Figure 2 shows that perceived discrimination within the NHS is higher in all ethnic minorities compared

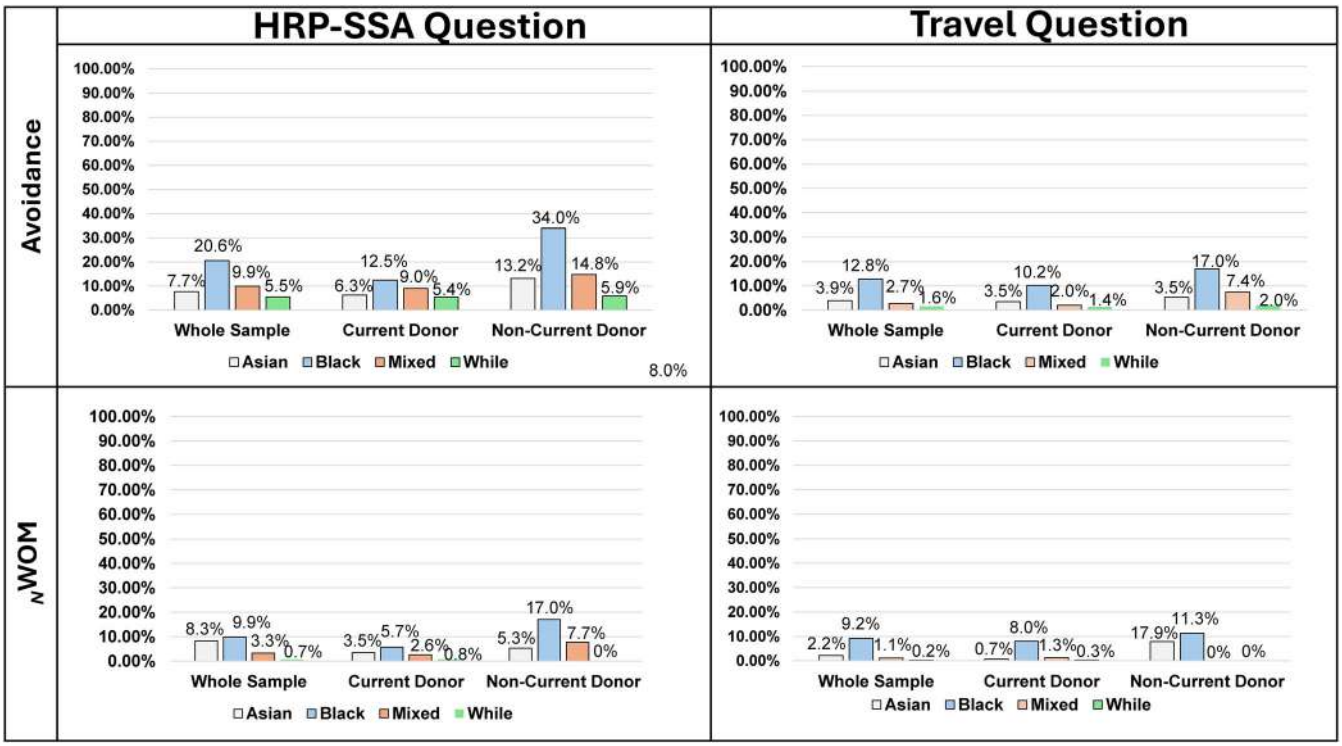

FIGURE 1 Percentages endorsing strongly agree/agree by ethnicity and donor status. HRP-SSA, higher risk partner from sub-Saharan Africa question $_N$WOM, negative word-of-mouth.

TABLE 1 Seemingly unrelated regression models for avoidance and negative word-of-mouth for sexual behaviour.

	Coefficient	Robust SE	*p* value	95% CI Lower	95% CI Upper
Outcome: avoidance					
Gender (male)	0.0727778	0.0644469	0.259	−0.0535358	0.1990913
Age	0.0026539	0.0026017	0.308	−0.0024454	0.0077532
Need to explain why the question is included	**0.0887023**	**0.0242322**	**0.000**	**0.041208**	**0.1361966**
The question is to ensure safe blood	**−0.5562526**	**0.0481966**	**0.000**	**−0.6507163**	**−0.461789**
Ethnicity (comparison is White)					
Mixed	0.1410763	0.0934134	0.131	−0.0420106	0.3241632
Asian	0.1166425	0.0847232	0.169	−0.049412	0.282697
Black	**0.4077688**	**0.1092357**	**0.000**	0.1936707	0.6218669
Not aware that ethnic blood is needed	**0.1597154**	**0.0779128**	**0.040**	0.007009	0.3124217
Current donor (yes)	**−0.3362416**	**0.0813097**	**0.000**	**−0.4956056**	**−0.1768777**
Social inclusion	−0.244049	0.1418597	0.085	−0.5220889	0.0339909
Racial discrimination within NHS	0.1983081	0.1698497	0.243	−0.1345912	0.5312074
Family member has donated blood (yes)	**0.176632**	**0.0627878**	**0.005**	**0.0535701**	**0.2996939**
Friends has donated blood (yes)	0.0773226	0.0739411	0.296	−0.0675993	0.2222444
Work colleague has donate blood (yes)	−0.0763502	0.0683174	0.264	−0.2102499	0.0575494
Neighbour has donated blood (yes)	−0.0634793	0.084325	0.452	−0.2287533	0.1017947
Constant	**3.84191**	**0.3300852**	**0.000**	**3.194955**	**4.488865**
R^2	0.29				
Outcome: negative word-of-mouth					
Gender (male)	0.0666061	0.0520117	0.200	−0.0353349	0.1685471
Age	**0.0055602**	**0.0021063**	**0.008**	**0.0014318**	**0.0096885**
Need to explain why the question is included	0.0203251	0.0179531	0.258	−0.0148624	0.0555127
The question is to ensure safe blood	**−0.3599031**	**0.0429607**	**0.000**	**−0.4441046**	**−0.2757016**
Ethnicity (Comparison is White)					
Mixed	**0.2015463**	**0.0753653**	**0.007**	0.053833	0.3492597
Asian	**0.2118931**	**0.0676564**	**0.002**	0.079289	0.3444972
Black	**0.4621369**	**0.0899145**	**0.000**	0.2859077	0.6383662
Not aware that ethnic blood is needed	0.09908	0.0584038	0.090	−0.0153894	0.2135493
Current donor (yes)	**−0.2778558**	**0.0688061**	**0.000**	**−0.4127133**	**−0.1429982**
Social inclusion	**−0.3395684**	**0.1274423**	**0.008**	**−0.5893507**	**−0.089786**
Racial discrimination	0.0293386	0.1378402	0.831	−0.2408232	0.2995005
Family member has donated blood (yes)	**0.1535602**	**0.0539083**	**0.004**	**0.0479019**	**0.2592184**
Friends Has donated blood (yes)	0.0427983	0.0545869	0.433	−0.06419	0.1497867
Work colleague has donate blood (yes)	−0.0682494	0.0549802	0.214	−0.1760086	0.0395098
Neighbour has donated blood (yes)	0.0230505	0.0759652	0.762	−0.1258384	0.1719395
Constant	2.946199	0.2618483	0.000	2.432986	3.459413
R^2	0.29				

Note: Breusch–Pagan test of independence: $\chi^2(1) = 268.702$, $p = 0.0000$ ($n = 853$).
Note: Figures in bold highlight the statistically significant effects. Coefficients in bold are all significant effects.
Abbreviations: CI, confidence interval; NHS, National Health Service; SE, standard deviation.

with White people and higher in Black people than people of Asian and Mixed ethnicities. Social inclusion is lowered in people of Black and Mixed ethnicities compared to White people, with social inclusion lower in Black compared with Asian people.

Hypothesis 3a,b. Mediation by perceived racial discrimination and social isolation.

Figure 3 shows the parallel mediation models for avoidance and $_N$WoM. Consistent with H3a, for people from ethnic

TABLE 2 Seemingly unrelated regression models for avoidance and negative word-of-mouth for travel abroad.

		Robust		95% CI	
	Coefficient	SE	*p* value	Lower	Upper
Outcome: avoidance					
Gender male	0.0786634	0.0497895	0.114	−0.0189222	0.1762489
Age	0.0014635	0.001945	0.452	−0.0023487	0.0052757
Need to explain	**0.0548282**	**0.0169459**	**0.001**	**0.0216148**	**0.0880415**
The question is to ensure safe blood	**−0.6196312**	**0.0485242**	**0.000**	**−0.7147368**	**−0.5245255**
Ethnicity (comparison is White)					
Mixed	0.0709655	0.0608869	0.244	−0.0483705	0.1903016
Asian	0.047944	0.0680526	0.481	−0.0854367	0.1813247
Black	**0.2804929**	**0.084801**	**0.001**	**0.114286**	**0.4466998**
Not aware that ethnic blood is needed	0.0368851	0.049103	0.453	−0.0593549	0.1331251
Current donor (yes)	**−0.2600327**	**0.0676046**	**0.000**	**−0.3925352**	**−0.1275302**
Social inclusion	−0.1897651	0.1242415	0.127	−0.433274	0.0537437
Racial discrimination within NHS	**0.2751077**	**0.1308768**	**0.036**	**0.0185938**	**0.5316216**
Family member has donated blood (yes)	0.0475548	0.0465199	0.307	−0.0436225	0.1387322
Friends has donated blood (yes)	0.0210827	0.0508747	0.679	−0.0786298	0.1207952
Work colleague has donate blood (yes)	−0.0634191	0.0459313	0.167	−0.1534428	0.0266046
Neighbour has donated blood (yes)	−0.0235403	0.0545463	0.666	−0.1304491	0.0833684
Constant	**4.229541**	**0.291407**	**0.000**	**3.658394**	**4.800689**
R^2	0.37				
Outcome: negative word-of-mouth					
Gender (male)	0.0334401	0.0461587	0.469	−0.0570293	0.1239096
Age	**0.0056782**	**0.0019385**	**0.003**	**0.0018787**	**0.0094777**
Need to explain	0.0091406	0.017519	0.602	−0.0251961	0.0434773
The question is to ensure safe blood	**−0.5002299**	**−0.0543796**	**0.000**	**−0.6068119**	**−0.3936478**
Ethnicity (comparison is White)					
Mixed	0.0704963	0.054624	0.197	−0.0365648	0.1775574
Asian	**0.1490424**	**0.0601033**	**0.013**	**0.0312421**	**0.2668427**
Black	**0.4367506**	**0.0924292**	**0.000**	**0.2555927**	**0.6179085**
Not aware that ethnic blood is needed	0.0125074	0.0438354	0.775	−0.0734085	0.0984233
Current donor (yes)	**−0.1644094**	**0.0649275**	**0.011**	**−0.2916649**	**−0.0371538**
Social inclusion	−0.1327164	0.1149905	0.248	−0.3580936	0.0926608
Racial discrimination within NHS	0.2428332	0.1325711	0.067	−0.0170014	0.5026677
Family member has donated blood (yes)	**0.0892877**	**0.0451744**	**0.048**	**0.0007475**	**0.1778278**
Friends has donated blood (yes)	−0.0844568	0.0470661	0.073	−0.1767045	0.007791
Work colleague has donate blood (yes)	−0.0249093	0.0441789	0.573	−0.1114984	0.0616798
Neighbour has donated blood (yes)	0.047215	0.0566966	0.405	−0.0639082	0.1583383
Constant	3.495889	0.2959177	0.000	2.915901	4.075877
R^2	0.31				

Note: Breusch–Pagan test of independence: $\chi^2(1) = 277.549$, $p = 0.0000$ ($n = 850$).
Note: Figures in bold highlight the statistically significant effects. Coefficients in bold are all significant effects.
Abbreviations: CI, confidence interval; NHS, National Health Service; SE, standard deviation.

minorities, there was a significant indirect effect on both avoidance and $_N$WoM via perceptions of higher racial discrimination. H3b was not supported as there was no indirect effect via social inclusion (Supplementary File S7 for more details).

Hypothesis 4a–c. Ameliorates effects of awareness.

Figure 4 shows that family, friends and colleagues are more likely to be blood donors than neighbours. For White people, compared with

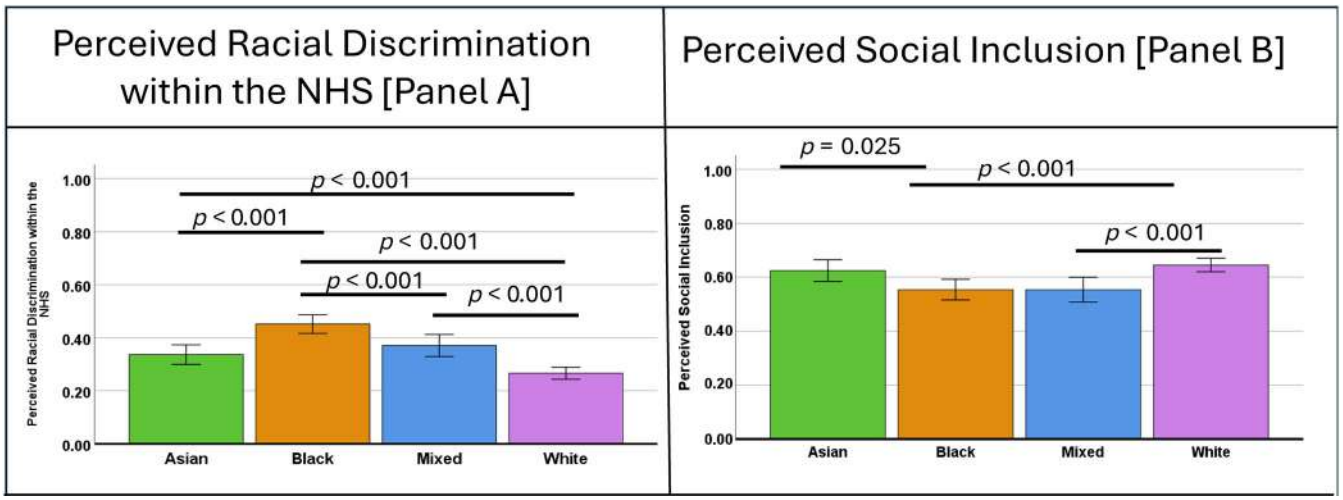

FIGURE 2 Levels of perceived racial discrimination within the NHS (Panel A) and social inclusion (Panel B). Panel (A) reports levels of perceived racial discrimination in the NHS on a standardized 0–1 scale where 1 is 100% complete discrimination and 0 is little to none. Panel (B) reports on perceived social inclusion on a standardized 0–1 scale where 1 is 100% inclusion 0 is little to none (exclusion). For Panel (A), the non-overlapping 95% confidence interval (CI) indicates that all ethnic groups are significantly different from each other in terms of perceived racial discrimination in the NHS (except Asian and Mixed group). For Panel (B), the pattern of the 95% CI indicates that people from Black and Mixed ethnicities are not significantly different from each other, nor are Asian and White people, but both Asian and White people are significantly different from Black and also White people are significantly different from people with Mixed ethnicity. Differences in Panel (A) and (B) are reported using Bonferroni post hoc tests.

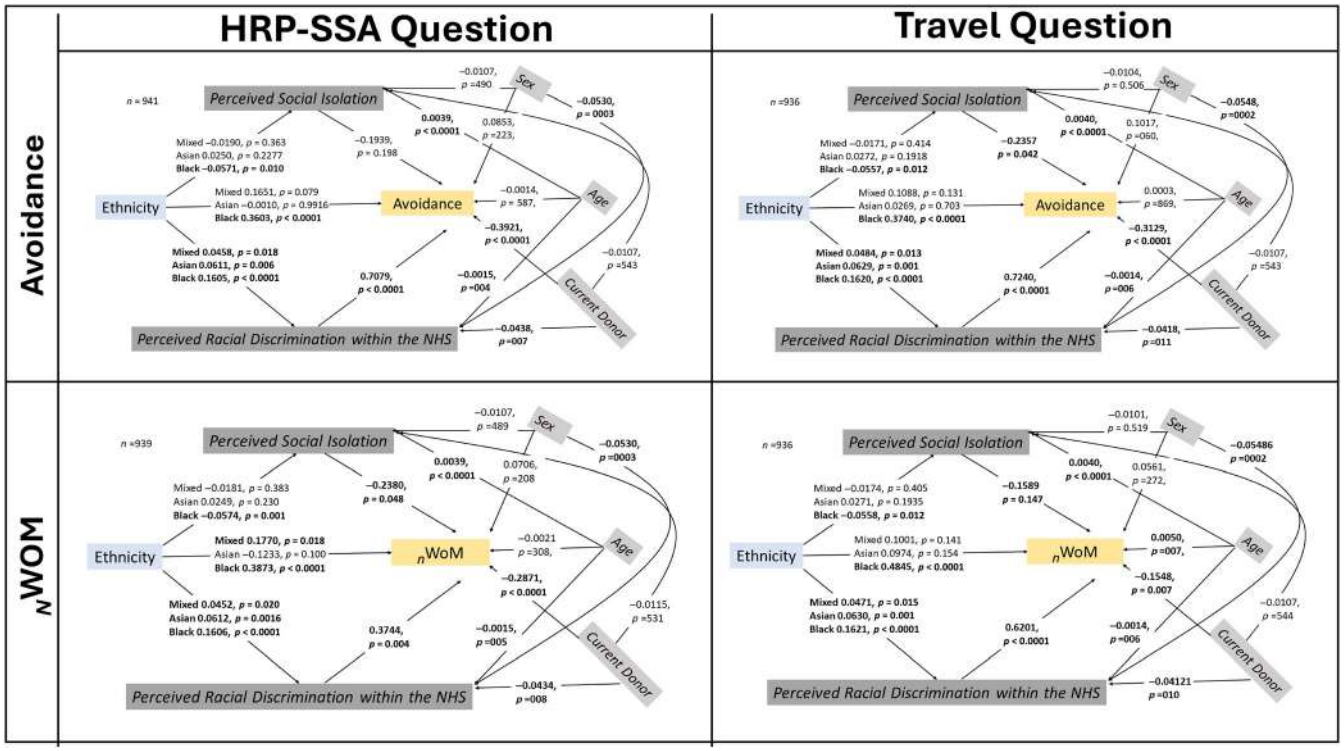

FIGURE 3 Parallel mediation models for ethnicity on avoidance and negative feedback via perceived ethnicity: White = comparison; sex (0 = female, 1 = male), current donor (0 = non-donors, 1 = current donor). HRP-SSA, higher risk partner from sub-Saharan Africa question; $_N$WoM, negative word-of-mouth.

all ethnic communities, family members are more likely to be blood donors. White people are also more likely to have friends as donors than Black people, work colleagues who donate compared with people of mixed ethnicity and neighbours who donate compared to Asian people.

Table 1 shows that avoidance was also *higher* if people (i) were less aware of the need for blood from ethnic minorities (supporting H4c) and (ii) knew a family member who has donated blood (not supporting H4a). Avoidance was *lower* if people believed the question was included to

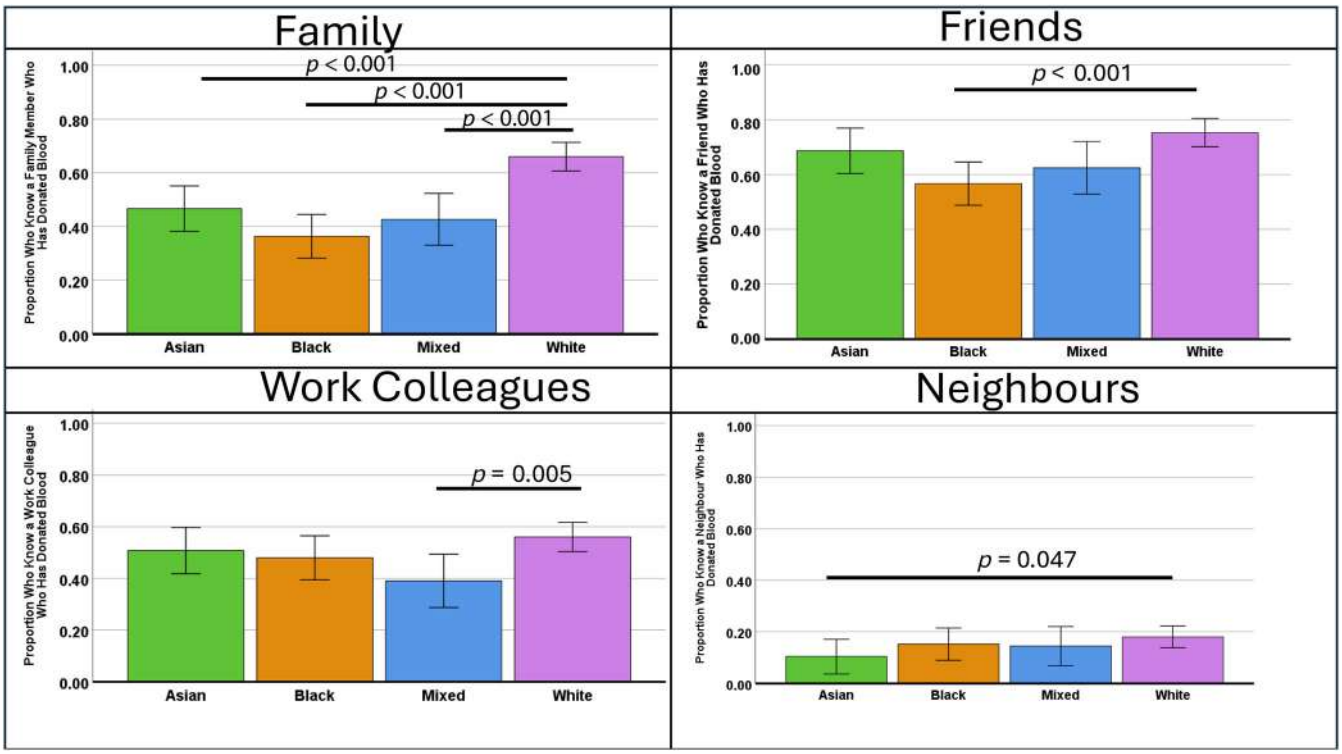

FIGURE 4 Percentage of family, friends, work colleagues and neighbours who are blood donors by ethnicity.

ensure safety (supporting H4b). $_N$WoM was *higher* if people (i) knew a family member who had donated blood (not supporting H4a) and lower if they believed the question was included to ensure safety of blood (supporting H4b). For the Travel question (Table 2) both avoidance and $_N$WOM were lower when people believed the question was included to ensure blood safety (supporting H4b), and $_N$WOM was higher if people knew a family member who had donated blood (not suppressing H4a).

There were no moderating effects of knowing a family member who donates (see Supplementary files S8–S10 for details). However, there is a significant interaction between ethnicity and donor status on $_N$WoM, such that Black and Mixed current donors are less likely to tell others not to donate than Black and Mixed non-donors (see Supplementary File S8; Figure S1).

STUDY 2: EPIDEMIOLOGY OF HRP-SSA ON HIV RATES AND TRAVEL QUESTIONS ON DEFERRAL

Methods

All UK donations are tested for markers of HIV and other blood-borne viruses. Based on this routine surveillance for the four UK blood services, data for each donor with confirmed HIV identified through donation screening in the United Kingdom from 1996 to 2019 were extracted from the joint NHSBT/UK Health Security Agency (UKHSA) Epidemiology Unit database [29]. Data fields included the confirmatory testing results, index donation date, most recent previous donation, gender, age, ethnicity, country of birth, probable exposure route and compliance. Data on partners giving samples to NHSBT and deferral data from donation sessions were provided on a one-off basis by each blood service where available from their donor management system. Annual data on malaria deferrals advised by the National Call Centre between 2015 and 2019 were provided by ethnic background and compared with annual data on donors making whole blood donations, calculating the deferrals as a percentage of donations made by each ethnic background (as in [29]).

Results

The detailed results are in Table 3. The proportion of UK donors with HIV attributed to HRP-SSA has decreased over time, with HRP-SSA being assigned as the possible exposure in 24% of donors with HIV between 1996 and 2019 and 10% (5/49) of donors with HIV between 2015 and 2019. Looking at recent HIV acquired within 12 months, 14% (19/132) reported HRP-SSA for 1996–2019 and 8% (1/12) for 2015–2019. Of these 19 donors with recent HIV, 6 reported a regular partner who may have had sex in Africa as their only risk. Of the 132 donors with recent HIV, 6 were detected in the window period i.e. HIV antibody negative, RNA positive, indicating that HIV was acquired extremely recently. Three of these

TABLE 3 HIV in blood donors, all and recent infection, UK 2015–2019.

	HIV all	HIV recent	% which are recent	% of recent infections
Total	49	12	24.5	
NAT pick up	-	1		
Seroconversion	-	10		
Gender				
Male	31	11	35.5	91.7
Female	18	1	5.6	8.3
Donor type				
New	24	2	8.3	16.7
Repeat	25	10	40.0	83.3
Age				0.0
Age-range	18–71	28–60		
Median age	37	42.5		
Ethnicity				
Asian	5	1	20.0	8.3
Black	2	0	0.0	0.0
Not known	1	0	0.0	0.0
Other	2	0	0.0	0.0
White	39	11	28.2	91.7
Born				
United Kingdom	31	4	12.9	33.3
Europe	6	2	33.3	16.7
Asia	2	1	50.0	8.3
Africa	1	0	0.0	0.0
Other	2	0	0.0	0.0
Not known	7	5	71.4	41.7
Acquired infection				
United Kingdom	29	8	27.6	66.7
Europe	5	2	40.0	16.7
Asia	3	1	33.3	8.3
Africa	1	0	0.0	0.0
Other	0	0	-	
Not known	9	1	11.1	8.3
Risk group				
GBM	13	6	46.2	50.0
Heterosexual sex	23	3	13.6	25.0
HRP-SSA	5	1	20.0	8.3
HRP-other	3	1	20.0	8.3
Other	1	0		0.0
Not known	4	1	25.0	8.3

Abbreviations: HIV, human immunodeficiency virus; HRP-SSA, higher risk partner from sub-Saharan Africa; GBM, gay and bisexual men; NAT, nucleic acid test.

reported HRP-SSA, including one with another possible exposure, the most recent in 2008. (Supplementary File S11 for additional data). The number of partners of potential donors who gave samples, allowing their partner to donate, was small, 60 in England in 2020, but none were found to be living with HIV. Other countries within the UK deferred without an option for partner testing. There were around 50 and 16 deferrals on session annually in Scotland and Wales, respectively.

> **Hypothesis 5.** People from ethnic minorities were more likely to be deferred after travel.

From 2015 to 2019, the average percentage of Asian-Indian people who were advised not to donate out of those making a donation was 8.2% (range = 6.3%–14.9%), Asian-Pakistani 9.3% (range = 6.8%–12.3%), Black-African 8.0% (range = 5.2%–9.9%) and White 1.7% (range = 1.5%–2.0%). These are similar to the figures recorded in 2015 when the malaria deferral was six months [25].

DISCUSSION

The results are clear: those from ethnic minorities are more likely to be put off donating and discourage others after reading the HRP-SSA and travel questions used in the United Kingdom in 2019. These effects were mediated through perceived racial discrimination within the NHS. Thus, there are clear negative behavioural effects associated with these 2019 questions. The epidemiology data showed that the HRP-SSA question was linked to a small proportion of HIV+ donations and was part of a downward trend. Thus, the combined behavioural and epidemiology data indicated that the removal of the HRP-SSA question was justified and safe, and indeed based, in part, on these data, this question was removed from Scotland, Wales, Northern Ireland and England in 2021 as part of the FAIR project (https://www.blood.co.uk/news-and-campaigns/news-and-statements/fair-steering-group/).

The spread of avoidance and $_N$WoM

The impact of avoidance and $_N$WoM in the community can spread quickly. Avoidance can result in the lone-wolf effect, whereby observing others choosing not to act sends a social signal that not donating is preferred [30]. $_N$WoM also spreads quickly through communities [8]. Thus, the summative effect of the lone-wolf effect and $_N$WoM, reinforced by perceived racial discrimination within the NHS, creates a complex social milieu for recruitment.

We initially hypothesized that knowing a family member who donates would mitigate these negative effects. However, we observe the opposite: knowing family members directly enhances the negative impact. There are several possibilities for this. First, people may feel these questions are unjustified and, as such, are upset on behalf of their families. Second, as they already know others who donate, they may feel less need to donate or encourage others. Third, they may know a family member who has been deferred.

Practical and clinical implications

In terms of the Travel question, malaria antibody testing was introduced consistently in England from 2001 as a way of reducing the deferral burden on Black and Asian donors. This deferral and testing strategy has been reviewed and reduced to the shortest deferral time that is thought safely possible under the current antibody testing strategy at 4 months post-travel [31]. In terms of people calling to check eligibility on the grounds of travel before donation, the NHSBT National Call Centre data showed that between 2015 and 2019, Asian and Black donors were more likely to be advised not to donate due to travel than White donors, with figures consistent with a previous observation made under 6-month deferral [25].

Providing a rationale for the pre-donation questions can reduce their negative impact. Thus, blood services must provide clear information about why these questions are needed and that they only remain if the evidence supports them. Each UK blood service explains on its website that the questions are to keep recipients and donors safe. Further work is underway to simplify the travel questions and study ways to prompt donors to disclose relevant history. Services may encourage $_{P}$WoM as a potential to counter-act $_{N}$WoM; however, people acting altruistically are reluctant to use $_{P}$WoM publicly [16], and it may not be effective anyway (see [12]). An optimal strategy, however, may be to intervene earlier downstream to create a positive experience for all donors, not just in terms of the social ambience of the centres and staff but more structurally in terms of how and when deferral questions are asked, what is asked and the ethnicity of staff.

Finally, perceived racial discrimination within the NHS was an important mechanism supporting $_{N}$WoM in ethnic minorities. It is beyond the capacity of blood services to address this wider sociopolitical issue. However, this discrimination should be recognized and publicly acknowledged in terms of openness and transparency.

Caveats

As these findings are UK-specific, generalizability should not be assumed. Blood services with similar questions should evaluate them for similar negative impacts, considering the appropriate local HIV epidemiology and the behavioural impact. We did not assess donor knowledge and attitudes, and further work should explore how these influence $_{P}$WoM as a function of ethnicity and other demographics. Finally, it should also be noted that the number of non-donors is small.

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E.F., S.R.B., K.D. and C.R. designed Study 1; E.F., S.R.B., K.D., C.R., D.E. and Z.K. designed and conducted Study 2; K.D. and C.R. analysed data for study 1. E.F. and E.D.L. analysed the data for study 1. E.F. drafted the first version with E.D.L., D.E., K.D., C.R., N.O.H., R.M., R.S., Z.K., R.D., S.R.B., N.E. and M.C., providing detailed feedback and revisions to the paper. All authors reviewed the manuscript and approved the final version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data reported in this paper are available from the first author on request.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Extending the post-thaw shelf-life of cryoprecipitate when stored at refrigerated temperatures

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Abstract

Background and Objectives: The post-thaw shelf-life of cryoprecipitate is 6 h, leading to high wastage. Storage of thawed cryoprecipitate at refrigerated temperatures may be feasible to extend the shelf-life. This study aimed to evaluate the quality of thawed cryoprecipitate stored at 1–6°C for up to 14 days.

Materials and Methods: Cryoprecipitate (mini- and full-size packs derived from both apheresis and whole blood [WB] collections) was thawed, immediately sampled and then stored at 1–6°C for up to 14 days. Mini-packs were sampled at 6, 24, 48 and 72 h, day 7 and 14; full-size cryoprecipitate was sampled on day 3, 5 or 7. Coagulation factors (F) II, V, VIII, IX, X and XIII, von Willebrand factor (VWF) and fibrinogen were measured using a coagulation analyser. Thrombin generation was measured by calibrated automated thrombogram.

Results: FVIII decreased during post-thaw storage; this was significant after 24 h for WB ($p = 0.0002$) and apheresis ($p < 0.0001$). All apheresis and eight of 20 WB cryoprecipitate met the FVIII specification (≥ 70 IU/unit) on day 14 post-thaw. Fibrinogen remained stable for 48 h, and components met the specification on day 14 post-thaw. There were no significant differences in VWF (WB $p = 0.1292$; apheresis $p = 0.1507$) throughout storage. There were small but significant decreases in thrombin generation lag time, endogenous thrombin potential and time to peak for both WB and apheresis cryoprecipitate.

Conclusion: Whilst coagulation factors in cryoprecipitate decreased after post-thaw storage, the thawed cryoprecipitate met the Council of Europe specifications when stored at refrigerated temperatures for 7 days.

Keywords

coagulation factors, cryoprecipitate, fibrinogen, re-precipitation, von Willebrand factor

Highlights

- Cryoprecipitate stored at 1–6°C for 7 days meets quality specifications for coagulation factor (F) VIII (≥70 IU/unit), von Willebrand factor (>100 IU/unit) and fibrinogen (≥140 mg/unit).
- Fibrinogen concentrations remained stable for the first 48 h during post-thaw storage, with a gradual decrease over 14 days of storage (41% for apheresis- and 26% for WB-derived cryoprecipitate).
- The data from this study support extending the post-thaw shelf-life of cryoprecipitate from 6 to 72 h when stored at 1–6°C.

INTRODUCTION

Cryoprecipitate is a blood component produced from WB- and apheresis-derived fresh-frozen plasma (FFP). It contains high concentrations of fibrinogen, von Willebrand factor (VWF), factor (F) XIII, fibronectin and FVIII [1]. Due to natural variation between donors, there is a broad range of coagulation factor concentrations in cryoprecipitate [2]. Cryoprecipitate is primarily used for fibrinogen replacement in patients with acquired fibrinogen deficiency, such as in severe trauma [3]. In particular, there is high demand for group AB cryoprecipitate as this component is used early in massive transfusion protocols [4]. More recently, cryoprecipitate transfusion has been replaced with fibrinogen concentrate. However, this is not available in all parts of Australia, and some hospitals prefer to use cryoprecipitate due to lower cost or because of a lack of data to support fibrinogen concentrate superiority [5]. Therefore, demand for cryoprecipitate is still high.

Cryoprecipitate is produced by slowly thawing FFP at 1–6°C overnight, then collecting and refreezing the insoluble precipitate as cryoprecipitate. It can be stored frozen (<−25°C) for up to 3 years [6]. However, in Australia, the frozen shelf-life is 12 months. Cryoprecipitate can be derived from either whole blood (WB) or apheresis FFP. A recommended adult dose of cryoprecipitate is 10 × WB- or 4 × apheresis-derived cryoprecipitate units [4], and, therefore, pools of cryoprecipitate are more commonly used. However, pooled cryoprecipitate is not available in Australia. Upon thawing, cryoprecipitate is stored at ambient temperature (20–24°C) for up to 6 h [7], or 4 h in the case of pooled cryoprecipitate product [8]. The short shelf-life of thawed cryoprecipitate leads to high wastage of this component [9]. Australian Red Cross Lifeblood reported that approximately 8% of all units issued between December 2022 and November 2023 were discarded [10]. Cryoprecipitate wastage in other countries varies with reported rates ranging from 3% to 33% [8, 9, 11], depending on the type of hospital (e.g., trauma centre, teaching facility, private) and location (e.g., regional versus city). An audit into cryoprecipitate wastage at five hospitals in South Australia found avoidable causes of cryoprecipitate wastage accounted for 92.2% of total wastage, with the main reasons being that units were thawed but not used (69.1%) or post-thaw time had expired (13.2%) [12].

The limited post-thaw shelf-life of cryoprecipitate is due to concerns regarding coagulation factor degradation, particularly FVIII levels declining rapidly upon thawing [13, 14], and the risk of bacterial proliferation. There have been a number of studies investigating the effect of extension of cryoprecipitate storage on factor stability, including one from our laboratory that found the quality of thawed cryoprecipitate was maintained out to 5 days when stored at ambient temperature [15]. Others have also demonstrated that thawed pooled cryoprecipitate remains stable up to 72 h when maintained at ambient temperature [16]. Although it has also been reported that room temperature (RT) storage results in less factor degradation than cold storage [13, 14, 17, 18], the risk of bacterial proliferation with RT storage is of concern.

Cold (1–6°C) storage of thawed cryoprecipitate is a promising alternative to RT storage, as it would minimize the rate of any potential bacterial proliferation [19–21]. Studies have shown that despite significant decreases in FVIII and fibrinogen concentrations, these were still within an acceptable range for up to 5 days [13, 20]. Others have investigated cold storage of cryoprecipitate for up to 35 days, after which adequate factor levels and sterility were maintained [21]. However, cryoprecipitate was stored in tubes rather than blood storage bags in this study, which does not replicate typical storage conditions and therefore the findings are not directly applicable to hospital settings.

Association for the Advancement of Blood & Biotherapies (AABB) standards, Council of Europe (CoE) and Australian and New Zealand Society of Blood Transfusion (ANZSBT) guidelines currently allow the use of thawed FFP that has been stored for up to 5 days at 1–6°C, which carries the same risk profile for bacterial contamination as cold storage of thawed cryoprecipitate [6, 22, 23].

Extending the post-thaw shelf-life of thawed cryoprecipitate would reduce in-hospital product wastage, thus reducing the number of cryoprecipitate components required to meet demand. To change current recommendations and practice, it must be demonstrated that the components meet quality standards and that coagulation factor integrity can be maintained. Therefore, the purpose of this study was to evaluate the post-thaw quality of cryoprecipitate stored at 1–6°C for 14 days by assessing levels of fibrinogen, coagulation factors (FII, FV, FVIII, FIX, FX and FXIII), VWF, complement components and potential for thrombin generation, thus demonstrating efficacy.

MATERIALS AND METHODS

This study was approved by the Australian Red Cross Lifeblood Human Research Ethics Committee (2015#19-LNR).

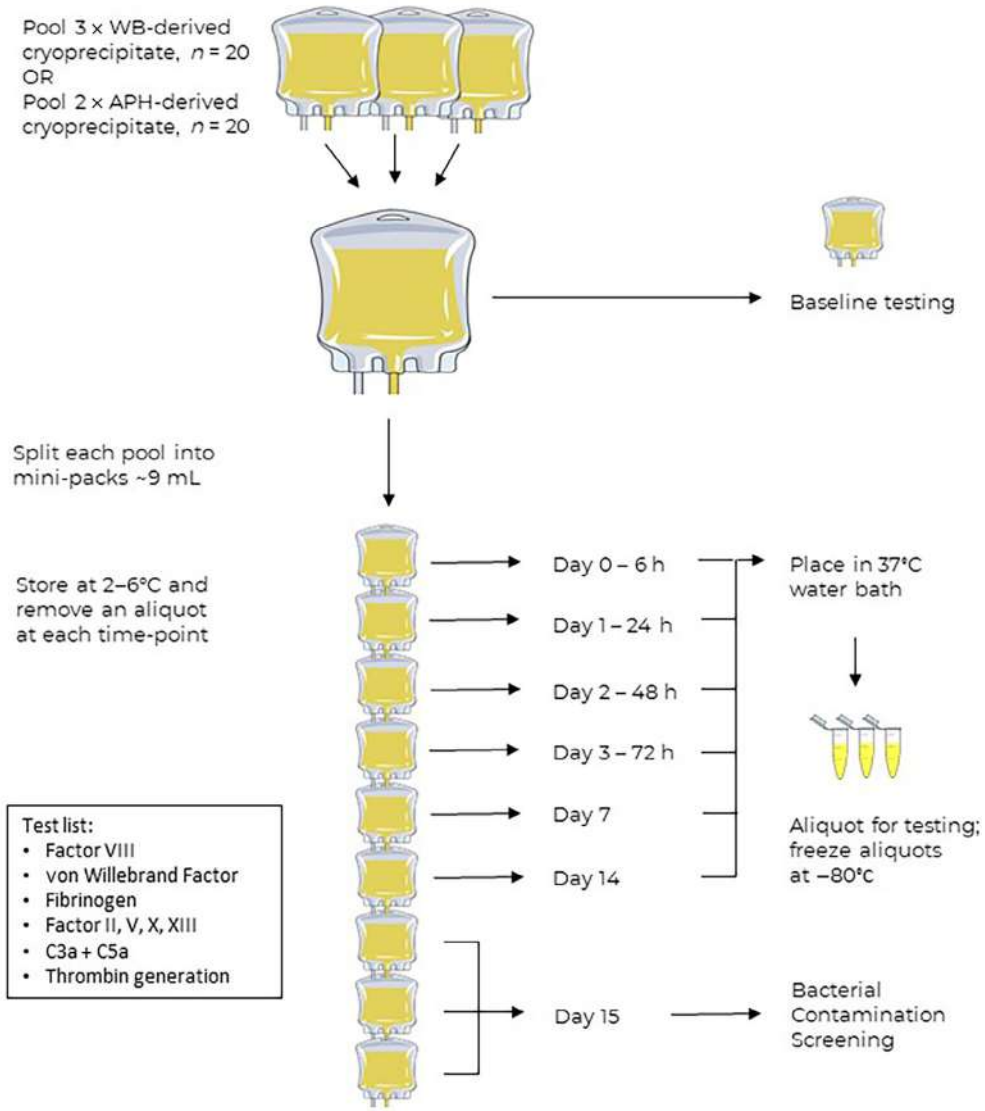

FIGURE 1 Diagrammatic representation of the pool, split and sampling design of the study. APH, apheresis; WB, whole blood.

Mini-packs

Sixty WB- and 40 apheresis-derived cryoprecipitate components were obtained from the frozen inventory in our processing centre, with an equal mixture of group O and non-group O components. Cryoprecipitate was thawed in a 37°C water bath, then visually inspected for turbidity, clots, fibrin strands, red cell contamination or any other discolouration; if present, cryoprecipitate was excluded from the study. Thawed cryoprecipitate was pooled and split as per Figure 1 to create mini-packs (150 mL paediatric transfer bag, Terumo, Tokyo, Japan), each containing approximately 9 mL of cryoprecipitate. Immediately after pooling and splitting, a mini-pack of cryoprecipitate was sampled (baseline), aliquoted (~1 mL) in Eppendorf tubes and frozen at −80°C until subsequent testing was performed. The remaining bags were refrigerated at 1–6°C for up to 14 days. At each time point, 6, 24, 48, 72 h, day 7 and day 14 post-thaw, a mini-pack was removed from 1 to 6°C storage and warmed in a 37°C water bath for 5 min to solubilize any precipitate, then further aliquoted (~1 mL) in Eppendorf tubes and frozen at −80°C until subsequent testing was performed.

Full-size packs

Twelve WB- and 12 apheresis-derived cryoprecipitate components were obtained from inventory (all blood group A). The components were randomly allocated to one of three groups: day 3, 5 or 7 ($n = 4$ per group). Cryoprecipitate was thawed in a 37°C water bath, each component was sampled immediately upon thawing (baseline), then stored at 1–6°C. On the allocated day, the cryoprecipitate was visually inspected for precipitate and placed in a 37°C water bath for 5 min to re-dissolve the precipitate. The cryoprecipitate was

sampled, and the aliquots (~1 mL) in Eppendorf tubes were frozen at −80°C for subsequent coagulation testing. Full-size packs were tested for fibrinogen, FV, FVIII and VWF activity only.

Filtering

Four units each of WB- and apheresis-derived full-size cryoprecipitate components were further filtered on day 3, 5 and 7 using an Infusomat Space Line filter (Braun; Melsungen, Germany), to mimic intravenous administration, and remove any re-precipitated material that could not be re-dissolved. In a clinical setting, this filtration would be performed prior to transfusion, and it was therefore necessary to determine whether the cryoprecipitate components retained minimum required coagulation properties for transfusion after thawing, storage and filtration. Unfiltered and filtered cryoprecipitate samples were tested for fibrinogen, FV, FVIII and VWF activity only.

Testing

Coagulation factors (FII, FV, FVIII, FIX, FX and FXIII), fibrinogen and VWF antigen levels were measured using an automated coagulation analyser (STA Compact; Diagnostica Stago Ltd, Asnieres, France). These factors were tested using one stage clotting assays or two-point immune-turbidimetric assay for FXIII and VWF, with STA and Kamiya Biomedical reagents according to the manufacturer's instructions and standardized using reference plasma.

Complement components, C3a and C5a, were measured using a solid-phase sandwich enzyme-linked immunosorbent assay (BD Biosciences; San Jose, CA, USA) according to the manufacturer's instructions.

Thrombin generation was measured using a calibrated automated thrombogram (Thrombinoscope BV, Maastricht, The Netherlands) and the PPP reagent (Thrombinoscope). The lag time, endogenous thrombin potential (ETP), peak height and time to peak (TTP) were calculated by the thrombogram software.

Bacterial contamination screening

Additional mini-packs were created for terminal bacterial burden sampling after day 14 of sampling. These were pooled and tested using an automated bacterial contamination screening system (Bac T/Alert Virtuo; BioMérieux, Durham, NC, USA) with both aerobic and anaerobic bottles, as per manufacturer's instructions.

Statistical analysis

Data are presented as the mean ± standard deviation (SD). A one-way repeated measure analysis of variance was used to compare data from within the two groups (apheresis or WB-derived cryoprecipitate) using Prism 9.4.1 (GraphPad Software, Inc., La Jolla, CA, USA), with post hoc Bonferroni tests used to determine differences within the groups at each time point. A paired *t*-test was used to determine differences in full-size cryoprecipitate components between baseline and selected time points using Excel (Microsoft 365, Redmond, WA, USA). A paired *t*-test was also used to determine differences between filtered and unfiltered cryoprecipitate. A p-value <0.05 was considered significant using Excel.

RESULTS

Mini-packs

The mean volumes immediately after thawing were 37 ± 2 mL and 60 ± 2 mL for WB- and apheresis-derived cryoprecipitate, respectively. Following pooling and splitting into the mini-packs, the mean volume was 8.4 ± 1.9 and 9.7 ± 1.7 mL for WB- and apheresis-derived cryoprecipitate, respectively. The mean volume of the original parent packs, rather than the volume of the mini-packs, was used to calculate the concentration per unit to determine if the components met specifications.

FVIII concentrations decreased in both WB- and apheresis-derived cryoprecipitate during post-thaw storage at 1–6°C and were significantly lower than baseline after only 24 h, as shown in Figure 2a,b. Fibrinogen concentrations remained stable for the first 48 h during post-thaw storage, after which there was a gradual decrease over 14 days of storage (Figure 2c,d). Figure 2e,f demonstrates there was little difference in VWF concentrations of thawed cryoprecipitate during 14-day storage at 1–6°C. Importantly, all apheresis-derived cryoprecipitate components met the CoE specifications at all time points [6], and the WB-derived cryoprecipitate met these specifications up to day 7 post-thaw (≥70 IU/unit for FVIII, ≥140 mg/unit for fibrinogen and >100 IU/unit for VWF) [6].

There were small but significant decreases in FII concentrations and significant decreases in FV concentrations in both WB- and apheresis-derived post-thaw cryoprecipitate (Figure 3a–d). There was little difference in FX and FXIII concentrations in WB-derived cryoprecipitate during storage, whilst there were significant increases in FX and decreases in FXIII concentrations in apheresis-derived cryoprecipitate (Figure 3e–h).

Complement components C3a and C5a both significantly increased in post-thaw cryoprecipitate during storage at 1–6°C (Figure 4a–d). C3a concentrations were up to ninefold higher in WB- and fivefold higher in apheresis-derived cryoprecipitate by day 14 of storage.

There were no significant differences in thrombin peak height over storage in WB- and apheresis-derived cryoprecipitate (Figure 5a,b). However, there was a small but significant decrease in lag time, ETP and TTP, with a more pronounced decline in lag time and TTP in apheresis-derived cryoprecipitate (Figure 5c–h).

Bacterial growth was not detected in any of the pooled aliquots.

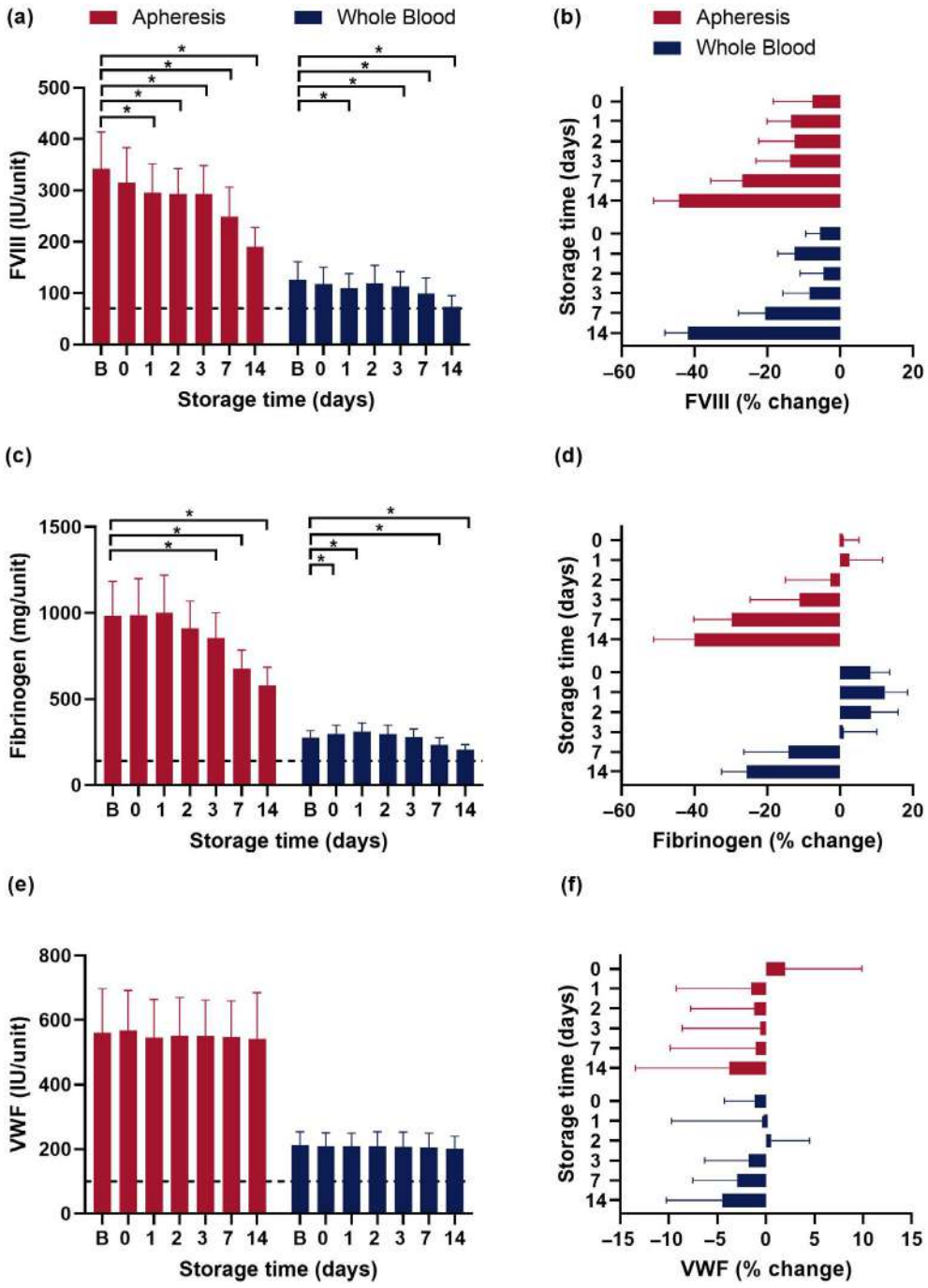

FIGURE 2 Factor (FVIII), fibrinogen and von Willebrand factor (VWF) concentrations in apheresis- and whole blood-derived cryoprecipitate following thawing and subsequent storage at 1–6°C. (a) FVIII, (c) fibrinogen and (e) VWF concentrations in cryoprecipitate were measured by coagulation analyser and calculated per unit based on mean cryoprecipitate volumes. (b) FVIII, (d) fibrinogen and (f) VWF percentage change to baseline. Black dotted lines represent minimum specifications (FVIII ≥70 IU/unit, fibrinogen ≥140 mg/unit, VWF > 100 IU/unit). Bar graphs represent the mean ± standard deviation (SD) ($n = 20$, except for D2 $n = 16$). D0 denotes 6 h post-thaw. B, baseline. *Time point significantly differs from baseline using a one-way ANOVA.

Full-size packs

There was a large variation in coagulation factor results for full-size WB- and apheresis-derived cryoprecipitate, due to the small sample size in each group ($n = 4$; Table 1). There were small, and in some cases significant, decreases in concentrations of fibrinogen, FV and FVIII in the post-thaw cryoprecipitate after 3, 5 or 7 days. VWF remained stable during the post-thaw storage.

Filtration of precipitated cryoprecipitate did not significantly alter the fibrinogen, FV, FVIII and VWF concentrations in WB- and

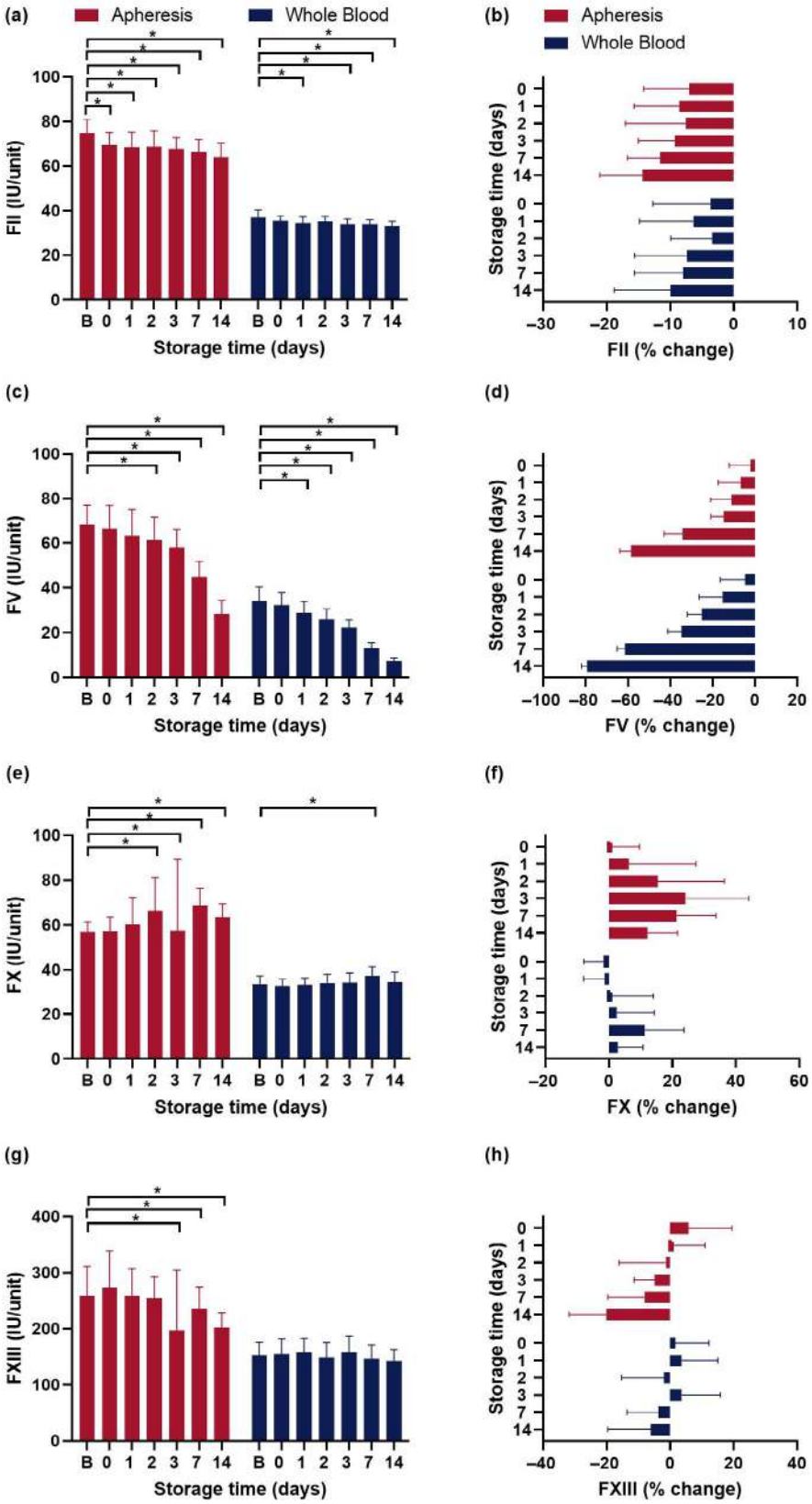

FIGURE 3 Factor (F) II, FV, FX and FXII concentrations in apheresis- and whole blood-derived cryoprecipitate following thawing and subsequent storage at 1–6°C. (a) FII, (c) FV, (e) FX and (g) FXIII concentrations in cryoprecipitate were measured by coagulation analyser and calculated per unit based on mean cryoprecipitate volumes. (b) FII, (d) FV, (f) FX and (h) FXIII percentage change to baseline. Data points represent the mean ± standard deviation (SD) ($n = 20$, except for D2 $n = 16$). D0 denotes 6 h post-thaw. B, baseline. *Time point significantly differs from baseline using a one-way ANOVA.

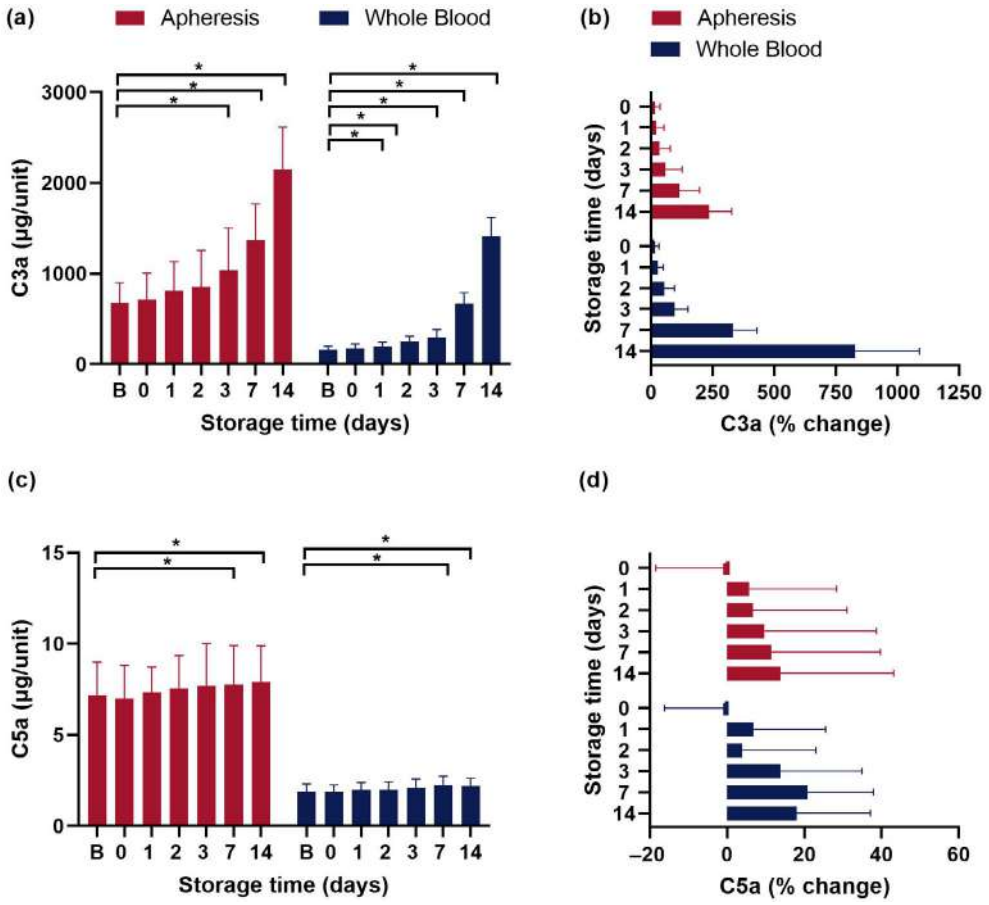

FIGURE 4 Complement components C3a and C5a concentrations in apheresis- and whole blood-derived cryoprecipitate following thawing and subsequent storage at 1–6°C. (a) C3a and (c) C5a concentrations in cryoprecipitate were measured by enzyme-linked immunosorbent assay and calculated per unit based on mean cryoprecipitate volumes. (b) C3a and (d) C5a percentage change to baseline. Data points represent the mean ± standard deviation (SD) ($n = 20$, except for D2 $n = 16$). D0 denotes 6 h post-thaw. B, baseline. *Apheresis time point significantly differs from baseline using a one-way ANOVA.

apheresis-derived cryoprecipitate stored at 1–6°C for 3, 5 or 7 days post-thaw (Table 2). The exception was observed in WB-derived cryoprecipitate on day 7, where filtration reduced the concentration of VWF from 215 ± 33 to 199 ± 25 IU/unit ($p = 0.0464$).

DISCUSSION

This study has determined the quality of thawed cryoprecipitate stored at 1–6°C for up to 14 days. The coagulation properties of the cryoprecipitate gradually deteriorated over extended storage, whilst the thrombin generation potential was maintained. VWF concentrations were also consistent over the 14 days of storage at 1–6°C. Fibrinogen concentrations were stable for the first 2 days then decreased significantly during extended post-thaw storage.

FVIII concentrations decreased by 15% and 21%, respectively, for apheresis- and WB-derived cryoprecipitate between 6 h (current expiry) and day 7 in our study. These data are consistent with a previous study, where a 14%–22% decline in FVIIIC concentrations between 6 h and day 5 for RT and cold-stored cryoprecipitate was observed [20]. Other studies have reported much greater decreases in FVIII concentrations; by 17% after only 24 h [22]; however, this study stored the cryoprecipitate at RT for 6 h before transferring to 1–6°C for up to 24 h, suggesting the additional RT storage has escalated FVIII loss, with a 50% decrease by day 3 post-thaw [21] for both RT and cold-stored cryoprecipitate. This is particularly evident where the cryoprecipitate was pools of six and the sample size was small, resulting in large error bars.

Cryoprecipitate is most commonly transfused for the fibrinogen content [5]. Fibrinogen concentration was maintained during extended storage, demonstrating it is still suitable for transfusion when fibrinogen is needed. Our results concur with other studies, which showed that fibrinogen concentrations remain unchanged from 0 to 24 h post-thaw when stored at refrigerated temperatures [20–22].

In our study, there was a 5% decrease in FXIII concentration in apheresis-derived cryoprecipitate by day 3, with an 8% decrease by day 7. FXIII was maintained in WB-derived cryoprecipitate until day 3 and had decreased by 4% at day 7. Thomson et al. reported a 4%

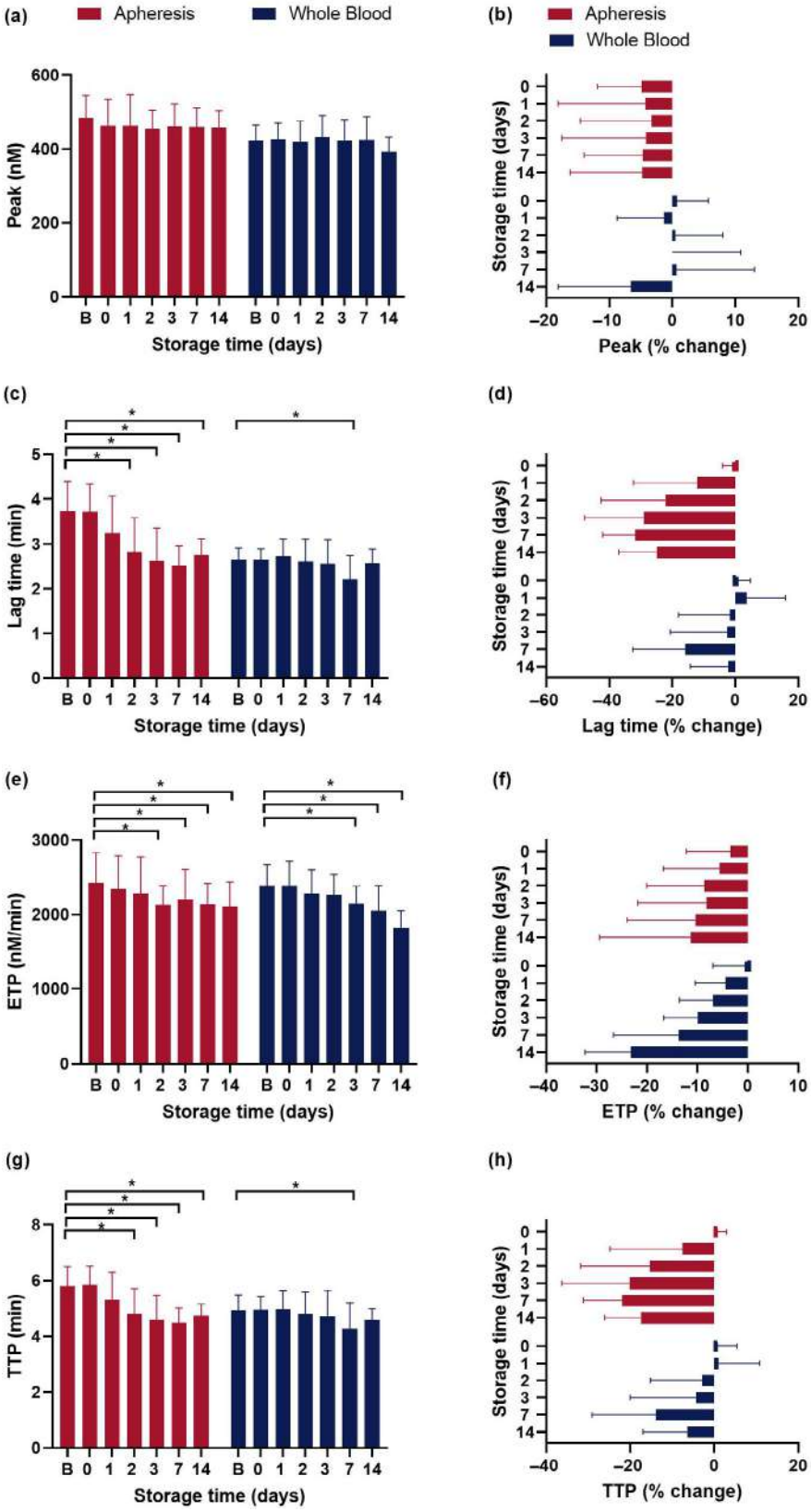

FIGURE 5 Thrombin generation potential of apheresis- and whole blood-derived cryoprecipitate following thawing and subsequent storage at 1–6°C. (a) Peak height (c) lag time, (e) endogenous thrombin potential (ETP) and (g) time to peak (TTP) were measure by calibrated automated thrombogram. (b) Peak height, (d) lag time, (f) ETP and (h) TTP percentage change to baseline. Data points represent the mean ± standard deviation (SD) ($n = 20$, except for D2 $n = 16$). D0 denotes 6 h post-thaw. B, baseline. *Apheresis time point significantly differs from baseline using a one-way analysis of variance.

TABLE 1 Coagulation factors in full-size apheresis- and whole blood-derived cryoprecipitate following storage at 1–6°C.

	Baseline	Day 3	*p*-Value	Baseline	Day 5	*p*-Value	Baseline	Day 7	*p*-Value
Apheresis									
Fibrinogen (mg/unit) (% change)	1006 ± 372	812 ± 140 (−15 ± 16)	0.2119	817 ± 116	544 ± 51 (−32 ± 16)	**0.0391**	931 ± 140	543 ± 78 (−41 ± 6)	**0.0045**
Factor V (IU/unit) (% change)	59 ± 11	51 ± 6 (−12 ± 7)	0.0630	68 ± 10	56 ± 10 (−18 ± 7)	**0.0227**	72 ± 15	49 ± 16 (−31 ± 15)	**0.0310**
Factor VIII (IU/unit) (% change)	322 ± 57	269 ± 73 (−18 ± 11)	0.0550	262 ± 35	208 ± 27 (−20 ± 3)	**0.0027**	322 ± 61	258 ± 42 (−19 ± 8)	**0.0332**
VWF (IU/unit) (% change)	626 ± 155	606 ± 181 (−4 ± 7)	0.3305	498 ± 135	478 ± 130 (−4 ± 7)	0.3748	659 ± 134	636 ± 117 (−3 ± 4)	0.2043
Whole blood									
Fibrinogen (mg/unit) (% change)	310 ± 118	336 ± 107 (10 ± 8)	0.1315	281 ± 66	253 ± 31 (−8 ± 12)	0.2707	262 ± 28	202 ± 29 (−23 ± 9)	**0.0208**
Factor V (IU/unit) (% change)	35 ± 3	27 ± 2 (−22 ± 2)	**0.0015**	26 ± 7	16 ± 4 (−39 ± 5)	**0.0095**	28 ± 7	17 ± 4 (−40 ± 8)	**0.0095**
Factor VIII (IU/unit) (% change)	184 ± 22	151 ± 18 (−18 ± 3)	**0.0032**	146 ± 21	128 ± 30 (−13 ± 11)	0.0650	109 ± 35	86 ± 26 (−20 ± 9)	**0.0478**
VWF (IU/unit) (% change)	364 ± 16	374 ± 33 (3 ± 6)	0.4465	245 ± 33	247 ± 38 (1 ± 3)	0.7228	243 ± 76	237 ± 85 (−4 ± 8)	0.5933

Note: Data are mean ± SD, $n = 4$. % change is the difference at time point compared to baseline. *p*-value determined using a paired *t*-test between baseline and selected time point. A *p*-value <0.05 is considered significant and highlighted in bold.
Abbreviation: VWF, von Willebrand factor.

decrease in FXIII concentration during 5 days of extended storage; however, this was not significant [20]. The most significant decrease in coagulation factors was observed in FV, reaching significance by 24 h in WB-derived cryoprecipitate and by 48 h post-thaw in the apheresis-derived cryoprecipitate. This observed reduction is not unexpected, as FV is a known labile factor and has been reported to decline rapidly in plasma during the time to freezing and in liquid stored plasma [24, 25].

C3a concentrations increased during storage at 1–6°C; by 24 h, it was significantly higher than baseline for WB-derived cryoprecipitate and similarly by 72 h for apheresis-derived cryoprecipitate. C3a is known to be activated in liquid stored plasma, up to threefold by day 7 [26] and 10-fold by day 35 day in stored plasma and WB [27, 28]. C3a is also rapidly cleared from the circulation after transfusion [26]. Trauma has been shown to activate the complement system, whereby the degree of activation is dependent upon the severity of injury [29–31]. Transfusion of cryoprecipitate with a high concentration of C3a can either have an immunosuppressive effect or exaggerate the activation of the complement system [26]. Elevated C3a levels can lead to multiple organ failure and thus C3a could be used as a predictor of prognosis [32]. Transfusion of autologous stored plasma (14 days) that had a threefold increase in C3a concentration during storage did not lead to any adverse transfusion reactions in patients [26], thus suggesting the patient trauma severity rather than the blood transfusion was a greater contributor.

Thrombin generation is also an important property of cryoprecipitate and its function. Our data indicated a slight decrease in thrombin generation potential during post-thaw storage of cryoprecipitate at 1–6°C. This was not different to that reported by others [21], although the degradation was more pronounced in our study.

A key observation made during this study was the re-precipitation of the cryoprecipitate during cold storage. Despite warming the components to 37°C, not all the precipitate could be re-dissolved or stayed dissolved. The degree of re-precipitation was dependent on the post-thaw storage duration, the individual donation and the source of plasma used for the cryoprecipitate (WB- or apheresis-derived). Apheresis-derived cryoprecipitate was more susceptible to re-precipitation, presumably due to higher fibrinogen content. The precipitate in most of the cryoprecipitate components could be easily dissolved until day 3, after which it became increasingly difficult to re-dissolve. Similar issues have been mentioned in published literature, although an extensive discussion of the problem was not provided [20–22]. Whilst we are unsure of the composition of the re-precipitation, we observed over 30% loss of fibrinogen after day 5 of cold storage, suggesting that the precipitate is likely composed of fibrinogen/fibrin, which slowly precipitates during extended cold storage.

It is possible that a cryoprecipitate component that is not fully re-dissolved could still be transfusible following in-line filtration at the time of transfusion. A small sub-set of cryoprecipitate components that underwent filtration showed there were no significant

TABLE 2 Coagulation factor concentrations for apheresis- and whole blood-derived cryoprecipitate before and after filtration.

	Apheresis			Whole blood		
	Unfiltered	Filtered	*p*-Value	Unfiltered	Filtered	*p*-Value
Fibrinogen (mg/unit)						
Day 3	881 ± 159	858 ± 195	0.4998	284 ± 65	288 ± 65	0.7193
Day 5	897 ± 228	864 ± 215	0.3634	279 ± 85	262 ± 51	0.4130
Day 7	745 ± 138	745 ± 138	1.0000	225 ± 43	231 ± 28	0.5886
Factor V (IU/unit)						
Day 3	56 ± 8	60 ± 6	0.3575	20 ± 1	19 ± 2	0.2152
Day 5	51 ± 8	47 ± 7	0.0689	15 ± 1	14 ± 1	0.2152
Day 7	41 ± 7	42 ± 6	0.2394	12 ± 1	12 ± 1	0.3910
Factor VIII (IU/unit)						
Day 3	300 ± 63	289 ± 52	0.4362	117 ± 20	117 ± 18	1.0000
Day 5	282 ± 49	293 ± 66	0.3541	102 ± 16	101 ± 17	0.4765
Day 7	267 ± 73	282 ± 81	0.2532	103 ± 26	99 ± 11	0.6286
von Willebrand factor (IU/unit)						
Day 3	538 ± 135	468 ± 165	0.1209	218 ± 28	221 ± 30	0.3769
Day 5	542 ± 148	525 ± 210	0.7633	218 ± 28	213 ± 31	0.1849
Day 7	519 ± 117	533 ± 173	0.6453	215 ± 33	199 ± 25	**0.0464**

Note: Data are mean ± SD, $n = 4$. *p*-value determined using a paired *t*-test for unfiltered and filtered samples at each time point. A *p*-value <0.05 is considered significant and highlighted in bold.
Abbreviation: SD, standard deviation.

differences in the pre- and post-filter groups, and all filtered cryoprecipitate would meet specifications for fibrinogen, FVIII and VWF.

One limitation of the study was the sample volume; it was not feasible to use a standard single or pooled cryoprecipitate component for each time point. This component is always in high demand and availability is limited. Although the sample size for full-size cryoprecipitate components was small, they had similar coagulation properties and re-precipitation after 3, 5 and 7 days, as observed for the mini-packs. A further limitation of the study was freezing the aliquots for coagulation testing; whilst this meant an additional freeze–thaw cycle, it eliminated any inter-assay variation.

In developed countries, the clinical implications of decreased in FVIII in cold-stored cryoprecipitate are minimal, as cryoprecipitate is no longer transfused to increase FVIII. The recommended adult dose of cryoprecipitate is 10 units of WB-derived cryoprecipitate or 4 apheresis units of cryoprecipitate, which should equate to 3–4 g of fibrinogen [4]. The loss of fibrinogen concentration up to 15% after 72 h is high, however cryoprecipitate fibrinogen concentrations well exceed the minimum requirement, meeting the specification even after 72 h of storage.

In conclusion, the data from this study demonstrates that the quality of cryoprecipitate stored at 1–6°C for up to 7 days post-thaw meets specifications for cryoprecipitate. However, a 72 h post-thaw-shelf-life for cryoprecipitate is recommended as being optimal, due to the re-precipitation that occurs when stored for longer periods at refrigerated temperatures.

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CONFLICT OF INTEREST STATEMENT

Denese C. Marks has received research funding from Cryogenics Holdings in the past 2 years. Other authors have no conflict of interests to declare.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Introduction of 7-day amotosalen/ultraviolet A light pathogen-reduced platelets in Honduras: Impact on platelet availability in a lower middle-income country

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Abstract

Background and Objectives: Honduras became the first lower middle-income country (LMIC) to adopt amotosalen/UVA pathogen-reduced (PR) platelet concentrates (PCs) as a national platelet safety measure in 2018. The Honduran Red Cross (HRC) produces ~70% of the national platelet supply using the platelet-rich plasma (PRP) method. Between 2015 and 2018, PCs were screened with bacterial culture and issued as individual, non-pooled PRP units with weight-based dosing and 5-day shelf-life. PR PCs were produced in six-PRP pools with a standardized dose ($\geq 3.0 \times 10^{11}$), no bacterial screening and 7-day shelf-life. Gamma irradiation and leukoreduction were not used.

Materials and Methods: PC production and distribution data were retrospectively analysed in two periods. Period 1 (P1) included 3 years of PRP PCs and a transition year (2015–18). Period 2 (P2) included 5 years of PR PCs (2019–23). PC doses were standardized to an equivalent adult dose for both periods. Descriptive statistics were calculated.

Results: HRC produced 10% more PC doses per year on average in P2 compared to P1. Mean annual waste at HRC declined from 23.9% in P1 to 1.1% in P2. Two urban regions consumed 96% of PC doses in P1 and 88.3% in P2. PC distributions increased in 14/18 regions.

Conclusion: Standardized dosage, PR and 7-day shelf-life increased PC availability, reduced waste, eliminated bacterial screening and avoided additional costs for arboviral testing, leukoreduction and irradiation. Access to PC transfusion remains limited in Honduras; however, the conversion to pooled PR PCs illustrates the potential to sustainably expand PC distribution in an LMIC.

Keywords

amotosalen, Honduras, pathogen reduction, platelets

Highlights

- Amotosalen/UVA pathogen-reduced (PR) platelet concentrates (PCs) have been used sustainably as the national standard in Honduras since 2018.
- Standardized pooling and dosing reduced PC waste.
- The 7-day shelf-life of PR PCs allowed expanded distribution of platelets to rural areas of a lower middle-income country.

INTRODUCTION

Platelet transfusion is an important supportive therapy for thrombocytopenic patients, including haematology/oncology patients, to prevent or treat bleeding associated with various chemotherapies, organ transplantation and haematopoietic stem-cell transplantation (HSCT); trauma patients with active haemorrhage or patients with bleeding disorders [1]. Platelets are collected and transfused worldwide, however, platelet supplies vary dramatically between countries, most notably between upper income and lower middle income countries (LMIC) [2]. The World Bank defines LMIC as countries with per capita gross national income (GNI) between $1136 and $4465 [3].

Honduras is an LMIC with a population of approximately 10 million people in Central America. Approximately half of the population resides in three large urban areas, including the capital city, Tegucigalpa (Figure 1).

A number of transfusion-transmissible arboviral and other vector-borne diseases are endemic to Honduras, including dengue virus, chikungunya virus and Chagas disease [4, 5]. Data on arboviral prevalence in Honduran blood donors are not available; however, arboviral risk in blood donors has been established in endemic areas elsewhere in Latin America and the Caribbean [6–8]. Other blood-borne pathogens, including human immunodeficiency virus (HIV), hepatitis B and C viruses (HBV, HCV), human T-lymphotropic virus (HTLV) and

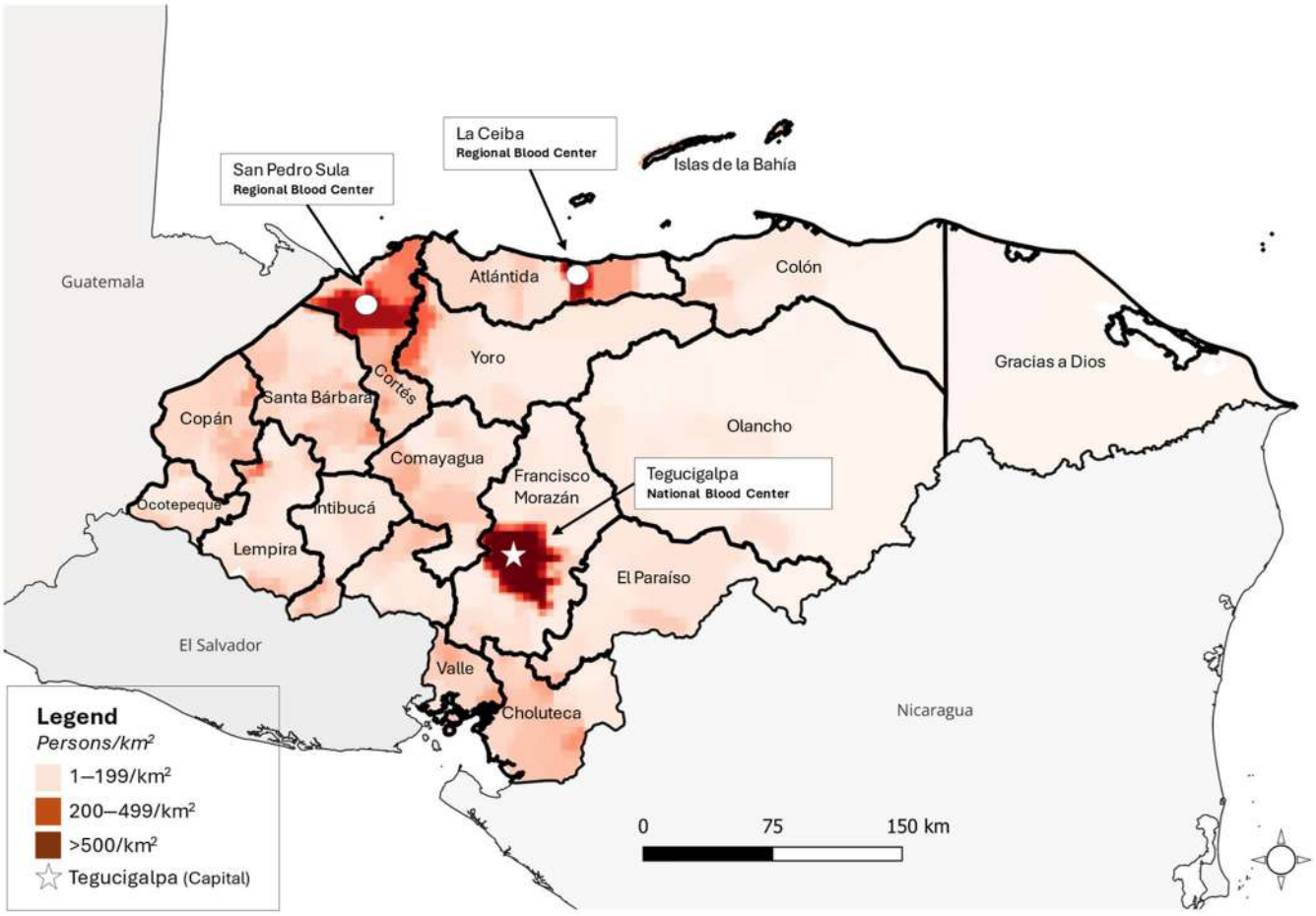

FIGURE 1 Geographic distribution of Honduran population expressed as persons per square kilometre; identification of high and low population areas and locations of Red Cross blood centres in relation to population centres.

syphilis have been documented in Honduran blood donors at relatively high levels. A serosurvey of Honduran blood donors conducted between 2014 and 2016 found that >2% of 48,567 donors were infected with a transfusion-transmissible infection (TTI) [9]. Bacterial contamination of platelets is not widely documented in Latin America due to limited haemovigilance [10]; however, hospital-based haemovigilance systems have documented transfusion-transmitted bacterial infections (TTBIs) in Brazil and Colombia [11, 12]. A 2021 review estimated that actual TTBI rates in Latin American countries could be 7–29 times higher than reported estimates [13].

The Honduran Red Cross (HRC) is responsible for collecting, preparing and distributing ~70% of the national blood supply, including platelets. The balance of labile blood products are produced by hospital blood banks. HRC is reimbursed for blood components transfused in public hospitals from governmental budgets assigned to the Secretary of Health and the Secretary for Social Security, which operate the national network of public hospitals. Out-of-pocket reimbursements are paid by patients transfused in the private sector.

HRC operates three blood centres: The National Blood Centre in Tegucigalpa and Regional Blood Centres in La Ceiba (Atlántida region) and San Pedro Sula (Cortés region) (Figure 1). HRC collects >47,000 whole blood donations per year, mostly (>70%) from family-replacement donors [2, 9]. Two of the three HRC blood centres (Tegucigalpa and San Pedro Sula) have been accredited by the Association for the Advancement of Blood and Biotherapies (AABB) since 2000. A small number of apheresis platelets are produced each year; the majority of the national platelet supply is produced from platelet-rich plasma (PRP). Before the introduction of pathogen reduction, platelets were produced as individual PRP units (PRPs), which were prescribed using a weight-based formula.

Leukoreduction and gamma irradiation were not used for platelet concentrate (PC) production in Honduras during the entire study period.

All whole blood donations are screened for HIV, HBV and HCV with an electro-chemiluminescence assay (ECLIA). To reduce the risk of window period infections, HRC uses nucleic acid testing (NAT) to re-screen donations with a negative ECLIA result. Serological assays are also used to screen donations for syphilis, HTLV-1 and 2 and hepatitis B core antigen. HRC introduced bacterial culture screening with the BacT/Alert system (bioMérieux, Marcy l'Étoile, France) for all platelets in 2015. Aerobic bottles were inoculated with a 7–10 mL sample between 12 and 24 h post-collection and incubated for at least 12 h prior to release. In 2016, challenges with reagent procurement and concerns about emerging and other non-bacterial infectious risks such as Zika virus [14] led the HRC to evaluate the amotosalen/UVA pathogen reduction technology (INTERCEPT® Blood System, Cerus Corporation, Concord, California, USA) as an enhanced safety measure for platelets. The HRC implemented amotosalen/UVA pathogen reduction for 100% of the PCs produced by HRC blood centres in 2018. This article describes the Honduran experience as the first LMIC to implement pathogen-reduced (PR) PCs at a national scale and sustain the practice for 5 years.

MATERIALS AND METHODS

Whole blood collections, PC production and distribution data were retrospectively extracted from HRC databases and stratified by year, region and type of PC. Data were further stratified into 2 discrete time periods. Period 1 (P1) covered 3 years of conventional PRP PC production (2015–2017) plus the transition year (2018). Period 2 (P2) covered the first 5 complete years of pooled PR PC production and distribution (2019–2023). PC doses were standardized to an equivalent adult dose for both periods: PRP doses in P1 were based on a 1 PRP per 10 kg dosing formula and a mean adult (male and female) weight estimate of 68 kg [15]. Pooled PR PC doses produced in P2 were prepared with six non-leukoreduced, non-irradiated PRPs and a standardized target dose of $\geq 3.0 \times 10^{11}$ (Figure 2).

HRC conducted a validation study in 2018 to assess the quality of PCs prepared and stored with the INTERCEPT Blood System for Platelets. The study measured PC volume, platelet concentration ($\times 10^9$/L), platelet dose ($\times 10^{11}$) and pH through storage day 7.

A cost–benefit analysis was performed based on the model of anticipated savings associated with pathogen reduction technology described by McCullough et al. [16]. Baseline costs included the use of a quadruple bag collection system for PRP production, consumables for bacterial culture screening and estimated costs associated with false positive results. Pathogen reduction costs assumed the use of a triple bag collection system with pooling sets and consumables, for example, sterile docking seals; the elimination of bacterial screening; the use of INTERCEPT large volume (LV) processing sets and savings associated with reduced processing and transfusion reaction costs. Additional savings included the avoidance of implementing gamma irradiation to prevent transfusion-associated graft-versus-host disease (TA-GVHD) and leukoreduction to reduce the risk of cytomegalovirus (CMV) infection, alloimmunization and clinical refractoriness [17]. Future cost-savings associated with avoided future testing costs were also estimated. All costs (in US Dollars) were based on actual 2017 prices and estimated prices listed by McCullough et al.

Population estimates (national and for 18 regions) were derived from the Honduran National Institute of Statistics [18]. Descriptive statistics (means, ranges) were calculated for two-period comparisons and multi-year trends. No statistical testing was performed. Maps were produced with QGIS version 3.36.1 (QGIS.org).

RESULTS

A total of 28,449 equivalent PRP PC doses were produced during the 9-year study period (11,865, 41.7% in P1; 16,584, 58.3% in P2). On average, 2996 PC doses were produced annually in P1 versus 3317 produced per year in P2 (+11.8%). The proportion of PC doses distributed for transfusion increased from 64.6% (1844 / 2854) in 2015 when shelf-life was limited to 5 days (>35% waste) to 98.8% (3126 / 3164) in 2023 after the extension of shelf-life to 7 days (~1.2% waste). Annual PC waste at HRC decreased from 23.9% per year on

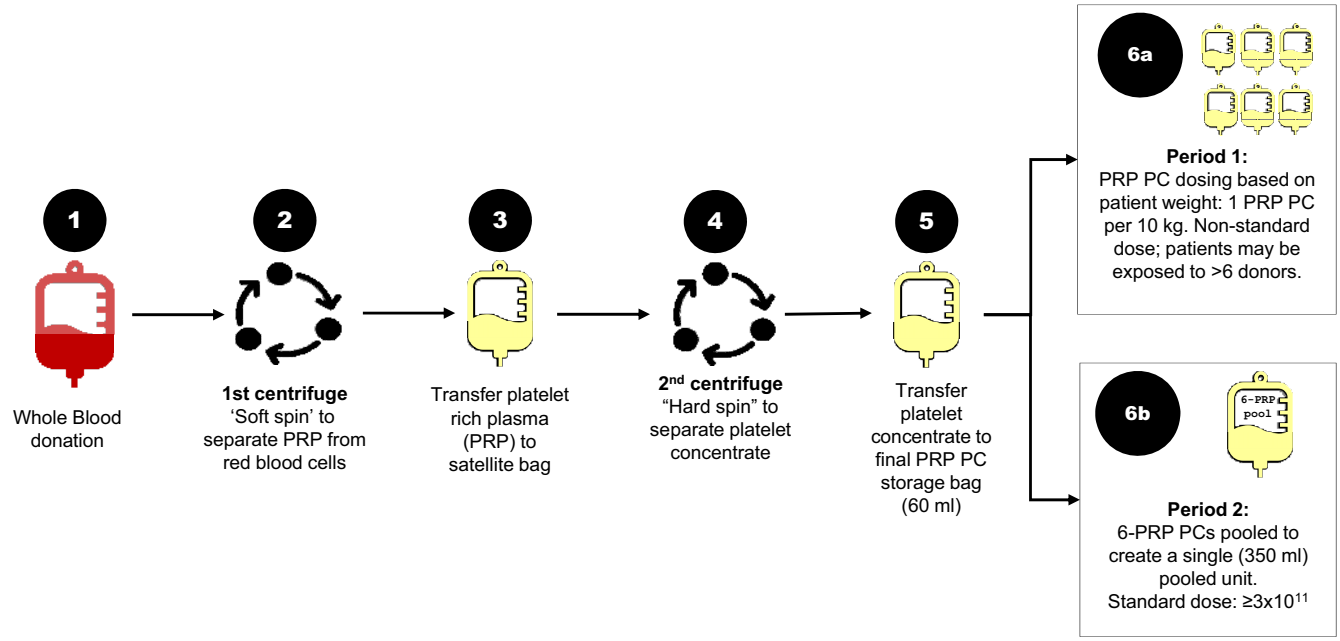

FIGURE 2 Procedure used to produce platelet components in Honduras: The platelet-rich plasma (PRP) method. PC, platelet concentrate.

TABLE 1 Nine-year summary of PC production, distribution and waste, Honduran Red Cross, 2015–2023.

		Individual PRPs + 2018 transition (P1)				Pooled amotosalen/UVA PCs (P2)				
		2015	2016	2017	2018[a]	2019	2020	2021	2022	2023
PC doses produced	*N*	2854	3283	2910	2818	3316	2826	3914	3364	3164
	Mean	2966				3317				
PC doses distributed	*N*	1844	2223	2166	2752	3278	2791	3884	3330	3126
	Mean	2246				3282				
Estimated waste (%)	%	35.4%	32.3%	25.6%	2.3%	1.1%	1.2%	0.8%	1.0%	1.2%
	Mean	23.9%				1.1%				
% PR		0%	0%	0%	~10%	100%	100%	100%	100%	100%
Platelet shelf-life (days)		5			5/7	7				

Abbreviations: PC, platelet concentrate; PR, pathogen-reduced; PRP, platelet-rich plasma.
[a]Pathogen reduction transition year. The grey shading is meant to highlight 2018 as the year HRC transitioned to pathogen reduction, that is, in this year the data reflect ~10% PR and 90% conventional, vs. 100% of conventional in 2015-2017 and 100% PR in 2019-2023.

average in P1 to 1.1% per year in P2 (Table 1). The majority of expirations in P1 occurred at the HRC production centre before release. Most expirations in P2 occurred at the receiving hospital after distribution from the HRC production centre. Platelets were released on the afternoon of Day 1 post-collection on average in both periods (Figure 4).

No cases of TA-GVHD were documented in either period.

Whole blood collections increased by 49% in 2023 compared with 2015. This increase was driven in large measure by overall population growth between 2015 and 2023. On a national basis, mean annual whole blood collections (~450 mL) did not change substantially when factored against population growth: 4.0 whole blood (WB) collections per 1000 population in P1 versus 4.4 per 1000 population in P2 (Table 2).

Population growth and geographic distribution of PC doses

The national population of Honduras grew 13.7% between 2015 and 2023, increasing from approximately 8,574,532 in 2015 to 9,743,373 in 2023, according to estimates derived from the 2013 Honduran Census [18]. Annual population growth by region averaged 1.6% (range: 1.2%–2.5%). Urban areas in three regions (Francisco Morazán, Cortés and Atlántida) accounted for ~42% of the national population throughout the 9-year study period.

Two regions (Francisco Morazán and Cortés) accounting for ~37% of the national population consumed 96% of PC doses in P1 and 88.3% in P2 (Table 3). In 2015, only 10 of 18 regions reported receiving one or more PRP doses per year. By 2023, 14 of 18 regions

TABLE 2 National RBC and PC production by population.

	Period 1 (P1)				Period 2 (P2)				
	2015	2016	2017	2018	2019	2020	2021	2022	2023
Population (pop.)	8,574,532	8,701,014	8,866,351	9,012,229	9,158,345	9,304,380	9,450,711	9,597,042	9,743,373
RBCs[a]	32,055	33,347	36,332	38,267	41,103	30,354	41,593	46,733	47,956
PC doses	2854	3283	2910	2818	3316	2826	3914	3364	3164
RBC per 1000 population (pop.)	3.7	3.8	4.1	4.2	4.5	3.3	4.4	4.9	4.9
PC per 1000 pop.	0.33	0.38	0.33	0.31	0.36	0.30	0.41	0.35	0.32
Mean PC/1000 pop.	0.23				0.34				
Mean RBC/1000 pop.	4.0				4.4				

Abbreviation: PC, platelet concentrate.
[a]Red blood cells (RBCs) are used as a proxy for whole blood (WB) collections as 97%–99% of WB collections were processed into RBCs each year.

received at least 1 PR PC dose per year. Two low population regions (Lempira [~360,000 pop.] and Ocotepeque [~168,000 pop.]) received no PC doses in either period (Figure 3).

Local validation of the amotosalen/UVA system and operational changes

The HRC validation study ($n = 30$ pools of six PRPs) confirmed that locally produced PR PCs met production parameters and acceptance criteria established by the manufacturer [19] and AABB Standards [20] (Table 4). Routine swirling and visual inspection procedures were also in place during both study periods. Certain workflow changes were required to accommodate the pathogen reduction process, which included PRP pooling, sterile docking to the INTERCEPT LV processing kit, illumination in the INTERCEPT Illuminator device and a 16-h hold for the adsorption of residual amotosalen and photo-products. These additional steps delayed PR PC release times by approximately 3 h compared with the routine process used to prepare individual PRP units; however, workflow changes had minimal impact on blood centre shift schedules and remained aligned with the HRC NAT and serology testing schedules (Figure 4).

Cost–benefit analysis

As predicted by the McCullough et al. model, per dose costs were ~$100 higher in the pathogen reduction scenario compared with the baseline scenario ($74/dose baseline vs. $175/dose pathogen reduction). The increased costs were all associated with the addition of pooling sets and INTERCEPT Processing Sets. However, these increases were off-set in the model by immediate savings achieved through the elimination of false-positive bacterial screening results and savings associated with the avoidance of adding Zika virus screening for platelets. Additionally, the model captured savings associated with a reduction in the number of WB units required to produce PR PCs compared with the number of WB donations to produce single PRP units for a comparable number of 'doses' using the previous weight-based dosing formula, reduced costs associated with TTIs and increased cost-recovery linked to reduced waste. When these additional savings were accounted for, per dose costs only increased by $39. The avoidance of future costs associated with the potential introduction of gamma irradiation or CMV screening (neither of which were available in Honduras), and tests for certain emerging infectious diseases also contributed to a conclusion that pathogen reduction could be introduced and sustained in a cost-neutral manner.

DISCUSSION

The introduction of PR PCs with the amotosalen/UVA technology allowed the HRC to provide more PCs to patients in a wider geographical area with reduced waste, and no impact on overall whole blood collection requirements. The amotosalen/UVA pathogen reduction technology also added evidence-based protection against bacterial [21, 22] and arboviral TTIs [23] without additional laboratory testing. For example, a 2023 meta-analysis by Giménez-Richarte et al. summarized pathogen inactivation studies showing the amotosalen/UVA technology achieved high levels of inactivation (≥4 $\log_{10}$, as recommended by the World Health Organization [24]) against Chikungunya (≥6.29 $\log_{10}$), Dengue (≥4.33 $\log_{10}$) and Zika (≥6.29 $\log_{10}$) viruses, three endemic arboviruses with epidemic potential in Latin America [25]. Likewise, in 2021 McDonald et al. demonstrated the amotosalen/UVA technology's capacity to achieve full inactivation of nine bacterial species commonly associated with TTBI through the end of 7-days' storage [22].

As the first LMIC blood centre to adopt pathogen reduction as the standard of care for the majority of PCs produced, HRC has demonstrated the feasibility of implementing and sustaining pathogen reduction as a replacement for bacterial culture screening on a national scale. The HRC experience also shows how an LMIC may accrue additional safety benefits from pathogen reduction while

TABLE 3 Platelet Distribution by Region, Honduras, 2015–23.

	Period 1 (P1)				Period 2 (P2)					Mean by period		
Region	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**	**2023**	**P1**	**P2**	**Change (+/−)**
Atlántida	23	19	41	44	259	215	254	161	142	32	206	+
Choluteca	0	0	0	0	0	5	0	0	7	0	2	+
Colón	0	0	1	0	6	30	1	1	8	0	9	+
Comayagua	0	6	3	8	7	15	4	1	24	4	10	+
Copán	0	2	4	0	1	7	8	30	23	2	14	+
Cortés	964	1122	1169	1215	1318	1367	1847	1579	1589	1117	1540	+
El Paraíso	1	0	1	1	12	10	31	8	0	1	12	+
Francisco Morazán	805	1036	886	661	1448	977	1556	1541	1275	847	1359	+
Gracias a Dios	2	0	0	0	5	3	11	0	1	0	4	+
Intibucá	0	1	0	0	0	0	3	0	1	0	1	+
Islas de la Bahía	3	1	7	2	4	6	13	4	8	3	7	+
La Paz	1	0	0	0	0	0	8	1	0	0	2	+
Lempira	0	0	0	0	0	0	0	0	0	0	0	nc
Ocotepeque	0	0	0	0	0	0	0	0	0	0	0	nc
Olancho	1	1	0	1	0	2	0	0	9	1	2	+
Santa Bárbara	38	32	46	4	5	28	4	8	35	30	16	−
Valle	0	0	0	0	0	0	1	1	2	0	1	+
Yoro	7	1	2	2	4	8	2	5	2	3	4	+
National	**1844**	**2221**	**2158**	**1938**	**3069**	**2673**	**3743**	**3340**	**3126**	**2040**	**3190**	

Abbreviation: nc, no change.
Note: The bold values represent the national totals of each year.

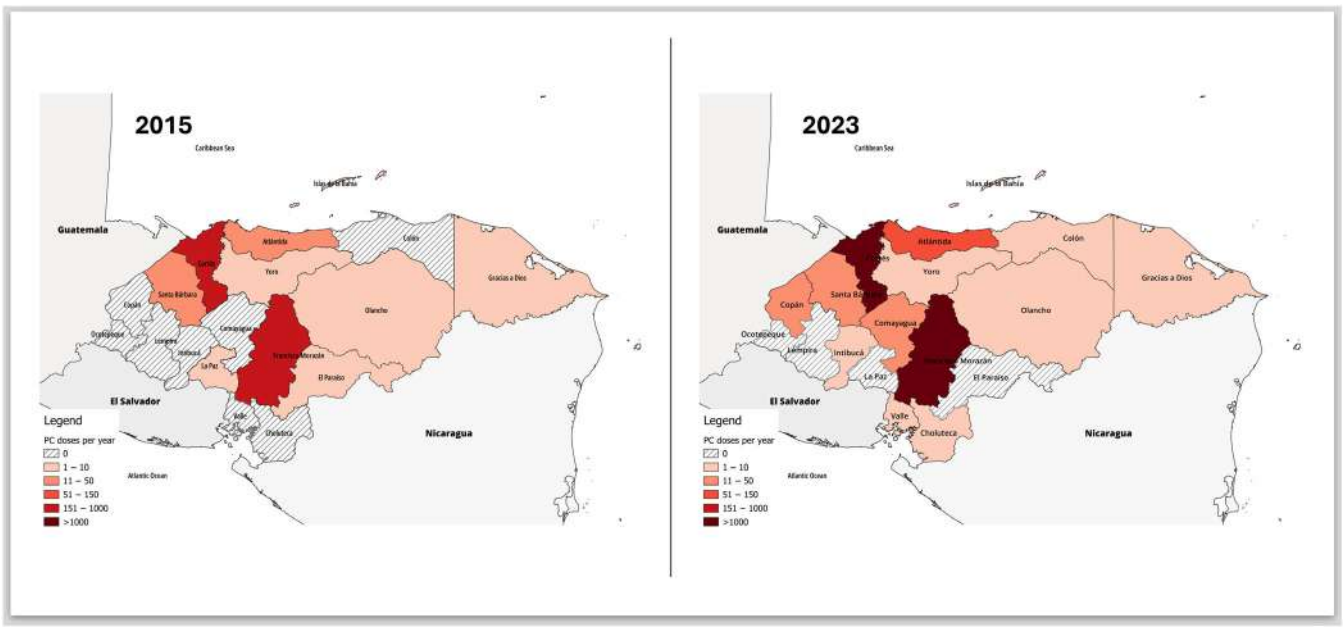

FIGURE 3 Evolution of national platelet distribution by region in Honduras, 2015 and 2023. PC, platelet concentrate.

avoiding future costs. Specifically, leukoreduction, gamma irradiation and CMV screening were not used in Honduras prior to pathogen reduction and have not been introduced since the adoption of pathogen reduction. In addition to reduced risk of viral, bacterial and protozoan threats, the amotosalen/UVA pathogen reduction may be used as an alternative to gamma irradiation for the prevention of TA-

TABLE 4 Results of HRC PR PC validation study, 2018.

N = 30 pools of six PRPs	Sample size	Volume (mL)[a]	Platelet concentration ($\times 10^9$/L)[a]	Platelet dose ($\times 10^{11}$)[a]	pH[a,b]	RBC
Acceptance criteria (ranges)	Variable (20–60)	255–420	1050–2100	2.5–7.0	6.4–8.0	<4 × 10^6/mL for 300–420 mL units or <4 × 10^5/mL for 255–420 mL units
Mean (high/low daily range)	N = 30 pools of six PRPs	304 (280–320)	1245 (1020–1690)	3.8 (3.1–5.1)	7.3 (7.0–8.0)	All units within acceptance criteria
Median (IQR)		304 (8)	1188 (207)	3.6 (0.6)	7.0 (0.4)	

Abbreviations: IQR, interquartile range; PC, platelet concentrate; PR, pathogen-reduced; PRP, platelet-rich plasma; RBC, red blood cells.
[a]Results of the validation study fell within acceptance ranges established by the manufacturer and AABB Standards for the production of pathogen-reduced platelets.
[b]pH values were measured daily post-collection up to day 7.

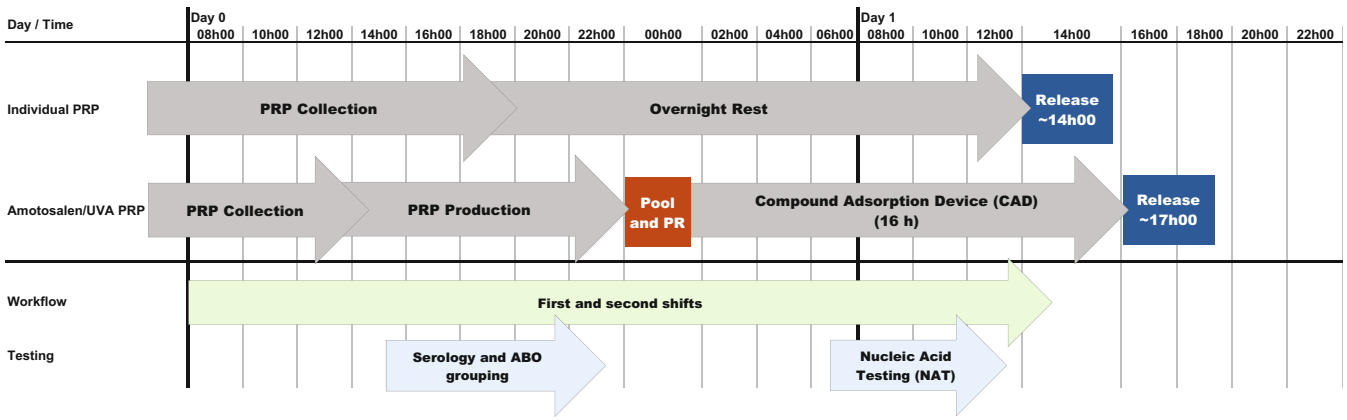

FIGURE 4 Honduran Red Cross (HRC) platelet production workflow and timeline before and after implementation of 100% pathogen-reduced (PR) platelets. NAT, nucleic acid testing; PRP, platelet-rich plasma.

GVHD and may replace CMV testing and leukoreduction for prevention of transfusion transmitted CMV infection [26–28]. The successful elimination of bacterial culture screening and irradiation with the introduction of amotosalen/UVA PR PCs for HSCT patients was described in 2019 by Sim et al. in a setting where leukoreduction was not used for platelets. A total of 33 patients received 76 non-leukoreduced and non-irradiated PR PCs without bacterial culture screening. Thirty-one (31) control patients were transfused with 89 bacterial screened and irradiated PCs. The primary efficacy endpoint—1-h corrected count increment (CCI)—was comparable in both cohorts which were followed for 100 days post-transfusion. The rate of transfusion reactions (the primary safety endpoint) was reduced in the test cohort, but not significantly. The study also found that clinical refractoriness and refractory transfusions were significantly lower in the test cohort ($p = 0.05$ and $p = 0.02$, respectively), with no TA-GVHD in either cohort and comparable rates of 100 day engraftment, mortality and infectious disease incidence in both cohorts [17].

Platelets remain a scarce commodity in Honduras, where <1 PC dose is available per 1000 population (by comparison up to eight PC doses are distributed per 1000 population per year in the United States [29]). PC distribution remains focused on urban areas, and barriers to PC distribution including inclement weather, poor roads and mountainous terrain were present in both P1 and P2. However, with up to 2 days' of additional shelf-life, the introduction of pathogen reduction allowed a measurable increase in the availability of PCs in rural areas (~4% in P1 vs. ~12% in P2), where the Honduran Ministry of Health operates a network of general and basic hospitals [30].

While the true burden of cancer and other oncological conditions that may require acute or prophylactic platelet transfusion is unknown in Honduras, the incidence of multiple types of cancers (and need for supportive therapies) is projected to increase in LMICs in the coming decades [31]. Limited access to healthcare commodities and procedures and challenges with transportation have already been identified as key factors in delayed cancer diagnoses and treatments [32], abandonment of cancer therapy [33, 34] and poor cancer outcomes [35] in Honduras. Increased availability of PR PCs with a 7-day shelf-life in regional hospitals may help mitigate the impact of barriers to healthcare in rural areas [36, 37] and reduce pressure on urban hospitals where medical indications for transfusion compete with transfusion requirements for high levels of trauma due to violence and traffic accidents which may also require blood products [38, 39].

The introduction of amotosalen/UVA PR PCs in Honduras was accompanied by a major clinical shift in platelet dosing guidelines and an extension of PC shelf-life from 5 to 7 days. The move from presumptive PC dosing based on patient weight to a standardized platelet dose per unit was well received by clinicians and has been shown in other LMIC settings to improve transfusion practice [40]. A clinical audit of platelet transfusion practice with standardized dosing is needed to understand the full clinical impact of this change in Honduras. A similar audit conducted over a 6-year period in a large academic hospital in Mexico found that up to 25% of platelet transfusions were 'inappropriate' when assessed against British Society for Haematology guidelines, despite local training [41].

The HRC decision to adopt pathogen reduction for all PCs was driven by concerns about the limitations of culture screening as a guard against non-bacterial TTIs [23] and made in the context of economic and logistical barriers to sustaining bacterial culture as a safety measure.

Since adopting pathogen reduction, HRC has worked with the amotosalen/UVA technology's manufacturer to limit year-on-year price increases and ensure a continuous supply of processing kits (since 2018 no PR stock-outs have occurred). The introduction of pathogen reduction in Honduras also occurred during a period of substantial growth in government expenditure on health, driven in large part by the coronavirus disease 2019 (COVID-19) pandemic. Between 2020 and 2023, public spending on hospital services increased >30%, with spending on materials and supplies rising by ~50% [42]. A fuller financial analysis is required to describe the impact of pathogen reduction within the overall growth of public sector spending on healthcare, validate savings accrued from the elimination of bacterial culture screening and estimate the avoidance of future costs associated with gamma irradiation, leukoreduction and CMV testing. Pending such an analysis real world cost savings or cost-recovery opportunities may be inferred given the clinical and operational changes achieved in Honduras and cost modelling done in Canada and with a different pathogen reduction technology in another LMIC [43, 44]. Additional cost savings may include, for example the following:

1. Cost-recovery from the higher number of transfusable units due to reduced waste.
2. Reduced healthcare costs associated with extended hospitalizations and specialty care associated with TTBI and emerging infectious diseases.

Additional research is also needed to assess patient safety trends in Honduras and compare the Honduran experience with positive clinical and safety trends described in other countries where the amotosalen/UVA technology has been adopted as the standard of care [45–48].

This study is subject to several limitations. First, retrospective data may be subject to uncontrollable reporting biases, especially related to the distribution of blood components. It was beyond the scope of this study, for example, to track each 'distributed' unit to confirm transfusion. As a result, wastage rates presented here may underestimate levels of un-used PC doses at the bedside. Second, the use of a mean adult weight (68 kg) to calculate the number of PRP PC doses in P1 may have produced under- or over-estimates depending on the actual ratios of male and female adults transfused in a given region or hospital. This method also underestimates paediatric PC transfusions. While standardized dosing with six-pool PRP PCs reduced the number of WB donations required to meet annual PC production quotas, the extent of this reduction is difficult to quantify. Third, individual patient outcomes were not available to assess the clinical benefit of increased access to PC transfusion in rural hospitals. Fourth, while clinicians reported preferring the new standard dosing system, data were not available to determine the number of PCs transfused per patient or other measures of appropriate PC transfusion practices in either period. Fifth, the economic analysis performed in 2017 does not account for inflation or other macroeconomic changes during the ensuing years; a fuller accounting of routine operating costs is warranted.

Despite these limitations, the HRC experience provides real world evidence of the feasibility of implementing and sustaining the amotosalen/UVA pathogen reduction technology with standardization of PC production and dosing, elimination of bacterial screening, avoidance of gamma irradiation and leukoreduction and 7-day shelf-life extension in an LMIC. Sustaining pathogen reduction as a routine blood centre process over a 5-year period is particularly striking in Honduras, where per capita spending on health by the government is the lowest in the Latin America region. While public spending on health in Honduras has increased since 2010, Honduras continues to lag its neighbours in the region by a substantial margin [49]. This experience may provide insights and reassurance to other LMIC that pathogen reduction is not a cost-prohibitive intervention when considered in the context of immediate cost savings, opportunities for cost-recovery and the avoidance of future costs, especially those associated with the introduction of gamma irradiation and leukoreduction technologies or severe clinical outcomes in patients.

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M.P. and G.A. conceived of the study and designed the manuscript with J.P.P. All authors contributed to data analysis and preparation and review of the manuscript.

CONFLICT OF INTEREST STATEMENT

J.P.P. is an employee and shareholder of Cerus Corporation.

DATA AVAILABILITY STATEMENT

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

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ORIGINAL ARTICLE

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Evaluation of the progress of a decade-long haemovigilance programme in India

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Abstract

Background and Objectives: Implementation of national haemovigilance programmes has significantly improved donor and recipient safety. Recently, India completed a decade of successful implementation of its national haemovigilance programmes. The national programme is still enrolling more blood centres. This study aimed to highlight the strengths and weaknesses of Haemovigilance Programme of India (HvPI), thereby providing valuable insights for future initiatives.

Materials and Methods: The National Coordinating Centre (NCC) conducted a multicentre, cross-sectional questionnaire-based survey among the reporting blood centres (January to April 2022). The survey consisted of three sections with a total of 27 questions focusing on the demographics of the participant blood centre as well as the impact on the recipient and donor haemovigilance. The survey was sent to 733 blood centres regularly reporting to the donor and recipient HvPI through Donor and Hemovigil Software.

Results: Total 296 responses were received (response rate of 40.4%) with maximum participation of private non-teaching hospital-based blood centres (33.8%). After their involvement in recipient HvPI, 85.7% of the respondents reported changes in their blood centre's work procedures, with the maximum improvement seen in the documentation of transfusion reactions (92.7%). Out of the 278 respondents who participated in donor HvPI, 89.9% (250) found that their blood centre's policies or work process changed as a result of their involvement in the programme.

Conclusion: In conclusion, our haemovigilance programme facilitates national collaboration for learning and sharing experiences, leading to improved policies and practices in reducing adverse reactions for both recipients and donors.

Keywords

effectiveness, feedback, haemovigilance, improvement

Highlights

- The implementation of national haemovigilance programmes has notably enhanced donor and recipient safety, reflecting a decade of successful execution in India.
- A cross-sectional questionnaire-based survey conducted among reporting blood centres revealed substantial changes in work procedures, particularly in documentation of transfusion reactions, following involvement in the recipient haemovigilance programme.
- The study demonstrates a high percentage of respondents reporting changes in their blood centre's policies or work processes due to participation in the haemovigilance programme, highlighting its effectiveness in driving tangible improvements for both recipients and donors.

INTRODUCTION

Haemovigilance encompasses surveillance procedures that cover the entire transfusion process, from blood collection to recipient follow-up, to collect and evaluate information on unexpected or unfavourable effects of labile blood products [1]. The ultimate goal of any haemovigilance programme is to provide evidence-based guidelines to prevent adverse reactions in donors or recipients. By analysing haemovigilance data, it is possible to understand the cause, frequency and clinical outcomes of adverse events, leading to changes in policies, practices and products that enhance donor and recipient safety.

To ensure patient safety and promote public health, a centralized haemovigilance programme, the Haemovigilance Programme of India (HvPI), was launched in the country in December 2012 [2]. The primary goal of HvPI was to develop guidelines and policies aimed at reducing and preventing adverse transfusion reactions by increasing awareness and promoting the reporting of these reactions. The National Blood Donor Vigilance Programme (NBDVP) was also launched on 14 June 2015 to monitor adverse reactions or incidents during the blood collection process [3]. Similar to HvPI, NBDVP was also designed to prevent adverse reactions associated with blood donation. The National Institute of Biologicals (NIB), Noida, serves as the National Coordinating Centre (NCC) for both programmes [2]. Currently, both the recipient and donor haemovigilance programmes have enrolled 1448 blood centres each. NCC has already published several articles on implementing the national programme and adverse transfusion and donor reactions [4, 5]. Through national and international haemovigilance programmes, various changes have been noted worldwide, which have helped to enhance donor and recipient safety.

Although haemovigilance programmes have been implemented for over a decade, not all blood centres in the country have enrolled in HvPI. To assess the programme's progress, HvPI designed a survey to evaluate the impact of its implementation in enrolled blood centres. The survey was aimed to identify policy changes in participant blood centres to reduce adverse reactions in donors and recipients, suggest areas for improving data collection and accuracy and determine the future course of action based on the survey results.

This study aimed to know whether the programme is achieving its intended goals such as awareness and acceptability of the programme and making a positive impact in reducing adverse events and to gather feedback from participants and stakeholders to identify areas where the HvPI programme can be improved including understanding the challenges and target enhancements. Through this comprehensive assessment, we aimed to highlight both the programme's strengths and weaknesses, thereby providing valuable insights for future initiatives.

MATERIALS AND METHODS

The NCC conducted a multi-centre, cross-sectional survey in India between January and April 2022 on behalf of HvPI. The survey was questionnaire-based, consisting of three sections and a total of 27 questions. The first section collected demographic information from participating sites with eight questions. The second section had 11 questions related to recipient haemovigilance, and the third section had eight questions related to donor haemovigilance. The survey was sent to 733 blood centres in India actively participated in the donor and recipient HvPI and regularly reported the data. The survey was distributed by NCC through Google form. Participation was voluntary, and two reminders were sent to complete the survey. The collected data were anonymized to protect individuals' identification, and descriptive statistics were applied, expressing variables in numbers and percentages.

RESULTS

Out of the 733 blood centres surveyed, 296 provided a comprehensive response, with a response rate of 40.4%. The data showed a notably higher response from private non-teaching (33.8%) and teaching (30.1%) hospital-based blood centres, compared with government teaching (19.2%) and non-teaching (4.4%) hospital-based blood centres. Additionally, standalone blood centres accounted for 12.5% of the responses (Figure 1).

Of the total respondents ($n = 296$), 91.5% (271) participated in both donor and recipient HvPI, whereas 6.1% [16] were only in recipient and 2.4% [7] were only in donor HvPI. Maximum participation in HvPI was reported in the last 3–5 years for both recipient (46.7%) and donor (47.8%) (Table 1).

The majority of respondents (87.5%) reported that the doctor in-charge or resident doctors were responsible for data validation for submission in HvPI, while only 52.7% of them entered the data directly (Figure 1).

Recipient haemovigilance

Since enrolment, 85.7% of blood centre reported improvements, whereas 85.1% reported policy changes in their blood centres after the implementation of recipient HvPI (Tables 2 and 3).

We observed that almost 96% of the respondents took various measures to reduce the time gap between the issue and transfusion of blood components, with 69.6% of them stopping the bulk issue of components altogether at a time (Figure 2). For motivating clinicians to improve reporting of transfusion reactions to blood centres, the 74.1% respondents discussed the issue in hospital transfusion committees and sent messages to the clinicians (Figure 3).

Donor haemovigilance

Out of the 278 respondents who participated in donor HvPI, 89.9% (250) found that their blood centre's policies or work process changed as a result of their involvement in the programme (Table 4). Around 75.5% respondents found recently published donor haemovigilance

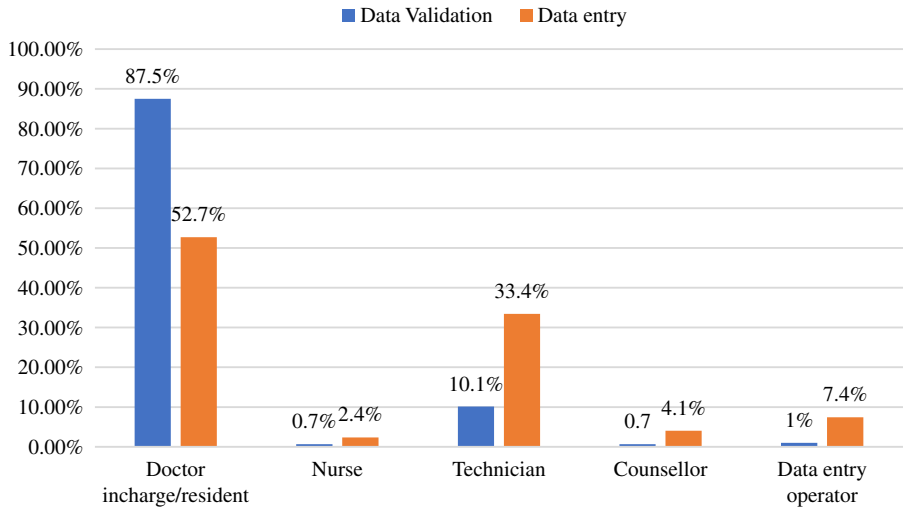

FIGURE 1 Responsibility for validation and entry of haemovigilance data.

TABLE 1 Years of participation in recipient and donor haemovigilance.

S. no.	Number of years	Recipient haemovigilance ($n = 289$)	Donor haemovigilance ($n = 278$)
1.	0–2	74 (25.6%)	98 (35.3%)
2.	3–5	135 (46.7%)	133 (47.8%)
3.	6–10	80 (27.7%)	47 (16.9%)

data to be beneficial in changing policies at their blood centre, and 96.8% believed that delayed or long-term effects of blood donation should be studied and included in the donor HvPI (Figure 4). Overall, respondents provided several suggestions to improve both the recipient and donor HvPI mentioned in Table S1.

DISCUSSION

Haemovigilance data can improve awareness of adverse reactions and inform preventative strategies. The programme was initiated in France in 1994 and has been adopted globally in various forms such as voluntary or mandatory [6]. In India, a voluntary haemovigilance programme began in 2012, expanding to include donor-related adverse reactions in 2015. Almost a decade after the implementation of HvPI programme, this was the first online survey to assess the effectiveness of the HvPI programme and gather feedback for future improvement.

This kind of survey helps in making evidence-based decisions about the HvPI programme and allocating resources effectively. Surveys enable organizations to compare their programme's effectiveness against industry benchmarks or best practices. Sharing survey results allows for open communication about the programme's outcomes, successes and areas in need of attention.

In our survey, only 40.4% of blood centres participated in both donor and recipient haemovigilance. The low participation rate underscores the need for increased awareness and advocacy regarding the importance of haemovigilance programme among blood centres. Most of the participation was from private hospital-based blood centres, which are registered in HvPI as a mandatory requirement for accreditation. In almost 90% of the participants, doctor in-charge or resident doctors validated adverse reactions, and technical staff were responsible for data validation and entry. This finding underscores the importance of doctors in accurately diagnosing and validating adverse reactions. Doctors' involvement in the validation process ensures that adverse events are properly identified and classified, allowing for appropriate management and follow-up measures to be implemented. Additionally, the involvement of technical staff in data validation and entry highlights the collaborative nature of haemovigilance efforts, where interdisciplinary teams work together to ensure the reliability and quality of data collected.

We have observed various improvements in recipient haemovigilance in participating blood centres after enrolment in HvPI. Blood centres conveyed the importance of reporting adverse transfusion reactions, which can help in developing transfusion policies, documentation and investigation of reactions [7,8]. Approximately 85% of respondents reported significant changes in the policies and procedures of their blood centres. Respondents implemented active haemovigilance at their workplaces, resulting in increased reporting of adverse reactions, reduced near-miss errors and improved patient safety. To increase reporting of adverse reactions, institutions should raise awareness, use HTC assistance and designate existing nurses as specialized haemovigilance nurses to oversee every transfusion and upload adverse reaction data to the NCC regularly [9].

TABLE 2 Improvement observed and policy changes by respondents after establishment of recipient HvPI.

Improvements observed ($n = 289$)	Policy changes ($n = 248$)
Better documentation of a transfusion reaction ($n = 230$) (79.6%)	Enhance the donor medical screening programme ($n = 175$) (70.6%)
Increased awareness and assistance through Hospital Transfusion Committee ($n = 156$) (54%)	Enhance donor arm disinfection ($n = 132$) (53.2%)
Implementation of active haemovigilance programme ($n = 143$) (49.5%)	Improve the cleanliness of blood and blood component storage areas ($n = 83$) (33.5%)
Improve technical resources and facilities for transfusion reaction workup ($n = 125$) (43.3%)	Improve technical resources and facilities for transfusion reaction workup ($n = 125$) (50.4%)
Increased reporting of adverse transfusion reactions ($n = 124$) (42.9%)	Blood component quality check is performed on a regular basis ($n = 116$) (46.8%)
Reduction in near miss errors ($n = 88$) (30.5%)	Establishment of a bacterial screening facility for blood components ($n = 74$) (29.8%)
Modifications in blood and blood components for prevention of adverse transfusion reaction ($n = 77$) (26.6%)	100% donor antibody screening ($n = 81$) (32.7%)
Reduction of ABO incompatible transfusion reaction ($n = 58$) (20.1%)	100% antibody screening testing on patients ($n = 66$) (26.6%)
Reduction of septic transfusion reactions ($n = 46$) (15.9%)	From manual cross-matching to automated cross-matching ($n = 57$) (23%)
Recruitment/designation of specialized haemovigilance nurse ($n = 27$) (9.3%)	
Others (*quality assurance department and director BTS, implement blood safety officer post, improvement in donor vigilance, most of practices already in place and training of hospital staff on ATR*) ($n = 5$) (1.7%)	Others (*better documentation, practices /policies already in place, revised transfusion reaction forms, increased awareness, temperature and times monitoring*, etc.) ($n = 27$) (10.9%)

Abbreviations: ATR, adverse transfusion reactions; BTS, blood transfusion services; HvPI, Haemovigilance Programme of India.

TABLE 3 Steps taken for the prevention of adverse transfusion reactions after implementation of recipient HvPI.

S. no	Steps taken	Participant responses
A.	Prevention of pulmonary complications ($n = 289$)	
1.	Refrain from utilizing multiparous female plasma for transfusions	138 (47.8%)
2.	Restrictive transfusion policy for high-risk patients	83 (28.7%)
3.	Advice diuretics for high-risk patients	40 (13.8%)
4.	Start using paediatric bag aliquots for small volume patients	63 (21.8%)
5.	Others (use of inline filter, use of PAS, plasma reduction in apheresis platelets)	22 (7.6%)
6.	None of the above	78 (29.9%)
B.	Prevention of febrile complications ($n = 289$)	
1.	Start using leukoreduced blood components	130 (45%)
2.	Advise premeditations to high-risk patients	81 (28%)
3.	Start bacterial screening facility for monthly quality control	108 (37.4%)
4.	Increase frequency in cleaning of the blood storage area	120 (41.5%)
5.	Others (improve documentation, pre-transfusion vital check, education of staff)	20 (6.9%)
6.	None of the above	53 (18.3%)
C.	Prevention of haemolytic transfusion reaction ($n = 289$)	
1.	Start donor antibody screening facility	113 (39.1%)
2.	Start patient antibody screening facility	94 (32.5%)
3.	More vigilance for near miss events	141 (48.8%)
4.	Improve donor and patient identification system	161 (55.7%)
5.	Clinicians are being trained to prevent incorrect storage, warming or transfusion of blood components, which can result in non-immune haemolysis	159 (55%)
6.	Improve bedside transfusion practices	155 (53.6%)
7.	Others (implementation of RFID system, barcode-labelled boxes, buffycoat removal)	14 (4.8%)
8.	None of the above	29 (10%)
D.	Prevention of the allergic or anaphylactic reactions ($n = 289$)	
1.	Advise premedication for high-risk patients	185 (64%)
2.	Issue of washed red cells or platelet components	59 (20.4%)
3.	Advise to use plasma alternatives in large volume transfusion	93 (32.2%)
4.	Others (PAS in SDAP, close monitoring of transfusion)	24 (8.3%)
5.	None of the above	12 (4.2%)

Abbreviations: HvPI, Haemovigilance Programme of India; PAS, platelet additive solution; RFID, radiofrequency identifications; SDAP, single-donor apheresis platelets.

We have also observed that blood centres took appropriate steps to reduce the adverse reactions. Numerous haemovigilance programmes on a global scale have already recommended avoiding the utilization of plasma multiparous women to prevent transfusion-related acute lung injury (TRALI) [10]. Nearly half of respondents stopped using multiparous female plasma for transfusion. Almost 30% of respondents adopted a restrictive transfusion strategy to prevent transfusion-associated circulatory overload (TACO), as a previous study has shown that restrictive transfusion policies lead to comparable clinical outcomes when compared with liberal policies, with the added benefit of causing less volume overload in recipients [11].

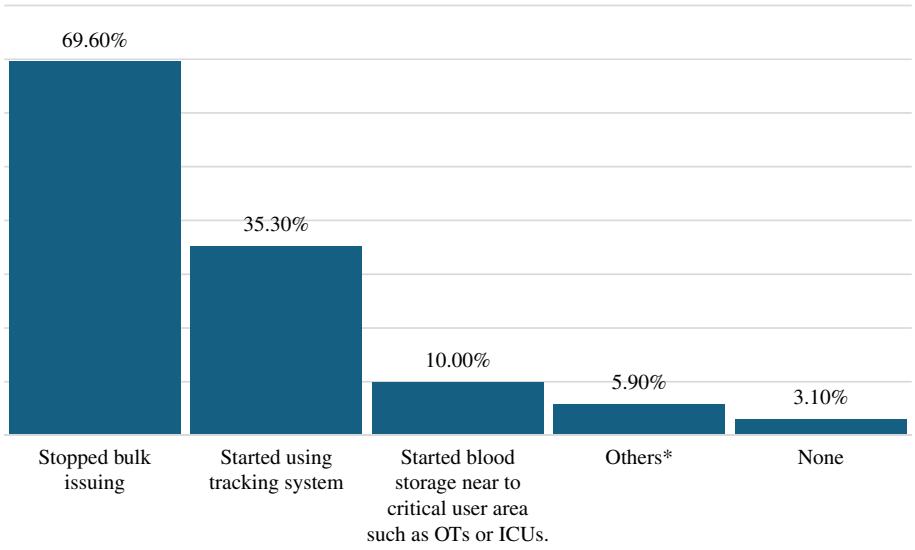

FIGURE 2 Steps taken to reduce the time gap between the issue and transfusion of blood component. *Others: Practice already in place, clinician education, random clinical audits training of staff. ICUs, intensive care units; OTs, operation theatres.

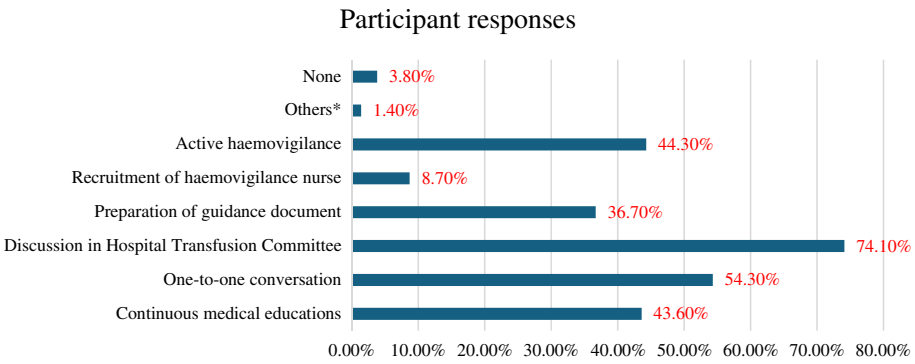

FIGURE 3 Measures taken for motivation of the clinicians to improve reporting of adverse reactions. *Others: arranging lectures, formation of hospital haemovigilance committee and mandatory fill-up of transfusion reaction form.

Our study found that the implementation of HvPI led to improved identification systems, increased vigilance for near-miss errors, enhanced immune-haematological practices and reduced non-immune causes for the prevention of haemolytic transfusion reactions. Blood centres achieved this using manual or automated identification methods, pre-transfusion antibody screening, staff education on proper storage and warming of blood components and safe transfusion practices.

Allergic or anaphylactic reactions arise from allergens, specifically protein contents in the blood or blood components, that the recipient is allergic to [12]. On the findings of previous HvPI reports, 64% blood centres advised recipients to be pre-medicated to prevent allergic or anaphylactic reactions caused by protein contents in the blood and blood components. Additionally, 8.3% employ modern solutions such as plasma additive solutions.

Encouraging clinicians to report adverse reactions to the blood centre is the most critical aspect of haemovigilance programme implementation and administration [13]. In our study, we found that blood centres used tailored approaches such as organizing continous medical education (CME), one-on-one conversations, discussions in hospital transfusion committees, preparing guidance documents, recruiting haemovigilance nurses and actively monitoring each transfusion.

Several valuable suggestions were made by participants for improving the country's haemovigilance programme in the future. These included continuing education or certificate courses for clinicians and blood centre personnel, recruitment of a haemovigilance nurse and initiate an active haemovigilance programme. Some responders suggested mandating adverse reaction reporting in HvPI and implementing additional programmes for reporting stem cell donation adverse reactions and haemovigilance in blood storage centres and district-level hospitals. Others suggested defining adverse reactions objectively and collecting data online for cross-comparison between different blood centres.

We have also observed various appropriate improvements in blood centres to reduce the adverse reactions in blood donor. Adverse reactions in donors discourage future giving, especially among young

TABLE 4 Measures taken by respondents for reduction of donor adverse reactions after implementation of NBDVP.

S. no.	Changes	Participant responses ($n = 250$)
1.	More stringent donor medical questionnaire	170 (68%)
2.	Make the blood donation centre environment more pleasant for donors	160 (64%)
3.	Donor anxiety reduction measures, that is, multimedia visuals measure, appointment of counsellor for bedside counselling of donor etc.	141 (56.4%)
4.	Implementation of prevention strategies such as pre-donation water intake, applied muscle tension or both	163 (65.2%)
5.	Special care to donors who had already history of adverse reaction in previous donation	171 (68.4%)
6.	Increased and regular training of the staff for phlebotomy techniques	169 (67.6%)
7.	Improve post-donation care and vigilance for at least up to 30 min from donation	157 (62.8%)
8.	Early identification and management of first reaction to prevent secondary reaction or injury	139 (55.6%)
9.	None	3 (1.2%)
10.	Others[a]	5 (2%)

Abbreviation: NBDVP, National Blood Donor Vigilance Programme.
[a]Others: Already in practice.

and first-time donors [14]. Almost 90% of respondents reported policy changes from their participation in the NBDVP. Strategies to reduce adverse reactions include strict medical questionnaires, donor centre improvements, prevention techniques and better aftercare [15]. Blood centres also offer extra care for donors with past reactions and expanded staff training.

Almost 75% of respondents used NBDVP statistics to change protocols and reduce donor adverse reactions. Regular publishing of haemovigilance data is critical for exchanging ideas and experiences. Almost 97% of respondents want an investigation into the delayed or long-term effects of blood donation. One-third initiated post-donation follow-up to track delayed reactions, and 80% are aware of donor adverse reaction severity grading tools. Respondents suggested improving the NBDVP by focusing on apheresis, disseminating guidelines based on data, implementing a severity grading tool and improving reporting forms and CME for participating centres.

Haemovigilance has shown significant improvements over time, revealing gaps in understanding of transfusion-related adverse events and prompting enhanced donor health selection and screening protocols [16,17]. Data from our study suggested priority topics for future research and emphasized the importance of rigorous clinical decision-making to achieve the best outcomes for patients. Based on the suggestions by participants. we will plan to increase the clinician's awareness by CME or personal interventions. We will also plan to increase international collaboration for universalising the adverse reactions definitions and imputability criteria. We will also increase the enrolment of the blood centres in HvPI, by creating more awareness and trying to make it mandatory with the regulatory interventions.

Our study had limitations that may have affected the generalizability of our findings. Email as a distribution method and unclear qualifications of respondents may have influenced the results. Subjective bias due to complex phrasing, as well as selection, recall and desirability bias, may have also played a role. Additionally, incomplete responses from some respondents resulted in varying denominators for different calculations. Although many centres were enrolled, only 733 were actively reporting to the nodal centre at the time of the study. Therefore, the survey was sent only to these actively participating members. Although the programme was implemented a decade ago, initial participation was limited due to a lack of awareness. As most participants joined during these recent years, this may slightly skew the results, leading to a one-sided distribution curve.

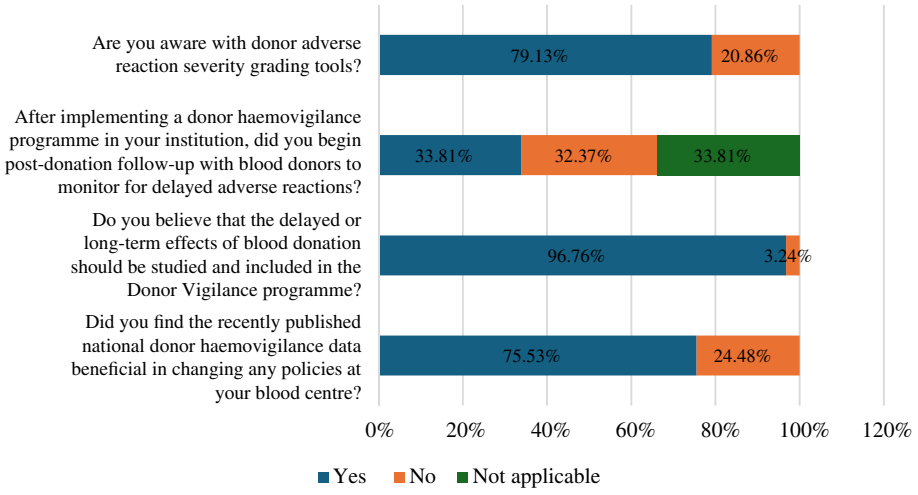

FIGURE 4 Various responses.

In conclusion, our haemovigilance programme facilitates national collaboration for learning and sharing experiences, leading to improved policies and practices in reducing adverse reactions for both recipients and donors. Survey findings indicate about blood centres efforts to improve awareness and reporting of transfusion-related reactions among clinicians. Respondents suggest mandatory haemovigilance programme, certificate courses and inclusion of missing fields like delayed reactions. Blood centres aim to raise awareness among clinicians and recruit haemovigilance nurses. Hence evaluation of our national haemovigilance programme and reporting centres feedback has made a positive impact both in the blood centres and future expansion policy at the national level.

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N.M. conceptualized the study; A.B. distributed the survey questionnaires to blood centres; G.K.P. and S.A. analysed the data; G.K.P. drafted the initial manuscript; and all authors contributed to reviewing and finalizing the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data for this study are held by the corresponding author and can be made available upon request. If deemed necessary, the data will be submitted to the journal for review.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Autoantibodies to ADAMTS13 in human immunodeficiency virus-associated thrombotic thrombocytopenic purpura

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Abstract

Background and Objectives: Thrombotic thrombocytopenic purpura (TTP) is a potentially fatal thrombotic microangiopathic disorder that can result from human immunodeficiency virus (HIV) infection. The pathogenesis involves a deficiency of the von Willebrand factor (vWF) cleaving protease ADAMTS13 (a disintegrin and metalloprotease with thrombospondin motifs member 13) and the presence of anti-ADAMTS13 autoantibodies. However, there is insufficient information regarding the epitope specificity and reactivity of these autoantibodies. This study aimed to perform epitope-mapping analysis to provide novel insights into the specific epitopes on ADAMTS13 domains affected by autoantibodies.

Materials and Methods: The study analysed 59 frozen citrate plasma samples from HIV-associated TTP patients in South Africa, measuring ADAMTS13 activity using Technozyme® ADAMTS13 activity test, total immunoglobulin (Ig) M and IgA antibodies levels using ELISA kit and purifying IgG antibodies using NAb™ Protein G spin columns. A synthetic ADAMTS13 peptide library was used for epitope mapping.

Results: Overall, 90% of samples showed anti-ADAMTS13 IgG autoantibodies, with 64% of these antibodies being inhibitory, as revealed by mixing studies. Samples with ADAMTS13 antigen levels below 5% showed high anti-ADAMTS13 IgG autoantibody titres (≥50 IU/mL), whereas those with 5%–10% levels had low autoantibody titres (<50 IU/mL).The metalloprotease, cysteine-rich and spacer domains were 100% involved in binding anti-ADAMTS13 IgG antibodies, with 58% of samples containing antibodies binding to the C-terminal part of the ADAMTS13 disintegrin-like domain, indicating different pathogenic mechanisms.

Conclusion: The metalloprotease, cysteine-rich and spacer domains are the primary targets for anti-ADAMTS13 IgG autoantibodies in patients with HIV-associated TTP. These findings suggest potential effects on the proteolytic activity of ADAMTS13, highlighting the complex nature of the pathogenic mechanisms involved.

Keywords
ADAMTS13, autoantibodies, HIV, thrombotic thrombocytopenic purpura

Highlights

- The metalloprotease, cysteine-rich and spacer domains of ADAMTS13 (a disintegrin and metalloprotease with thrombospondin motifs member 13) were constantly (100%) involved in binding anti-ADAMTS13 immunoglobulin (Ig) G antibodies in human immunodeficiency virus (HIV)-associated thrombotic thrombocytopenic purpura (TTP).
- All HIV-associated TTP patients showed IgG autoantibody binding to amino acid residues 645–684 from the spacer domain, suggesting an epitope area with the amino acid sequence 'QEDADIQVYRRYGEEYGNLTRPDITFTYFQ' at positions 650–669.
- Anti-ADAMTS13 IgG antibodies were found in 90% of patients with HIV-associated TTP (53/59), whereas anti-ADAMTS13 IgM antibodies were found in 30% of HIV-associated TTP patients and 64% contained anti-ADAMTS13 IgA antibodies.

INTRODUCTION

Thrombotic thrombocytopenic purpura (TTP), a rare but severe haematologic disease, is a member of a closely related group of disorders, thrombotic microangiopathies (TMA). TMA is a group of disease that has microangiopathic haemolytic anaemia and thrombocytopenia due to the formation of microvascular platelet rich thrombi, which causes ischaemic organ dysfunction such as reduction in kidney function and neurological symptoms. TTP is a prevalent systemic TTP that significantly affects the central nervous system and kidneys, although to a lesser extent [1]. Recent investigations reveal that one of the major abnormalities in chronic relapsing TTP is the absence function of a metalloproteinase enzyme, ADAMTS13 (a disintegrin and metalloprotease with thrombospondin motifs member 13). This enzyme controls the generation of specific large forms of von Willebrand factor (vWF) known as ultra-large vWF multimers (UL-vWF), which interacts with platelets for haemostasis. Defects or deficiencies (<10%) of ADAMTS13 leads to the accumulation of UL-vWF multimers in the circulation, eventually forming vWF-platelet-rich thrombi under high shear stress conditions manifesting phenotypically as TTP [2–5]. Autoantibodies to ADAMTS13 can either inhibit or increase the protease's clearance from the circulation, binding to various protease domains, with the cys-rich/spacer domain being consistently active. A study on patients with acquired TTP found anti-spacer autoantibodies target three hotspot areas [6–8].

The characteristically described forms of TTP are rare but a similar condition is now frequently observed in patients infected with the human immunodeficiency virus (HIV) in Sub-Saharan Africa since the 1980s, the country with the highest HIV infection incidence and also high HIV-associated with TTP [9, 10]. Recurrent episodes have been identified in HIV-related TTP at a rate of up to 60% and a mortality rate of between 10% and 30%, which is high, compared with non-HIV TTP patients. These high mortality rates can be explained by situations such as diagnostic uncontrollable, inability to identify patients at risk and inadequate resources. Hence, diagnostic and prognostic biomarkers need to be established in HIV-associated TTP [11, 12]. TTP is seen in acquired immunodeficiency syndrome patients with a low helper T cells (CD4+) count (<200 cells/μL) and high viral loads, and the incidence of HIV-associated TTP was expected to decline with widespread access to anti-retroviral therapy (ART). However, cases of TTP in HIV infection are still prevalent in South Africa, despite increased access to ART [13–15]. Recently, TTP is being observed even in HIV infected patients with viral loads below the detectable limit on ART, but the exact primary pathogenesis is not clear [16, 17]. The transmission of HIV-associated TTP is prospective linked to various mechanisms related to the viral infection. The HIV endothelial cell dysfunction has been considered as important in the pathogenesis of HIV-associated TTP. Although some studies suggested that endothelial dysfunction may not be the primary cause of TTP, rather that vascular agitation may be the consequence of TTP. The autoimmune dysfunction with autoantibody production and abnormal T-cell responses may contribute significantly to the reduction of ADAMTS13 in HIV-associated TTP. HIV infection with a low CD4+ lymphocyte count and a high viral load are associated with an increased incidence of ADAMTS13 autoantibodies [18, 19]. Furthermore, the presence of ADAMTS13 autoantibodies may contribute to severe ADAMTS13 deficiency and trigger HIV-associated TTP. Several studies have confirmed the importance of autoantibodies to ADAMTS13 in the pathogenesis of HIV-associated TTP. In some HIV-associated TTP cases, acquired ADAMTS13 deficiency may occur in the absence of detectable autoantibodies/ autoantibodies that inhibit ADAMTS13 [20]. Even though many reports are discussed in autoantibodies to ADAMTS13 in HIV-associated TTP as well as in HIV infected people without TTP, but the binding specificity of these autoantibodies however remains unknown. The detection of ADAMTS13 autoantibodies and defining their epitopes on the ADAMTS13 protein in HIV-associated TTP patients may be of clinical value with disease prognostication and treatment efficacy assessment.

METHODS AND MATERIALS

HIV-associated TTP plasma samples

A study in South Africa examined 59 frozen, anonymized citrate plasma samples from HIV-associated TTP patients. The samples were collected from across the country and sent to the Specialized Haemostasis laboratory at the University of the Free State. The samples were collected before treatment, and it is stored at −80°C for at least a year for further research. The study received ethical approval from the University of the Free State's Health Science reserach Ethics Committee (UFS-HSD2019/0027/3007). The study involved National Health Laboratory Service samples, identified by unique laboratory numbers, de-identified using a double-blind technique and given random research numbers. The research was conducted with permission from the Free State Department of Health Provincial Research Committee and blood samples from the National Health Laboratory Service (FS-201903-005).

Mixing study

The study aimed to identify neutralizing immunoglobulin (Ig) G autoantibodies to ADAMTS13 in HIV-associated TTP plasma samples with ADAMTS13 activity levels below 10%. The Technozyme® ADAMTS13 activity test was used to measure ADAMTS13 activity in samples and pooled normal plasma (PNP). The results showed normal ADAMTS13 activity levels in PNP, ranging from 50% to 150%. However, no correction was shown in the mixing test, indicating the presence of an inhibitor, resulting in less than 50% ADAMTS13 activity.

The Bethesda technique was used to measure the potency of neutralizing anti-ADAMTS13 antibodies. A Bethesda unit (BU) is the concentration of an inhibitor in plasma that reduces ADAMTS13 activity by 50% in PNP. Residual activity of 25%–75% indicates an inhibitor, whereas over 75% activity indicates the absence of a clinically significant inhibitor. The strength of neutralizing anti-ADAMTS13 antibodies was evaluated using a modified Bethesda test [21].

The study utilized HIV-associated TTP plasma samples treated at 56°C for an hour to remove endogenous ADAMTS13 activity. Samples with strong inhibitors were diluted with saline and PNP and incubated at 25°C for 2 h; PNP served as a negative control. ADAMTS13 activity was measured using the Technozyme® ADAMTS13 activity assay.

Total IgM and IgA antibodies

The study examined IgM and IgA antibodies in samples with a positive titre of anti-ADAMTS13 IgG antibodies. Total plasma Ig levels were measured using a commercially available ELISA kit from Bethyl Laboratories. The test was conducted in two separate studies using ELISA 96-well plates pre-coated with either IgM or IgA anti-human antibodies. The standard and samples was diluted using dilution buffer according to the manufacturer's instructions. The study involved duplicate wells with standard and sample solutions, incubating them at room temperature for an hour, washing them four times with wash buffer, adding anti-human IgM/IgA detection antibody, incubating for 1 h, adding horseradish peroxidase (HRP) solution and adding tetramethylbenzidine substrate solution. The reaction was stopped with adding stop solution, and the absorbance was measured at 450 nm using a microplate reader. The manufacturer estimated IgM and IgA concentrations in samples using an extrapolated standard curve, with representative reference ranges for healthy individuals for IgA is 1.1–2.6 mg/mL and for IgM is 0.23–1.4 mg/mL in sodium citrate plasma [6].

Extraction of IgG autoantibodies

The study purified total IgG antibodies from 53 HIV-associated TTP plasma samples using NAb™ Protein G spin columns. Positive anti-ADAMTS13 IgG antibody titres were used for analysis. Protein content and purity of eluted IgG fractions were measured using a BioDrop spectrophotometer. Fractions with an absorbance ratio of less than 0.6 showed no nucleic acid contamination. The purified antibodies were dialyzed in phosphate buffered saline (PBS) to eliminate low molecular weight compounds and salt contamination. The eluted pure IgG protein was pooled and stored at 4°C. The absorbance of the samples were measured at 260/280.

Epitope mapping studies of anti-ADAMTS13 IgG antibodies

Synthetic peptides

GenScript™ has developed a synthetic peptide library from the ADAMTS13 protein, containing 105 biotinylated peptides. The library was screened for linear B-cell epitopes using an ELISA-based method. The peptide library includes domains influencing ADAMTS13 function and vWF binding under static conditions. The N terminus of the synthesized peptides was biotinylated. To minimize costs, the library included domains that significantly contribute to ADAMTS13 function and vWF binding under static conditions. The peptides were designed in a 20-mer/15-mer overlapping format with an offset of 5 amino acids (Figure 1). The peptides were extracted as a lyophilized powder with over 75% purity and stored at −20°C. Peptide names were derived from domain names, and the amino acid locations were compared to the full-length ADAMTS13 protein coding region (Table 1).

Developing a peptide ELISA

A peptide ELISA was developed using artificial peptides from a library and a monoclonal antibody specific to the Fc-region of the

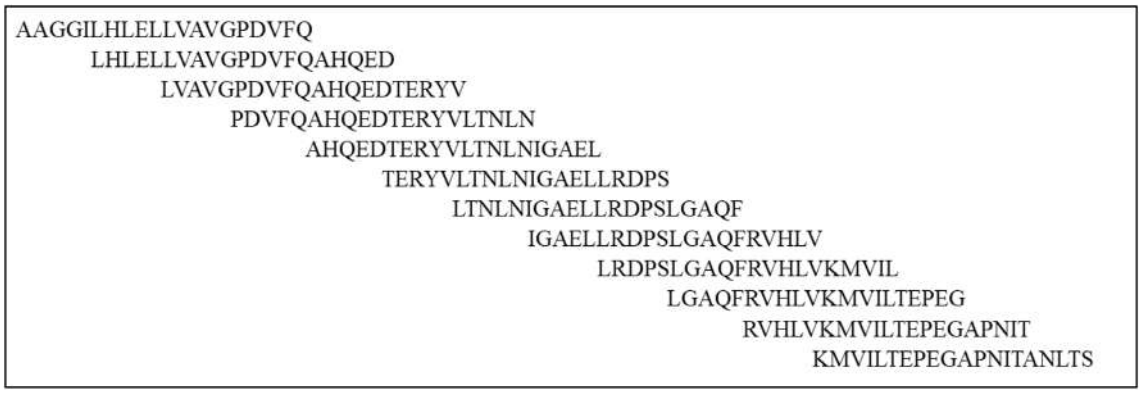

FIGURE 1 Overlapping linear peptide sequences from the metalloprotease domain 75–150. The designed peptides are 20 amino acids long with 15 overlapping amino acids and an offset of 5 amino acids.

TABLE 1 The ADAMTS13 domain groupings selected for designing a peptide library.

Domain grouping	Position in ADAMTS13 amino acid sequence	Structure–function
Metalloprotease–disintegrin domains	75–383	Catalytic domains.
Cysteine-rich spacer domains	440–680	Critical role in substrate recognition and binding, promoting proteolysis of vWF by ADAMTS13

Abbreviations: ADAMTS, a disintegrin and metalloprotease with thrombospondin motifs member 13; vWF, von Willebrand factor.

TABLE 2 Laboratory inclusion criteria for HIV-associated TTP diagnosis.

Laboratory test (units)	Laboratory findings: Normal values/ranges	Laboratory findings: HIV–TTP patients
Full blood count		
Schistocytes on morphological examination of slide	Absent	Present
Platelet count (10^9/L)	150–450	<100
Haemoglobin (g/dL)	12.1–17.2	<12.1
Lactate dehydrogenase (LDH) (U/L)	100–190	Elevated and frequently >800
Creatinine (μg/L)	49–90	>90
Coombs test	Negative	Negative
ADAMTS13 antigen levels (%)	50–150	Usually <5
ADAMTS13 activity (%)	50–150	Usually <10
ADAMTS13 autoantibodies	Negative	Positive
HIV status	Negative	Positive
Anti-retroviral therapy (ART) at presentation	N/A	Either ART naïve or on ART therapy
Helper T cells (CD4+ count, cells/mm^3)	500–1500	219.372

Abbreviations: ADAMTS, a disintegrin and metalloprotease with thrombospondin motifs member 13; HIV, human immunodeficiency virus; TTP, thrombotic thrombocytopenic purpura.

anti-human IgG antibody. Overall, 53 HIV-associated TTP plasma samples were used to generate purified ADAMTS13 IgG autoantibodies, which were used to identify potential epitope sites.

Peptide ELISA

Epitope mapping studies were conducted on 105 overlapping biotinylated peptides in a peptide library to monitor binding events in each patient's purified IgG sample using Peptide ELISA, with all synthetic peptides tested individually, with a purity of over 75%.

A 96-well ELISA plate first pre-coated with 100 μL/well of streptavidin (200 ng/mL, GenScript®, USA) was diluted in PBS buffer and incubated overnight at 4°C, and the plates were washed four times using washing buffer (PBS/0.05% Tween-20 [pH 7.4]). After washing, the plates were blocked with 200 μL/well of blocking buffer for 2 h at 37°C. After incubation, the plates were washed again and 100 μL/well of each diluted peptide was added to the precoated plate and incubated at 37°C for 2 h. After incubation, the plates were washed. Then the plates were blocked again with 200 μL/well of blocking buffer for 2 h at 37°C. Following another washing step, 100 μL/well of the purified IgG antibody from each patient was diluted in duplicate to each plate. The plates were incubated for 1 h at 37°C and then washed again. Then, 100 μL/well of an HRP-conjugated monoclonal anti-human IgG antibody (Abcam®) was added for detection. The plates were incubated for 1 h at room temperature, followed by another wash. Finally, 100 μL/well of the O-phenylenediamine dihydrochloride substrate was added and incubated for 10 min at room temperature; thereafter 30 μL/well stop solution (4 M H_2SO_4) was added to stop the reaction, and the absorbance was measured at 490–630 nm.

The study involved subtracting the mean optical density at 490 nm (OD_{490}) value of two blanks from all other OD_{490} values, with

TABLE 3 The concentration of anti-ADAMTS13 IgG antibody in HIV-associated TTP plasma samples.

Anti-ADAMTS13 IgG antibodies	Number of samples	Median anti-ADAMTS13 IgG antibody titre (μg/mL)	Median Bethesda unit (BU/mL)
Non-inhibitory	17/53	26 (17–223)	<0.5
Low inhibition <5 BU	17/53	42 (18–86)	1.85 (0.64–4.54)
Strong inhibition >5 BU	19/53	96 (32–175)	9.74 (5.10–17.92)

Note: Results are expressed as mean ± SD, $p < 0.05$; median anti-ADAMTS13 IgG antibodies concentration units are expressed as μg/mL and median Bethesda unit is expressed as BU/mL.
Abbreviations: ADAMTS, a disintegrin and metalloprotease with thrombospondin motifs member 13; HIV, human immunodeficiency virus; Ig, immunoglobulin; TTP, thrombotic thrombocytopenic purpura.

each plate having a negative control. Samples with OD_{490} values greater than the cut-off value were considered positive binding to the peptide, whereas those with OD_{490} values less than the cut-off value were considered no binding to the respective peptide.

Data analysis

The data were assessed using GraphPad Prism software version 6. Statistical analysis involved the application of a student-*T* test, and a *p*-value of <0.05 was deemed to indicate statistical significance.

RESULTS

Laboratory inclusion criteria for HIV-associated TTP diagnosis

The Table 2 shows that the diagnostic criteria for HIV-associated TTP include laboratory findings of HIV infection, thrombocytopenia, microangiopathic haemolytic anaemia and elevated serum lactate dehydrogenase levels. A creatinine test was also used to assess renal function, with renal impairment considered when creatinine levels were above 90.0 μg/L.

Anti-ADAMTS13 IgG antibody concentration and Bethesda inhibitory (BU) activity were measured in HIV-associated TTP plasma samples

Mixing tests were conducted on HIV-associated TTP patient samples with ADAMTS13 activity below 10% and positive anti-ADAMTS13 IgG antibody titre. Overall, 53 samples showed inhibitory anti-ADAMTS13 IgG antibodies, with non-inhibitory 17, low inhibition 17 and high inhibition 19, respectively. Table 3 shows concentrations and Bethesda Units (BU) for non-inhibitory, mild inhibitory and high inhibitory of autoantibody titres.

TABLE 4 Determination of total IgM and IgA in HIV-associated TTP plasma samples.

Parameter	HIV-associated TTP ($n = 53$)
Median IgM level (ranges) Mean ± SD	1.6 (1.05–2.35) mg/mL 1.59 ± 0.27
Median IgA level (ranges) Mean ± SD	1.85 (1.06–2.98) mg/mL 2.10 ± 0.60

Note: Results are expressed as mean ± SD, $p < 0.05$, IgM and IgA antibodies concentration expressed as mg/mL.
Abbreviations: HIV, human immunodeficiency virus; Ig, immunoglobulin; *n*, number of samples; TTP, thrombotic thrombocytopenic purpura.

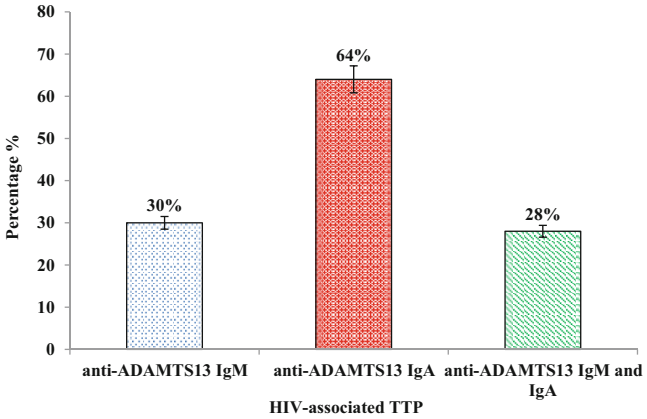

FIGURE 2 Percentage of human immunodeficiency virus (HIV)-associated thrombotic thrombocytopenic purpura (TTP) patients with positive anti-ADAMTS13 (a disintegrin and metalloprotease with thrombospondin motifs member 13) immunoglobulin (Ig) M, IgA and both IgM and IgA autoantibody levels: Results are expressed as percentage (%).

Determination of total IgM and IgA in HIV-associated TTP plasma samples

Table 4 shows the total IgM and IgA antibody concentrations in HIV-associated TTP patient (53) samples.

Determination of anti-ADAMTS13 IgM and IgA antibodies

Figure 2 shows the percentage of patients with anti-ADAMTS13 IgM and IgA antibodies in HIV-associated TTP plasma samples.

TABLE 5 ADAMTS13 domains with reactivity towards IgG antibodies of individual HIV-associated TTP plasma samples.

ADAMTS13 domains	HIV-associated TTP plasma ($n = 53$)
Metalloprotease domain only	22/53 (42%)
Disintegrin-like domain only	0/53 (0%)
Both metalloprotease and disintegrin-like domains together	31/53 (58%)
Cysteine-rich domain only	0/53 (0%)
Spacer domain only	1/53 (2%)
Both cistein-rich and spacer domains together	52/53 (98%)
All four ADAMTS13 domains together	31/53 (78%)

Note: All results units are expressed as percentages (%).
Abbreviations: ADAMTS, a disintegrin and metalloprotease with thrombospondin motifs member 13; HIV, human immunodeficiency virus; Ig, immunoglobulin; *n*, number of samples; TTP, thrombotic thrombocytopenic purpura.

Binding of purified IgG antibodies to linear overlapping ADAMTS13 peptides using peptide ELISA

Table 5 reveals that 94% of HIV-associated TTP patient plasma samples have IgG autoantibodies that bind to linear peptides from all four ADAMTS13 proximal domains. In total, 42% of samples have IgG autoantibodies bound to the metalloprotease domain, whereas 58% are bound to both metalloprotease and disintegrin-like domains. None respond solely to the disintegrin-like domain, but 98% respond to cysteine-rich and spacer domains.

Figure 3 shows the percentage of HIV-associated TTP samples with ADAMTS domain-specific autoantibodies, with ADAMTS13 metalloprotease, disintegrin-likes, cysteine-rich and spacer domains as predominant antibody binding targets. Table 6 summarizes immunoglobulin IgG autoantibody binding data and linear ADAMTS13 peptide epitopes.

Table 7 shows potential antigenic regions in metalloprotease and disintegrin-like domains, while Table 8 shows potential antigenic regions in cysteine-rich and spacer domains.

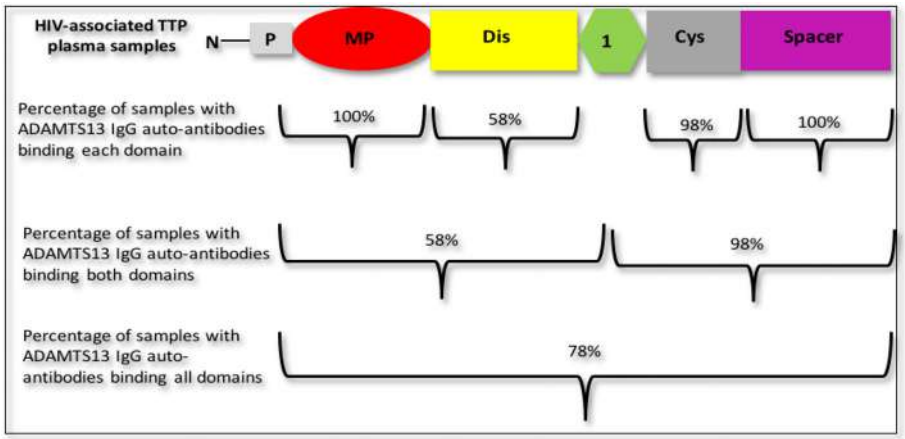

FIGURE 3 Percentage of human immunodeficiency virus (HIV)-associated thrombotic thrombocytopenic purpura (TTP) samples with ADAMTS13 (a disintegrin and metalloprotease with thrombospondin motifs member 13) domain-specific autoantibodies: Cys, cysteine-rich domain; Dis, disintegrin-like domain; MP, metalloprotease domain; N, N-terminal side; P, propeptide; Spacer, Spacer domain; TSP1, thrombospondin motif 1.

TABLE 6 Shared and non-shared linear ADAMTS13 peptide epitope regions that bind to IgG autoantibodies isolated from HIV-associated TTP patients.

Binding domains	Metalloprotease domain aa epitope regions	Disintegrin-link domain aa epitope regions	Cysteine-rich domain aa epitope regions	Spacer domain amino aa regions
Shared epitope regions	75–80	169–189	445–479	595–619
	125–139	320–345	455–469	625–649
	200–224	350–379	475–504	650–669
	260–284	340–379		
Non-shared epitope regions	80–124	275–304	505–529	560–586
	220–244	290–324	540–564	
	240–26	355–376		
		365–374		

Abbreviations: aa, amino acid; ADAMTS, a disintegrin and metalloprotease with thrombospondin motifs member 13; HIV, human immunodeficiency virus; Ig, immunoglobulin; *n*, number of samples; TTP, thrombotic thrombocytopenic purpura.

TABLE 7 Linear peptides with potential antigenic regions in the metalloprotease (MP) and disintegrin (Dis)-like domains.

Peptide name	Peptide sequence	Position	HIV-associated TTP patient group ($n = 53$)
MP1	AAGGILHLELLVAVGPDVFQ	75–94	53/53 (100%)
MP1–MP8	LHLELLVAVGPDVFQAHQEDTERYVLTNLNIGAELLR DPSLGAQFRVHLV	80–129	0/53
MP9–MP10	LRDPSLGAQFRVHLVKMVILTEPEG	115–139	53/53 (100%)
MP9–MP12	LRDPSLGAQFRVHLVKMVILTEPEGAPNITANLTS	125–149	0/53
MP18–MP21	TINPEDDTDPGHADLVLYITRFDLELPDGNRQVRG	160–194	15/53 (28%)
MP19–MP20	DDTDPGHADLVLYITRFDLELPDGN	165–189	37/53 (70%)
MP19–MP21	DDTDPGHADLVLYITRFDLELPDGNRQVRG	165–194	4/53 (8%)
MP25–MP28	VTQLGGACSPTWSCLITEDTGFDLGGVTIAHEIGHS	195–229	10/53 (19%)
MP29–MP34	GFDLGVTIAHEIGHSFGLEHDGAPGSGCGPSGHVMASDGAAPRAG	215–249	0/53
MP33–MP36	DGAPGSGCGPSGHVMASDGAAPRAGLAWSPCSRRQ	235–269	3/53 (6%)
MP37–MP39	APRAGLAWSPCSRRQLLSLLSAGRARCVWD	255–284	26/53 (49%)
MP37–MP/Dis40	APRAGLAWSPCSRRQLLSLLSAGRARCVWDPPRPQ	255–289	25/53 (47%)
MP40–MP/Dis44	LLSLLSAGRARCVWDPPRPQPGSAGHPPDAQPGLY YSANE	270–304	0/53
MP/Dis41–Dis44	SAGRARCVWDPPRPQPGSAGHPPDAQPGLYYSANE	275–309	0/53
MP/Dis43–Dis46	PPRPQPGSAGHPPDAQPGLYYSANEQCRVAFGPKA	285–319	1/53 (2%)
Dis44–Dis47	PGSAGHPPDAQPGLYYSANEQCRVAFGPKAVACTF	290–324	2/53 (4%)
Dis45–Dis48	HPPDAQPGLYYSANEQCRVAFGPKAVACTFAREHL	295–334	1/53 (2%)
Dis49–Dis52	FGPKAVACTFAREHLDMCQALSCHTDPLDQSSCSR	315–349	4/53 (7%)
Dis49–Dis/TSP1 59	FGPKAVACTFAREHLDMCQALSCHTDPLDQSSCSR LLVPLDGTECCGVEKWCSKGRCRSLVELTPIAAVH	315–383	5/53 (9%)
Dis53–Dis58	LSCHTDPLDQSSCSRLLVPLLDGTECCGVEKWCSKGRCRSLVELTP	335–379	5/53 (9%)
Dis53–Dis/TSP1 59	LSCHTDPLDQSSCSRLLVPLDGTECCGVEKWCSKGRCRSLVELTPIAAVH	335–383	5/53 (9%)
Dis55–Dis/TSP1 59	SSCSRLLVPLLDGTECGVEKWCSKGRCRSLVELTP IAAVH	345–383	2/53 (4%)
Dis56–Dis/TSP1 59	LLVPLLDGTECGVEKWCSKGRCRSLVELTPIAAVH	350–383	2/53 (4%)
Dis57–Dis/TSP1 59	LDGTECGVEKWCSKGRCRSLVELTPIAAVH	355–383	1/53 (2%)
Dis58–Dis/TSP1 59	CGVEKWCSKGRCRSLVELTPIAAVH	360–383	253 (4%)
Dis/TSP1 59	WCSKGRCRSLVELTPIAAVH	365–33	11/53 (21%)

Note: Amino acid residues in the overlapping regions of antigenic peptides in red. Peptide names are derived from the relevant domain names and amino acid position relative to the coding region of full-length ADAMTS13 protein.
Abbreviations: ADAMTS, a disintegrin and metalloprotease with thrombospondin motifs member 13; HIV, human immunodeficiency virus; *n*, number of samples; TTP, thrombotic thrombocytopenic purpura.

DISCUSSION

TTP is a prevalent type of TTP in Sub-Saharan Africa; it is primarily caused by HIV infection. The ADAMTS13 protein plays a central role in the pathogenesis of acquired TTP, with autoantibodies targeting this enzyme often being the primary cause of severe ADAMTS13 deficiency in HIV-associated TTP [9]. Several studies have underscored the importance of measuring autoantibodies to ADAMTS13 in managing patients with TTP. However, the ADAMTS13 autoantibody status in HIV-associated TTP patients has not yet been fully investigated [20, 22].

The laboratory inclusion criteria used for this diagnosis are summarized from multiple published studies [23, 24]. TTP is a common condition in patients with advanced HIV disease the severe deficiency of ADAMTS13, lower platelet, haemoglobin, CD4+ T-cell count and high level of plasma lactate dehydrogenase (LDH) and creatinine levels

TABLE 8 Linear peptides with potential antigenic regions detected from the Cysteine-rich (Cys) and Spacer (Spa) domains.

Peptide name	Peptide sequence	Position	HIV-associated TTP group $n = 53$
Cys1–Cys4	KTQLEFMSQQCARTDGQPLRSSPGGASFYHWGAAV	440–474	2/53 (4%)
Cys3–Cys6	CARTDGQPLRSSPGGASFYHWGAAVPHSQGGDALCR	450–484	8/53 (15%)
Cys2–Cys5	FMSQQCARTDGQPLRSSPGGASFYHWGAAVPHSQG	445–479	23/53 (43%)
Cys3–Cys5	CARTDGQPLRSSPGGASFYHWGAAVPHSQG	450–479	6/53 (11%)
Cys3–Cys10	CARTDGQPLRSSPGGASFYHWGAAVPHSQGDALCR HMCRAIGESFIMKRGHMCRAIGESFIMKRGDSFLD	450–504	4/53 (8%)
Cys7–Cys10	WGAAVPHSQGDALCRHMCRAIGESFDALCRHMCR AIGESFIMKRGDSFLD	470–504	11/53 (21%)
Cys13–Cys16	DSFLDGTRCMPSGPREDGTLSLCVSGSCRTFGCDG	500–534	7/53 (13%)
Cys17–Cys/ Spa20	SLCVSGSCRTFGCDGRMDSQQVWDRFCQVCGGGDNST	520–554	29/53 (55%)
Cys18–Cys/ Spa20	GSCRTFGCDGRMDSQQVWDRCQVCGGDNST	525–554	1/53 (2%)
Cys13–Cys/ Spa20	DSFLDGTRCMPSGPREDGTLSLCVSGSCRTFGCDG RMDSQQVWDRCQVCGGDNST	500–554	5/53 (9%)
Spa24–Spa 28	CSPRKGSFTAGRAREYVTFLTVTPNLTSVYIANHRPLFTH	555–594	1/53 (2%)
Spa31–Spa 34	PLFTHLAVRIGGRYVVAGKMSISPNTTYPSLLEDG	590–624	40/53 (75%)
Spa31–Spa39	PLFTHLAVRIGGRYVVAGKMSISPNTTYPSLLEDGRVEYR VALTEDRLPRLEEIRIWGPL	590–649	2/53 (4%)
Spa37–Spa40	LLEDGRVEYRVALTEDRLPRLEEIRIWGPLQEDAD	620–654	27/53 (51%)
Spa42–Spa/ TSP2 46	IWGPLQEDADIQVYRRYGEEYGNLTRPDITFTYFQPKPRQ	645–684	53/53 (100%)

Note: Amino acid residues in the overlapping regions of antigenic peptides are highlighted in red. Peptide names are derived from the relevant domain names and the relevant amino acid position relative to the coding region of full-length ADAMTS13 protein.
Abbreviations: ADAMTS, a disintegrin and metalloprotease with thrombospondin motifs member 13; HIV, human immunodeficiency virus; *n*, number of samples; TTP, thrombotic thrombocytopenic purpura.

[25, 26]. This study also find similar representing severe deficiency of ADAMTS13 antigen and activity levels, lower platelet count, decreased haemoglobin, low CD4+ T-cell counts and elevated LDH and creatinine levels in HIV-associated TTP patients.

The frequency of autoantibodies in acquired TTP patients suggesting that an immune-mediated activity against ADAMTS13 is present in almost all patients presenting with HIV-associated TTP [22, 27]. Increased anti-ADAMTS13 IgG antibody titres are associated with poor prognosis in TTP patients [20]. In concurrence with the previous reports, in the current investigation, the anti-ADAMTS13 IgG concentration was assessed in 90% of the positive anti-ADAMTS13 IgG antibodies in this HIV-associated TTP (53/59) plasma, this indicates the immune-mediated activity against HIV-associated TTP patients.

This study also investigated the laboratory evidence of autoimmunity in HIV-associated TTP plasma samples by also measuring IgM and IgA titres to emphasize the relationship between the disease and the immunological parameters. A relationship between autoantibodies and CD4+ T-lymphocyte count has previously been documented [19, 23, 24]. It is reported that autoantibodies can trigger T-cell apoptosis by crosslinking Ig-related T-cell membrane molecules and envelope glycoprotein, a glycoprotein on the HIV envelope, resulting in CD4+ T-cell reduction with loss of integrity of the immune system [25, 26]. Emerging data demonstrate that the HIV-associated TTP plasma samples were detected with slightly increased plasma IgM and IgA antibodies and had a CD4+ count of less than normal ranges. HIV infection therefore prompts patients to autoimmune responses. Autoantibodies have prognostic significance in infectious diseases such as infections with HIV and have diagnostic value in HIV-associated TTP.

The IgG antibodies are primarily involved in the pathogenesis of other acquired forms of TTP by causing decreased ADAMTS13 protein in plasma, but these antibodies have not been characterized in acquired HIV-associated TTP. The selected ADAMTS13 proximal domains include the metalloprotease, disintegrin-like, cysteine-rich and spacer domains interact with unravelled vWF substrate and are necessary for the proteolytic activity of ADAMTS13. Furthermore, their activity towards vWF fragments under static conditions has been evaluated and representing the specific effects on ADAMTS13 activity [6, 27]. The results of the current study show that all IgG antibodies isolated from 53 HIV-associated TTP plasma samples had multiple binding sites on the four functional domains of ADAMTS13 probed. Our epitope-mapping studies indicated that anti-ADAMTS13 IgG antibodies identified similar immuno-dominant epitopes in the HIV-associated TTP group but additional binding sites were also identified in this group. We also observed that the cysteine-rich and spacer domains strong major binding sites in the HIV-associate TTP patients.

The propeptide metalloprotease domain is reported to function as a molecular safeguard of ADAMTS13 and does not affect the enzymatic action of the protein or its expression levels [28], and it also detected IgG antibodies binding the propeptide domain in 20% of acquired TTP plasma samples [7]. This study also identified the first immune-dominant epitope regions in the metalloprotease domain. All the HIV-associated TTP samples showed reactivity to the first peptide of the metalloprotease domain. The potential epitope region was identified and comprised of amino acids 'AAGGI' at position 75–80 in the full ADAMTS13 nucleotide sequence. These amino acid residues are located on the C-terminal part of the propeptide domain on the ADAMTS13 protein.

The metalloprotease domain regions contain the ADAMTS13 catalytic sequence as well as ADAMTS13 sub-sites, which are important for ADAMTS13 interaction with vWF and disintegrin-like domain has been reported to significantly increase the cleavage efficiency and specificity of ADAMTS13 [29]. In concurrence with the previous reports, we observed the disintegrin-like domain contains exocites at Arg349 and Leu350 residues that form weaker interactions with the unravelled vWF A2 domain residues at Asp1614 and Ala1612 close to the cleavage site. Thus, antibodies that bind to both the metalloprotease domain and the disintegrin-like domain may affect the ability of the ADAMTS13 protease to interact with the vWF substrate. Furthermore, the metalloprotease domain was identified as the most antigenic region when compared to the disintegrin-like domain in HIV-associated TTP group.

The cysteine-rich and spacer domains are constantly involved in antibody binding in patients with acquired TTP [7, 8, 30, 31]. The cysteine-rich and spacer domain have been found valuable for efficient in vivo vWF ADAMTS13 proteolysis. The spacer domain is essential for proteolysis of full-length vWF under flow conditions [29, 32, 33], and data in the present study also agree with the previous studies also found that 98% of HIV-associated TTP patient samples had IgG autoantibodies that bind to both the cysteine-rich as well as the spacer domains. This IgG autoantibodies that bind to these domains may interfere with ADAMTS13–vWF interaction.

Limitation of this study includes the overlapping peptide library derived from the ADAMTS13 protein, synthesized by GenScript (USA), consisting of 105 biotinylated peptides. These peptides were designed to identify linear B-cell epitopes using an ELISA-based method. Due to the high costs of peptide libraries for large proteins, only specific domains of ADAMTS13—the metalloprotease, disintegrin-like, cysteine-rich and spacer domains were selected, based on their functional significance and binding to vWF under static conditions.

In conclusion, this study showed that the ADAMTS13 proximal domains contain various epitope regions for anti-ADAMTS13 IgG autoantibody interaction in HIV-associated TTP patients. Therefore, it is evident that a polyclonal mixture of anti-ADAMTS13 IgG antibodies is present in HIV-associated TTP patients with similar binding patterns interacting with specific epitopes in the ADAMTS13 proximal domains. These include amino acid residues 125–139, 169–189, 200–224, 220–244 and 260–284 in the metalloprotease domain, 445–504 and 505–564 in the cysteine-rich domain and 650–669, 590–624 and 625–649 in the spacer domain. This study has improved our understanding of the immunological response potentially involved in HIV-associated TTP. This study also recommends screening for inhibitory anti-ADAMTS13 IgG autoantibodies to characterize the pathophysiology of HIV-associated TTP.

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M.M., M.K. and S.L. contributed to the project's design; M.M. and M.K. performed the overall experiments and analysed the data; M.M. and P.K. wrote the manuscript. All authors contributed to manuscript proofreading.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

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ORIGINAL ARTICLE

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Frequency of human platelet antigens (HPA) in the Greek population as deduced from the first registry of HPA-typed blood donors

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Abstract

Background and Objectives: Human platelet antigens (HPA) play a central role in foetal and neonatal alloimmune thrombocytopenia (FNAIT), post-transfusion purpura and some cases of platelet therapy refractoriness. The frequency distribution of HPA had not been studied in the Greek population before we started to create a registry of HPA-typed apheresis platelet donors. The aim of this study was the determination of the frequency of various HPA in the Greek population, through the establishment of a registry of typed donors.

Materials and Methods: Here, we report on the first 1000 platelet donors of Greek origin who gave informed consent and were genotyped for 12 pairs of antithetical HPA by Single Specific Primer-Polymerase Chain Reaction (SSP-PCR), including HPA-1, HPA-3, HPA-5 and HPA-15. Antigen frequencies are reported, and allele frequencies were calculated and compared with other European and non-European populations. Tested donors cover all ABO and Rhesus D antigen spectrum.

Results: Antigen and allele frequencies are very similar to other White populations. The frequency of HPA-1bb is 2.9% in our study, and the frequency of HPA-2b, HPA-4b, HPA-9b and HPA-15b is also slightly higher than in other literature reports, while the frequency of HPA-15b was found higher than that of HPA-15a.

Conclusion: We report antigen and allele frequencies for a large array of clinically significant HPA for the first time in the Greek population. Frequencies are consistent with other European populations. This registry of HPA-typed platelet donors, available to donate on demand, is an important asset for the treatment of FNAIT cases in Greece.

Keywords

alloimmune thrombocytopenia, donor registry, FNAIT, HPA frequency distribution, HPA genotyping, platelet antigens

Highlights

- This is the first study, to the best of our knowledge, investigating antigen and allele frequencies of human platelet antigens (HPA) in the Greek population.

- A registry of HPA-typed apheresis platelet donors was created in Greece, in order to assist in the timely provision of platelets in cases of neonatal alloimmune thrombocytopenia and refractoriness to platelet transfusion therapy.
- The allele frequencies found in this registry are similar to other European populations; however, a slightly higher frequency of HPA-1bb could indicate an increased risk of foetal and neonatal alloimmune thrombocytopenia cases in Greece due to HPA-1a alloimmunization.

INTRODUCTION

Human platelet antigens (HPA) are specific antigens found on the surface of platelets. They are formed as a result of various single-nucleotide variations (SNV) in genes that encode proteins found on platelet membranes [1]. According to the established nomenclature, all human platelet antigens identified to date are numbered and organized into 35 biallelic systems of antithetical antigens (HPA-1 to HPA-35), in which the high-frequency antigen is designated by the superscript 'a' and the low frequency antigen with the superscript 'b' [2]. In case an alloantibody against the antithetical antigen has not been reported, a 'w' designation is added after the antigen name [3–7].

Exposure to alloantigens, mainly through pregnancy or transfusion, can lead to the formation of antibodies. Alloantibodies against these antigens can be clinically significant in foetal and neonatal alloimmune thrombocytopenia (FNAIT), post-transfusion purpura (PTP) and non-human leukocyte antigen (HLA) refractoriness to platelet transfusion therapy [8, 9]. In such cases, transfusion of HPA-compatible platelets donated by previously typed donors can have very significant clinical impact [10].

Furthermore, the determination of the distribution of human platelet antigens and the frequency of the respective gene alleles can be very interesting in the context of genetic population studies, as differences among various populations may reflect natural selective pressure and could guide public health policies, such as population screening programmes for the prevention and management of FNAIT [11–13]. No such extended study has been performed to date in the population of Greece, except for small scale studies on the frequency of HPA-1a and HPA-1b [14, 15].

Establishing a registry of voluntary non-remunerated platelet donors typed for a vast selection of HPA is a very crucial component in the timely and successful treatment of FNAIT cases, as these donors can be summoned to donate upon request, according to their optimal combined HPA, ABO and Rhesus D antigen (RhD) compatibility [8–10, 16, 17]. In the same way, cases of PTP and refractoriness to platelet transfusion therapy not due to anti-HLA antibodies may also be supported with transfusion of HPA-compatible platelets [18–22].

MATERIALS AND METHODS

Since September 2019, all donors visiting our centre for platelet apheresis were informed about the aims of the registry we intended to create. Most of them gave informed written consent to be HPA-typed and provided an additional blood sample for that cause. They were genotyped using a commercial Single Specific Primer-Polymerase Chain Reaction (SSP-PCR) kit. Seventy-seven samples (7.7% of the dataset) were kindly provided by the blood bank departments of other hospitals that perform platelet apheresis in the wider area of Northern Greece, provided that donors had given informed consent.

Each donor was genotyped for a total of 12 sets of two antithetical antigens, specifically HPA-1a/b, HPA-2a/b, HPA-3a/b, HPA-4a/b, HPA-5a/b, HPA-6a/b, HPA-8a/b, HPA-9a/b, HPA-11a/b, HPA-15a/b, HPA-21a/b and HPA-27a/b. Results were recorded both in the hospital laboratory information system (LIS) and in Microsoft Excel spreadsheet; all agarose gels were photographed under ultraviolet light, and images were saved in electronic files.

Antigen frequencies were calculated and also allele frequencies were calculated based on antigen frequencies. Results were compared with literature reports regarding other European populations.

DNA extraction

The commercial kit Ready DNA Isolation Spin Kit (Inno-train, Kronberg im Taunus, Hessen, Germany) was used according to the manufacturer's instructions. Sample material was 200 µL Proteinase K-treated whole blood, and the expected yield was 5–10 µg of genomic DNA, given that all blood samples were analysed before the platelet apheresis procedure and contained 5–10 $\times$ 10^3 white blood cells/µL. All DNA samples were stored frozen at −70°C.

Single specific primer-polymerase chain reaction

The commercial kit HPA-Ready Gene plus (inno-train, Kronberg im Taunus, Hessen, Germany) was used according to the manufacturer's instructions. For each reaction, 1 µL of extracted DNA (concentration 25–50 ng/µL) was added to the Mastermix. The PCR program was 94°C for 2 min, 5 cycles of 94°C for 20 s and 70°C for 60 s, 10 cycles of 94°C for 20 s followed by 65°C for 60 s and 72°C for 45 s, 20 cycles of 94°C for 20 s followed by 61°C for 50 s and 72°C for 45 s and finally 72°C for 5 min.

Agarose gel electrophoresis

Band evaluation was performed by agarose gel electrophoresis. For each donor sample that included 24 reactions, a negative control and

molecular weight marker per row of samples, a 2% agarose gel with 1× Tris/Borate/EDTA buffer was prepared. Ethidium bromide was added to a final concentration of 0.7 μg/mL. Bands were visualized and evaluated under ultraviolet (UV) light.

Ethics statement

In this article, we exclusively address anonymized data collected as part of genotyping of platelet donors after written informed consent.

RESULTS

The results of 1000 genotyped Greek donors are reported here. Donations and sample analysis were performed during the period September 2019–December 2023. Results in which one or more reactions failed (internal control was negative) or were inconclusive were filtered out (22 donors were filtered out for these reasons). Moreover, given that the scope of this report is to reflect the Greek population, results coming from donors who were not of Greek origin are not reported here. As nationality is not always formally recorded during donor examination, Greek origin was assumed based on combination of full name, fathers' name, mother's name and place of birth as recorded on donors' formal identification documents, (49 donors were filtered out because of assumed non-Greek origin of at least one parent). A total of 1071 donors were genotyped. Platelet donors that were genotyped were randomly selected with no bias. Results are shown in Table 1.

Allele frequencies were calculated and Hardy–Weinberg analysis for each pair of antithetical antigens is shown in Table 2.

All donors were also tested for ABO and RhD phenotype, results are shown in Table S1. Phenotype distribution is very similar to published results for the Greek population [24], which is an indirect indication that tested donors were selected without bias. Nevertheless, given that the *ABO* and *RHD* genes are located in chromosomes 9 and 1, respectively, and none of the genes that express the tested HPA antigens are located in those chromosomes, the distribution of ABO and RhD is not expected to have any impact on the frequencies of HPA in our population.

TABLE 1 Frequency of human platelet antigens (HPA) in Greek platelet donors ($n = 1000$).

HPA ($n = 1000$)	Positive	Homozygous	Heterozygous	Negative	White people [23]
HPA-1a	97.1%	69.0%	28.1%	2.9%	98%
HPA-1b	31.0%	2.9%	28.1%	69.0%	28%
HPA-2a	97.9%	74.5%	23.4%	2.1%	99%
HPA-2b	25.5%	2.1%	23.4%	74.5%	15%
HPA-3a	85.0%	37.4%	47.6%	15.0%	85%
HPA-3b	62.6%	15.0%	47.6%	37.4%	63%
HPA-4a	99.9%	99.8%	0.1%	0.1%	>99.9%
HPA-4b	0.2%	0.1%	0.1%	99.8%	<0.1%
HPA-5a	97.7%	77.3%	20.4%	2.3%	98%
HPA-5b	22.7%	2.3%	20.4%	77.3%	21%
HPA-6a	99.9%	99.1%	0.8%	0.1%	>99%
HPA-6b	0.9%	0.1%	0.8%	99.1%	<1%
HPA-8a	100.0%	100.0%	0.0%	0.0%	>99%
HPA-8b	0.0%	0.0%	0.0%	100.0%	<1%
HPA-9a	100.0%	98.9%	1.1%	0.0%	>99%
HPA-9b	1.1%	0.0%	1.1%	98.9%	<1%
HPA-11a	100.0%	100.0%	0.0%	0.0%	>99%
HPA-11b	0.0%	0.0%	0.0%	100.0%	<1%
HPA-15a	72.7%	22.6%	50.1%	27.3%	77%
HPA-15b	77.4%	27.3%	50.1%	22.6%	65%
HPA-21a	100.0%	100.0%	0.0%	0.0%	>99%
HPA-21b	0.0%	0.0%	0.0%	100.0%	<1%
HPA-27a	100.0%	100.0%	0.0%	0.0%	>99%
HPA-27b	0.0%	0.0%	0.0%	100.0%	<1%

Note: Literature reports on the frequency of each human platelet antigen (HPA) for people of European ancestry who live in North America is shown for reasons of comparison.

TABLE 2 Distribution of allele frequencies and Hardy–Weinberg analysis (n/a = not applicable).

HPA	Allele frequency	χ^2	*p* value
HPA-1a	0.8305	0.0037	0.998
HPA-1b	0.1695		
HPA-2a	0.8620	0.2704	0.874
HPA-2b	0.1380		
HPA-3a	0.6120	0.0052	0.997
HPA-3b	0.3880		
HPA-4a	0.9985	n/a	n/a
HPA-4b	0.0015		
HPA-5a	0.8750	4.5466	0.103
HPA-5b	0.1250		
HPA-6a	0.9950	n/a	n/a
HPA-6b	0.0050		
HPA-8a	1.0000	n/a	n/a
HPA-8b	0.0000		
HPA-9a	0.9945	0.0306	0.9848
HPA-9b	0.0055		
HPA-11a	1.0000	n/a	n/a
HPA-11b	0.0000		
HPA-15a	0.4765	0.0178	0.991
HPA-15b	0.5235		
HPA-21a	1.0000	n/a	n/a
HPA-21b	0.0000		
HPA-27a	1.0000	n/a	n/a
HPA-27b	0.0000		

Abbreviation: HPA, human platelet antigens.

Antigen frequencies were compared with literature reports regarding other populations, as shown in Table S2. The same comparison was performed for allele frequencies, as shown in Table S3.

DISCUSSION

A registry of voluntary non-remunerated platelet donors typed for a vast selection of HPA is a very important asset for a blood centre, as it can contribute significantly to the proper and timely treatment of FNAIT, refractoriness to platelet transfusion therapy and PTP [8, 18, 19]. It could also provide platelet panel donors useful for platelet serology assays. In our study, over the course of approximately 4 years, more than 1000 platelet donors spanning all ABO and RhD phenotypes were typed for HPA, and the majority of these donors are expected to be available to donate on demand for many years to come. Several cases of suspected FNAIT and refractoriness to platelet transfusion therapy were investigated (7 and 2 cases, respectively) and HPA-compatible platelets were administered. The treatment of neonatal alloimmune thrombocytopenia and the prevention of its potential devastating effects on affected neonates makes the registry invaluable for the national healthcare system.

Moreover, this registry offered the possibility to study antigen and allele frequencies of HPA in the Greek population for the first time. The large number of tested individuals ($n = 1000$ subjects) is particularly significant, as most similar reports on other European populations have examined much fewer individuals, and this has perhaps allowed for the detection of low frequency antigens. Comparison of our results with other populations demonstrates the genetic similarity of Greeks with other White populations.

Although the frequency of most antigens is very similar to other literature reports, there are some interesting deviations. Low frequency antigens such as HPA-2b, HPA-4b and HPA-9b are detected in higher frequency compared to other European populations. In addition to that, the frequency of HPA-15b is higher than that of HPA-15a, which is contradictory not only to many other literature reports but also to the nomenclature convention that dictates the attribution of the superscript 'a' to the high-frequency antigen. On the other hand, despite the large number of samples, no samples were found positive for HPA-8b, HPA-11b, HPA-21b and HPA-27b. Proper statistical analysis and perhaps larger scale studies could indicate whether these differences are actually statistically significant in terms of population genetics.

The main limitation of this study was that all tested individuals reside in North Greece and the vast majority of them (98.5%) are located in one major city, Thessaloniki. As a result, this distribution may not be precisely representative of the entire Greek population, especially of the southern part of the country. Moreover, although all tested donors are of Greek origin, their detailed ancestral background was not further explored. Given the historical background of massive internal migration within the territory of Greece over the last century and the mixed population makeover of Thessaloniki, we believe that this distribution can be quite representative of an extended mixture of people of Greek origin.

Focusing on the frequency of HPA-1a, in this study, we found a slightly higher frequency of HPA-1a negative individuals than in many other European populations, which is important as most FNAIT cases are caused by HPA-1a alloantibodies. Previous studies have calculated the risk of antibody formation in HPA-1a negative women during reproductive age to be around 8.6%–9.7% [25, 26]. The incidence of severe FNAIT (defined as platelet count $<50 \times 10^9$/L) was estimated to be 0.04%, whereas the incidence of intracranial haemorrhage (ICH) varies from 9.9%–25% of the severe FNAIT cases, based on antenatal and postnatal screening studies [27]. The frequency of HPA-1a-negative individuals (thus also pregnant women potentially at risk for FNAIT) in those White populations was 2.1% [10], so a frequency of 2.9% HPA-1bb in the Greek population could potentially indicate higher risk of severe FNAIT in this population. Unfortunately, the incidence of FNAIT cases in Greece remains unknown to date as only scarce cases have been reported [28], so this assumption is only theoretical.

Increased risk of HPA-1a alloimmunization has been reported in women carrying certain human leucocyte antigens class II, more

importantly HLA-DRB3*01:01 but also HLA-DRB4*01:01 and HLA-DQB1*02:01 [29–31], so further studies on the frequency distribution of HLA class I and II alleles in the Greek population could offer further valuable insight in the estimation of FNAIT risk in this population [32].

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G.K. designed the study, contributed to sample analysis, performed data analysis and wrote the first draft of the manuscript; E.B., D.P. and D.S. contributed to sample analysis; F.G. contributed to writing the draft manuscript; E.H.-M. conceived the study and reviewed the manuscript; all authors reviewed and approved the final manuscript.

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.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Ethnic diversity in Chilean blood groups: A comprehensive analysis of genotypes, phenotypes, alleles and the immunogenic potential of antigens in northern, southern and central regions

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Abstract

Background and Objectives: The available information on blood groups in the Chilean population is derived from studies on aboriginal cohorts and routine serological test results. The purpose of this study is to conduct a comprehensive analysis of genotypes, phenotypes and blood group alleles in donors from northern, central and southern Chile using molecular methods.

Materials and Methods: Overall, 850 samples from donors in northern, central and southern Chile were genotyped. Allelic, genotypic and antigenic frequencies were calculated and compared among regions. Of these, 602 samples were analysed by haemagglutination, and discrepancies found between phenotypes and genotypes were investigated. The immunogenic potential of antigens was calculated by the Giblett equation, using the antigenic frequencies of donors from Santiago and the alloantibody frequencies of patients from the same region.

Results: Alleles of low prevalence, variant alleles and those responsible for the absence of high-prevalence antigens were found. Significant differences were observed between the antigenic frequencies of the three regions. Discrepancies between serologic and molecular results were mostly attributed to the molecular background affecting antigen expression. In the calculation of the immunogenic potential of antigens, the highest value was attributed to the Di^a antigen.

Conclusion: These findings represent the first molecular characterization of blood group antigens in Chileans. Our results highlight the necessity of using molecular tools to explore the genotypes underlying variant phenotypes, low-frequency antigens and antigens lacking specific antisera that cannot be detected by haemagglutination. Additionally, they emphasize the importance of understanding the distribution of blood groups among different populations.

Keywords
antigenic frequencies, blood group antigen, genotype blood groups

Highlights
- Antigens, alleles and genotypes of clinical importance not previously described were found.
- The presence of these phenotypes impacts transfusion safety, leads to problems in pre-transfusion studies and increases the difficulty in finding compatible red blood cell units.
- There are significant statistical differences among the three areas studied, which could have an impact on alloimmunization.
- In the calculation of the immunogenic potential of antigens, the highest value was attributed to the Di^a antigen.

INTRODUCTION

There are more than 300 polymorphic antigens on the red blood cell (RBC) membrane that can be incompatible between a blood donor and a transfusion recipient or between a mother and her child during pregnancy, potentially causing alloimmunization and eventually leading to haemolytic transfusion reaction (HTR) or haemolytic disease of the fetus and newborn (HDFN), respectively [1]. Blood transfusion plays a crucial role in the treatment of various diseases; approximately 15% of inpatients undergo blood transfusion, and about 1% of transfused products result in severe HTR in alloimmunized patients due to blood group incompatibility [2]. Between 2017 and 2021, the US Food and Drug Administration (FDA) reports that 21% of transfusion-related deaths were caused by HTR resulting from blood group incompatibility [3]. Despite existing strategies to mitigate this adverse transfusion effect, prevention of RBC alloimmunization remains an unsolved challenge.

Alloimmunization is a complex adverse event related to transfusion, resulting from diverse factors such as donor and patient antigen mismatches, the recipient's immunological status, underlying inflammation, the immunomodulatory effects of transfused erythrocytes on the recipient's immune system and genetic factors.

In Chile, patients undergoing transfusion therapy are at high risk of alloimmunization since most receive only ABO and RhD-matched blood [4]. The ethnic background of both patients and donors can impact transfusion outcomes. Therefore, understanding of the blood groups present in the population is critical for implementing extended phenotype/genotype matching, aiming to reduce alloimmunization rates and improve patient outcomes [4].

No molecular studies of blood groups have been performed in the Chilean population to detect clinically significant genotypes and to predict phenotypes for which there are no antisera. The existing information comes from prospective studies carried out mainly in small groups of aborigines, which have anthropological objectives or from retrospective information gathered from the results of routine serological studies conducted in different blood banks, these studies are limited by the variable availability of reagents between blood banks of different health centres, and serological studies are restricted to identifying antigenic specificities for which antisera exist. Additionally, they are conditioned by the sensitivity of the technique, which may not detect weak variants [5–9]. Knowledge of the blood groups present in a population is essential for selecting the most appropriate reagents and phenotyping techniques to ensure patient safety. As serological reagents have limitations, it is relevant to conduct studies using molecular methods.

Chileans have varying proportions of European, Native American and, to a lesser extent, West African and East Asian ancestry [10]. Given the ethnic background of the Chilean population and the recent increase in immigration to Chile, it is possible to find previously undescribed genotypes, alleles and phenotypes due to the lack of molecular methods, and it is also plausible that there are significant differences in blood group frequencies in different regions of the country, variations that could increase the risk of alloimmunization [11].

As a comprehensive molecular analysis of blood group genotypes, alleles and phenotypes in the Chilean population has not yet been performed, this study represents the first in-depth examination using genotyping techniques. Consequently, the objectives of this study were to provide data on the genotypic, allelic and antigenic frequencies of 11 blood systems in a cohort of Chilean blood donors from the Northern, Central and Southern regions, to compare the frequencies between regions and with those documented in Europeans and Afro-descendants, to evaluate the concordance between the serological phenotype and the phenotype predicted from the genotype.

Additionally, we estimated the immunogenic potential of clinically relevant antigens.

MATERIALS AND METHODS

Blood samples

Three different groups of Chilean donors were enrolled in this study. A total of 850 blood samples were randomly collected from three regions: 212 from Arica (Northern), 515 from Santiago (Central) and 123 from Punta Arenas (Southern). Two etylenediaminetetra-acetic acid (EDTA) samples were collected from each donor, samples from cities in northern and southern Chile were shipped by air for processing in the Immunohematology and Blood Group Molecular Laboratories of the Santa María Clinic Blood Bank, located in Santiago, in the Central zone, Metropolitan region.

The inclusion criteria were (1) to be Chilean, considering Chilean nationality and fixed residence in Chile, (2) to meet the requirements to donate blood according to the general technical regulation NGS146 that regulates the care of donors in Chile and (3) to have signed the informed consent form agreeing to participate in the study.

Ethical approval was obtained from the ethics committee at the Tarapacá University, Arica, I region (North zone); Santa María Clinic, Metropolitan region (Central zone); Magallanes University, Punta Arenas, XII region (South zone) and the Chile University, Santiago to Chile.

Molecular methods

DNA was extracted from the EDTA-anticoagulated whole blood samples using the QIAamp DNA mini kit (Qiagen, USA). DNA concentration was measured on the Qubit fluorometer and was acceptable in the range between 10 and 100 ng/μL. Extracted DNA was stored at −80°C until testing.

Two commercial platforms were employed to genotype the samples: HEA BeadChip (Bioarray Solutions, Immucor, Warren, NJ, USA) and ID CORE XT (Grifols, Spain) [12, 13]. A total of 602 samples (212 from Arica, 328 from Santiago and 123 from Punta Arenas city) were genotyped by the HEA Beadchip, which analyses 38 RBC antigens of the following 11 blood group systems: RH, KEL, JK, FY, MNS, DI, DO, CO, LU, LW and SC using 24 single-nucleotide polymorphism (SNPs). The 187 remaining samples from Santiago were genotyped by IDCORE XT, utilizing Luminex xMAP technology that includes 29 SNPs responsible for the expression of 37 RBC antigens of the following 10 blood group systems (RH, KEL, JK, FY, MNS, DI, DO, CO, YT and LU).

RHD and *RHCE* gene variants from samples exhibiting discrepancies between molecular and serological methods were analysed by the RHD and RHCE BeadChips (Bioarray Solutions, Immucor) [14] in a reference laboratory.

Results for all blood group systems were expressed in accordance with the International Society of Blood Transfusion (ISBT) nomenclature of blood groups, alleles and phenotypes [15]. Rare blood group donors are defined as those that are negative for high-prevalence antigens (<1:1000).

Serological methods

The 602 samples genotyped with the HEA BeadChip platform [12] were previously serologically phenotyped by tube or using the automated NeoGalileo equipment (Immucor, Norcross, GA, USA) with monoclonal and polyclonal antisera according to the manufacturer's instructions for the following blood group antigens: Rh (C, c, E, e), KEL (k), JK (Jk^a, Jk^b), FY (Fy^a, Fy^b), DI (Di^a), MNS (M, N, S, s) and LU (Lu^a, Lu^b). The 187 samples that were genotyped using ID CORE XT [13] were not phenotyped through serological methods because we did not have all the necessary antisera available when the samples were processed.

Predicted phenotypes based on genotypes were compared with results from serological phenotyping, and any discrepancies were analysed. A discrepancy was defined as a situation where genotype results were positive but serology results were negative, or vice versa.

Statistical analysis

Antigenic and allelic frequencies for each region were estimated using direct counting and presented as percentages. Fisher's test was employed to assess whether statistically significant differences existed among antigenic frequencies of Chilean blood donors in the three regions studied. Furthermore, the RBC antigenic frequencies of each region were compared with those reported for individuals of Caucasian and Afro-descendant backgrounds. p-values <0.05 were considered indicative of significance.

Calculation of the immunogenic potential of antigens

For this calculation, the 'Giblett equation' was applied [16]. This equation included in the analysis only those RBC antigens for which both antigen and clinically significant antibody frequency data were available. RBC antigen frequencies predicted from the genotype data collected in Santiago were used. Alloantibody frequencies were obtained from 502 patients with one or more irregular antibodies identified at the same centre. Data were obtained from the statistics module of the Hematos software used in the Blood Bank during the same period as the donors' genotyping. The alloimmunized patients included in the study were from the same region as the donors.

The 'Giblett equation' is a widely used method for estimating the immunogenic potential of blood group antigens. This calculation involves dividing the total number of antibodies of a specific type by

the probability that an antigen-negative individual will receive transfusions of antigen-positive red blood cells. The resulting value is then normalized relative to the immunogenic potential of K antigen by dividing it by the corresponding value for K [16].

RESULTS

In the 850 donors studied from the 3 zones of the country, we found 55 different genotypes and 35 alleles from 11 blood group systems. Within these, uncommon alleles coding for low-frequency antigens (LFA), absence of high-frequency antigens (HFA) and partial and weakly expressed antigens were found.

In the Rh system, 14 genotypes were identified, 6 of which included the *RHCE*01.20.01* allele or the *RHCE*01.20.03* allele, all of which were in heterozygosis. Table 1 displays the number of donors and genotypes in which each allele was found, along with the corresponding nucleotide, amino acid changes and associated phenotypes.

Eight genotypes were found in the FY system. Among these, five genotypes were observed in 54 (6.3%) donors with the *FY*02W.01* allele or the *FY*02N.01* allele, of which 13 (1.5%) were carriers of the *FY*02W.01* allele, and 41 (4.8%) of the *FY*02N.01* allele, two (0.2%) of the latter had a homozygous genotype (*FY*02N.01/FY*02N.01*) and were therefore classified as carriers of the Fy null phenotype. Table 2 displays the donors and their respective genotypes containing either the *FY*02N.01* or *FY*02W.01* alleles.

In the other systems studied, donors with infrequent genotypes and alleles were also identified. Table 3 shows the homozygous genotypes responsible for the absence of HFA in KEL, LU, DI, YT blood group systems, found in Chilean donors. Additionally, other rare alleles associated with LFA were detected. Table 4 displays the alleles, genotypes and LFA occurrences found in the donors studied.

None of the alleles, genotypes and phenotypes described in Tables 1–4 had been previously described in Chileans. Details of the genotypes and alleles identified in all donors studied and the frequencies at which they were found in the three areas studied are shown in Tables S1 and S2.

Antigens predicted from genotypes: Frequencies and statistical analysis

Table 5 displays the frequencies of blood system antigens: RhCE, KEL, JK, FY, MNS, LU, DI, CO, DO, LW and SC obtained from molecular studies of blood donors from the northern (Arica), central (Santiago) and southern (Punta Arenas) regions of Chile. To the left of Table 5 is

TABLE 1 Nucleotide and amino acid change, phenotypes and genotypes associated with *RHCE*01.20.01* and *RHCE*01.20.03* alleles from the Chilean donors.

Allele name ISBT	Nucleotide change	Predicted amino acid change	Phenotypes	Donors (*n*)	Genotypes with *RHCE*01.20* alleles
*RHCE*01.20.01*	733 C>G	Leu245Val	c + partial, e + partial, V + VS+, hr^B pos., weak or neg.	15	*RHCE*01/RHCE*01.20.01* *RHCE* 02/RHCE*01.20.01* *RHCE*03/RHCE*01.20.01* *RHCE*04/RHCE*01.20.01*
*RHCE*01.20.03*	48 G>C 733 C>G 1006 G>T	Trp16Cys Leu245Val Gly336Cys	c + partial, e + partial, V−VS+, hr^B−	4	*RHCE*01/RHCE*01.20.03* *RHCE*03/RHCE*01.20.03*

TABLE 2 Nucleotide and amino acid change, phenotypes and genotypes associated with *FY*02W.01* and *FY*02N.01* alleles from the Chilean donors.

Allele name ISBT	Nucleotide change	Predicted amino acid change	Phenotypes	Donors (*n*)	Genotypes
*FY*02W.01*	265 C>T	Arg89Cys	Fy^b weak	8	*FY*01/FY*02W.01*
				5	*FY*02/FY*02W.01*
*FY*02N.01*	-67 T>C	0	Fy^a	29	*FY*01/FY*02N.01*
			Fy^b	10	*FY*02/FY*02N.01*
			Silent	2	*FY*02N.01/FY*02N.01*

TABLE 3 Rare phenotypes of the KEL, LU, DI and YT blood systems present in Chilean donors.

System	Phenotypes	Genotypes	Nucleotide change	Predicted amino acid change	Donors (*n*)
KEL	K+k−	*KEL*01.01/KEL*01.01*	578 C>T	Thr193Met	1
DI	Di(a+b−)	*DI*01/DI*01*	2561 C>T	Pro854Leu	2
LU	Lu(a+b−)	*LU*01/LU*01*	230 G>A	Arg77His	1
YT	Yt(a−b+)	*YT*02/YT*02*	1057 C>A	His353Asn	2

TABLE 4 Alleles encoding LFA, antigens and genotypes detected in Chilean donors.

System	Allele encoding LFA	LFA	Genotypes	Donors (*n*)
RhCE	*RHCE*01.20.01*	V+VS+	*RHCE*03/01.20.01*	15
			*RHCE*01/01.20.01*	
			*RHCE*02/01.20.01*	
			*RHCE*04/01.20.01*	
	*RHCE*01.20.03*	VS+	*RHCE*01.39/01.20.03*	4
			*RHCE*03/01.20.03*	
KEL	*KEL*01.03*	Kp^a	*KEL*01.03 /KEL*01.04*	18
	*KEL*02.06*	Js^a	*KEL*02.06/KEL*02.05*	2
MNS	*GYP*501*	Mi^a	*GYPB*03/GYP*501*	1
DI	*DI*01*	Di^a	*DI*01/DI*02*	2
			*DI*01/DI*01*	28
LU	*LU*01*	Lu^a	*LU*01/LU*01*	1
			*LU*01/LU*02*	25
CO	*CO*02*	Co^b	*CO*01/CO*02*	40
SC	*SC*02*	Sc2	*SC*01/SC*02*	5

Abbreviation: LFA, low-frequency antigen.

a map of Chile, highlighting in red the areas from which the samples were obtained.

In the 187 samples studied from Santiago donors by the ID CORE platform, RBC antigen frequencies were also calculated for: Mi^a (0.5%), Yt^a (98.9%) and Yt^b (10.7%).

As expected, due to the heterogeneous nature of the miscegenation that formed the Chilean population across the country, significant statistical differences were observed in the frequencies of eight antigens across five blood group systems (RhCE, FY, JK, MNS and DO) among Chilean blood donors residing in the three regions studied. Table 6 presents the *p*-values resulting from the statistical comparisons, with statistically significant differences highlighted in bold. Antigenic frequencies with values of 0 or 100% were excluded from the analysis.

In all the statistical analyses performed, significant statistical differences were found between all the frequencies obtained in the three areas studied, and those reported in Caucasians and Afro-descendants. The above except for the LU and DO blood system antigens between Punta Arena–Caucasians and Punta Arenas–Afro-descendants and in the DO system antigens between Arica–Afro-descendants' frequencies.

Discrepancies between genotyping and phenotyping

Six hundred and six samples from the three areas genotyped by the HEA Bead Chip platform were subsequently phenotyped with antisera. Discrepancies between genotype and phenotype were observed in 10 samples from Arica and Santiago for four antigens (Fy^b, C, e and M). All identified discrepancies were attributed to negative results in the serological method and positive results in the molecular method. The concordance rate between serological and molecular methods is presented in Table 7. In three of the six donors with discrepancies in C, the cause could not be identified with the studies conducted. In a subsequent sample from donor 4, the serological study was repeated with 2 anti-C clones, yet the C antigen remained undetected. Consequently, sequencing is required to resolve this discrepancy.

The *RH*01.01 allele present in donor 5 encodes a weak e antigen. However, the serological test returned a positive result, likely because the RH*01.01* allele can lead to weaker expression of the e antigen, depending on the serological methodology employed. For donors 5 and 6, no additional samples were obtained to repeat the serological study, and the discrepancies with the *RHCE* genotype (RHCE Beadchip) were left unexplained. In both cases, sequencing is necessary to resolve the discrepancies.

The immunogenic potential of antigens

Figure 1 illustrates the frequencies of antigens and antibodies specific to the antigens K, Di^a, E, C, e, c, Fy^a, Kp^a, Jk^a, Lu^a, Jk^b, S, Fy^b and s, which were used for calculating immunogenicity. The corresponding values, multiplied by 1 factor of 100 are depicted in Figure 2, with the highest value attributed to the Di^a antigen.

DISCUSSION

This study represents the first implementation of molecular methods to characterize the genotypes, alleles and antigens of blood groups in donors from the Northern, Central and Southern zones of Chile. Within this investigation, previously unreported antigens lacking

TABLE 5 Antigenic frequencies of the blood systems: RH, KEL, JK, FY, MNS, LU, DI, CO, DO, LW and SC studied in donors from northern, central and southern Chile.

The RBC antigen frequencies (%)																		
Arica	**C**	**E**	**c**	**e**	**V**	**VS**	**K**	**k**	**Kp^a**	**Kp^b**	**Js^a**	**Js^b**	**Fy^a**	**Fy^b**	**Jk^a**	**Jk^b**	**M**	**N**
***n* = 212**	73.1	49.1	74.1	87.7	0.9	1.9	4.2	100	1.4	100	0.5	100	84.9	57.5	67	84.9	88.2	55.7
	S	**s**	**U**	**Lu^a**	**Lu^b**	**Di^a**	**Di^b**	**Co^a**	**Co^b**	**Do^a**	**Do^b**	**Hy**	**Jo^a**	**LW^a**	**LW^b**	**Sc1**	**Sc2**	
	42	92.5	100	1.4	100	4.2	100	100	3.8	51.9	92	100	100	100	0	100	0.5	
Santiago	**C**	**E**	**c**	**e**	**V**	**VS**	**K**	**k**	**Kp^a**	**Kp^b**	**Js^a**	**Js^b**	**Fy^a**	**Fy^b**	**Jk^a**	**Jk^b**	**M**	**N**
***n* = 515**	60.2	38	74.9	95.5	1.7	2.1	6.0	99.8	1.2	100	0.2	100	73.6	68	70.7	82.7	86	61.5
	S	**s**	**U**	**Lu^a**	**Lu^b**	**Di^a**	**Di^b**	**Co^a**	**Co^b**	**Do^a**	**Do^b**	**Hy**	**Jo^a**	**LW^a**	**LW^b**	**Sc1**	**Sc2**	
	51.8	92.6	100	3.5	99.8	3.9	99.6	100	6.2	59.4	88.5	100	100	100	0	100	0.2	
Punta Arenas	**C**	**E**	**c**	**e**	**V**	**VS**	**K**	**k**	**Kp^a**	**Kp^b**	**Js^a**	**Js^b**	**Fy^a**	**Fy^b**	**Jk^a**	**Jk^b**	**M**	**N**
***n* = 123**	82.1	43.1	81.3	94.3	3.3	3.3	3.3	100	1.6	100	0	100	80.5	74	63.4	90.2	85.4	57.7
	S	**s**	**U**	**Lu^a**	**Lu^b**	**Di^a**	**Di^b**	**Co^a**	**Co^b**	**Do^a**	**Do^b**	**Hy**	**Jo^a**	**LW^a**	**LW^b**	**Sc1**	**Sc2**	
	32.5	93.5	100	4.1	100	0.8	100	100	1.6	60.2	86.2	100	100	100	0	100	2.4	

Abbreviation: RBC, red blood cell. In the gray rows are the names of blood group antigens included in the study.

TABLE 6 *p*-Value obtained from statistical analyses between antigenic frequencies of Arica–Santiago, Santiago–Punta Arenas and Arica–Punta Arenas.

p-Value								
Antigens	**C**	**E**	**e**	**Fy^a**	**Fy^b**	**Jk^a**	**S**	**Do^b**
Arica–Santiago	0.472	**0.006**	**0.005**	**0.005**	**0.006**	0.379	**0.018**	**<0.001**
Santiago–Punta Arenas	**0.007**	0.34	0.64	0.287	0.277	**<0.001**	**0.0001**	**0.027**
Arica–Punta Arenas	**0.031**	**<0.001**	0.058	0.362	**0.003**	**<0.001**	0.103	0.095

Note: Statistically significant differences are highlighted in bold.

TABLE 7 Concordance rate between serological and molecular antigen detection.

Antigen	Donors (*n*)	Concordance rate (%)	Genotypes
Fy^b	3	99.5	*FY*01/FY*02W.01*
C Donors 1, 2 and 3 are also VS+ and e antigen discrepant	6	99.0 (c) 99.5 (e)	Donors 1 and 2:*RHCE*03/RHCE*01.20.03* Donor 3: *RHCE*01/RHCE*01.20.03* Donor 4: *RHCE*01/RHCE*01* Donor 5: *RHCE*01/RHCE*01.01* Donor 6: *RHCE*03/RHCE*01*
M	1	99.8	It was not possible to obtain a new sample to repeat.

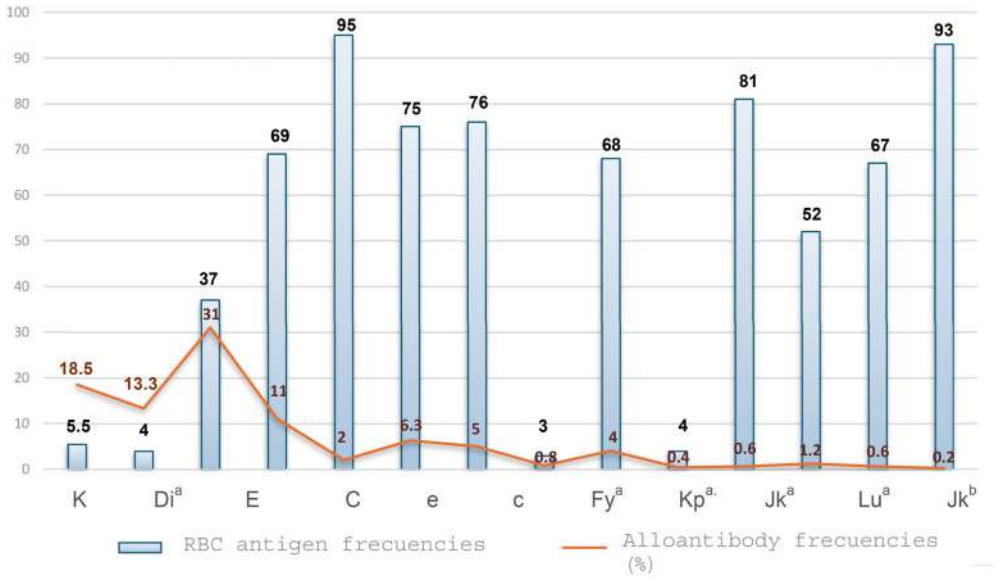

FIGURE 1 Erythrocyte antigen frequencies in Santiago donors and alloantibody frequencies in Santiago patients.

corresponding antisera, as well as LFA, weak variants, null phenotypes and rare blood group phenotypes, were recognized. Notably, genotypes harbouring alleles that affect antigen expression or inactivate antigen expression in the RH and FY systems were identified. Specifically, alleles *RHCE*01.20.01* and *RHCE*01.20.03* were identified, producing partial phenotypes of the c and e antigens on the RhCE protein. Additionally, these alleles are responsible for the expression of the LFA V and VS. *RHCE*01.20.01* also associated with the expression of hr^B antigen, unlike *RHCE*01.20.03*, which lacks hrB expression [17]. The *FY*01W.01* allele, identified in 13 donors, resulted in weak antigen expression, which may not always be detected by serological methods. Conversely, the *FY*02N.01* allele, present in 41 donors, is a silent allele. This allele features a T>C change within the promoter region of the gene, located 33 bp upstream of the erythroid transcription start point and 67 bp upstream of the major translation start codon (position −67). This mutation occurs within a GATA consensus sequence, disrupting the binding of the erythroid-specific GATA-1 transcription factor and consequently preventing gene expression in erythroid cells while maintaining expression in other cell types [18]. Furthermore, two donors exhibited the Fy null phenotype

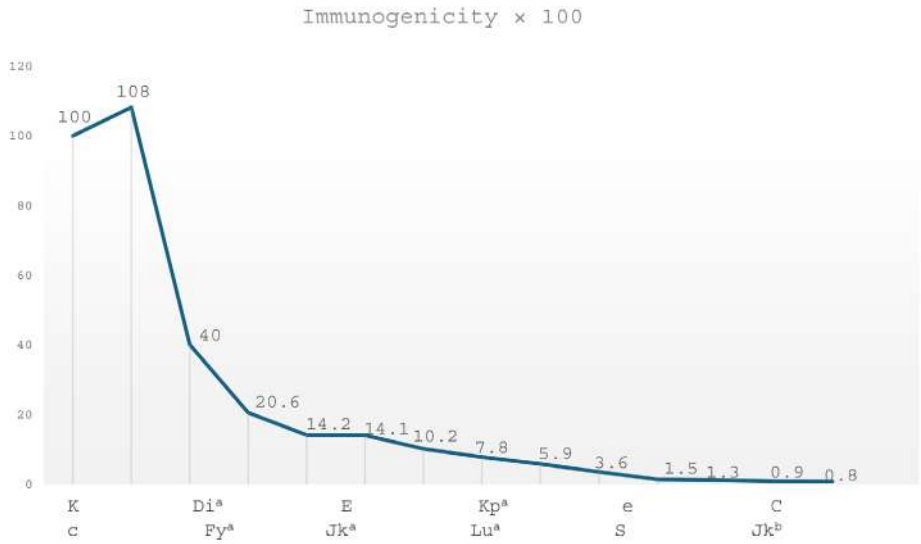

FIGURE 2 The calculation of the immunogenic potential of antigens performed with the antigenic frequencies of donors from Santiago and the frequency of antibodies of the same specificity from patients treated at the same institution.

due to a homozygous genotype for the *FY*02N.01* allele. While this phenotype is common among Africans, it is rare in other populations.

In the blood systems KEL, LU, DI, YT homozygous genotypes responsible for the absence of HFA, also known as rare, were identified. The identification and recruitment of blood donors with rare phenotypes, is crucial for providing safe transfusion therapy to patients with these phenotypes. While there are highly effective rare blood donor registry programmes worldwide, not all regions have the same level of availability. In Chile, only the Santa Maria Clinic has such a registry, which is partly formed by the donors identified in this study. Additionally, there is an Ibero-American initiative to establish a registry of rare blood donors capable of supplying Latin American countries and providing rare blood to patients in the region who need it [19].

The molecular study facilitated the detection of LFA, for which corresponding antisera are unavailable. All identified LFAs have been implicated in acute or delayed HTR or HDFN and had not been previously described in Chileans. The presence of the V, VS and Js^a antigens can be attributed to the West African ancestry of Chileans which accounts for close to 2.5% [10]. The donor who presented the Mi^a antigen indicated that he is unaware of having any Asian ancestry, noting that his parents, grandparents and great-grandparents are Chilean, with the exception of his great-grandmother, who was Italian. However, it is important to consider that Chileans have approximately 1.7% East Asian ancestry, likely a result of the Asian individuals who arrived during the colonization of Chile [10]. Similarly, the presence of the Di^a antigen is attributed to the Native American ancestry of the Chilean people, which comprises 38.7% [10], the higher frequency of this antigen in the northern zone aligns with previous findings reported by Etcheverry et al. [5] who observed that only the Atacameños (Amerindian-natives of the northern zone) presented the Di^a antigen, with a frequency of 12.5%.

LFA Sc2 was detected in all three regions studied, with the frequency found in Punta Arenas (2.4%) being higher than those reported in other countries, such as Canada, England, Germany, Czech Republic and Japan, where frequencies range from 0.5% in Japan to 1.7% in Canada [1]. While there are few reports of HTR and HDFN caused by anti-Sc2, it is noteworthy that one of the three reports of HDFN originated from Chile [20]. Due to the limited availability of molecular methods to detect these antigens in Chile, the role of LFA in HTR and HDFN may be underestimated. Additionally, identifying specific antibodies against LFA poses a challenge, as these antigens are rarely present in commercial RBCs. Failure to detect them may result in HTR in a previously alloimmunized patient.

Statistically significant differences were found between the frequencies of certain antigens in the RhCE, FY, MNS, DI, JK and DO blood systems across the three regions. These differences can be attributed to the heterogeneity of the Chilean population, characterized by a mixture in different proportions throughout the country of various native Amerindian peoples, European and African ancestry [5, 10].

Disparities in antigen frequencies among the three regions of the country increase the risk of alloimmunization. This risk is particularly significant given Chile's geography layout—a long and narrow country—where the most advanced healthcare facilities are concentrated in Santiago, located in the Central zone. Consequently, patients requiring organ transplants, haematopoietic progenitor transplants, cardiovascular surgery and other transfusion-dependent procedures from the Northern and Southern zones, are often transferred to Santiago. This find may suggest that patients from the North and South of the country face an increased risk of alloimmunization when transfused with RBC from donors in the Central zone, although haemovigilance data are required to confirm this.

Discrepancies between serological and molecular studies in 10 donors revealed the presence of weakly expressed antigens that are not detectable by serology. Three of these donors presented the allele *FY*01W.01*, which encodes a weak Fy^b antigen. This highlights that for certain antigens with weak expression, the serological method lacks the necessary sensitivity. Additionally, three discrepant samples in the C

antigen revealed the *RHCE*01.20.03* variant allele of the *RHCE* gene that had not been previously documented in Chileans, underscoring the importance of molecular methods to identify such variants.

Understanding immunogenicity is crucial for prioritizing which antigens should be routinely studied and determining the level of compatibility that RBCs should have in transfusion. In assessing the immunogenic potential of antigens, the Di^a antigen yielded the highest value, which is a significant finding, especially given that this antigen and its corresponding antibody are not routinely identified in Chile. This result strongly supports the inclusion of Di^a antigen testing in the typing of Chilean donors and underscores the need for mandatory use of red blood cells capable of detecting anti-Di^a antibodies.

In conclusion, this study conducted using molecular methods represents the first comprehensive and invaluable dataset on the phenotype, genotype and alleles present in Chileans, which cannot be studied by serological methods alone. This information can play crucial roles, the provision of compatible blood for alloimmunized patients, guiding the prioritization of antigens for further study and potentially establishing a rare donor programme in Chile. The insights gained from this research significantly contribute to enhancing transfusion practices and blood banking strategies in the Chilean context.

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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.

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SUPPORTING INFORMATION

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

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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

Outpatient elective intravenous hydration therapy: Should blood donors be deferred for medical spa hydration?

Intravenous (IV) hydration therapy (i.e., IV drip therapy) may be broadly defined as non-medically indicated infusion and/or injection of fluid that may contain supplements, vitamins and/or minerals into the venous peripheral system. This elective procedure has gained significant popularity in the United States and is being increasingly offered at various sites including medical spas, aesthetic clinics and IV hydration clinics, as well as through mobile on-demand services. These establishments market IV hydration therapy for various 'medical applications', including the amelioration of the aftereffects of alcohol intoxication (i.e., 'hangover cures'), detoxification of the body, libido enhancement, immunologic boosting, vitamin supplementation and improvement of mood and energy levels. Despite their geographic ubiquity and therapeutic claims, these entities are not federally regulated. Regulatory oversight of IV drip therapy among US states varies considerably, including whether it is defined as a medical practice [1–4].

The US Food and Drug Administration (FDA) has raised concerns about IV hydration therapy practices given the lack of evidence-based benefit in the setting of legitimate, potentially fatal, risks, particularly infection [5]. Specifically, the FDA has noted that sterile compounding activities, such as adding vitamins to IV infusion bags, are commonly performed at IV hydration therapy clinics and medical spas. However, as these establishments offering IV hydration therapy are not registered with the FDA, it is not known (1) how many of these establishments exist; (2) whether they are preparing, packing or storing the IV products under sanitary conditions; (3) if a licensed practitioner is on-site to evaluate patients and write prescriptions for the products or (4) whether their compounding practices comply with section 503A of the Food, Drug, and Cosmetic (FD&C) Act or applicable state regulations [6]. Any facility performing these processes under insanitary conditions significantly increases the risk of product contamination, which may cause—and has already resulted in—serious patient illness, hospitalization and/or death [6].

In addition to the risks for the IV hydration therapy recipient, there is the potential for transmissible risk to other individuals if the recipient later donates blood. Individuals who are inadvertently inoculated with bacterial or fungal organisms during the IV hydration therapy procedure would likely display signs of illness resulting in blood donation deferral. However, there is the theoretical risk of transmission of HIV and viral hepatitis if sterility and aseptic administration practices between individuals and by the provider are not maintained. The possibility of harm underlying this theoretical risk is akin to an individual donating blood after receipt of a tattoo or body piercing from an unregulated establishment, particularly if the components used to administer the IV hydration therapy are re-used and/or are not sterilized between recipients. Conceivably, individuals undergoing IV hydration therapy could present for blood donation during the window periods in which these high-risk viral infections cannot be reliably detected.

At present, there are no uniform federal regulations, and consolidation of state-by-state legislative oversight for IV hydration is limited. Variations in state regulations regarding who can own an IV hydration establishment, as well as who can order, initiate and oversee an IV hydration procedure further complicate the myriad of practices across the United States. For example, the state of Kentucky differentiates between 'ambulatory infusion agencies', which are regulated, and 'IV hydration clinics' (mobile or freestanding), which are not regulated [7]. In Florida, although a physician licence is required for an individual to serve as 'medical director', anyone can own an IV hydration clinic, and a variety of non-physician individuals are allowed to compound and administer the therapy [8]. As such, readers are encouraged to consult individual state statutes pertaining to the practice of medicine to ascertain the details of IV hydration therapy practices in their state.

Given the variability in IV hydration therapy practices and the lack of consistent regulatory oversight, there may be confusion among individuals and blood collection organizations regarding if and when an individual can donate blood following receipt of IV hydration therapy. These questions are not explicitly addressed by current Association for the Advancement of Blood and Biotherapies (AABB) Standards for donor eligibility or the blood donor history questionnaire (DHQ). AABB Standards require inspection of the donor for stigmata of parenteral drug use, as well as assessment for the 'use of a needle to administer nonprescription drugs' [9]. The DHQ assesses for risk factors associated with transfusion-transmitted infections by querying if a donor has 'used needles to inject drugs, steroids, or anything not prescribed by [their] doctor' within the past 3 months [10]. In US states that permit elective IV hydration therapy without prescriptions or licensed supervision, it is possible for individuals to receive unlicensed, non-prescription IV hydration therapies, which may affect their blood donation eligibility—perhaps for 3 months or indefinitely. In conjunction with the longstanding blood donor physical examination of the antecubital region for signs of intravenous puncture site requirement [10], a potential new question for the AABB

DHQ my take the form of 'In the past 3 months have you received recreational intravenous (IV) hydration therapy?'. This or a similar question may assist in identifying individuals who may pose a higher risk due to receipt of IV hydration therapy.

In addition to a lack of explicit questions in the AABB DHQ, no currently available state-by-state regulatory clearinghouse exists to guide blood donation centres for IV hydration. Candidate donors may also be unaware of the conditions, oversight and regulations pertaining to their individual recreational practices. Unlike tattoo practices wherein blood collection centres have proactively identified which states do and do not regulate themselves [11], no such resource exists for IV hydration therapy. Thus, the blood collection industry would greatly benefit from real-time expert consensus data, including a comprehensive document of state regulations that could be immediately made available if a candidate blood donor affirmed a recent history of IV hydration.

The IV hydration therapy market within the cosmetic field is a multibillion-dollar industry [12]. With increasing access to consumers through mobile and in-house IV hydration therapy services, the market is poised to grow in the next several years [12]. As such, the number of blood donors who may receive IV hydration therapy could increase. This parallels other contemporary practices in this homeopathic and 'holistic' space, such as the use of platelet-rich plasma (PRP), which may be administered either via venipuncture or even intravenously. Presently, wide variability exists in what is deemed allogeneic PRP and the potential medical benefit [13]. Despite uncontrolled trials addressing medical efficacy of allogeneic PRP, we believe receipt of blood products/derivatives and other 'IV therapies' should fall under current blood deferral practices.

Public trust in the blood supply is an area of intense scrutiny, and historic precedent dictates that blood centres and regulatory bodies take all necessary steps to ensure the safety of blood products. Therefore, we suggest that increased regulatory oversight of both IV hydration products themselves, and the establishments that provide these services, as well as clarification from regulatory bodies and the blood collection industry regarding the suitability of blood donors who have received IV hydration therapy, are warranted. In the interim, we advocate for a 3-month donation deferral for individuals after their most recent IV hydration therapy from any establishment that is not currently regulated at the US state level.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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EVENTS

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

21 December 2024	International Symposium on Neonatal & Pediatric Transfusion. https://docs.google.com/forms/d/e/1FAIpQLSd7fKVNOVVO-wTP9B3czRjRe7-J1EWw0entl_NPw6x97St4_A/viewform
14–15 January 2025	EDQM Blood Conference: Innovation in Blood Establishment Processes. https://www.edqm.eu/en/edqm-blood-conference

