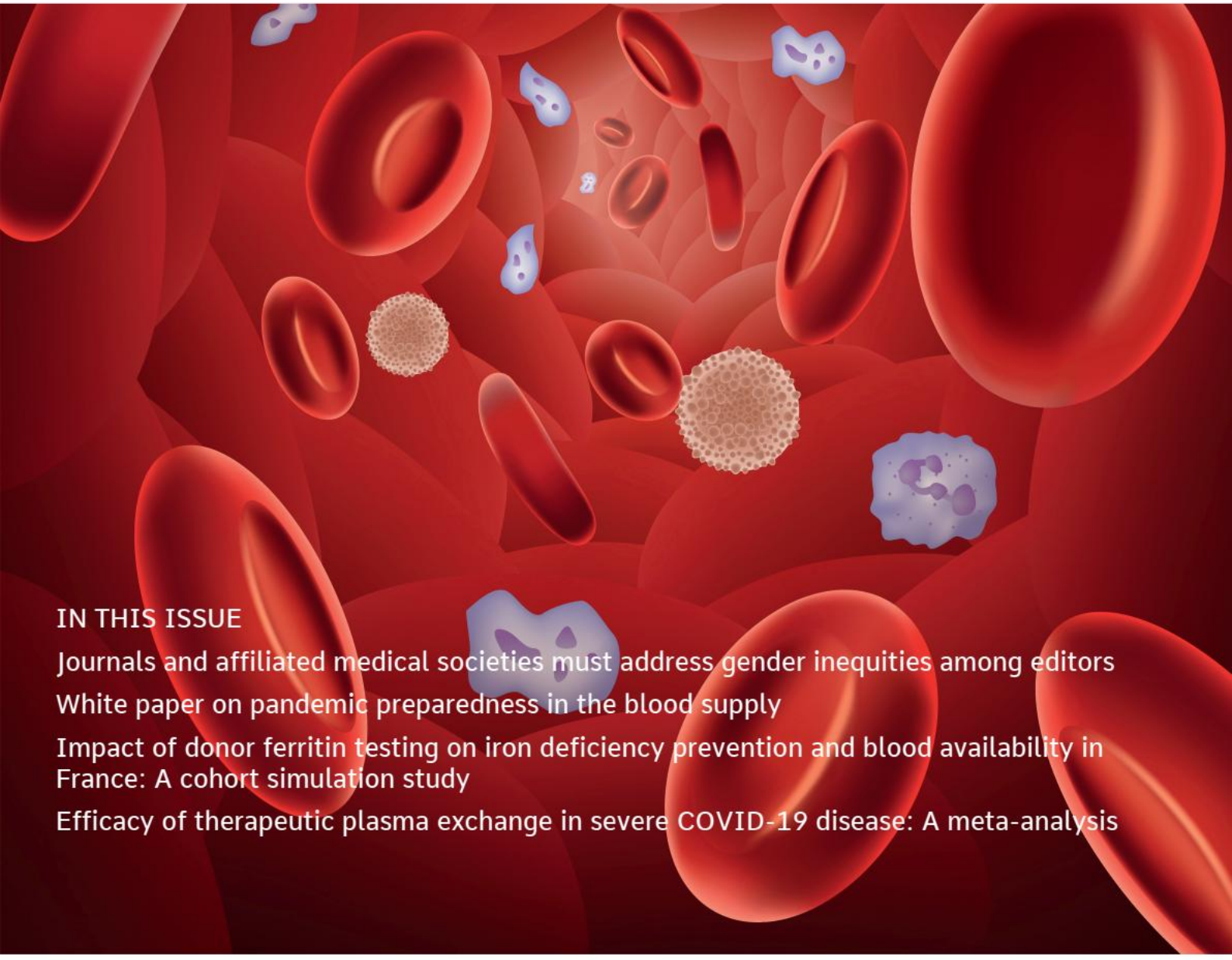
ISSN 0042-9007 (Print)
ISSN 1423-0410 (Online)

Volume 119 Number 4 APRIL 2024

Vox Sanguinis

The International Journal of Transfusion Medicine



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ISBT International Society of Blood Transfusion

Vox Sanguinis

International Journal of Blood Transfusion

Official Journal of the International Society of Blood Transfusion

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

Vox Sanguinis

International Journal of Blood Transfusion

Aims and Scope

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

1. Donors and Donations: Donor recruitment and retention; Donor selection; Donor health (vigilance, side effects of donation); Big data analysis and blood donation.
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.

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

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VOX SANGUINIS (Online ISSN: 1423-0410 Print ISSN: 0042-9007) is published monthly. Postmaster: Send all address changes to VOX SANGUINIS, Wiley Periodicals LLC, C/O The Sheridan Press, PO Box 465, Hanover, PA 17331, USA. For submission instructions, subscription and all other information visit: www.wileyonlinelibrary.com/journal/vox. Printed in the UK by Hobbs the Printers Ltd.

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

Received: 18 March 2024 | Accepted: 18 March 2024

DOI: 10.1111/vox.13623

EDITORIAL

Vox Sanguinis ISBT International Society of Blood Transfusion

The response of *Vox Sanguinis* to a call for action for the planet

In the face of unprecedented environmental challenges, the need to address climate change has never been more pressing. The scientific consensus is clear: the Earth is warming at an alarming rate, driven by the excessive emission of greenhouse gases, such as carbon dioxide (CO_2) and methane, into the atmosphere. These emissions result from human activities, including the burning of fossil fuels for energy, deforestation, industrial processes and agricultural practices. It is crucial to acknowledge our role in causing this crisis, as only through acceptance can we begin to implement effective solutions.

The effects of climate change are becoming increasingly evident, with rising global temperatures, melting ice caps, extreme weather events and disruptions to ecosystems. The Intergovernmental Panel on Climate Change of the United Nations warns that, without swift and substantial action, the consequences will be irreversible, leading to catastrophic impacts on biodiversity, food security and human well-being [1].

In November 2022, coinciding with the 27th Conference of the Parties (COP) of the United Nations Framework Convention on Climate Change, *Vox Sanguinis* published, in conjunction with over 200 journals around the world, an editorial [2] calling for 'urgent actions to ensure it is the COP that finally delivers climate justice for Africa and vulnerable countries. This is essential, not just for health of those countries, but also for the health of the whole world.'

As the editorial stated, the 'health of the world' is not just metaphorical, it is also the health of the people who live on it because one of the consequences of the increase in temperatures is changes in the ecology of the vectors able to transmit infections to humans. The current outbreak of dengue is a case in point. Since the beginning of 2023, an unexpected spike in dengue cases has resulted in a close-to-historic high of over 5 million cases, with more than 5000 dengue-related deaths reported in five World Health Organization regions. Close to 80% of these cases have been reported in the region of the Americas, 69% of them in Brazil [3]. In January 2024, the number of dengue cases in Brazil surged to three times the amount recorded in the same period last year, with the country reporting 217,841 probable cases of the disease. Fifteen deaths from dengue have been confirmed, while 149 are under investigation [4].

In the aforementioned editorial, we also noted that any changes in the field of infectious diseases might ultimately impact the field of transfusion medicine (through potential transmission of infectious disease via transfusion or through severely reducing the blood donor population), and this is a further justification (the first being that we are all part of the human race that inhabits this planet) for *Vox Sanguinis*, one of the voices of the transfusion medicine community, to join the fight against climate change. As evidence of *Vox*'s commitment to this effort, we reported that the printing of the journal would be moved from Singapore to the United Kingdom as of January 2023, and that, as a result, we would reduce the carbon footprint of our publication and distribution process by 41.4%.

This editorial is to announce a step further. From April 2024, *Vox Sanguinis*, reflecting the values of the different stakeholders involved in its publication (the Editorial Board, the Board of Directors of the International Blood Transfusion Society [ISBT] and the publisher, Wiley), will no longer publish the journal in printed form for ISBT members. Only paying subscribers will continue to receive the printed journal until December 2024, after which, we will cease all print publication and distribution to subscribers. This will mean that the 19.1 tons of CO_2 footprint generated by the print distribution of *Vox* will be completely eliminated by December 2024. The impact associated with the journal's print publication (the paper used, the printing process and the plastic packaging) will also be removed, representing a further significant reduction of *Vox Sanguinis'* carbon footprint.

It is hoped that the *Vox Sanguinis* community will support this important step forward by the journal in the fight against climate change. We think this is the right response to the call for action we published in November 2022.

ACKNOWLEDGEMENT

The critical reading of the text by Maria Davie is gratefully acknowledged.

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REFERENCES

1. IPCC. Climate change 2022: Impacts, adaptation and vulnerability. Intergovernmental Panel on Climate Change report. 2022. Available

from: https://www.ipcc.ch/report/ar6/wg2/. Last accessed 14 Mar 2024.
2. Lozano M. Climate change: a call for action, even for *Vox Sanguinis*. Vox Sang. 2022;117:1249–50.
3. World Health Organization. Dengue—Global situation. Disease outbreaks news. Available from: https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON498. Last accessed 14 Mar 2024.
4. Agência Brasil Reporter–Brasília. Dengue cases triple at beginning of year in Brazil. Confirmed fatalities from the disease have reached fifteen. Available from: https://agenciabrasil.ebc.com.br/en/saude/noticia/2024-01/dengue-cases-triple-beginning-year-brazil. Last accessed 14 Mar 2024.

Received: 18 September 2023 | Revised: 5 December 2023 | Accepted: 5 December 2023

DOI: 10.1111/vox.13578

REVIEW

Vox Sanguinis ISBT International Society of Blood Transfusion

Notification and follow-up of blood donors reactive for transfusion-transmitted infections: A narrative review of the literature from India

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Funding information
The authors received no specific funding for this work.

Abstract

Background and Objectives: Notifying blood donors of their reactive status for transfusion-transmitted infections (TTIs) plays a vital role in enabling early diagnosis and management while also preventing these donors from making future donation and transmission of the infectious agent. Given the limited data on donor notification processes in India, a narrative review was conducted to assess the existing notification process and identify areas requiring enhancement.

Materials and Methods: We conducted literature searches using PubMed, Google Scholar and Scopus, employing various keywords. The review included data on the year of the study, study design, donor numbers, TTI screening methods, sero-reactive donor confirmation, notification frequency and methods, donor responses, post-test counselling and risk factor assessment.

Results: Out of the 29 identified articles, 16 studies were included in the analysis. Repeat testing for initially reactive results was conducted in nine studies for 24.3% reactive donors. Phone calls were the primary notification method in most studies (8; 50%), with letters sent in cases of no response. Only 12 studies provided data on notified donors, revealing a notification rate of 71.2%. Of all initially reactive donors, 33.3% sought post-test counselling. Data from six studies indicated that 74.3% of responsive donors had identifiable TTI risk factors.

Conclusion: Our review revealed significant variability in the notification processes across different studies. To enhance the management of TTI-reactive donor notifications and responses, we recommend the establishment of universal protocols encompassing pre-donation counselling, repeat/confirmatory testing, notification methods and comprehensive follow-up and treatment.

Keywords

donor notification, post-test counselling, reactive blood donors, transfusion-transmitted infections

Highlights

- In this review, we found significant variability in the donor notification process, especially with regard to repeat testing of transfusion-transmitted infection (TTI)-reactive blood donors, notification methods, post-notification counselling and follow-up.

- Sub-optimal notification rates and responses from TTI-reactive blood donors were observed.
- Challenges are identified and recommendations provided on how we can improve the notification process for improvement of donor health and overall transfusion safety.

INTRODUCTION

Screening of blood donors for transfusion transmissible infections (TTIs) is a mandatory step towards ensuring the safety of the blood transfusion. During TTI screening of the blood donor samples, if the results for either of the TTI markers are found to be reactive, blood collected from such donors is discarded and the donor is deferred in accordance with the regulatory standards of the region. Majority of these donors who are TTI reactive are first-time donors or are unaware of their infectious status [1]. Thus, it is the duty of the transfusion services to notify such donors, counsel them and refer them to specialty clinics for further investigations and management. This also helps in preventing reactive donors (or their spouses) from future donations and further transmission of the incriminated infectious agent. Although notification of TTI-reactive status has several benefits for blood donors and the donor centre, sometimes an inappropriate approach to disclosure of reactive status can lead to emotional and social distress amongst the donors, which may escalate into medicolegal issues between the donor and the blood centre.

In India, disclosure of TTI-reactive status to the blood donors was not mandated until 2002, when the Government of India adopted National Blood Policy, which allowed for notification, counselling and referral of TTI-reactive blood donors, to ensure safety of the blood supplies [2]. Blood centres are now required to obtain written consent from donors prior to blood donation for donor notification in case of a reactive screening tests. Thereafter, consenting donors are notified after repeat testing and requested to report back to the blood centre for further counselling and referral to appropriate facilities [3]. Although donor notification process is now mandatory, the data regarding the donor notification rates, their responses or return to the blood centres, visit to the referral facilities and their follow-up at the treatment facility are highly variable. The aim of this narrative review was to evaluate the blood donor notification for TTI reactivity in terms of repeat testing, methods of notifications, response to notifications, follow-up after notification and the challenges for the same.

MATERIALS AND METHODS

This narrative review was based on the literature search and analysis of the studies evaluating the donor notification and follow-up of TTI-reactive donors in India.

Literature search

Search engines including PubMed, Google Scholar and Scopus were used for searching available literature. The keyword used were 'blood donor' AND 'notification' OR 'notification rate' OR 'response rate' OR 'counselling' in India. No filter for date or publication type was applied.

Inclusion and exclusion criteria

Studies published in English language with full text available, observational studies (both prospective and retrospective studies), having detailed description of donor notification and their response to notification of sero-reactive donors for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and syphilis were included for evaluation and data analysis. The included studies were also evaluated for retraction statements and any errata published. Non-English studies, grey literature, such as posters, abstracts, supplements, book chapters, case studies, intervention-based studies, letter to editor, review, editorials, redundant or retracted/errata published, were excluded from the analysis. As the screening for malarial parasite was reported by very few studies, with very low reactivity/positivity rates, it was also excluded for further sub-analysis.

Study eligibility and data extraction

The initial search and data extraction were done by one author (R.C.), which was then screened, reviewed and verified independently by two authors (G.K.P. and H.C.P.) for essential steps in the donor notification process (shown in Figure 1). These steps were then assessed for each study included in this narrative review.

The details of the data extracted are as follows:

1. *Study characteristics*: Year of study, nature/design of study, number of donors, methods/techniques for TTI screening, repeat

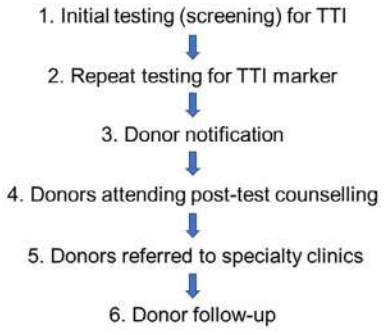

FIGURE 1 Essential steps in the donor notification process. TTI, transfusion-transmitted infection.

TABLE 1 Detailed characteristics of the included studies.

Author/year of publication	Study design	Number of subjects (*n*)	Total reactives % (*n*)	Testing method (viral markers/ syphilis)	Common age group (years)	Gender (M/F)	Type of donor (voluntary/ replacement)	Status of blood donor (first time/ repeat)	Re-testing prior to notification	Notification method	Donor notified % (*n*)	Donors attending counselling centre % (*n*)	Repeat testing during counselling	History of risk behaviour (*n*)	Donor follow-up (*n*)
Patel [4] 2012	Prospective	20,865	1.9 (391)	ELISA/RPR	21–35	388/3	-	-	No	Only phone	NM	60.4 (236)	No	-	-
Kaur [5] 2013	Prospective	15,844	1.1 (172)	ELISA/RPR	26–35	165/7	138/34	-	Yes (same test kit, duplicate sample)	Letter and phone both	89.5 (154)	34.9 (60)	No	-	-
Agarwal [6] 2014	NM	48,386	0.9 (416)	ELISA/RPR	-	-	-	-	Yes (different test kit, single sample)	Letter and phone both	NM	59.9 (249)	No	-	249
Chaurasia [7] 2014	NM	113,014	2.5 (2838)	ELISA/rapid card	-	-	275/2563	-	No	Letter or phone	NM	23.3 (662)	Yes (Same test kit, single sample)	-	373
Sachdev [8] 2015	Retrospective	83,865	0.9 (787)	ELISA/rapid card	-	764/23	562/225	327/460	No	Only phone	78.4 (617)	21.2 (167)	Yes (different test kit, single sample)	142/167	167
Kaur [9] 2015	Prospective	6065	3.4 (204)	ELISA/NM	-	-	-	-	No	Letter and phone both	88.7 (181)	27.5 (56)	No	-	-
Kotwal [10] 2015	Prospective	15,322	3.0 (464)	ELISA[a]/RPR	26–35	457/7	-	-	No	Letter and phone both	49.4 (229)	48.5 (225)	No	182/225	217
Kumari [11] 2017	Prospective	4281	2.7 (116)	ELISA/NM	-	112/4	104/12	55/61	Yes (same test kit, duplicate sample)	Letter f/b phone	100 (116)	35.3 (41)	No	22/41	-
Raturi [12] 2018	Prospective	30,343	0.4 (129)	ELISA/RPR	25–34	128/1	-	89/40	Yes (same test kit, duplicate sample)	Letter and phone both	88.4 (114)	58.1 (75)	No	-	-
Handa [13] 2019	NM	12,621	2.7 (343)	ELISA/RPR	25–35	-	-	-	Yes (same test kit, duplicate sample)	Only phone	53.1 (182)	21.0 (72)	Yes (same test kit, duplicate sample)	-	-
Basnotra [14] 2019	Retrospective	34,204	1.1 (375)	ELISA/rapid card	-	-	-	-	No	Letter and phone both	NM	31.2 (117)	Yes (same test kit, single sample)	-	-
Sabari Priya [15] 2019	Retrospective	4768	1.5 (73)	ELISA/ELISA	-	-	-	-	Yes (same test kit, single sample)	Only phone	56.2 (41)	30.1 (22)	No	-	-
Sardeshpande [16] 2019	NM	8172	1.9 (156)	ELISA/VDRL	26–35	153/3	-	-	No	Letter and phone both	85.9 (134)	86.5 (135)	Yes (same test kit, single sample)	120/135	130

(Continues)

TABLE 1 (Continued)

Author/year of publication	Study design	Number of subjects (*n*)	Total reactives % (*n*)	Testing method (viral markers/ syphilis)	Common age group (years)	Gender (M/F)	Type of donor (voluntary/ replacement)	Status of blood donor (first time/ repeat)	Re-testing prior to notification	Notification method	Donor notified % (*n*)	Donors attending counselling centre % (*n*)	Repeat testing during counselling	History of risk behaviour (*n*)	Donor follow-up (*n*)
Bala Bhasker [17] 2021	Retrospective	17,025	1.1 (183)	CLIA/NM	18–30	182/1	183/0	-	Yes (different test kit, single sample)	Only phone	58.5 (107)	26.8 (49)	No	14/49	-
Dholakiya [18] 2022	Retrospective	36,162	0.6 (212)	CLIA/RPR	18–30	211/1	-	-	Yes (same test kit, duplicate sample)	Only phone	71.7 (152)	52.8 (112)	No	-	-
Syal [19] 2022	Retrospective	1345	2.2 (29)	ELISA and NM	26–35	28/1	-	25/4	Yes (same test kit, single sample)	Only phone	51.7 (15)	48.3 (14)	Yes (same test kit, single sample)	7/14	14

Abbreviations: CLIA, chemiluminescent immunoassay; ELISA, enzyme linked immunosorbent assay; NM, not mentioned; RPR, rapid plasma reagin test; VDRL, venereal disease research laboratory test.
[a]Additional nucleic acid testing (NAT) was performed for testing of viral markers.

testing/confirmation of sero-reactive donors, frequency and method of notification, responsive donors (donors reporting back to blood centres), post-test counselling done/or not for reported donors, history for assessment of high-risk groups (such as history of injectable treatment, promiscuous behaviour, jaundice, tattooing or piercing, blood transfusion).

2. *Details of TTI-reactive donors*: Demographics such as age, gender, type of donor (voluntary/replacement), status of blood donors (first time/repeat), and so on. Total number and marker wise breakout for reactive, notified, responsive and referred donors.

Statistical analysis

The data available were collected and entered Microsoft Excel (MS Office 365). This was followed by quantitative analysis of the common variables across the included studies using percentage and/or proportions as applicable.

RESULTS

Study characteristics

A total of 29 studies were identified, with the initial study being published in 2006. Of these, 13 studies were excluded, due to the publication type (1) or the intervention-based study (3), inclusion of fewer infectious markers (5) and rest due to lack of detailed data for the said infectious markers (4). Because no published statements for retraction or errata were found, no studies were excluded for this. Amongst the remaining 16 studies included in the analysis, a total of 452,282 donors were screened for TTI markers, of which 6888 (1.52%) were found to be initially reactive. The detailed description of the included study characteristics and TTI-reactive donors are shown in Table 1.

Repeat testing prior to donor notification

Repeat testing for initial reactive results was performed in nine studies with 1673 (24.3%) reactive donors. Of these, repeat testing was same testing as the initial screening tests were done for 1444 (86.3%) reactive donors. It was also noticed that the repeat testing was performed in duplicate for 972 (58.1%) reactive donors. The details of reactive donors notified were available for 1257 (75.1%) in eight studies.

Notification of reactive donors

Most of the studies (8; 50%) notified the donors initially by phone calls, and if no response was received, letters were sent, followed by seven (43.8%) studies where only phone calls were used for

TABLE 2 Comparison of response rates to various markers.

	HIV		HBV		HCV		Syphilis		Co-infections/malaria positive	
Author	Reactives	Response rate (%)	Reactives	Response rate (%)	Reactives	Response rate (%)	Reactives	Response rate (%)	Reactives	Response rate (%)
Patel [4]	59	31 (52.5)	176	113 (64.2)	28	11 (39.3)	128	81 (63.3)	0	-
Kaur [5]	6	3 (50.0)	64	26 (40.6)	65	25 (38.5)	37	6 (16.2)	0	-
Agarwal [6]	17	9 (52.9)	225	154 (68.4)	76	42 (55.3)	98	44 (44.9)	0	-
Chaurasia [7]	311	96 (30.9)	1557	313 (20.1)	612	182 (29.7)	358	71 (19.8)	0	-
Sachdev [8]	68	9 (13.2)	449	101 (22.5)	268	56 (20.9)	2	1 (50.0)	0	-
Kaur [9]	11	2 (18.2)	58	19 (32.8)	79	20 (25.3)	56	15 (26.8)	0	-
Kotwal [10]	47	27 (57.4)	284	114 (40.1)	49	28 (57.1)	84	56 (66.7)	10[a]	-
Kumari [11]	12	5 (41.7)	41	14 (34.1)	61	22 (36.1)	0	-	2[a]	-
Raturi [12]	18	14 (77.8)	94	48 (51.1)	2	1 (50.0)	13	10 (76.9)	2[a]/2[b]	-/2[b]
Handa [13]	23	9 (39.1)	90	10 (11.1)	230	53 (23.0)	0	-	0	-
Basnotra [14]	26	15 (57.7)	166	48 (28.9)	40	14 (35.0)	138	40 (29.0)	5[a]	-
Sabari Priya [15]	7	4 (57.1)	51	9 (17.6)	1	-	14	9 (64.3)	0	-
Sardeshpande [16]	17	12 (70.6)	91	86 (94.5)	47	36 (76.6)	1	1 (100.0)	0	-
Bala Bhasker [17]	29	11 (37.9)	106	30 (28.3)	38	6 (15.8)	10	2 (20.0)	1[a]	-
Dholakiya [18]	46	46 (100.0)	86	30 (34.9)	44	22 (50.0)	36	14 (38.9)	0	-
Syal [19]	3	2 (66.7)	6	4 (66.7)	16	7 (43.8)	2	1 (50.0)	2[a]	-
Total	700	295 (42.1)	3544	1119 (31.6)	1656	525 (31.7)	977	351 (35.9)	22[a]/2[b]	-/2[b]

[a]Co-infections.
[b]Malaria.

TABLE 3 Comparison of response rate amongst various subgroups in the included studies.

Author	Groups compared	Reactives (*n*)	Responders (*n*)	Remarks
Patel [4]	Gender (male/female)	388/3	234/0	Male donors responded better
Chaurasia [7]	Type of donor (voluntary/replacement)	275/2563	120/542	Better response amongst voluntary donors
	Gender (male/female)	764/23	161/6	Female donors responded better
Sachdev [8]	Type of donor (voluntary/replacement)	562/225	160/7	Better response amongst voluntary donors
	Donation status (first time/repeat)	327/460	60/107	Better response amongst repeat donors
	Age (<25/26–35/>35 years)	117/227/120	64/109/56	Better response amongst younger donors
Kotwal [10]	Location (near/far off places)	375/89	189/40	Better response amongst donors from nearby areas
	Method of notification (phone/letters)	229/235	225/0	No donor responded to letters
	Age (18–30/31–40/41–60) years	66/33/17	24/10/7	Better response amongst younger donors
	Gender (male/female)	112/4	40/1	Male donors responded better
	Type of donor (voluntary/replacement)	104/12	36/5	Better response amongst voluntary donors
Kumari [11]	Donation status (first time/repeat)	55/61	24/17	Better response amongst first-time donors
	Marital status (married/unmarried)	69/47	30/11	Better response by married donors
	Occupation (farmer/private job/students/ business/labourer/driver/others)	40/27/20/11/8/5/5	9/10//11/5/1/4/1	Response was highest amongst drivers > students > business
Raturi [12]	Donation status (first time/repeat)	89/40	48/27	Better response amongst first-time donors
	Age group (<25/25–35/>35 years)	128/164/51	20/39/13	Better response amongst older donors
Handa [13]	Location (Faridkot/others)	69/113	33/39	Better response amongst donors from nearby areas
	Age group (<25/26–35/>35 years)	45/92/19	37/80/18	Better response amongst younger donors
Sardeshpande [16]	Location (Nagpur/others)	118/38	115/20	Better response amongst donors from nearby areas
	Method of notification (phone/letter)	134/22	125/10	Donors notified using telephone responded better
	Age group (18–25/26–35/>35 years)	6/10/13	4/7/1	Better response amongst younger donors
Syal [19]	Gender (male/female)	28/1	14/1	Male donors responded better
	Donation status (first time/repeat)	25/4	15/0	Better response amongst first-time donors
	Location (urban/rural)	19/10	11/4	Better response amongst donors from urban areas

TABLE 4 Assessment of high-risk behaviour during post-test counselling.

Author	Donors with risk factors (%)	Risk category					Remarks
		H/O injectable treatment	H/O high-risk behaviour	H/O jaundice (self/family/close contacts)	H/O tattooing or ear piercing	H/O blood transfusion	
Sachdev [8]	124 (74.3)	37	34	27	22	4	18 donors were aware of their infectious status or received notification for earlier donations
Kotwal [10]	182 (80.9)[a]	-	-	-	-	-	
Kumari [11]	22 (53.7)	6	12	4	-	2	Some donors with multiple risk
Sardeshpande [16]	120 (88.9)[a]	-	-	-	-	-	
Bala Bhasker [17]	14 (28.6)[a]	-	-	-	-	-	3 donors were aware of their infectious status
Syal [19]	7 (50)	1	4	1	1	-	1 donor was aware of his infectious status

Abbreviation: H/O, history of.
[a]Details not available.

TABLE 5 Follow-up of referred donors.

Author	Donors counselled	Follow-up at referral centre	Long-term follow-up			
			Time of follow-up	Donors receiving treatment	Donors turned negative/medication not started	Donors lost to follow-up
Agarwal [6]	249	249	6 months	180	2 + 52[a]	15
Chaurasia [7]	662	373	6 months	176	NA	NA
Sachdev [8]	167	167	4 months	51	59	57
Kotwal [10]	225	217	-	NA	NA	NA
Sardeshpande [16]	135	130	-	NA	NA	NA
Syal [19]	14	14	At 6 months	7	3	4

Abbreviation: NA, data not available.
[a]Two donors were negative and 52 did not continue treatment.

notification and last one study where either letters or phone calls were used for notification. Of these, data for the notified donors were furnished in 12 studies, which included 2042 (71.2%) donors of 2868 reactive donors.

Response rates

Of the total 6888 reactive donors, 2292 (33.3%) reactive donors reported back to the blood centres for post-test counselling and referral. Details of the responsive donors of these studies amongst various sub-groups are shown in Tables 2 and 3.

Post-test counselling

During post-test counselling, repeat testing for the said infectious marker was done in six studies for 1167 (50.9%) donors of 2292 responsive donors. This repeat testing was mainly performed as a single test using the same test kits (as used during initial screening tests) by most studies (5; 83.3%). Reassessment for the high-risk behaviours during post-test counselling was performed in six studies, the details of which are shown in Table 4.

Donor referral

Only six studies furnished follow-up information of 1150 (50.2%) donors of 2292 responsive donors. This follow-up included their first attendance at the specialty clinical or any consequent follow-up. The details of the follow-up of referred donors are shown in Table 5.

DISCUSSION

In India, the prevalence for TTI reactivity amongst blood donors is quite high when compared with the developed countries [20].

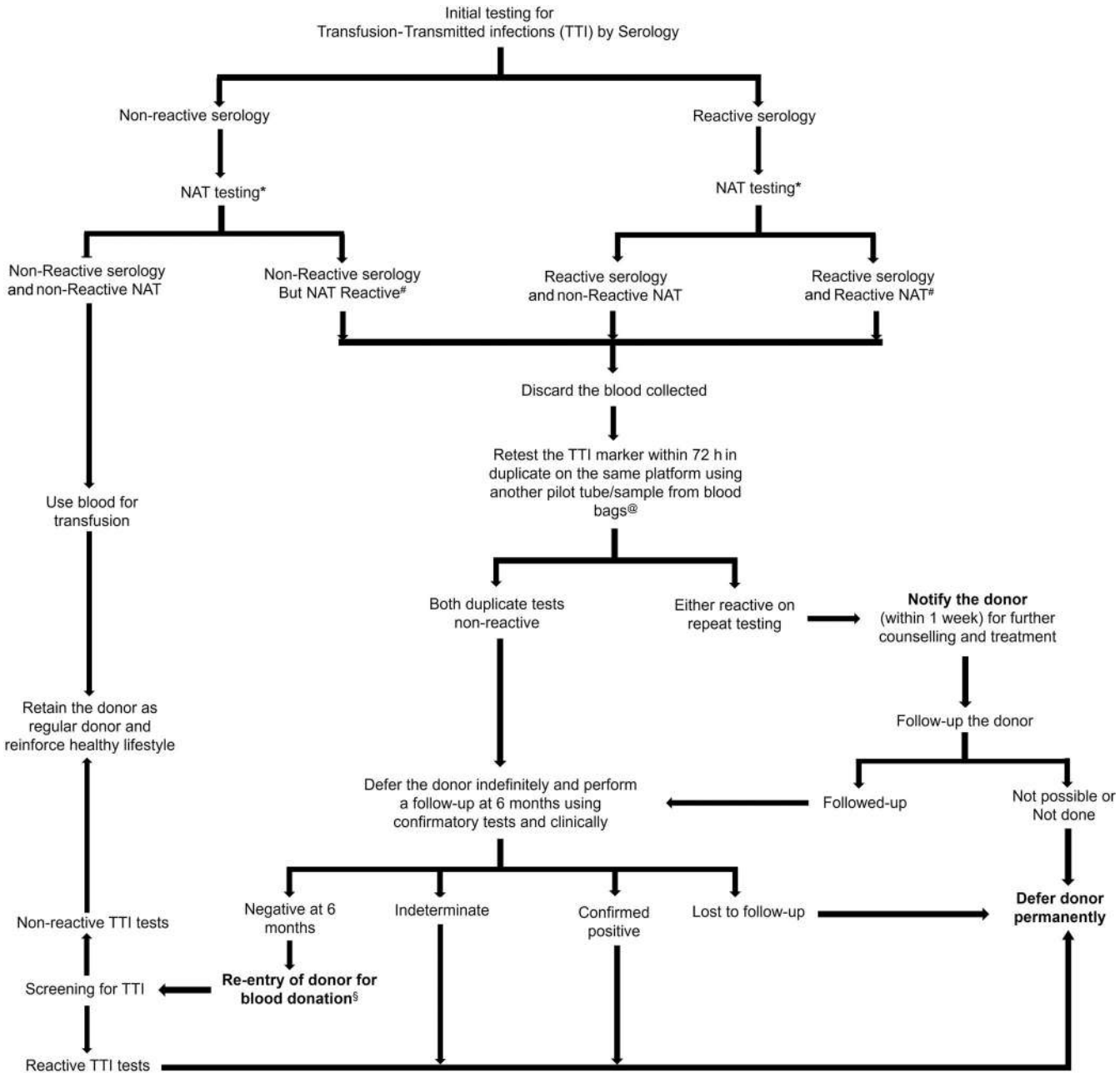

FIGURE 2 Proposed generic flow for donor notification process. NAT, nucleic acid testing; TTI, transfusion-transmitted infection. *NAT is and optional assay; #results marked with sign is not valid for laboratories using only serology-based assays; @retesting should be done using both NAT and serology if NAT is used during initial testing; §not a current policy but can be included in future if regulatory authorities allow).

Notification and follow-up of large number of potentially infected (reactive) individuals place a huge burden on the transfusion services. In this narrative review, we evaluated the algorithms for donor notification and method of notification and their responses, along with the history of risk factors during post-test counselling and the follow-up of referred donors. Notification for TTI-reactive status apprises the blood donors of their potential infectious status, which helps them in early consultation, investigations and treatment. Because notification for false infectious status can lead to an adverse social and emotional impact, it is necessary to ensure the results of screening tests are true and accurate [21, 22]. In 2017, published results of an International forum for donor notification and counselling showed that repeat testing (or confirmatory testing in some countries) is a part of the protocol for TTI testing necessary for donor notification in several developed and developing countries [23]. Although repeat testing was done by majority of the studies included in the review, we also observed considerable variation amongst the algorithms, with regard to the number, type and timings for repeat testing. For the uniformity of the notification process throughout the country, we need to have a defined algorithm for repeat testing with detailed description about the test kits, timing of repeat testing and the number and the type of sample to be used (same tube/fresh or sample from blood unit) for repeat testing or confirmation of the initial results.

TABLE 6 Recommendations to improve donor notification, counselling and referral to treatment facilities.

1. Explaining donor, the importance of TTI testing and implications of reactive status (during pre-donation information/education/counselling) on their health or for their spouses, for the patients and for blood centres.
2. Verifying the details such as address/phone number are correct.
3. Raising awareness and an option for confidential unit exclusion (CUE).
4. Need for confirmation of the sero-reactive results or possibly an algorithm for eliminating false-positive results.
5. Notification should be in a time-bound and expedited manner.
6. Linking or creating a network of referral facilities.
7. Trained and experienced counsellors (especially for pre-donation and post-test counselling donors).
8. Creation of donor clinics (for timely investigation and consultation in the same premises).
9. Creating a mechanism for immediate and long-term follow-up of such donors.
10. Algorithms for re-entry of false positives/treated or immune blood donors back into the donor pool.
11. For better understanding, reporting and universal acceptance, notified donors and responsive donors (donors reporting to blood centre/referral centre) should be re-defined.

Abbreviation: TTI, transfusion-transmitted infection.

During the analysis, we also observed that the detailed results and interpretation of the repeat testing were missing from all the included studies, thereby underrepresenting the false positivity rates observed during initial testing or other technical errors associated with the TTI screening. Compared to other TTI markers, screening for HCV has been reported to yield high false positivity, permanent deferral of such false reactive donors, without any confirmatory/supplemental assays, will lead to significant losses of blood donor from the eligible donor pool, especially in countries where the donation rates are low [24–27].

The observed notification rates were highly variable amongst the included studies, which could be due to difference information provided to the donors during pre-donation counselling, awareness of the donors with regard to TTI or the notification methods or its timing. To ensure that all reactive donors are notified, it is essential to provide proper pre-donation information and counselling to the donors about the nature of the tests performed at blood centres for screening test, the outcomes and its implication of the donor's health (or their family members) through the mode of communication desired by the blood donor [28]. Although proper pre-donation counselling may help solve these issues, practical problems regarding the skill and adequacy of the number of staff involved for pre-donations counselling are far from optimal in India. Although a recent amendment in the regulatory requirement has mandated trained counsellors at blood centres, many busy centres including ours have fewer counsellors, which does not allow sufficient time for each donor, especially during outdoor blood donation drives [29].

Unlike the other parts of the world, where donors are generally notified using postal letter (except for HIV-reactive cases) within 1 week after complete testing, we observed that notification using telephone calls (with or without postal letters) was most common in India [30]. Although, telephonic notifications seem to be the preferred method of notification by many donors and have a better response. However, relying entirely on the phone calls can also be a disadvantage in some situations, as donors are often unable to respond while they are busy or if the phones (mobiles) are not-reachable or switched off, and so on [31]. In the study by Agarwal et al., wherein the authors first sent letter, followed by phone call to confirm the receipt of the letter posted seemed to be an appropriate mechanism for donor notification [6]. Similar strategies using dual method of notification can also be implemented for optimal notification of the reactive donors.

Almost all included studies in this review lacked description of any timelines within which donor notification should be completed. While timely notification can help the donor to seek early counselling and treatment. It may also be convenient for many donors while they can still recall their recent donation and respond/return to the blood centre.

In India, following notification, TTI-reactive donors are asked to return to the blood centres for further counselling without informing the results of TTI screening. We observed a variable and lower response rates when compared with other reports from Malaysia or Israel [32, 33]. Lower responses in our review were mainly attributed to the busy schedule of blood donors or increased time and distance required for reporting back to the blood centre, as majority of the donors in India are family/replacement who often travel long distances to hospitals/health facilities for blood donation when required by their relatives/friends and acquaintances.

It was also felt that during telephonic notification, many reactive donors wanted to visit a clinician of their choice for confirmation of their health status. This clearly outlines the lack of information and counselling regarding notification during pre-donation counselling. It also highlights the lack of trust towards the blood centres or fear of social stigma or fear of ridicule by the blood centres, which would reveal/uncover some of their personal/sexual life during post-test counselling. Building up a network of counselling cum referral facility, along with a confidential data sharing mechanism should be able to overcome the reasons for not attending the post-test counselling.

The overall response rates for HIV-reactive donors were considerably higher for HIV infection and syphilis than hepatitis B and C, which could be due to increased social stigma associated with the HIV infection and/or sexual routes for its transmission. Contrary to this,

return rates for other sub-groups such as age, gender, location (near/far off places), method of notification (phone/letters), type of donor (voluntary/replacement), donation status (first time/repeat), marital status (married/unmarried) and occupation were variable, thus warranting a larger study with increased focus on the demographics, for a targeted approach.

It was also evident from the available data that many of the reactive donors who reported to the blood centre had risk factors for TTI or were already aware of their infectious status, which was overlooked by them during pre-donation screening and counselling [28]. Presence of a centralized database will help preventing such donors from further blood donation. Alternatively, an option for confidential unit exclusion should also be provided to donors during pre-donation counselling, whereby they may give information anonymously of their at-risk blood donation without any peer/societal pressure [34].

Follow-up of reactive donors at the referral centre is to ensure that donors are undergoing clinical consultation, investigation and treatment as necessary. We observed that during follow-up, many donors were confirmed as negative, whereas others were lost to follow-up, which could be due to their false results during initial screening tests. Other reasons for loss of follow-up may be time consuming/inconvenient visits at the referral centres or need for second opinion/advice for confirmation of results notified by the blood centres. Alternatively, 'Donor Clinics' can be instituted at the blood centres for a fast track and complete evaluation of reactive blood donors as recommended by Tiwari et al. [35].

Every year, millions of reactive donors are deferred permanently, some of which may be later confirmed to be negative, after clinical assessment and investigations [26]. Such confirmed negative donors may be allowed to donate blood after a certain follow-up period and repeat investigations [36]. Responses received in an international forum for donor notification and counselling showed that several developed and developing countries already have an algorithm in-place for accepting confirmed negative donors for future blood donations [30]. Although the preparation of similar algorithm in our context requires detailed prospective evidence, a generic process flow addressing these issues has been included as Figure 2.

Another area that needs attention are the blood donors who have hepatitis B or hepatitis C infections and undergo proper treatment with newer medicine and achieve complete remission/sustained viral response [37–40]. Centralized database with evidence from long-term follow-up of such donors can be performed to ensure safety of such donors. This will not only add to the trustiness of the transfusion services but also will increase the base of the available donor pool.

There were two limitations of this study: first, a comprehensive meta-analysis for all aspects was not performed, as the data from the included studies were highly heterogeneous; second, studies evaluating only fewer TTI markers were excluded from analysis due to risk of assessment bias.

To conclude, there is limited quality evidence on donor notification in India. We observed significant variations in the donor notification process, with respect to the repeat testing prior to notification, mechanism of notification, return to blood centre, referral and their follow-up. Longitudinal studies are therefore needed to evaluate the essential steps in the donor notification process and the long-term follow-up of donors in order to determine false-positivity rates, deferral status for donors and the likelihood that these donors will re-enter the eligible donor pool. Furthermore, we recommend the strategies outlined in Table 6 for improvement of the overall notification process.

ACKNOWLEDGEMENTS

R.C. contributed to the conceptualization, performed initial review of the articles searched, designed the methodology, performed data analysis and wrote the initial draft of the article. G.K.P. re-reviewed the articles searched, performed formal analysis, drafted the final article and verified the data entered. H.C.P. re-reviewed the articles searched, designed the methodology, performed formal analysis and reviewed the article. R.M. contributed to data entry, performed final analysis and reviewed the article.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The compilation of the results from the included studies in the review shall me made available as and when necessary.

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REFERENCES

1. Shrivastava M, Mishra S, Navaid S. Time trend and prevalence analysis of transfusion transmitted infections among blood donors: a retrospective study from 2001 to 2016. Indian J Community Med. 2023;48:274–80.
2. *National Blood Policy*. New Delhi: National AIDS Control Organisation, Ministry of Health & Family Welfare, GoI; 2007. Available from: http://nbtc.naco.gov.in/page/policies_guidelines/. Last accessed 16 Dec 2023.
3. NACO (NBTC) Ministry of Health & Family Welfare. Guidelines for blood donor selection and blood donor referral. New Delhi, India: NACO (NBTC) Ministry of Health & Family Welfare, GoI; 2017. Available from: http://nbtc.naco.gov.in/page/policies_guidelines/. Last accessed 16 Dec 2023.
4. Patel P, Patel S, Bhatt J, Bhatnagar N, Gajjar M, Shah M. Evaluation of response to donor notification of reactive transfusion transmitted infections (TTIs) result. Natl J Integr Res Med. 2012;3:20–5.
5. Kaur G, Kaur P, Basu S, Kaur R, Sharma S. Donor notification and counseling – experience and challenges. Transfus Apher Sci. 2013; 49:291–4.
6. Agarwal N. Response rate of blood donors in the Uttarakhand region of India after notification of reactive test results on their blood samples. Blood Transfus. 2014;12:s51–s53.
7. Chaurasia R, Zaman S, Das B, Chatterjee K. Screening donated blood for transfusion transmitted infections by serology along with NAT and response rate to notification of reactive results: an Indian experience. J Blood Transfus. 2014;2014:412105.

8. Sachdev S, Mittal K, Patidar G, Marwaha N, Sharma RR, Duseja AK, et al. Risk factors for transfusion transmissible infections elicited on post donation counselling in blood donors: need to strengthen pre-donation counselling. Indian J Hematol Blood Transfus. 2015;31:378–84.
9. Kaur H, Mannan R, Manjari M, Piplani S, Arora S. Notification rate and counseling for seropositive donors in a tertiary care teaching hospital at Amritsar (Punjab), India. GJMR. 2015;15:13–6.
10. Kotwal U, Doda V, Arora S, Bhardwaj S. Blood donor notification and counseling: our experience from a tertiary care hospital in India. Asian J Transfus Sci. 2015;9:18–22.
11. Kumari S. Reactive donor notification and counseling: reveals concealed risk factors. Indian J Soc Psychiatry. 2017;33:38–43.
12. Raturi M, Shastry S, Raj P. Response rate of sero-reactive blood donors to notification and counselling. JTM. 2018;11:144–50.
13. Handa A, Kukar N, Aggarwal D, Maharishi RN. Donor notification and response rate of sero-reactive blood donors: a challenge and long way to go. Int J Community Med Public Health. 2019;6:2794–7.
14. Basnotra RM, Sidhu MD. Donor notification in reactive donors and their response to communication. Int J Res Med Sci. 2019;7:1088–92.
15. Sabari PE. Retrospective analysis of patterns of donor deferral among blood donors in a tertiary care hospital. Int J Contemp Med Res. 2019;6:A6–9.
16. Sardeshpande AM, Parate SN. Donor notification & counseling – our experience from a tertiary care hospital in central India. J Med Sci Clin Res. 2009;7:679–84.
17. Bhasker PMB, Aluri A. Donor notification and counseling: experiences and challenges from a private multi-specialty hospital in South India. Asian J Transfus Sci. 2021;15:166–71.
18. Dholakiya S, Nandani S, Vachhani N. Donor notification and response rate in a stand-alone blood center in Western India. Glob J Transfus Med. 2022;7:159.
19. Syal N, Kukar N. Impact of notification and counselling of reactive blood donors at a super-speciality hospital. Int J Community Med Public Health. 2022;9:853–7.
20. Hillary EJ, Saurabh S, Rachita S, Nakul R, Raman K, Ravindranath T, et al. Access to safe blood in low-income and middle-income countries: lessons from India. BMJ Glob Health. 2017;18:e000167.
21. Kleinman S, Wang B, Wu Y, Glynn SA, Williams A, Nass C, et al. The donor notification process from the donor's perspective. Transfusion. 2004;44:658–66.
22. Kiely P, Hoad VC, Wood EM. False positive viral marker results in blood donors and their unintended consequences. Vox Sang. 2018;113:530–9.
23. Sharma RR, Lozano M, Fearon M, Bigham M, Djoudi R, Gallian P, et al. Vox Sanguinis International Forum on donor notification and counselling strategies for markers of transfusion-transmissible infections. Vox Sang. 2017;112:e1–e21.
24. Fletcher GJ, Raghavendran A, Sivakumar J, Samuel P, Abraham P. Diagnostic reliability of Architect anti-HCV assay: experience of a tertiary care hospital in India. J Clin Lab Anal. 2018;32:e22245.
25. Lucey O, Acana S, Olupot-Olupot P, Muhindo R, Ayikobua R, Uyoga S, et al. High false discovery rate of the Architect anti-HCV screening test in blood donors in Uganda and evaluation of an algorithm for confirmatory testing. Vox Sang. 2022;117:1360–7.
26. Katz L, Strong DM, Tegtmeier G, Stramer S. Performance of an algorithm for the reentry of volunteer blood donors deferred due to false-positive test results for antibody to hepatitis B core antigen. Transfusion. 2008;48:2315–22.
27. Grégoire Y, Germain M, Delage G. Factors associated with a second deferral among donors eligible for re-entry after a false-positive screening test for syphilis, HCV, HBV and HIV. Vox Sang. 2018;113:339–44.
28. Polizzotto MN, Wood EM, Ingham H, Keller AJ. Reducing the risk of transfusion-transmissible viral infection through blood donor selection: the Australian experience 2000 through 2006. Transfusion. 2008;48:55–63.
29. Drugs and cosmetics (second amendment) rules, 2020 (GSR 166E Blood centres) Department of Health and Family Welfare, New Delhi, GoI; 2020.
30. Sharma RR, Lozano M, Fearon M, Bigham M, Djoudi R, Gallian P, et al. Vox Sanguinis International Forum on donor notification and counselling strategies for markers of transfusion-transmissible infections: summary. Vox Sang. 2017;112:388–96.
31. Porto-Ferreira FA, de Almeida-Neto C, Murphy EL, Montebello SC, Nogueira FA, Koga da Silva EM, et al. A randomized trial to evaluate the use of text messaging, letter, and telephone call reminders to improve return of blood donors with reactive serologic tests. Transfusion. 2017;57:102–7.
32. Roshan TM, Rosline H, Ahmed SA, Rapiaah M, Khattak MN. Response rate of Malaysian blood donors with reactive screening test to transfusion medicine unit calls. Southeast Asian J Trop Med Public Health. 2009;40:1315–21.
33. Kerzman H, Green MS, Shinar E. Predictors for non-compliance of notified hepatitis C virus-positive blood donors with recommendation to seek medical counselling. Vox Sang. 2009;96:20–8.
34. Rad F, Sajjadi SM, Azizi A, Maghsudlu M. Confidential unit exclusion (CUE): double edged sword for blood transfusion center: a ten-year study on volunteer blood donors in Kohgiluyeh and Boyer Ahmad province. Tran Transfus Clin Biol. 2019;26:253–6.
35. Tiwari AK, Bhardwaj G, Dara RC, Arora D, Aggarwal G, Bhargava R, et al. Notification and counselling of hepatitis positive blood donors, their immediate emotional response, contact-testing and their follow-up: study from a tertiary care hospital! Transfus Apher Sci. 2018;57:391–7.
36. Deng X, Zang L, Candotti D. Re-entry evaluation of Chinese blood donors with unconfirmed hepatitis B screening results. Viruses. 2022;14:2545.
37. Peng J, Cao J, Yu T, Cai S, Li Z, Zhang X, et al. Predictors of sustained virologic response after discontinuation of nucleos(t)ide analog treatment for chronic hepatitis B. Saudi J Gastroenterol. 2015;21:245–53.
38. Sonneveld MJ, Park JY, Kaewdech A, Seto W-K, Tanaka Y, Carey I, et al. Prediction of sustained response after nucleo(s)tide analogue cessation using HBsAg and HBcrAg levels: a multicenter study (CREATE). Clin Gastroenterol Hepatol. 2022;20:e784–93.
39. Carrat F, Fontaine H, Dorival C, Simony M, Diallo A, Hezode C, et al. Clinical outcomes in patients with chronic hepatitis C after direct-acting antiviral treatment: a prospective cohort study. Lancet. 2019;393:1453–64.
40. Bhattacharya D, Aronsohn A, Price J, Lo Re V, AASLD-IDSA HCV Guidance Panel. Hepatitis C guidance 2023 update: AASLD-IDSA recommendations for testing, managing, and treating hepatitis C virus infection. Clin Infect Dis. 2023;25:ciad319.

How to cite this article: Chaurasia R, Patidar GK, Pandey HC, Meher R. Notification and follow-up of blood donors reactive for transfusion-transmitted infections: A narrative review of the literature from India. Vox Sang. 2024;119:289–99.

Received: 5 September 2023 | Revised: 1 December 2023 | Accepted: 6 December 2023

DOI: 10.1111/vox.13579

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Development and validation of a scoring system to predict vasovagal reaction upon whole-blood donation

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Funding information
The authors received no specific funding for this work.

Abstract

Background and Objectives: Risk factors for vasovagal reaction (VVR) have been extensively studied. With knowledge of the relative importance of these risk factors for VVR, collection staff could take care of blood donors from the same standpoint, leading to improved donor safety. We therefore developed a scoring system to predict VVR, which incorporates registration information.

Materials and Methods: Pre-syncopal and syncopal symptoms, as well as on- and off-site reactions, are included in this analysis as VVR. We defined the donor status as follows: first-time donors, repeat donors with no history of reaction and repeat donors with a history of reaction. We prepared two datasets: whole-blood donations at a blood donation site in Tokyo between January 2019 and December 2019 were included in training data ($n = 361{,}114$), and whole-blood donations between January 2020 and August 2020 were included in testing data ($n = 216{,}211$).

Results: The most important variable was the donor status, followed by age, estimated blood volume and height. We integrated them into a scoring system. Training and testing datasets were combined ($n = 577{,}325$), and VVR rates in groups with scores of 0, 1, 2, 3, 4 and 5 or more were 0.09% (95% CI: 0.081%–0.10%), 0.33% (95% CI: 0.31%–0.36%), 0.87% (95% CI: 0.78%–0.96%), 1.17% (95% CI: 1.05%–1.30%), 2.15% (95% CI: 1.98%–2.32%) and 3.11% (95% CI: 2.90%–3.34%), respectively.

Conclusion: The scoring system enables staff to significantly predict VVR and may help them to identify donors at increased risk of experiencing syncope, thereby mitigating the negative impact of VVR on donor safety and return by paying close attention to high-risk donors.

Keywords

age, donor status, estimated blood volume, height, risk factor, scoring system, vasovagal reaction, whole-blood donation

Tatsuma Hashizume and Gaku Kondo contributed equally to this work.

Highlights

- The most important vasovagal reaction (VVR) risk factor was donor status (first-time or repeat donors with or without history of reaction), followed by age, estimated blood volume and height.
- Integrating these risk factors into a scoring system facilitated the significant prediction of VVR.
- With this scoring system, collection staff could take care of blood donors from the same standpoint, leading to improved donor safety.

INTRODUCTION

Several mechanisms underlie a vasovagal reaction (VVR) in blood donors, which may be physiological and/or psychological [1, 2]. Identifying blood donors at an increased risk of experiencing syncope during voluntary donation is important in terms of donor return and safety, especially delayed reactions leading to critical consequences, including severe injuries. To alleviate the negative impact of the symptom, risk factors for VVR have been extensively studied. A recent systematic review [3] reported risk factors associated with the development of VVR among whole-blood donors. The following risk factors were associated with an increased risk of syncope: female sex [4–6], lower body weight [7–9], lower estimated blood volume (EBV) [5, 10, 11], young age [4, 6, 11, 12], first-time donor [4, 6, 9, 11, 12], low resting blood pressure [7], reduced sleep before donation [13, 14], donation location [11] and previous history of symptoms at blood donation [15, 16].

Knowing the relative importance of these key factors for VVR may allow for improved screening prior to donation and mitigate attrition due to negative experiences. The collection staff would be able to take care of blood donors from the same standpoint, and could pay more attention to high-risk donors, leading to improved donor safety. We, therefore, developed a scoring system for blood donors to predict VVR, which incorporates registration information such as the donor status, age and EBV. Pre-syncopal and syncopal symptoms as well as on- and off-site reactions are included in this analysis as VVR.

MATERIALS AND METHODS

Data collection

We prepared two datasets: training and testing data. Whole-blood donors who donated at a blood donation site in Tokyo of The Japanese Red Cross Society over a period of 1 year between January 2019 and December 2019 were included in the training data ($n = 361{,}114$), and over 8 months between January 2020 and August 2020 were included in the testing data ($n = 216{,}211$). There are two set volumes of whole-blood donations in Japan: 200 and 400 mL. Donors requesting 200 mL of whole-blood donation were excluded because they were fewer and less likely to develop VVR than those requesting 400 mL whole-blood donation. (The adjusted VVR rate using an inverse probability weighting method with propensity score was 0.35% and 0.54%, respectively, $p = 0.043$, based on a nationwide survey in 2013.) During the study period, there were multiple donations from some donors. We included all donations from each donor. Thus, the unit of analysis was 'donations' rather than 'donors'.

The donor characteristics collected from the database were age, sex, body weight and height, systolic blood pressure (sBP), diastolic blood pressure (dBP), pulse rate (PR), donation history (first-time or repeat), sleeping and fasting time, number of VVR experiences in past donations and donation site (mobile or fixed). Height, weight and sleeping time were self-reported by the donors at the time of donation. Fasting time was calculated by subtracting the self-reported last mealtime from the start time of blood collection. sBP, dBP and PR were measured before blood collection using fully automated sphygmomanometers.

Outcome measure

The objective variable was the occurrence of VVR, defined as a general feeling of discomfort and weakness with anxiety, dizziness and nausea related to blood donation, which may progress to loss of consciousness [17]. The presence of VVR was confirmed by the blood collection staff on the basis of symptoms and vital signs. Off-site reactions were recorded only if the donors notified us voluntarily. VVR in this analysis refers to all reactions including pre-syncopal symptoms and syncopal reactions, as previously described [13].

Permutation variable importance

Permutation variable importance (PVIMP) is defined as the decrease in the prediction performance of a model when the value of a single variable is randomly shuffled. In detail, for a given variable x, the dataset is randomly shuffled in x, leaving all other variables in place, and the prediction accuracy is calculated. PVIMP for x is calculated as the rate of decrease in predictive accuracy by perturbing. In principle, PVIMP is based on the idea that if a variable is highly relevant to the outcome, the degree of reduction in predictive accuracy will be greater by perturbing it.

Predictors

We introduced a novel variable called the donor status. The donor status consists of four groups: first-time donors (FT), repeat donors with no history of VVR (Rep 0), repeat donors with a history of one episode of VVR (Rep 1) and repeat donors with a history of two or more episodes of VVR (Rep 2). Additional variables included in this model were as follows: metric scale variables— age, EBV, height, sBP, dBP, PR, shock index (SI: pulse in bpm divided by sBP in mmHg), sleeping time and fasting time; and categorical scale variables—sex and blood donation venue (mobile or fixed). Categorical scale variables were converted to numerical data of 0 or 1 as dummy variables. At blood collection sites of the Japanese Red Cross Society, blood donors are asked how much sleep they had the night before, and the sleeping time is used as a criterion to assess the safety of blood donation [13]. EBV is calculated according to Ogawa's equation [13]. The fact that EBV is calculated from the sex, weight and height suggests that these three variables are correlated with EBV. The correlation coefficients with EBV were calculated as 0.68, 0.99 and 0.66, respectively. The correlation coefficient between EBV and body weight was high. Because the coexistence of variables that are highly correlated with each other leads to biased results, we decided to exclude body weight. On the other hand, we judged that sex and height were not correlated with EBV sufficiently to warrant exclusion from our analysis. Although SI is also calculated from sBP and PR, the correlation coefficients of sBP and PR with SI were calculated as 0.30 and 0.027, respectively, which were both low.

Estimating importance of the variable

We first estimated the importance of each variable considered to be associated with VVR using the training data. PVIMP was used as an indicator of the importance [18].

Specifically, the original sample is randomly partitioned into 10 equally sized sub-samples. Of these 10 sub-samples, a single sub-sample is retained as the validation data for testing the model, and the remaining 9 sub-samples are used as training data. The cross-validation process is then repeated 10 times, with each of the 10 sub-samples used exactly once as the validation data. For each cross-validation process, the predictive accuracy based on a sub-sample is recorded. Then, PVIMP is calculated for all the sub-samples and averaged to estimate the importance of each variable.

Random forest (RF) was used to create the predictive model from the dataset. RF is an ensemble learning method that fits many decision-tree classifiers on various bootstrap samples of the dataset and uses averaging to improve the predictive accuracy [19]. RF can calculate the predicted outcome and predicted probability. Our data were imbalanced, with markedly skewed class proportions for the objective variable (VVR occurrence: 1730 vs. VVR non-occurrence: 359,384). In such cases, machine learning, including RF, predicts that VVR will not occur in almost all cases, so precision (positive predictive value), recall (sensitivity) and so forth, which are based on the predicted outcome, are inappropriate as indicators of the model's predictive accuracy, which was used to calculate PVIMP. Based on the above facts, we adopted the area under the curve (AUC), which reflects the accuracy of predicted probabilities, as an indicator of the model's predictive accuracy in calculating PVIMP. Receiver operating characteristic (ROC) curves are plots of sensitivity and specificity by arranging samples on a straight line based on predicted probabilities and varying cut-off values. AUC of the ROC curve represents the degree to which the presence or absence of an outcome can be separated and discriminated between samples in a row based on predicted probabilities. Theoretically, AUC is an index that takes a value between 0 and 1; AUC = 1 means perfect classification, and AUC < 0.5 means classification that is worse than random.

Development of scoring system

First, the range of weight given to each variable was determined to reflect the magnitude of PVIMP. No further steps were taken for variables with the range of weight of zero. Next, using the predictive model, we graphically represented the predicted average marginal probability of VVR at a range of values of each predictor. An average marginal probability for a given predictor, which was a point on the graph, was calculated by taking the sum of the predicted probabilities of VVR for each array consisting of the values of the other predictors and averaging that sum. In this way, it is possible to calculate probabilities that remove the effect of the covariance. Using this graph for each predictor as a reference, cut-off points for the metric scale variables were determined, and weights for each class within the variables were calculated based on the predicted average marginal probability of VVR. Lastly, we performed variable selection by a backward selection method of maximizing R^2, which was calculated by the Cochrane–Armitage trend analysis. This analysis is an approach for determining the regression line using the weighted least squares method (regressing VVR rate by score). R^2 is a statistical measure of how well the regression predictions approximate the real data points. R^2 ranges from 0 to 1, and an R^2 of 1 indicates that the regression predictions perfectly fit the data. In the validation, we applied the scoring algorithm to the testing dataset, the performance of which was assessed by calculating R^2.

Finally, we applied the scoring algorithm to the overall dataset to improve the estimation accuracy by increasing the sample size.

Data analyses were conducted using scikit-learn (version 0.24.1), a library for Python for a machine-learning approach, and R version 3.6.1 for a statistical approach.

RESULTS

Population characteristics

Overall, VVR occurred in 0.48% and 0.52% of the population in training and testing datasets, respectively. There was no major difference in characteristics of donors between training and testing data (Table 1). We detected a small increase of VVR rates from the training

TABLE 1 Characteristics of study population.

Characteristics		Training data (%)	VVR (+)/(−)	Testing data (%)	VVR (+)/(−)
Total donation		361,114	1730/359,384	216,211	1129/215,082
Sex	Male	261,300 (72.4)	929/260,371	150,769 (69.7)	514/150,255
	Female	99,814 (27.6)	801/99,013	65,442 (30.3)	615/64,827
Age	17–19	17,927 (5.0)	337/17,590	6075 (2.8)	138/5937
	20–29	72,090 (20.0)	832/71,258	40,933 (18.9)	518/40,415
	30–49	160,253 (44.4)	455/159,798	98,122 (45.4)	378/97,744
	50–69	110,844 (30.7)	106/110,738	71,081 (32.9)	95/70,986
Donation history	First-time	36,387 (10.1)	798/35,589	17,250 (8.0)	436/16,814
	Repeat	324,727 (89.9)	932/323,795	198,961 (92.0)	693/198,268
Weight (kg)	50–59	96,414 (26.7)	922/95,492	58,822 (27.2)	593/58,229
	60–69	124,012 (34.3)	520/123,492	73,313 (33.9)	331/72,982
	70–79	86,756 (24.0)	204/86,552	51,366 (23.8)	143/51,223
	80–89	37,365 (10.3)	62/37,303	22,470 (10.4)	47/22,423
	≥90	16,567 (4.6)	22/16,545	10,240 (4.7)	15/10,225
Height (m)	≤1.59	49,512 (13.7)	471/49,041	31,789 (14.7)	355/31,434
	1.60–1.79	285,510 (79.1)	1197/284,313	169,391 (78.3)	742/168,649
	≥1.80	26,092 (7.2)	62/26,030	15,031 (7.0)	32/14,999
EBV (mL)	≤3499	6956 (1.9)	96/6860	4129 (1.9)	65/4064
	3500–4499	158,885 (44.0)	1152/157,733	96,204 (44.5)	736/95,468
	4500–5499	160,906 (44.6)	435/160,471	94,774 (43.8)	292/94,482
	≥5500	34,367 (9.5)	47/34,320	21,104 (9.8)	36/21,068
Systolic BP (mmHg)	90–99	9585 (2.7)	94/9491	5148 (2.4)	64/5084
	100–119	115,207 (31.9)	859/114,348	66,658 (30.8)	562/66,096
	120–139	161,886 (44.8)	629/161,257	97,738 (45.2)	414/97,324
	140–159	64,150 (17.8)	135/64,015	39,983 (18.5)	87/39,896
	≥160	10,286 (2.8)	13 / 10,273	6684 (3.1)	2/6682
Diastolic BP (mmHg)	≤59	15,443 (4.3)	172/15,271	7426 (3.4)	97/7329
	60–79	166,213 (46.0)	1126/165,087	94,683 (43.8)	685/93,998
	80–99	157,609 (43.6)	406/157,203	99,366 (46.0)	321/99,045
	≥100	21,849 (6.1)	26/21,823	14,736 (6.8)	16/14,720
Pulse (bpm)	≤59	20,314 (5.6)	83/20,231	10,126 (4.7)	59/10,067
	60–89	284,694 (78.8)	1303/283,391	165,459 (76.5)	783/164,676
	≥90	56,106 (15.5)	344/55,762	40,626 (18.8)	287/40,339
Fasting time (h)	≤3	220,921 (61.2)	1064/219,857	134,890 (62.4)	731/134,159
	4–6	125,234 (34.7)	572/124,662	73,138 (33.8)	353/72,785
	≥7	14,959 (4.1)	94/14,865	8183 (3.8)	45/8138
Sleeping time (h)	≤5	51,457 (14.2)	258/51,199	23,684 (11.0)	123/23,561
	6–8	295,899 (81.9)	1371/294,528	182,810 (84.6)	925/181,885
	≥9	13,758 (3.8)	101/13,657	9717 (4.5)	81/9636
Number of previous episodes of VVR	0	349,318 (96.7)	1529/347,789	208,078 (96.2)	984/207,094
	1	9728 (2.7)	167/9561	6591 (3.0)	119/6472
	≥2	2068 (0.6)	34/2034	1542 (0.7)	26/1516
Donation site	Mobile	148,890 (41.2)	815/148,075	77,465 (35.8)	407/77,058
	Fixed	212,224 (58.8)	915/211,309	138,746 (64.2)	722/138,024

Abbreviations: BP, blood pressure; EBV, estimated blood volume; VVR, vasovagal reaction.

data period (0.48%) to testing data period (0.52%) during the COVID-19 pandemic. A recent report suggested that VVR rates are elevated during the pandemic, probably because of restriction measures such as universal masking, regardless of the donation type [20].

Importance of variable

PVIMP showed that the most important variable was the donor status, followed (in order) by age, EBV, height, sBP, SI, dBP, PR, sex, donation site, fasting time and sleeping time (Figure 1). The predictive performance of RF used for these analyses was as high as 0.84 for AUC with five-fold cross-validation.

Development and performance of scoring system

The range of weight given to each variable according to the magnitude of PVIMP is shown in Table 2. The cut-off points for the metric scale variables determined from the graph of the average marginal probability of VVR are also shown in Table 2. As an example, Figure 2 shows the average marginal probability curve for height, on the basis of which we set the cut-off value at 1.65 m. The weights for each class within the variables were determined on the basis of the average marginal probability of VVR. For example, the predicted VVR rates of FT, Rep 0, Rep 1 and Rep 2, which were classes of the variable donor status, were calculated as 1.43%, 0.32%, 1.18% and 1.25%, respectively (Figure 3). Therefore, we determined the weights of the classes as 3, 0, 3 and 3, respectively. Variable selection by a backward selection method resulted in a scoring system with the donor status, age, EBV and height showing the highest R^2 (=0.94) (Table 3). Scores of 5 or more were grouped together, since scores of 6 and 7 were awarded to far fewer cases than other scores (number of cases with score exceeding 5 was 4113).

Figure 4a shows how the VVR rate changes as the score increases in the training data. There was a positive systematic linear relationship between the two variables, with higher scores indicating a higher incidence of VVR (p for trend <.001).

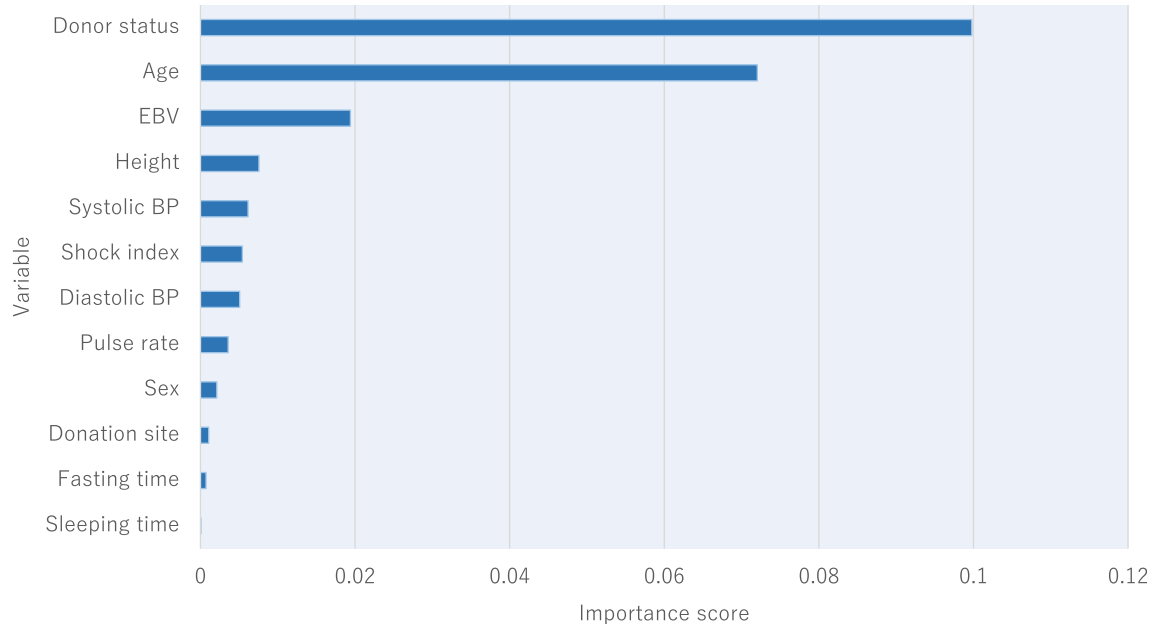


FIGURE 1 Permutation variable importance of each variable. How much the variable contributes to improving the predictive accuracy of the model is shown. BP, blood pressure; EBV, estimated blood volume.

TABLE 2 Range of weight and cut-off points for each variable.

Variable	Donor status	Age	EBV	Height	sBP	dBP	SI	PR	Sex	Donation site	Sleeping time	Fasting time
Range of weight	0–3	0–2	0–1	0–1	0–0.5	0–0.5	0–0.5	0–0.5	0–0.5	0	0	0
Cut-off points		20 and 30	3500	1.65	100	50	0.8	90				

Note: The range of weight given to each variable was determined to reflect the magnitude of permutation variable importance. The cut-off points for metric scale variables were determined from the graph of the average marginal probability of vasovagal reaction. Donation site, sleeping time and fasting time, of which the range of weight is 0, did not proceed to the next step.
Abbreviations: dBP, diastolic blood pressure; EBV, estimated blood volume; PR, pulse rates; sBP, systolic blood pressure; SI, shock index.

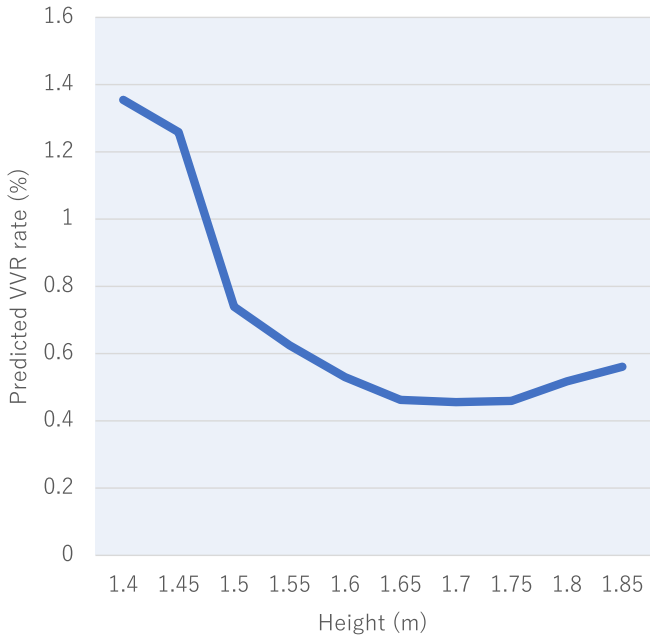


FIGURE 2 Average marginal probability curve for height. On the basis of the curve, the cut-off value for height was set at 1.65 m. VVR, vasovagal reaction.

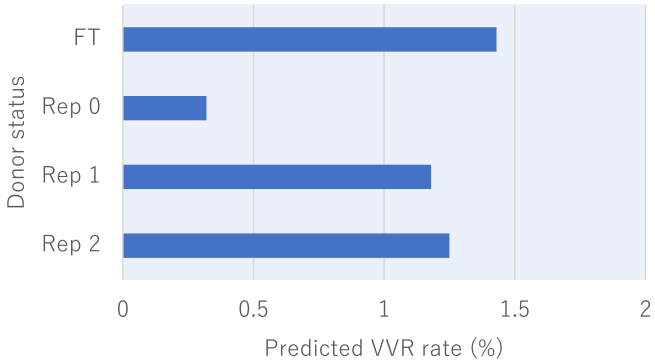


FIGURE 3 Predicted vasovagal reaction (VVR) rates of first-time donor (FT), repeat donor with no history of VVR (Rep 0), repeat donor with a history of one episode of VVR (Rep 1) and repeat donor with a history of two or more episodes of VVR (Rep 2). First-time donation and previous donation reactions equally influenced VVR.

A positive systematic linear relationship was observed between the score and VVR rate for the testing data as well (Figure 4b), and the calculated R^2 was 0.91, which was sufficiently high (p for trend <.001).

The training and testing datasets were combined ($n = 577{,}325$) to provide a more precise estimate of the VVR rate for each score. Overall, VVR occurred in 0.50% of donors. VVR rates in groups with scores of 0, 1, 2, 3, 4 and 5 or more were 0.09% (95% CI: 0.081%–0.10%), 0.33% (95% CI: 0.31%–0.36%), 0.87% (95% CI: 0.78%–0.96%), 1.17% (95% CI: 1.05%–1.30%), 2.15% (95% CI: 1.98%–2.32%) and 3.11% (95% CI: 2.90%–3.34%), respectively (Figure 4c). The calculated R^2 was 0.94, which was high (p for trend <.001).

TABLE 3 VVR scoring system.

Score	0	1	2	3
Donor status	Rep 0	-	-	FT/Rep 1/Rep 2
Age	≥30	20–29	17–19	-
EBV (mL)	>3500	≤3500	-	-
Height (m)	>1.65	≤1.65	-	-

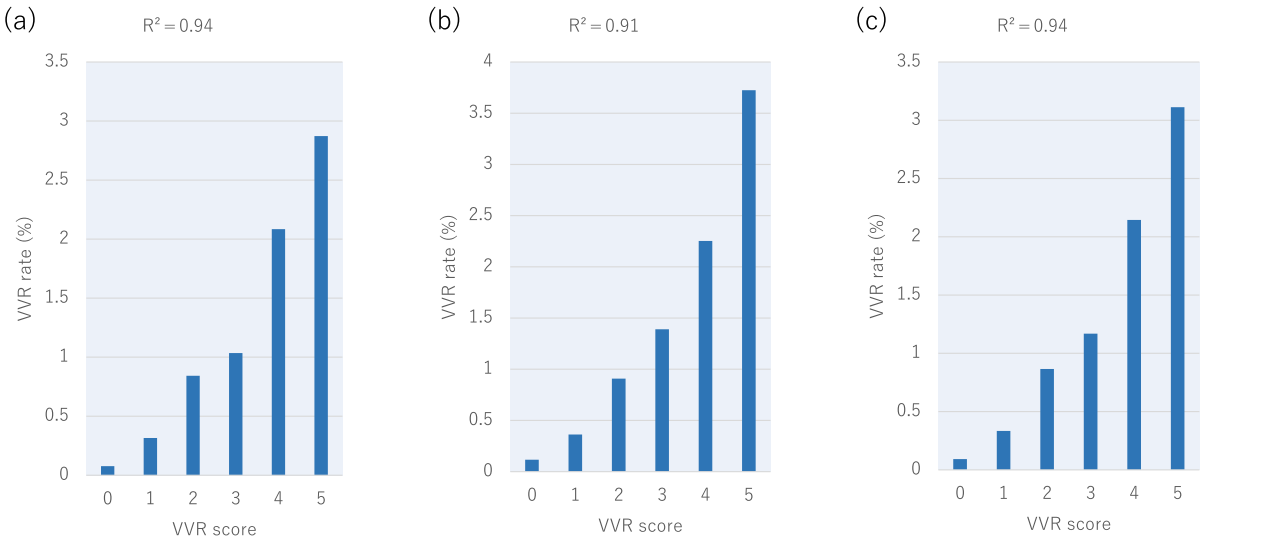
Abbreviations: EBV, estimated blood volume; FT, first-time donor; Rep 0, repeat donor with no history of VVR; Rep 1, repeat donor with a history of one episode of VVR; Rep 2, repeat donor with a history of two or more episodes of VVR; VVR, vasovagal reaction.

DISCUSSION

Previous reports had identified several key risk factors for VVR, including sex [4–6], EBV [5, 10, 11], age [4, 6, 11, 12] and first-time donation [4, 6, 9, 11, 12]. No published report compared the relative risk of each VVR factor; thus, some staff may believe that a first-time donor has the highest VVR risk, or other staff may believe that the highest risk is being female. We showed the relative importance of these factors and integrated them into a scoring system. The scoring system enables staff to significantly predict VVR and may help them to identify donors at increased risk of syncope, thereby mitigating the negative impact of VVR on donor safety by paying close attention to high-risk donors. One of the major strengths of the scoring system is that collection staff could take care of donors from the same standpoint. The collection staff would also be able to take care of high-risk donors with more attention, such as encouraging pre- and post-hydration, relaxing with communication, prompting applied muscle tension and recommending additional rest in the donor chair. A previous report had suggested that compliance with applied muscle tension was markedly improved with nurse supervision [21]. Even staff in the reception and refreshment area could pay close attention to donors if they looked pale or in distress, which could lead to a falling injury.

In our analysis, we defined the donor status as follows: first-time donors, repeat donors with no history of reaction and repeat donors with a history of reaction. The donor status was identified as the most important risk factor for VVR, and first-time donation and previous donation reactions equally influenced VVR. The importance of the first-time donor status is well-established [4, 6, 9, 11, 12]. On the other hand, a few previous studies reported that those who had experienced syncope during a previous donation were at an increased risk of recurrent syncope [15, 16]. As previously reported, our results showed that age was the next most important variable for VVR [4, 6, 11, 12].

Unexpectedly, we found that sex was not an important variable compared with donor status, age, and EBV. Female sex is a well-known risk factor for VVR [4–6]; however, previous analyses have given conflicting results [7, 22]. It is important to consider that the risk of VVR associated with being female can be incorporated into the risk of repeat donors with a history of VVR. However, the correlation coefficient between sex and the number of VVR experiences in past donations was 0.032, which makes this consideration irrelevant.



score	0	1	2	3	4	5
VVR	141	308	227	178	381	495
Total	183812	97632	26962	17197	18280	17231

score	0	1	2	3	4	5
VVR	129	224	145	145	235	251
Total	110896	61738	15972	10435	10432	6738

score	0	1	2	3	4	5
VVR	270	532	372	323	616	746
Total	294708	159370	42934	27632	28712	23969

FIGURE 4 Relationship between the score and vasovagal reaction (VVR) rate for (a) training data, (b) testing data and (c) overall data.

On the other hand, in our scoring system height remained as important as EBV, which is a well-established risk factor for VVR [5, 10, 11]. To our knowledge, there has been no report indicating height as an important variable for VVR. Although previous reports described weight as a risk factor for VVR [7–9], we did not include it as a variable in our analysis because weight and EBV are highly correlated. Since the correlation ratio between sex and height was calculated as 0.463, which is a moderate correlation, the effect of height and sex might overlap in some areas. Our finding suggests that upon whole-blood donation, height may replace sex as a risk factor for VVR; otherwise, our EBV formula needs to be corrected by height for VVR estimation. We may also need to devise a new EBV formula, especially for Asian populations [23], reflecting height more efficiently.

Our study had several limitations. First, we developed our scoring system using data from a single blood center, that is, the Japanese Red Cross Tokyo Metropolitan Blood Center. By using data from a single blood centre, we could avoid inter-blood centre variability, but it is unclear how well this scoring system will translate worldwide, considering factors of racial and ethnic diversity [24]. Second, it is important to analyse reactions across the time-course of blood donation; however, owing to the limited number of samples of off-site reactions, we were unable to analyse them separately. Our previous report had suggested that syncopal-type reactions tended to be delayed in female and elderly donors [25]. Third, psychological [26] and environmental [27] factors are not included in our scoring system. Incorporating these factors would further improve the VVR scoring system.

Our scoring system does not require the use of on-site variables such as blood pressure or pulse, as it simply uses registered information. At the reception, we are currently encouraging first-time donors to wear a neck-strap so that staff can easily recognize that they are high-risk donors. Based on the results of this scoring system, we should also recommend other donors with a high score to wear the neck-strap. Our scoring system warrants prospective evaluation and can be regarded as a basic framework to which newly discovered risk factors for VVR may be added.

ACKNOWLEDGEMENTS

The authors are grateful to the voluntary blood donors and staff of the Japanese Red Cross Tokyo Metropolitan Blood Center for their contribution to transfusion medicine.

T.H., G.K. and F.I. designed the study; T.H., G.K., Y.I., K.K. and N.K. collected the data; T.H., G.K., N.N., K.A. and Y.S. analysed the data; T.H., G.K. and F.I. wrote the paper; F.I. and S.M. reviewed and edited the paper.

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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REFERENCES

1. Thijsen A, Masser B. Vasovagal reactions in blood donors: risks, prevention and management. Transfus Med. 2019;29:13–22.
2. Hoogerwerf MD, Veldhuizen IJ, De Kort WL, Frings-Dresen MH, Sluiter JK. Factors associated with psychological and physiological

stress reactions to blood donations: a systematic review of the literature. Blood Transfus. 2015;13:354–62.
3. Donald SJ, McIntyre WF, Dingwall O, Hiebert B, Ponnampalam A, Seifer CM. Is donating blood for the faint of heart? A systematic review of predictors of syncope in whole blood donors. Transfusion. 2019;59:2865–9.
4. Eder A, Hillyer C, Dy B, Notari EP, Benjamin RJ. Adverse reactions to allogeneic whole blood donation by 16- and 17-year-olds. JAMA. 2008;299:2279–86.
5. Goldman M, Osmond L, Yi Q, Cameron-Choi K, O'Brien SF. Frequency and risk factors for donor reactions in an anonymous blood donor survey. Transfusion. 2013;53:1979–84.
6. France CR, Rader A, Carlson B. Donors who react may not come back: analysis of repeat donation as a function of phlebotomist ratings of vasovagal reactions. Transfus Apher Sci. 2005;33:99–106.
7. Trouern-Trend JJ, Cable RG, Badon SJ, Newman BH, Popovsky MA. A case-controlled multicenter study of vasovagal reactions in blood donors: influence of sex, age, donation status, weight, blood pressure, and pulse. Transfusion. 1999;39:316–20.
8. Kasprisin D, Glynn S, Taylor F, Miller KA. Moderate and severe reactions in blood donors. Transfusion. 1992;32:23–6.
9. Newman BH. Vasovagal reaction rates and body weight: findings in high- and low-risk populations. Transfusion. 2003;43:1084–8.
10. Wiltbank TB, Giordano GF, Kamel H, Tomasulo P, Custer B. Faint and prefaint reactions in whole-blood donors: an analysis of predonation measurements and their predictive value. Transfusion. 2008;48:1799–808.
11. Bravo M, Kamel H, Custer B, Tomasulo P. Factors associated with fainting: before, during and after whole blood donation. Vox Sang. 2011;101:303–12.
12. Zervou EK, Ziciadis K, Karabini F, Xanthi E, Chrisostomou E, Tzolou A. Vasovagal reactions in blood donors during or immediately after blood donation. Transfus Med. 2005;15:389–94.
13. Takanashi M, Odajima T, Aota S, Sudoh M, Yamaga Y, Ono Y, et al. Risk factor analysis of vasovagal reaction from blood donation. Transfus Apher Sci. 2012;47:319–25.
14. Ke S, Xu P, Xiong J, Xu L, Ma M, Du X, et al. Long-term poor sleep quality is associated with adverse donor reactions in college students in Central China: a population-based cross-sectional study. Vox Sang. 2023;118:455–62.
15. Eder A, Notari E, Dodd R. Do reactions after whole blood donation predict syncope on return donation? Transfusion. 2012;52:2570–6.
16. Rudokaite J, Ong LLS, Onal Ertugrul I, Janssen MP, Huis In't Veld EMJ. Predicting vasovagal reactions to needles with anticipatory facial temperature profiles. Sci Rep. 2023;13:9667.
17. Goldman M, Land K, Robillard P, Tomasulo P, Wiersum-Osselton J for the Working Group on Donor Vigilance of the International Society of Blood Transfusion Working Party on Haemovigilance. Standard for surveillance of complications related to blood donation. AABB, ISBT, IHN. 2014.
18. Altmann A, Toloşi L, Sander O, Lengauer T. Permutation importance: a corrected feature importance measure. Bioinformatics. 2010;26:1340–7.
19. Breiman L. Random forests. Mach Learn. 2001;45:5–32.
20. Wei W-H, Smith M, Vera A, Meads K, Hessell J, Reid L, et al. Novel risk patterns of vasovagal reactions in NZ blood donations complicated by COVID-19 restrictions. Front Public Health. 2023;11:1180279.
21. Morand C, Coudurier N, Rolland C, Thoret S, Legrand D, Tiberghien P, et al. Prevention of syncopal-type reactions after whole blood donation: a cluster-randomized trial assessing hydration and muscle tension exercise. Transfusion. 2016;56:2412–21.
22. Wiersum-Osselton J, Marijt-van der Kreek T, Brand A, Veldhuizen I, van der Bom JG, de Kort W. Risk factors for complications in donors at first and repeat whole blood donation: a cohort study with assessment of the impact on donor return. Blood Transfus. 2014;12:s28–s36.
23. Jia ZS, Xie HX, Yang J, Liu XM, Sun ZQ, Li Z, et al. Total blood volume of Asian patients undergoing cardiac surgery is far from that predicted by conventional methods. J Cardiovasc Surg (Torino). 2013;54:423–30.
24. Newman BH. Vasovagal reactions in high school students: findings relative to race, risk factor synergism, female sex, and non-high school participants. Transfusion. 2002;42:1557–60.
25. Namba N, Ishimaru F, Kondo G, Hashizume T, Kunii N, Shibata R, et al. Syncopal-type reactions tend to be delayed and result in falls among elderly blood donors. Vox Sang. 2021;116:36–41.
26. France CR, France JL, Frame-Brown TA, Venable GA, Menitove JE. Fear of blood draw and total draw time combine to predict vasovagal reactions among whole blood donors. Transfusion. 2016;56:179–85.
27. Hashizume T, Ishimaru F, Kondo G, Namba N, Miura Y, Aota S, et al. Comfortable environment for blood donation lowers the rate of vasovagal reactions. ISBT Sci Ser. 2020;15:261–5.

How to cite this article: Hashizume T, Kondo G, Ishimaru F, Ikeda Y, Kagawa K, Kunii N, et al. Development and validation of a scoring system to predict vasovagal reaction upon whole-blood donation. Vox Sang. 2024;119:300–7.

Received: 11 October 2023 | Revised: 18 December 2023 | Accepted: 20 December 2023

DOI: 10.1111/vox.13586

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Compliance of blood donors in Germany with non-sexual deferral criteria

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Funding information
The authors received no specific funding for this work.

Abstract

Background and Objectives: In addition to mandatory testing of blood donations, the deferral of donors in the case of various sexual and non-sexual risk exposures ensures the safety of blood products in Germany. The study aimed to quantify non-disclosure of non-sexual risk exposures, as no data are available so far.

Materials and Methods: We conducted an anonymous online survey among whole-blood donors with successful donations between January and March 2020. Data on travel to countries with endemic malaria, recent mild or febrile infections, tattoos or piercings and drug use were collected. We analysed non-compliance in relation to donor demographics by multivariable analyses.

Results: Altogether, 5.4% of the donors were non-compliant. Non-disclosure was highest for mild infection with 3.3% of donors, followed by febrile infections (1.4%), travel to malaria endemic countries (0.7%) and body modifications (0.5%). Intravenous drug use was negligible in our study population. Age was a predictor for all investigated risks, with higher prevalence in younger age groups. Prevalence ratios for non-disclosure of body modifications and mild infection were higher in females than males. Donation in blood establishments with mobile services was associated with higher non-disclosure of mild infections.

Conclusion: The considerable degree of non-compliance in some donor groups reflects the prevalence of risk factors in the underlying population (e.g., body modification) as well as probable tendency to socially desirable responding. Donor education should not focus exclusively on sexual risk behaviour, as undisclosed non-sexual exposures may bear risks for recipients and donors.

Keywords

blood donors, compliance, deferral, non-sexual risk exposures

Highlights

- In addition to compliance with selection criteria for sexual risk exposures, full disclosure of non-sexual risks is crucial for patient and donor safety. This is the first nationwide study in Germany that investigated compliance with donor deferral criteria.
- In our study, 1 in 20 donors did not disclose relevant travel to malaria endemic areas, mild or febrile infections, tattoos/piercings or drug use. Younger age was the strongest predictor of non-compliance for all risk exposures.

- Donations at mobile services might be more prone to non-compliance with donor selection criteria, probably because of fewer available donation appointments that donors do not want to miss.

INTRODUCTION

In order to prevent transfusion-transmissible infections (TTIs), testing of all blood donations for human immunodeficiency virus (HIV), hepatitis B, C, E virus (HBV, HCV, HEV) and syphilis is mandatory in Germany. Moreover, donors are deferred from donation in case of exposures associated with infection risks including risky sexual behaviour, recent travel to certain countries and invasive medical treatment, in order to prevent infections in the window phase or those not tested. Candidate donors can refer to the website of the respective blood service or the Federal Centre for Health Education to check for general eligibility criteria prior to donation. In Germany, donors can donate whole blood after qualifying at their first visit. Donors' risk exposures are determined using a donor health questionnaire and assessed by physicians, and all donors are informed in writing that they should report any illness that occurred shortly after donating to the blood service.

For maximum prevention of TTIs, a complete disclosure of potential risk exposures is essential, especially in case of untested pathogens. Therefore, compliance with deferral criteria is an important indicator for ensuring the safety of blood products. Most studies have focused on donors' compliance with deferral criteria for sexual risk behaviour, as sexually transmitted infections pose a threat to blood safety. However, the relevance of other risk factors for TTIs was shown in donor populations [1, 2]. So far, no data exist for the extent of non-disclosure of non-sexual-risk exposures among whole-blood donors in Germany. We have therefore included questions about recent travel, body modifications, intravenous drug use (IVDU) and mild and febrile infections in a compliance study, which primarily focused on sexual risks [3]. Data analysis aimed to identify donor populations that may need intensified donor education.

MATERIALS AND METHODS

We conducted an anonymous online survey among whole-blood donors in Germany, as has already been described in detail [3]. Briefly, 21 blood establishments (BEs), which represent approximately 80% of the German donor population, invited all non-deferred donors within an 8-week period between January and March 2020 by handing over an invitation flyer immediately after donation. The donors were asked to complete an anonymous online questionnaire including questions about drug use, recent travel, tattoo/piercings and (febrile) infections as well as socio-demographic characteristics.

According to the national hemotherapy guidelines, infection risks were defined as follows:

1. Persons with febrile illness or diarrhoea within 4 weeks prior to donation (hereafter: febrile illness).
2. Persons with a mild infection (e.g., respiratory) within 1 week prior to donation (mild infection).
3. Persons who travelled to a malaria endemic region 6 months prior to donation (malaria risk).
4. Persons with a new tattoo or piercing (ear, other) within 4 months prior to donation (body modification).
5. Persons who have ever injected drugs (IVDU).

Prevalence of non-compliance with selection criteria for infectious risk exposures is given with 95% confidence interval (95% CI). Prevalence estimates were post-stratified for sex and age group considering the cluster sampling in BE using data on invited blood donors as well as the total donor population in Germany in the study period (first quarter 2020) to check the representativeness of study results. Association of non-compliance with socio-demographic data was assessed using modified Poisson regression with robust error estimation providing prevalence ratios (PRs) in a univariate analysis [4].

Donor characteristics that are possibly relevant for the identification of donors with increased need for information about risk exposures were obtained from multivariable analyses of associations between non-disclosure of risks and socio-demographic items. For this purpose, modified Poisson regression models with stepwise backward elimination of variables with a p-level threshold of 0.05 were used. Only data that were known at the time of donation (age, sex, donor status, type of donation service, residence) were considered.

The Ethics Committee of the Berlin Chamber of Physicians decided that ethics approval was not required because the survey study was performed completely anonymously (Ref. Eth-oA 15/19). All participants had to provide informed consent through the survey website before starting the survey. The questionnaire could be cancelled at any time and the consent could be withdrawn.

RESULTS

BEs invited 290,834 donors whose demographic characteristics corresponded to the total donor population in Germany in the study period (Table 1). Altogether, 14,882 complete questionnaires could be analysed. Most of the study participants were repeat donors ($n = 14{,}426$; 97%) and male ($n = 9327$; 63%). Proportions of participating male and repeat donors were higher than for invited donors. Age distribution of participants was comparable to invited donors, with a median age of 46 years (interquartile range 31–55 years).

Altogether, 802 (5.4%) donors had not indicated their recent mild or febrile infection, recent invasive body modification, travel to malaria endemic region or IVDU in the donor health assessment.

Non-disclosure of infection risks was highest for mild infection with 3.3% of donors (Table 2). The non-disclosure of mild infections was strongly age-dependent with a clearly higher prevalence in the younger age groups, and was significantly higher in women than men (χ^2-test $p < 0.001$) (Table 3).

A similar pattern of non-disclosure—although with lower prevalence—was also observed for febrile illness (1.4%) as well as for body modification (0.5%). Travel-associated risk of malaria infection was not indicated at the time of donation by 0.7% of all donors without significant age or sex associations.

Young women under 35 years of age were found to have remarkably higher non-compliance than other donor populations for non-sexual risks. Besides the significant prevalence differences to older women for all risk exposures, we found a significantly higher prevalence compared to men in this age group for mild infections (5.2% vs. 3.9%, χ^2-test, $p < 0.05$) and body modifications (1.8% vs. 0.4%, χ^2-test, $p < 0.001$). The highest overall prevalence of non-compliance was found in women under 25 years for mild infections (5.4%, 95% CI: 4.2%–6.9%), febrile illness (3.4%, 95% CI: 2.4%–4.6%) and body modifications (2.0%, 95% CI: 1.3%–3.0%), and in women aged 25–34 years for malaria risk (1.2%, 95% CI: 0.7%–2.1%).

Non-disclosed IVDU was negligible in our study population, with only five donors reporting past IVDU, including one with IVDU within the last 12 months.

Overall, non-disclosure of any of the investigated non-sexual risk exposures was significantly higher in women (6.9%, 95% CI: 6.2%–7.6%) than in men (4.5%, 95% CI: 4.1%–5.0%).

Non-compliance was generally higher in new donors than in repeat donors, reflecting the age differences of the donor groups: 75% of the new donors were younger than 35 years, but only 30% of the repeat donors were.

Post-stratified non-compliance prevalences that consider possible age and gender biases in the study population as well as BE cluster sampling (Table 2) showed no differences from the study results. It can therefore be assumed that the study population represents the total whole-blood donors in Germany well, despite the somewhat lower proportions of participating new donors and women.

Variable selection for identification of socio-demographic factors that are associated with non-compliance showed that age was a predictor for all investigated risks (Table 4). Younger age groups carried a higher non-compliance risk for non-sexual exposures. Furthermore, sex dependence was found for non-disclosure of body modifications and mild infection, with higher PR for female donors. An additional association was found between the kind of blood service and non-disclosure of mild infections, with higher PR for blood donation at Red Cross Services.

TABLE 1 Demographic characteristics of participants, invited donors and the total donor population in Germany in the study period.

	Participants	Invited donors[a], $n = 290{,}834$ (%)	Total donor population, $n = 937{,}887$ (%)
Donor status			
FTD	455 (3.1%)	10.0	12.1
RD	14,426 (96.9%)	90.0	87.9
Sex			
Female	5555 (37.3%)	42.4	43.2
Male	9327 (62.7%)	57.6	56.8
Age			
18–24 y	1994 (13.4%)	15.4	15.9
25–34 y	2660 (17.8%)	17.0	18.2
35–44 y	2344 (15.8%)	14.2	14.6
45–54 y	3709 (24.9%)	23.2	22.8
55+ y[b]	4175 (28.1%)	30.2	28.5

Abbreviations: FTD, first-time donor; RD, repeat donor, y, years.
[a]Data provided by 19 BEs.
[b]Age groups 55–64 y and 65+ y were merged due to available strata for the total donor population.

DISCUSSION

Deferral of candidate donors with higher risk for TTIs reduces the transmission of pathogens that are either missed by mandatory

TABLE 2 Prevalence of non-compliance with non-sexual risk exposures—Numbers and proportions of participating donors and post-stratified proportions considering BE-specific FTD proportion and age and sex distribution of invited donors as well as of the total donor population in Germany in the study period.

	Study population			Post-stratified invited population		Post-stratified total population	
	n	%	95% CI	%	95% CI	%	95% CI
Malaria risk	100/14,858	0.7	0.6–0.8	0.6	0.4–1.0	0.7	0.4–1.1
Body modification	70/14,868	0.5	0.4–0.6	0.5	0.3–0.7	0.6	0.4–0.8
Mild infection	477/14,510	3.3	3.0–3.6	3.3	2.9–3.7	3.3	2.9–3.8
Febrile illness	203/14,694	1.4	1.2–1.6	1.4	1.2–1.8	1.5	1.2–1.8
IVDU[a]	5/14,853	0.03	0.01–0.08				

Abbreviations: BE, blood establishment; CI, confidence interval; FTD, first-time donor; IVDU, intravenous drug use.
[a]Post-stratified prevalence was not calculated due to zero prevalence in most BEs.

TABLE 3 Numbers and proportion of non-compliance in certain donors and univariate analysis of association between donor demographics and non-compliance.

	Malaria risk					Body modification				Mild infection					Febrile infection				
	n	%	95% CI	PR	95% CI	*n*	%	95% CI	PR	95% CI	*n*	%	95% CI	PR	95% CI	*n*	%	95% CI	PR	95% CI
Donor status																				
FTD	7/455	1.5	0.7–3.2	2.4	1.1–5.1	8/454	1.8	0.9–3.5	4.1	2.0–8.5	25/448	5.6	3.8–8.1	1.7	1.2–2.6	13/443	2.9	1.7–5.0	2.2	1.3–3.8
RD	93/14,402	0.6	0.5–0.8	Ref.		62/14,413	0.4	0.3–0.6	Ref.		452/14,061	3.2	2.9–3.5	Ref.		190/14,250	1.3	1.2–1.5	Ref.	
Sex																				
Female	37/5546	0.7	0.5–0.9	1.0	0.7–1.5	54/5549	1.0	0.7–1.3	5.7	3.2–9.9	217/5408	4.0	3.5–4.6	1.4	1.2–1.7	97/5484	1.8	1.5–2.2	1.5	1.2–2.0
Male	63/9249	0.7	0.5–0.9	Ref.		16/9319	0.2	0.1–0.2	Ref.		260/9102	2.9	2.5–3.2	Ref.		106/9210	1.2	1.0–1.4	Ref.	
Age																				
18–24 y	17/1993	0.9	0.5–1.3	2.3	0.8–6.7	29/1994	1.5	1.0–2.1	15.5	2.1–113.6	87/1929	4.5	3.7–5.5	4.3	2.3–8.0	54/1951	2.8	2.1–3.6	9.7	3.1–31.1
25–34 y	28/2658	1.1	0.7–1.5	2.8	1.0–8.0	21/2660	0.8	0.5–1.2	8.4	1.1–62.4	117/2599	4.5	3.8–5.4	4.3	2.3–7.9	63/2614	2.4	1.9–3.1	8.5	2.7–27.0
35–44 y	17/2341	0.7	0.5–1.2	1.9	0.7–5.7	10/2342	0.4	0.2–0.8	4.5	0.6–35.5	81/2285	3.5	2.9–4.4	3.4	1.8–6.3	29/2317	1.3	0.9–1.8	4.4	1.3–14.4
45–54 y	15/3697	0.4	0.2–0.7	1.1	0.4–3.2	7/3700	0.2	0.1–0.4	2.0	0.2–16.4	113/3609	3.1	2.6–3.8	3.0	1.6–5.5	31/3671	0.8	0.6–1.2	3.0	0.9–9.7
55–64 y	19/3105	0.6	0.4–1.0	1.6	0.6–4.8	2/3107	0.1	0.0–0.3	0.7	0.1–7.6	68/3039	2.2	1.8–2.8	2.1	1.1–4.0	23/3085	0.7	0.5–1.1	2.6	0.8–8.7
65+ y	4/1064	0.4	0.1–1.0	Ref.		1/1065	0.1	0.0–0.7	Ref.		11/1049	1.0	0.6–1.9	Ref.		3/1056	0.3	0.1–0.9	Ref.	
Highest professional degree																				
Poly-technic/university	56/6501	0.9	0.7–1.1	Ref.		21/6505	0.3	0.2–0.5	Ref.		206/6334	3.3	2.8–3.7	Ref.		77/6431	1.2	1.0–1.5	Ref.	
Vocational training	27/6548	0.4	0.3–0.6	0.5	0.3–0.8	24/6553	0.4	0.2–0.5	1.1	0.6–2.0	191/6423	3.0	2.6–3.4	0.9	0.8–1.1	65/6495	1.0	0.8–1.3	0.8	0.6–1.2
Current training	12/1476	0.8	0.5–1.4	0.9	0.5–1.8	20/1477	1.4	0.9–2.1	4.2	2.3–7.7	72/1431	5.0	4.0–6.3	1.5	1.2–2.0	50/1443	3.5	2.6–4.5	2.9	2.0–4.1
No training	1/103	1.0	0.1–6.6	1.1	0.2–8.1	2/103	1.9	0.5–7.4	6.0	1.4–25.3	4/100	4.0	1.5–10.2	1.2	0.5–3.2	6/98	6.1	2.8–13.0	5.1	2.3–11.5
Residence																				
<2000 inhabitants	14/2838	0.5	0.3–0.8	Ref.		14/2841	0.5	0.3–0.8	Ref.		94/2779	3.4	2.8–4.1	Ref.		43/2807	1.5	1.1–2.1	Ref.	
2000–20,000 inh.	31/5022	0.6	0.4–0.9	1.3	0.7–2.3	23/5024	0.5	0.3–0.7	0.9	0.5–1.8	161/4908	3.3	2.8–3.8	1.0	0.8–1.2	63/4970	1.3	1.0–1.6	0.8	0.6–1.2
20,000–100,000 inh.	21/3612	0.6	0.4–0.9	1.2	0.6–2.3	13/3616	0.4	0.2–0.6	0.7	0.3–1.5	106/3522	3.0	2.5–3.6	0.9	0.7–1.2	43/3567	1.2	0.9–1.6	0.8	0.5–1.2
100,000–500,000 inh.	12/1644	0.7	0.4–1.3	1.5	0.7–3.2	8/1644	0.5	0.2–1.0	1.0	0.4–2.3	59/1598	3.7	2.9–4.7	1.1	0.8–1.5	21/1623	1.3	0.8–2.0	0.8	0.5–1.4
>500,000 inh.	19/1476	1.3	0.8–2.0	2.6	1.3–5.2	11/1477	0.7	0.4–1.3	1.5	0.7–3.3	45/1445	3.1	2.3–4.1	0.9	0.6–1.3	26/1464	1.8	1.2–2.6	1.2	0.7–1.9
Donation service																				
Red Cross	72/10,845	0.7	0.5–0.8	1.0	0.6–1.7	51/10,851	0.5	0.4–0.6	1.0	0.5–1.8	363/10,606	3.4	3.1–3.8	1.2	0.9–1.5	144/10,729	1.3	1.1–1.6	0.7	0.5–1.0
University	15/2247	0.7	0.4–1.1	Ref.		11/2247	0.5	0.3–0.9	Ref.		64/2172	2.9	2.3–3.7	Ref.		41/2223	1.8	1.4–2.5	Ref.	
Private	11/1513	0.7	0.4–1.3	1.1	0.5–2.4	6/1515	0.4	0.2–0.9	0.8	0.3–2.2	43/1481	2.9	2.2–3.9	1.0	0.7–1.4	15/1490	1.0	0.6–1.7	0.5	0.3–1.0

Abbreviations: CI, confidence interval; FTD, first-time donor; PR, prevalence ratio; RD, repeat donor; Ref., reference; y, years.

TABLE 4 Prevalence ratios and 95% CI for demographic donor characteristics that are known at time of eligibility assessment and associated with non-compliance estimated in a multivariable Poisson regression model[a].

	Malaria risk	Body modification	Mild infection without fever	Febrile infection
Donor status	ni	ni	ni	ni
Sex	ni			ni
Female		4.9 (2.7–8.9)	1.3 (1.1–1.5)	
Male		Ref.	Ref.	
Age				
18–24 y	2.5 (1.2–5.1)	15.7 (3.7–65.7)	4.3 (2.2–8.3)	9.6 (3.0–30.6)
25–34 y	2.9 (1.5–5.6)	9.3 (2.2–39.6)	4.5 (2.4–8.6)	7.8 (2.5–25.0)
35–44 y	2.0 (1.0–4.2)	5.9 (1.3–26.7)	3.5 (1.8–6.7)	4.3 (1.3–14.1)
45–54 y	Ref.	2.6 (0.5–12.5)	3.1 (1.6–5.9)	2.7 (0.8–9.0)
55–64 y	1.7 (0.8–3.4)	-	2.2 (1.1–4.3)	2.3 (0.7–7.6)
65+ y	1.1 (0.4–3.3)	Ref.	Ref.	Ref.
Residence	ni	ni	ni	ni
Donation service	ni	ni		ni
Red Cross			1.4 (1.1–1.9)	
University			Ref.	
Private			1.1 (0.7–1.6)	

Abbreviations: CI, confidence interval; ni, not included; Ref., reference; y, years.
[a]Stepwise backward variable selection ($p < 0.05$).

testing (e.g., in the diagnostic window phase) or for which no routine testing is implemented (e.g., travel-related infection risks). Residual risk for possibly undetected (asymptomatic) infections is considered very low if deferrals are met.

In our compliance study, we found a considerable degree of non-compliance with deferral criteria for non-sexual exposures that may be associated with higher risk for transfusion-related transmission of pathogens. For all investigated issues, non-compliance depended on age, with significantly higher prevalence in donors younger than 35 years. Furthermore, women had significantly higher PRs for non-disclosure of recent body modifications and mild infections.

The observed relations of non-compliance to demographic characteristics of donors reflect the prevalence proportions of risk factors in the underlying population. For example, in Germany, tattoos and piercings are more common among women and among young adults (under 35 years) [5, 6]. As the personal perception of risk is essential for reporting a specific behaviour in the context of blood donations [7], consequently, non-compliance should be highest in these donor groups if invasive body modifications were perceived to be non-risky by this population [8].

Additionally, in women a tendency to over-report favourable behaviour and to be more prone to socially desirable responding is more common [9]. The somewhat more pronounced intention of women to help others with their blood donation compared to men [10] may also contribute to their more frequent non-disclosure of risk exposures.

The age-dependent non-compliance in our study is consistent with the observed higher non-disclosure of sexual risk exposures in donors younger than 35 years [3]. However, male and female donors differ in their compliance to sexual and non-sexual deferral criteria. Non-disclosure of sexual risks is more pronounced in male donors [3], whereas non-sexual risk exposures were less frequently indicated by women. Therefore, gender-specific donor education might help to reduce non-disclosure of risk exposures that are relevant for donor selection.

Incorrect recall and timing of relevant health risks may also contribute to non-compliance. However, there is no clear tendency towards underreporting of health issues—unbiased information, over-reporting as well as underreporting in certain recall periods is described [11–14]. Memory aids such as calendars improve the recall of travel and disease dates and may support the timing of health issues that are relevant for donor eligibility [15].

Furthermore, we found that donations at mobile services might be more prone to non-compliance with deferral criteria. In contrast to urban infrastructure with permanent access to blood donation centres, mobile services offer only a few donation appointments per year at suburban or rural locations. In consequence, motivated donors in rural areas cannot always choose a donation date that matches best with their risk-free periods. It should be noted that donors seem to be susceptible to non-disclosure of risks that they consider negligible in order not to miss their donation appointment. This could explain why non-disclosure of mild infection in the last week before donation was highest in BEs with mobile teams (Red Cross donation services).

The overall proportion of non-compliance is not insignificant; but it is difficult to quantify the impact of this non-compliance on related residual risks for infectious donations. Extrapolating the observed proportion of non-disclosed travel to malaria-endemic countries to the total donor population results in more than 10.000 donating

individuals per year in Germany who were probably at risk. Owing to an anti-Plasmodium antibody prevalence of 1.6% in deferred candidate donors in Switzerland and Germany [16] with travel to/living in malaria-endemic countries, possibly infectious donations cannot be excluded. Furthermore, the non-disclosure of travel may also increase the risk for other circulating transfusion-transmissible pathogens that are not tested for, for example, dengue virus or yellow fever virus. However, no arbovirus transmission and only one malaria transmission was reported to the German haemovigilance system since 1997, indicating an overall very low risk of transmission [17].

Because of the low residual risk, some deferral criteria are critically discussed, because temporary deferral of donors results in the drop-out of candidate donors [18–21] and requires increased efforts in motivation and reactivation of lapsed donors [22, 23]. Such reduced donor willingness could be an important issue in times of blood shortage. For example, the deferral of donors with new tattoos or piercings is questioned in some countries, as residual risk of transfusion-transmissible viral infections was not increased in recent studies [24, 25]. On the other hand, infection risks by tattooing still exists [26, 27]. Analysis of the reported HCV infections in the general population in Germany showed that of those infections with a reported transmission risk, 6% were attributed to tattooing and piercing in 2021 [28]. Therefore, the balance between protection of recipients and availability of blood products have to be carefully considered. This is true for all deferral criteria.

Moreover, the donor safety aspect of some deferral criteria has to kept in mind—a whole-blood donation during a mild infection is potentially unfavourable.

ACKNOWLEDGEMENTS

We thank all blood donation services that supported the study: University Hospital (UH) RWTH Aachen, Bavarian Red Cross, Centre for Transfusion Medicine and Cell Therapy Berlin (ZTB), UH Bonn, UH Düsseldorf, UH Essen, District Hospital Freiberg, UH Freiburg, UH Marburg-Gießen, UH Greifswald, Haema AG, UH Halle, Hannover Medical School (MHH), SLK Clinics Heilbronn, UH Jena, UH Leipzig, Südharz Hospital Nordhausen, Red Cross Baden-Wuerttemberg/Hesse, Red Cross DRK West, Red Cross North-East, Red Cross NSTOB, UH Saarland, and Klinikum Stuttgart.

K.P. organized the study, analysed the data and drafted the manuscript; S.A. customized the online survey and edited the paper; R. O. initiated and designed the study and edited the paper. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo at https://zenodo.org/10.5281/zenodo.10451630.

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REFERENCES

1. Preußel K, Offergeld R. Which infectious blood donors could be identified by the donor history questionnaire? – comparison of blood donors infected with HIV or HCV with notified cases from general population in Germany. Transfus Med Hemother. 2018;45:108–14.
2. Van Remoortel H, Moorkens D, Avau B, Compernolle V, Vandekerckhove P, De Buck E. Is there a risk of transfusion-transmissible infections after percutaneous needle treatments in blood donors? A systematic review and meta-analysis. Vox Sang. 2019;114:297–309.
3. Preußel K, Albrecht S, Offergeld R. Undisclosed sexual risk exposures: results of a nationwide compliance study among whole blood donors in Germany. Transfus Med Hemother. 2022;49:368–78.
4. Zou G. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol. 2004;159:702–6.
5. Stirn A, Hinz A, Brähler E. Prevalence of tattooing and body piercing in Germany and perception of health, mental disorders, and sensation seeking among tattooed and body-pierced individuals. J Psychosom Res. 2006;60:531–4.
6. Borkenhagen A, Mirastschijski U, Petrowski K, Brähler E. Tattoos in Germany: prevalence, demographics, and health orientation. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2019;62:1077–82.
7. Raimondo M, Facco G, Regine V, Pupella S, Grazzini G, Suligoi B. HIV-positive blood donors unaware of their sexual at-risk behaviours before donation in Italy. Vox Sang. 2016;110:134–42.
8. The German Federal Institut for Risk Assessment (BfR). BfR Consumer Monitor 2018 – Special Tattoos. 2018 Available from: https://www.bfr.bund.de/cm/364/bfr-consumer-monitor-2018-special-tattoos.pdf. Last accessed 3 Jan 2024.
9. Bernardi RA. Associations between Hofstede's cultural constructs and social desirability response bias. J Busin Ethics. 2006;65:43–53.
10. Suemnig A, Konerding U, Hron G, Lubenow N, Alpen U, Hoffmann W, et al. Motivational factors for blood donation in first-time donors and repeat donors: a cross-sectional study in West Pomerania. Transfus Med. 2017;27:413–20.
11. Lackner JM, Jaccard J, Keefer L, Firth R, Carosella AM, Sitrin M, et al. The accuracy of patient-reported measures for GI symptoms: a comparison of real time and retrospective reports. Neurogastroenterol Motil. 2014;26:1802–11.
12. Brusco NK, Watts JJ. Empirical evidence of recall bias for primary health care visits. BMC Health Serv Res. 2015;15:381.
13. Flynn KE, Mansfield SA, Smith AR, Gillespie BW, Bradley CS, Cella D, et al. Can 7 or 30-day recall questions capture self-reported lower urinary tract symptoms accurately? J Urol. 2019;202:770–8.
14. Miller VE, Faurot KR, Palssson OS, MacIntosh BA, Suchindran C, Honvoh G, et al. Comparing prospective headache diary and retrospective four-week headache questionnaire over 20 weeks: secondary data analysis from a randomized controlled trial. Cephalalgia. 2020;40:1523–31.
15. Ratnam I, Torresi J, Matchett E, Pollissard L, Luxemburger C, Lemoh CN, et al. Development and validation of an instrument to assess the risk of developing viral infections in Australian travelers during international travel. J Travel Med. 2011;18:262–70.
16. Niederhauser C, Tinguely C, Dreier J, Vollmer T, Marti HP, Nickel B, et al. Comparison of a new IgG-EIA for the detection of anti-plasmodium antibodies with two currently used assays. Transfus Med Hemother. 2021;48:265–71.
17. Funk MB, Heiden M, Müller S. Hämovigilanz-Bericht des Paul-Ehrlich-Instituts 2020: Auswertung der Meldungen von Reaktionen und Zwischenfällen nach § 63i AMG. 2021 Available from: https://www.pei.de/SharedDocs/Downloads/DE/newsroom/pflichtberichte/haemovigilanzberichte/haemovigilanz-bericht-2020.pdf?__blob=publicationFile&v=4. Last accessed 3 Jan 2024.

18. Klinkenberg EF, Romeijn B, de Kort WL, Merz EM. Reasons to end the donor career: a quantitative study among stopped blood donors in The Netherlands. Transfus Med. 2018;28:200–7.
19. Spekman MLC, van Tilburg TG, Merz EM. Do deferred donors continue their donations? A large-scale register study on whole blood donor return in The Netherlands. Transfusion. 2019;59:3657–65.
20. Clement M, Shehu E, Chandler T. The impact of temporary deferrals on future blood donation behavior across the donor life cycle. Transfusion. 2021;61:1799–808.
21. Quee F, O'Brien SF, Prinsze F, Steele WR, Grégoire Y, Cutajar A, et al. Whole blood donor return rates after deferral for tattooing or body piercing-survey across blood donation services: the BEST collaborative study. Vox Sang. 2022;117:1085–9.
22. Gemelli CN, Thijsen A, Van Dyke N, Kruse SP, Davison TE. Notifying donors when their deferral is ending: an effective donor retention strategy. Transfusion. 2021;61:2930–40.
23. Gemelli CN, Kruse SP, Thijsen A, Van Dyke N, Karki S, Davison TE. Improving donor retention following a temporary deferral: a cluster randomized controlled trial of deferral educational materials. Transfusion. 2022;62:1230–9.
24. Hoad VC, Guy RJ, Seed CR, Harley R. Tattoos, blood-borne viruses and blood donors: a blood donor cohort and risk assessment. Vox Sang. 2019;114:687–93.
25. Prinsze FJ, van de Laar T, Slot E, de Jong M, Bokhorst A, de Kort W, et al. No increased risk of transfusion-transmissible infections after tattooing, body piercing, or acupuncture among blood donors in The Netherlands. Transfusion. 2019;59:2575–83.
26. Cohen PR. Tattoo-associated viral infections: a review. Clin Cosmet Investig Dermatol. 2021;14:1529–40.
27. Tampa M, Mitran MI, Mitran CI, Matei C, Amuzescu A, Buzatu AA, et al. Viral infections confined to tattoos-a narrative review. Medicina (Kaunas). 2022;58:58.
28. Steffen G, Behnke AL, Dudareva S, Hommes F, Krings A, Kollan C, et al. Viral hepatitis C in 2021. Epid Bull. 2022;38:7–25.

How to cite this article: Preußel K, Albrecht S, Offergeld R. Compliance of blood donors in Germany with non-sexual deferral criteria. Vox Sang. 2024;119:308–14.

Received: 13 October 2023 | Revised: 1 January 2024 | Accepted: 2 January 2024

DOI: 10.1111/vox.13592

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

International review of blood donation nucleic acid amplification testing

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Funding information
Open access publishing facilitated by University of the Sunshine Coast, as part of the Wiley - University of the Sunshine Coast agreement via the Council of Australian University Librarians.

Abstract

Background and Objectives: Nucleic acid amplification testing (NAT), in blood services context, is used for the detection of viral and parasite nucleic acids to reduce transfusion-transmitted infections. This project reviewed NAT for screening blood donations globally.

Materials and Methods: A survey on NAT usage, developed by the International Society of Blood Transfusion Working Party on Transfusion-transmitted Infectious

For affiliations refer to page 323

Diseases (ISBT WP-TTID), was distributed through ISBT WP-TTID members. Data were analysed using descriptive statistics.

Results: Forty-three responses were received from 32 countries. Increased adoption of blood donation viral screening by NAT was observed over the past decade. NAT-positive donations were detected for all viruses tested in 2019 (proportion of donations positive by NAT were 0.0099% for human immunodeficiency virus [HIV], 0.0063% for hepatitis C virus [HCV], 0.0247% for hepatitis B virus [HBV], 0.0323% for hepatitis E virus [HEV], 0.0014% for West Nile virus [WNV] and 0.00005% for Zika virus [ZIKV]). Globally, over 3100 NAT-positive donations were identified as NAT yield or solely by NAT in 2019 and over 22,000 since the introduction of NAT, with HBV accounting for over half. NAT-positivity rate was higher in first-time donors for all viruses tested except WNV. During 2019, a small number of participants performed NAT for parasites (*Trypanosoma cruzi*, *Babesia* spp., *Plasmodium* spp.).

Conclusion: This survey captures current use of blood donation NAT globally. There has been increased NAT usage over the last decade. It is clear that NAT contributes to improving blood transfusion safety globally; however, there is a need to overcome economic barriers for regions/countries not performing NAT.

Keywords

blood, NAT, safety, transfusion, TTI, virus

Highlights

- Over the past decade, there has been increased adoption of nucleic acid amplification testing (NAT) to screen donations for transfusion-transmitted viruses.
- Globally, over 3100 NAT-positive donations were detected as NAT yield or solely by NAT in 2019 and over 22,000 since the introduction of NAT.
- NAT contributes to improving global blood safety.

INTRODUCTION

Nucleic acid amplification testing (NAT) detects targeted nucleic acid sequences in a sample with high sensitivity and specificity. NAT is used for screening blood donations for viruses and parasites globally, reducing the risk of transfusion-transmitted infectious diseases (TTIDs) and thereby providing an additional layer of blood safety [1]. NAT for blood donation was initially implemented for human immunodeficiency virus (HIV) and hepatitis C virus (HCV) in the 1990s, and soon after for hepatitis B virus (HBV) [2]. NAT is now also used in selected regions for other viruses including hepatitis E virus (HEV), West Nile virus (WNV) and/or Zika virus (ZIKV), as well as for parasites including *Babesia* spp. [3–9]. Given the detection of acute/incident infections, NAT is fundamental for tracking changes in the epidemiology and distribution of bloodborne infections over time.

Since the adoption of NAT for blood donation screening, there have been at least three international collaborative studies capturing global usage and yield of viral NAT in blood donations [2, 10, 11]. An increasing number of countries have participated, highlighting the increased adoption of NAT globally. The last survey was conducted by the International Society of Blood Transfusion (ISBT) Working Party on Transfusion-transmitted Infectious Diseases (WP-TTID) using data from donations collected during 2008 [2]. The findings of this previous survey provided evidence for increasing use of NAT to improve blood safety. Since the last survey, a number of changes impacting NAT have occurred, such as technological improvements in testing chemistries and automation. NAT has been expanded for use in molecular surveillance of infectious diseases and to screen for emerging pathogens transmitted by blood.

Over 10 years have passed since the last international NAT survey [2] and well over 20 years since NAT was first implemented [11]. Given this, the Virology and Surveillance, Risk Assessment and Policy subgroups of the ISBT WP-TTID developed and conducted a new survey, with the aim to capture the current use and safety benefits of NAT.

MATERIALS AND METHODS

This survey was based on questions used in the previous survey with appropriate modifications and additions (Data S1) [2]. Participants could complete the survey online through the Qualtrics flexible survey tool (qualtrics.com) or manually using a fillable PDF or Word document. The survey was executed in 2021–2023, but asked participants to provide data for 2019 (1 January–31 December). This year was selected because it was prior to the COVID-19 pandemic so as not to

capture any possible testing changes or impact on donor populations due to the pandemic. The survey focused on NAT of blood donations for clinical products but not plasma for fractionation.

The survey was first circulated through ISBT WP-TTID members on 13 May 2021, with two follow-up reminders (sent on 13 October 2021 and 2 February 2022). The major global suppliers of NAT assays for blood donation screening, Roche Diagnostics (Basel, Switzerland) and Grifols Diagnostic Solutions (Emeryville, CA, USA), were asked to encourage their customers to participate. The survey was publicized during the Global ISBT Virtual Congress in June 2022. Personal emails were sent in August 2022 to members of the WP-TTID who had not responded to the survey. Finally, the survey was again publicized during the 33rd Regional ISBT Congress in Gothenburg, Sweden, in June 2023. The data captured and presented here include all responses received up to 18 September 2023.

Duplicate responses were removed. Responses containing no answers to questions relating to NAT were also removed. A small number of responders provided incomplete answers to some questions or sections; in these instances, only responses that allowed interpretation (e.g., where both the number of donations tested by NAT and the number of NAT-positive donations were provided) were included in each analysis, hence differing numbers of responders throughout. Descriptive analyses were performed, with reported variables expressed as frequencies and percentages, and 95% confidence intervals (CI) calculated. Given that some regions within a country reported different responses to some questions, percentages were based on the proportion of survey responses, not the whole country. The incidence/prevalence of HIV, HCV and HBV for responder countries was obtained [12, 13]. Comparisons of incidence/prevalence between survey responders performing NAT and those not performing NAT were performed with a Mann–Whitney test, using GraphPad Prism.

This study was a review of operational processes and summary data without donation or donor identifiers, and therefore not considered research on human subjects. Therefore, ethical approval for human research was not required.

RESULTS

NAT usage, 2019

A total of 43 responses were received from 32 countries (Figure 1). The data from our survey represent results for 2019 from over 28 million donations and cover a population of over 1 billion people. There was a diverse geographical distribution of survey respondents, with the largest proportion from Europe ($n = 16$), followed by Asia and Western Pacific regions ($n = 14$), South America ($n = 5$), Africa ($n = 5$) and North America ($n = 3$).

Of the 43 survey responses, 38 indicated that they perform NAT for at least one virus (Table 1), representing 27 countries: Argentina, Australia, Belgium, Brazil, Canada, China, Colombia, Denmark, France, Germany, Greece, India, Ireland, Japan, New Zealand, Oman, Poland, Republic of Korea, Singapore, South Africa, Spain, Switzerland, Thailand, The Netherlands, United Kingdom, United States of America and Vietnam. HIV, HCV and HBV NAT was performed by the largest proportion of responders (88%, 84%, and 84%, respectively), followed by HEV and WNV (each 26%), and finally ZIKV (7%). Most participants used NAT that detected HIV-1 in combination with HIV-2; three responders performed NAT specifically for HIV-1, with one performing NAT for HIV-2 separately.

The five responders not performing NAT for HIV, HCV or HBV indicated economic reasons for the lack of testing. Responder countries not performing NAT had a higher incidence/prevalence of HCV and HBV compared to responder countries performing NAT (Table 2). One respondent not performing NAT indicated that implementation was planned for 2023.

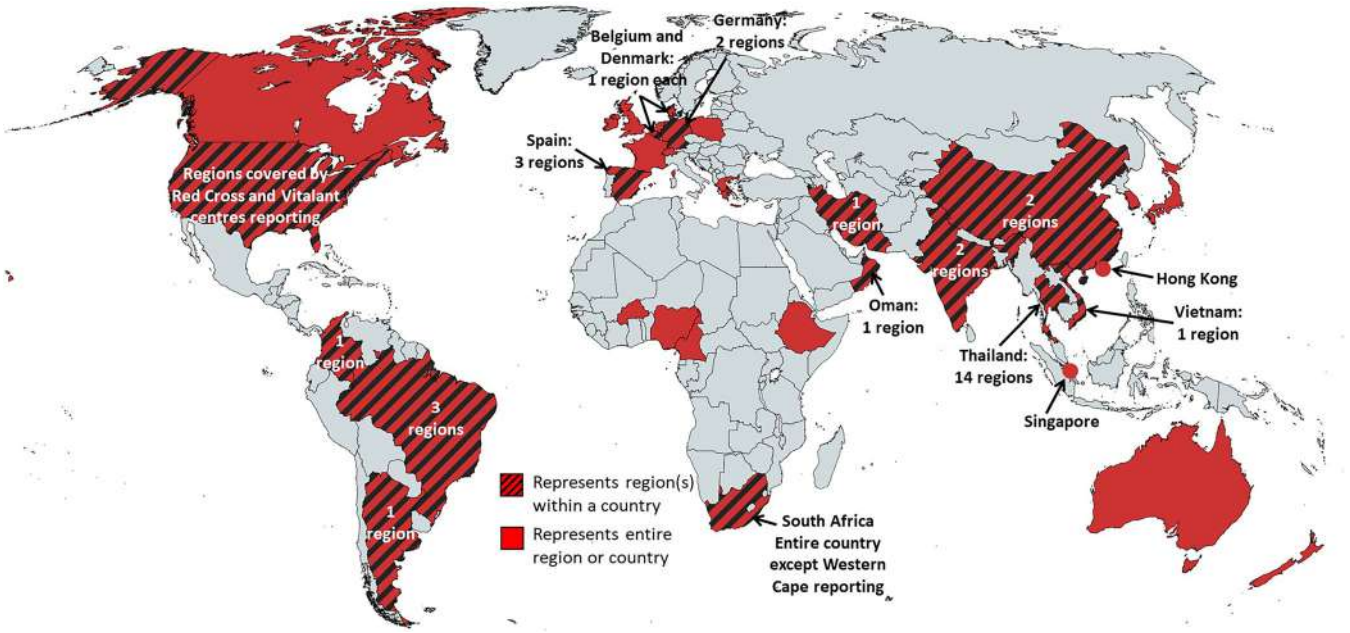

FIGURE 1 Geographical spread of survey respondents (prepared using mapchart.net).

TABLE 1 Survey responders performing blood donation NAT in 2019.

	HIV	HCV	HBV	HEV	WNV	ZIKV
Yes	38	36	36	11	11	3
No	5	5	5	30	30	38
No response	0	2	2	2	2	2
Proportion performing NAT	88%	84%	84%	26%	26%	7%

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; NAT, nucleic acid amplification testing; WNV, West Nile virus; ZIKV, Zika virus.

TABLE 2 Viral incidence/prevalence in the countries of survey responders performing NAT and those not performing NAT.

	HIV[a]	HCV[b]	HBV[c]
Incidence/prevalence (median)—NAT[d]	0.15% ($n = 23$)	0.30% ($n = 35$)	0.29% ($n = 34$)
Incidence/prevalence (median)—no NAT	0.19% ($n = 5$)	0.70% ($n = 5$)	1.32% ($n = 5$)
p-value	0.4375	0.0447	0.0073

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; NAT, nucleic acid amplification testing.
[a]HIV incidence, adults aged 15–49 per 1000 uninfected population, 2019 [12].
[b]Modelled viraemic prevalence, 2020 [13].
[c]Proportion of new cases of acute HBV in all sexes and ages per 100,000 people, 2019 [12].
[d]Data not available for all survey responder countries.

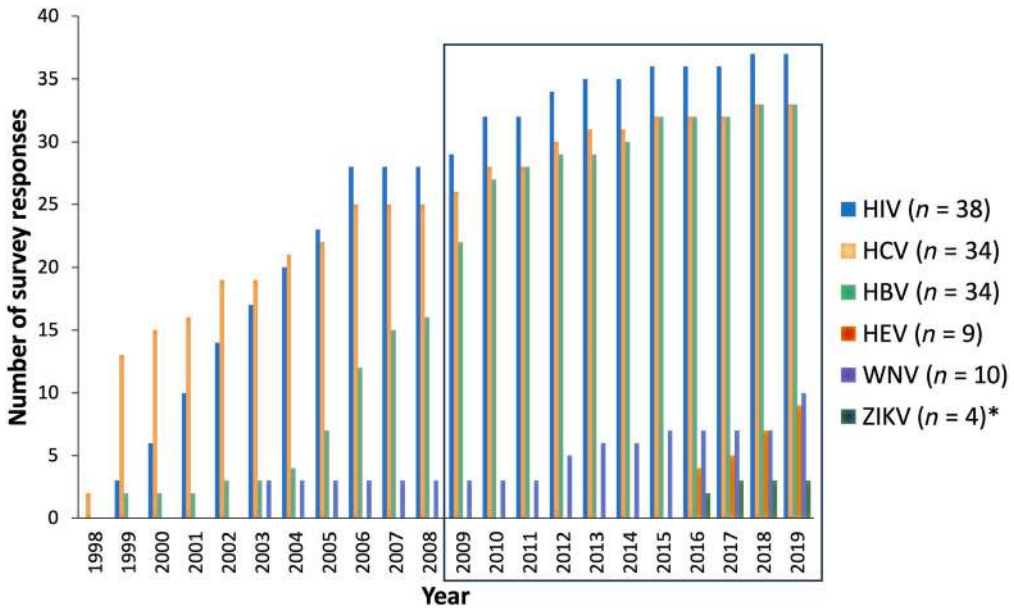

FIGURE 2 Implementation year of NAT for each virus. Box highlights time period since data were collected for the last ISBT NAT survey [2]. Data were not available from all respondents. *One survey responder indicated ZIKV NAT was used in 2016 only. HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; ISBT, International Society of Blood Transfusion; NAT, nucleic acid amplification testing; WNV, West Nile virus; ZIKV, Zika virus.

Date of NAT implementation

Data were collected for the previous NAT survey in 2008; since then, there has been an increase in the implementation of HIV, HCV and, later, HBV NAT among the survey responders (Figure 2). Since this time, only eight additional regions implemented NAT for these agents by 2019. Since 2008, there has been an increase in the adoption of NAT for other viruses; ZIKV, WNV and HEV testing have been implemented in four, seven and nine new regions or countries, respectively. The earliest adoption of ZIKV or HEV NAT was in 2016.

NAT-positive donations, 2019

In 2019, the proportion of donations positive by NAT (with or without detectable antibodies, if applicable) were 0.0099% (95% CI: 0.0095%–

TABLE 3 NAT-positive and NAT yield donations by region, 2019.

	Africa ($n = 1$)	Asia and Western Pacific ($n = 11$)	Europe ($n = 15$)	North America ($n = 3$)	South America ($n = 5$)	Total ($n = 35$)
Inhabitants supplied by blood operators (*n*)	54,000,000	>293,333,957[a]	250,543,947	366,156,716	>22,553,901[a]	>986,588,521[a]
HIV						
Donations tested (*n*)	949,121	11,118,151	8,764,993	6,668,100	349,295	27,919,660
NAT-positive donations (*n*)	2046	449	98	98	76	2767
NAT-positivity (rate[b])	2155.68	40.13	11.18	14.70	217.58	99.11
NAT yield[c] donations (*n*)	66	22	4	0	2	94
NAT yield[c] (rate[b])	69.54	1.97	0.46	-	5.73	3.37
HCV						
Donations tested (*n*)	949,121	11,183,633	8,764,973	6,668,100	349,295	27,915,122
NAT-positive donations (*n*)	90	847	269	498	48	1752
NAT positivity (rate[b])	94.82	75.74	30.69	74.68	137.42	62.76
NAT yield[c] donations (*n*)	3	38	1	4	0	46
NAT yield[c] (rate[b])	3.16	3.40	0.11	0.60	-	1.65
HBV						
Donations tested (*n*)	949,121	11,188,151	8,764,987	6,668,100	349,295	27,919,654
NAT-positive donations (*n*)	1088	4823	544	359	74	6888
NAT positivity (rate[b])	1146.32	431.08	62.07	53.84	211.86	246.71
NAT yield[c] donations (*n*)	227	1577	53	6	0	1863
NAT yield[c] (rate[b])	239.17	140.95	6.05	0.90	-	66.73
HEV						
Donations tested (*n*)	0	0	3,209,633	0	200	3,209,833
NAT-positive donations (*n*)	0	0	1037	0	1	1038
NAT positivity (rate[b])	-	-	323.09	-	5000.00	323.38
WNV						
Donations tested (*n*)	0	0	103,430	6,380,208	0	6,483,638
NAT-positive donations (*n*)	0	0	0	93	0	93
NAT positivity (rate[b])	-	-	-	14.58	-	14.34
ZIKV						
Donations tested (*n*)	0	129,983	0	5,779,697	0	5,909,680
NAT-positive donations (*n*)	0	2	0	1	0	3
NAT positivity (rate[b])	-	15.39	-	0.17	-	0.51

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; NAT, nucleic acid amplification testing; WNV, West Nile virus; ZIKV, Zika virus.

[a]Data were not available from all respondents.

[b]Rate is expressed per 1000,000 donations.

[c]NAT yield refers to samples that test positive by NAT only and not on other tests, if performed.

0.0103%; 2767/27,919,660) or 99 per million donations for HIV, 0.0063% (95% CI: 0.0060%–0.0066%; 1752/27,915,122) or 63 per million donations for HCV, 0.0247% (95% CI: 0.0241%–0.0253%; 6888/27,919,654) or 247 per million donations for HBV, 0.0323% (95% CI: 0.0304%–0.0343%; 1038/3,209,833) or 323 per million donations for HEV, 0.0014% (95% CI: 0.0011%–0.0017%; 93/6,483,638) or 14 per million donations for WNV and 0.00005% (95% CI: 0%–0.00011%; 3/5,909,680) or 1 per million donations for ZIKV (Table 3). The majority of donations tested by NAT were from repeat donors (Figure 3a). For HIV, HCV, HBV, HEV and ZIKV, there was a greater overall rate (per million donations) of NAT-positive donations from first-time donors, while the reverse was observed for WNV (Figure 3b); however, this pattern was not observed by all survey responders for HEV and WNV (data not shown).

NAT-yield donations, 2019

NAT yield refers to donations testing positive for NAT, but negative by serology, if performed, and can be reported for HIV, HCV and

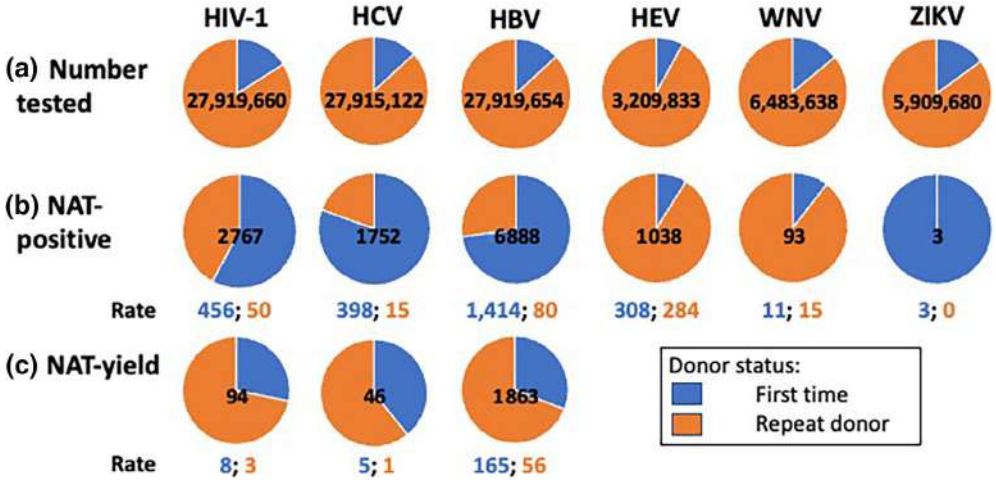

FIGURE 3 NAT-positive donations, 2019, by repeat and first-time donors, organized by (a) number of donations tested, (b) number of NAT-positive donations and (c) number of NAT-yield donations. Rates per million donations are provided. Data were not available from all respondents. HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; NAT, nucleic acid amplification testing; WNV, West Nile virus; ZIKV, Zika virus.

HBV. NAT-yield donations were identified for HIV ($n = 94$), HCV ($n = 46$) and HBV ($n = 1863$) (Table 3, Figure 3c). Although repeat donors made up the greatest proportion of NAT-yield donations, the NAT-yield rate per million donations was higher in first-time donors (Figure 3c).

Regional variation in NAT positivity and yield, 2019

Considerable variations in the NAT positivity and NAT-yield rates were observed between the different geographical regions (Table 3). The rate of HIV positivity was highest in donations from African donors, followed by those in South America, and the lowest in donations from Europe. HIV NAT-yield rates showed a similar trend; for 2019, none were detected in North America. For HCV, the NAT-positivity rate was highest in donations from South America, followed by those in Africa, and the lowest rate in donations from Europe. The HCV NAT-yield rate, however, showed a different trend, with no HCV NAT-yield donations from South America and the highest rate in the Asia and Western Pacific regions followed closely by Africa. The HBV NAT-positivity rate was highest in donations from African donors, followed by those from Asia and the Western Pacific, and the lowest rate in donations from North America. HBV NAT-yield rates showed a similar trend; however, none were detected in respondents from South America. The overall NAT positivity and NAT-yield rate were highest for HBV.

HEV NAT was performed on European and a very small number of South American donations, preventing yield to be compared by region. Nearly all WNV NAT was performed in North America; small numbers of donations were tested in Europe. All WNV NAT positives were observed in donations from North America. Again, nearly all ZIKV NAT was performed on donations from North American donors, with much smaller numbers performed in Asia and Western Pacific; however, the rates were higher in the Asia and Western Pacific regions compared to North America (albeit $n = 2$ vs. $n = 1$ positives per region, respectively).

NAT-positive donations and NAT yield since implementation

Similar to the 2008 survey, data were collected on NAT since its introduction until the end of 2019 in the regions and countries surveyed, to provide historical context on the value of NAT for blood screening (Table 4). Since implementation, over 517 million donations have been screened for HIV and HCV, with almost 370 million screened for HBV, reflecting its later implementation. HIV RNA was detected by NAT in 32,914 donations of which 1153 were NAT yields; HCV RNA was detected in 75,108 donations of which 1121 were NAT yields; and HBV DNA was detected in 68,096 donations of which 14,465 were NAT yields. The overall rate of NAT positivity and NAT yield was highest for HBV, which is similar to what was observed in 2008. The highest and lowest rates of NAT positivity and NAT yield since implementation were similar regionally to what was observed in 2008.

The number of donations tested for HEV, WNV and ZIKV were lower, reflecting their later date of implementation and regional and temporal use (Table 4). In Europe, where nearly all HEV NAT is performed, 1763 HEV NAT-positive donations were identified among nearly 8 million donations screened. Over 140 million donations, predominantly from North America, have been screened for WNV, with 3142 positive donations identified in North America and one in Europe. ZIKV NAT in Asia and Western Pacific, Europe and predominantly North America resulted in 589 positive donations from over 19 million donations.

TABLE 4 NAT-positive and NAT-yield donations by region, implementation to 2019.

	Africa ($n = 1$)	Asia and Western Pacific ($n = 11$)	Europe ($n = 15$)	North America ($n = 3$)	South America ($n = 5$)	Total ($n = 35$)
HIV						
Donations tested (*n*)	8,372,857	183,612,566	142,102,177	179,847,163	3,167,924	517,102,687
NAT-positive donations (*n*)	22,656	2575	1829	4794	1060	32,914
NAT positivity (rate[a])	2705.89	14.02	12.87	26.66	334.60	63.65
NAT yield[b] donations (*n*)	764	187	84	108	10	1153
NAT yield[b] (rate[b])	91.25	1.02	0.59	0.60	3.16	2.23
HCV						
Donations tested (*n*)	8,372,857	183,612,566	164,554,178	180,927,967	2,480,395	539,947,963
NAT-positive donations (*n*)	855	7926	10,968	54,153	1206	75,108
NAT positivity (rate[a])	102.12	43.17	66.65	299.31	486.21	139.10
NAT yield[b] donations (*n*)	50	262	191	614	4	1121
NAT yield[b] (rate[b])	5.97	1.43	1.16	3.39	1.61	2.08
HBV						
Donations tested (*n*)	8,372,857	152,221,471	102,928,968	103,272,077	2,418,536	369,213,909
NAT-positive donations (*n*)	12,462	33,093	15,975	5852	714	68,096
NAT positivity (rate[a])	1488.38	217.40	155.20	56.67	295.22	184.44
NAT yield[b] donations (*n*)	2318	11,116	897	124	10	14,465
NAT-yield[b] (rate[b])	276.85	73.03	8.71	1.20	4.13	39.18
HEV						
Donations tested (*n*)	0	0	7,721,643	0	337	7,721,980
NAT-positive donations (*n*)	0	0	1762	0	1	1763
NAT-positivity (rate[a])	-	-	228.19	-	2967.36	228.31
WNV						
Donations tested (*n*)	0	0	480,861	139,722,060	0	140,202,921
NAT-positive donations (*n*)	0	0	1	3142	0	3143
NAT positivity (rate[a])	-	-	2.08	22.49	-	22.42
ZIKV						
Donations tested (*n*)	0	383,148	19,800	18,898,123	0	19,301,071
NAT-positive donations (*n*)	0	10	147	432	0	589
NAT positivity (rate[a])	-	26.10	7424.24	22.86	-	30.52

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; NAT, nucleic acid amplification testing; WNV, West Nile virus; ZIKV, Zika virus.

[a]Rate is expressed per 1000,000 donations.

[b]NAT yield refers to samples that test positive by NAT only and not on other tests, if performed.

Residual risk estimates

To understand the different approaches used for calculating the residual transfusion-transmission risk for the different viruses tested by NAT, we asked whether such estimates were calculated in 2019 and, if so, which method was used. Approximately half of the participants indicated they did not perform such risk modelling calculations (Data S2). For those who did undertake these analyses, the classic incidence window-period model (based on repeat donor incidence and the pre-NAT infectious window period) [14] was used more often than the Weusten risk-day equivalent model [15] or the NAT yield and limiting antigen avidity (LAg) assay recent window-period ratio methods [16–18].

NAT for other agents

During 2019, NAT was performed for other agents (data not shown). Other TTIDs tested by NAT in 2019 included hepatitis A virus (HAV; $n = 6$), parvovirus B19 (B19; $n = 7$), cytomegalovirus (CMV; n = 1), human T-lymphotropic virus types 1 and 2 (HTLV-1/2; $n = 2$), *Trypanosoma cruzi* ($n = 1$), *Babesia* spp. ($n = 1$) and *Plasmodium* spp. ($n = 3$).

A number of responders indicated implementation or planned implementation of NAT for HAV, B19 and HTLV-1/2. Implementation of NAT for dengue, chikungunya and Zika viruses would be considered by a number of respondents if their regional epidemiological situation changes.

DISCUSSION

Our survey reports updated data on global blood donation NAT screening. We observed increased adoption of NAT for transfusion-transmitted viruses over the past decade, with an increase in HIV, HCV and HBV NAT usage and increased implementation of NAT for other viruses such as HEV, WNV and ZIKV. NAT-positive donations (including NAT yield and concordant NAT/antibody-positive donations) were identified for all viruses tested. Within the survey period of 2019, over 3100 NAT-positive donations were identified as NAT yield or solely by NAT. Since its introduction, over 519 million donations have been screened by NAT, with >22,000 donations identified as NAT yield or solely by NAT. HBV accounted for the majority of NAT-positive and NAT-yield donations, which is consistent with what has been reported previously in countries that do not perform anti-HBc testing [19]. Without NAT, these donations could have potentially resulted in TTIs in recipients of multiple components derived from each donation. NAT has thus been a significant contributor to improving blood transfusion safety globally. NAT, as an alternative to travel deferrals (e.g., for WNV in non-endemic settings), allows donations to be collected, rather than the deferral of donors, thus also contributing to sufficiency of supply. The main barrier for regions/countries not performing NAT was economic. For example, HIV, HCV and HBV NAT had previously been shown to be not cost effective in Zimbabwe [20]. Reducing cost and improving access to suitable assays for resource-limited countries may assist with adoption of blood donation NAT in such regions, further improving global blood transfusion safety, especially as incidence/prevalence of HCV and HBV was higher in responder countries/regions not performing NAT compared to those that do.

The overall HIV and HBV NAT-yield rates, per million donations, were higher in 2019 compared to 2008 (HIV: 3.37 vs. 1.93, respectively; HBV: 66.73 vs. 8.50, respectively), while HCV had a slightly lower NAT-yield rate (1.65 vs. 1.86, respectively). In Africa and the Asia and Western Pacific regions, the NAT-yield rates for all viruses was higher in 2019 than in previous periods, whereas for the other regions decreases were observed, for example, for HIV and HCV in Europe, as well as HCV and HBV in North America. Given that the global incidence of HIV, HBV and HCV decreased during this time [12, 13], increases in NAT-yield and NAT-positive rates (i.e., HBV) likely reflect improvements in NAT sensitivity. Significant regional variability in NAT-yield and positivity rates exists, reflecting differences in local viral epidemiology, highlighting the importance of tailoring blood safety initiatives to local situations.

During 2019, the majority of donations tested by NAT by survey responders were from repeat donors, reflecting the fact that this donor group makes up the majority of blood donors in survey responder regions. The overall NAT positivity rate for HIV, HCV and HBV was higher in first-time donors in 2019, similar to the previous study based on data from 2008 [2]. Although repeat donors make up the greatest number of HIV, HCV and HBV NAT-yield donations, which reflect their accounting for the greater proportion of donations tested, the NAT-yield rate per million donations was consistently higher in first-time donors for these three TTIDs. The large difference in NAT positivity between first-time and repeat donors suggests that repeat donors do self-risk assessments. Although the overall HEV NAT positivity rate in 2019 was higher in first-time donors, the reverse was observed by multiple responders; this appears to be driven by one survey responder that tested a large number of donations in 2019 and had a rate of HEV positivity higher in first-time donors. WNV was the only virus whose overall rate of NAT positivity in 2019 was higher in repeat donors (15 per million, compared to first-time donors, 11 per million), simply reflecting the fact that both first-time and repeat donors are at a comparable risk of being bitten by an infected mosquito; thus, since there are many more repeat donors, there are many more repeat WNV-positive donors. The number of ZIKV NAT-positive donations was small, with all three ZIKV NAT-positive donations in 2019 coming from first-time donors; however, repeat donors in North America were positive in previous years [8], and similar to WNV, donation status is not a contributor to positivity by a mosquito-borne agent.

Blood donation NAT was not restricted to these six viruses and was also performed on other agents in 2019, including HAV, B19, HTLV-1/2, *T. cruzi*, *Babesia* spp. and *Plasmodium* spp. A number of survey responders indicated planned implementation of NAT for other agents such as HAV, B19 and HTLV, or arboviral NAT, for some or multiple agents, if changes in their epidemiological situations occur. Moreover, laboratory-developed or research-use-only assays may be available in some jurisdictions, which were not captured in the present study. Such assays could be rapidly deployed in the initial response to emerging threats, negating the need to rely on commercial assays in such instances. With the emergence of different agents in different geographical regions, such in-house fit-for-purpose NAT assays may be the best first-line defence. In addition, there appears to be an increasing use of multiplex NAT assays, including those for emerging TTIDs. Given this ever-changing landscape, it is imperative that blood operators and TTID specialists continue to work together with commercial NAT assay manufacturers, such as through the activities of the ISBT WP-TTID, on a regular basis to ensure collaborative studies of performance of established and new NAT assays, such as in this report.

Our study has limitations. We report the results of NAT from 43 survey responders from 32 countries; other blood operators were invited to participate in this study, and many are performing blood donation viral NAT. For example, while we report no HIV NAT-yield donations in North America during 2019 among responders to our survey, such infections were detected during this time in donations given to organizations that did not contribute data to this survey and, in previous and subsequent years, for those in North America who did participate in this survey [21]. The results from our survey would be biased towards countries, regions or organizations actively involved in the ISBT WP-TTID and/or using Roche or Grifols NAT assays. We report the proportion of survey responders, rather than the country as a whole, given that some regions within a country reported different responses to some questions; but this does not affect NAT positivity or yield rates. Some countries or regions noted implementation or removal of NAT since 2019 (e.g., [22]), further highlighting the need to undertake surveys like this on a regular basis. We report "reactives"

and use that term as analogous to confirmed positives. Sensitivities of reported NAT assays and algorithms to reach a final consensus definition of positive were not defined by the survey or the ISBT, thus there will be differences for which we cannot control and may have an impact on our results. Thus, our results may have overestimated NAT yield, but even so, this should not have an impact on the trends that we reported here. Finally, we focused on blood donation viral NAT; given NAT is now also used for screening blood donations for parasites, such as *Babesia* spp. [9], future studies performed by the ISBT WP-TTID should be extended to cover all TTIDs.

To our knowledge, this is the largest survey of blood donation NAT to date and the only comprehensive snapshot of NAT usage in the past 10 years. Blood donation NAT usage has increased since its first introduction. Given the detection of over 22,000 NAT-only positive donations combined since its introduction, it is clear that NAT has played an important role in enhancing blood transfusion safety globally. Overcoming barriers in those countries/regions not performing NAT would undoubtedly offer the benefits of NAT, with potentially higher yield and impact on safety in low- and middle-income countries many of which have high burdens of TTIDs.

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ACKNOWLEDGEMENTS

We acknowledge the contribution of Clive Seed to the initial stages of this project and also Thi Thanh Dung, Konstantinos Stamoulis, Roberta Fachini, Ratti Ram Sharma, Abiy Belay Ambaye and Habtamu Taye Guyaho for suppling data. We thank ISBT WP-TTID members not listed as authors and also Susan Galel, Jean Stanley and Laura Fryza who facilitated distribution of the survey.

H.M.F., C.O., B.C., M.B. and S.L.S. conceived the study and prepared the survey. H.M.F. prepared the first draft of the manuscript. All authors contributed to study design, data analysis, data interpretation and manuscript editing and approved the final version of the manuscript. Open access publishing facilitated by University of the Sunshine Coast, as part of the Wiley - University of the Sunshine Coast agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST STATEMENT

Helen M. Faddy has received research funding and/or honoraria from Grifols Diagnostic Solutions Inc. and Roche Diagnostic Solutions in the past. Brian Custer and/or the organization he is employed by has received research funding from Grifols Diagnostic Solutions Inc. and Roche Diagnostic Solutions. Susan Stramer has received research funding and/or honoraria from Grifols Diagnostic Solutions Inc. and Roche Diagnostic Solutions in the past. Christian Erikstrup has received unrestricted research grants from Abbott Diagnostics and Novo Nordisk, which are administered by Aarhus University Hospital and Aarhus University, respectively. Christian Erikstrup has not received any personal fees from these or other entities. Silvia Sauleda has received research funding from Grifols Diagnostic Solutions in the past. The remaining authors have no relevant conflict of interest (COI) to declare.

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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REFERENCES

1. Busch MP, Bloch EM, Kleinman S. Prevention of transfusion-transmitted infections. Blood. 2019;133:1854–64.
2. Roth WK, Busch MP, Schuller A, Ismay S, Cheng A, Seed CR, et al. International survey on NAT testing of blood donations: expanding implementation and yield from 1999 to 2009. Vox Sang. 2012;102:82–90.
3. Boland F, Martinez A, Pomeroy L, O'Flaherty N. Blood donor screening for hepatitis E virus in the European Union. Transfus Med Hemother. 2019;46:95–103.
4. Sakata H, Matsubayashi K, Iida J, Nakauchi K, Kishimoto S, Sato S, et al. Trends in hepatitis E virus infection: analyses of the long-term screening of blood donors in Hokkaido, Japan, 2005-2019. Transfusion. 2021;61:3390–401.
5. Groves JA, Foster GA, Dodd RY, Stramer SL. West Nile virus activity in United States blood donors and optimizing detection strategies: 2014-2018. Transfusion. 2020;60:94–105.
6. O'Brien SF, Scalia V, Zuber E, Hawes G, Alport EC, Goldman M, et al. West Nile virus in 2006 and 2007: the Canadian Blood Services' experience. Transfusion. 2010;50:1118–25.
7. Beau F, Mallet HP, Lastère S, Broult J, Laperche S. Transfusion risk associated with recent arbovirus outbreaks in French Polynesia. Vox Sang. 2020;115:124–32.
8. Saá P, Proctor M, Foster G, Krysztof D, Winton C, Linnen JM, et al. Investigational testing for Zika virus among U.S. blood donors. N Engl J Med. 2018;378:1778–88.
9. Tonnetti L, Dodd RY, Foster G, Stramer SL. Babesia blood testing: the first-year experience. Transfusion. 2022;62:135–42.
10. Coste J, Reesink HW, Engelfriet CP, Laperche S, Brown S, Busch MP, et al. Implementation of donor screening for infectious agents transmitted by blood by nucleic acid technology: update to 2003. Vox Sang. 2005;88:289–303.
11. Engelfriet CP, Reesink HW. Implementation of donor screening for infectious agents transmitted by blood by nucleic acid technology. Vox Sang. 2002;82:87–111.
12. Our World in Data. Available from: http://ourwordindata.org/about. Last accessed 4 Oct 2023.
13. Blach S, Terrault NA, Tacke F, Gamkrelidze I, Craxi A, Tanaka J, et al. Global change in hepatitis C virus prevalence and cascade of care between 2015 and 2020: a modelling study. Lancet. Gastroenterol Hepatol. 2022;7:396–415.
14. Kleinman S, Busch MP, Korelitz JJ, Schreiber GB. The incidence/window period model and its use to assess the risk of transfusion-transmitted human immunodeficiency virus and hepatitis C virus infection. Transfus Med Rev. 1997;11:155–72.
15. Weusten JJ, van Drimmelen HA, Lelie PN. Mathematic modeling of the risk of HBV, HCV, and HIV transmission by window-phase donations not detected by NAT. Transfusion. 2002;42:537–48.
16. Busch MP, Glynn SA, Stramer SL, Strong DM, Caglioti S, Wright DJ, et al. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. Transfusion. 2005;45:254–64.
17. Grebe E, Busch MP, Notari EP, Bruhn R, Quiner C, Hindes D, et al. HIV incidence in US first-time blood donors and transfusion risk with a 12-month deferral for men who have sex with men. Blood. 2020;136:1359–67.
18. Vermeulen M, Chowdhury D, Swanevelder R, Grebe E, Brambilla D, Jentsch U, et al. HIV incidence in south African blood donors from 2012 to 2016: a comparison of estimation methods. Vox Sang. 2021;116:71–80.
19. Niederhauser C, Tinguely C, Stolz M, Vock M, El Dusouqui SA, Gowland P. Evolution of blood safety in Switzerland over the last 25 years for HIV, HCV, HBV and Treponema pallidum. Viruses. 2022;14:2611.
20. Mafirakureva N, Mapako T, Khoza S, Emmanuel JC, Marowa L, Mvere D, et al. Cost effectiveness of adding nucleic acid testing to hepatitis B, hepatitis C, and human immunodeficiency virus screening of blood donations in Zimbabwe. Transfusion. 2016;56:3101–11.
21. Steele W, Dodd R, Notari E, Haynes J, Anderson S, Williams A, et al. HIV, HCV, and HBV incidence and residual risk in US blood donors before and after implementation of the 12-month deferral policy for men who have sex with men. Transfusion. 2021;61:839–50.

22. Alkhazashvili M, Bloch EM, Shadaker S, Kuchuloria T, Getia V, Turdziladze A, et al. Advancing blood transfusion safety using molecular detection in the country of Georgia. Transfus Clin Biol. 2023;30:307–13.

SUPPORTING INFORMATION

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

How to cite this article: Faddy HM, Osiowy C, Custer B, Busch M, Stramer SL, Adesina O, et al. International review of blood donation nucleic acid amplification testing. Vox Sang. 2024;119:315–25.

Received: 15 July 2023 | Revised: 1 December 2023 | Accepted: 8 December 2023

DOI: 10.1111/vox.13583

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Patient-tailored platelet transfusion practices for children supported by extracorporeal membrane oxygenation

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Funding information
The authors received no specific funding for this work.

Abstract

Background and Objectives: Extracorporeal membrane oxygenation (ECMO) serves as cardiopulmonary therapy in critically ill patients with respiratory/heart failure and often necessitates multiple blood product transfusions. The administration of platelet transfusions during ECMO is triggered by the presence or risk of significant bleeding. Most paediatric ECMO programmes follow guidelines that recommend a platelet transfusion threshold of 80–100 $\times$ 10^9/L. To reduce exposure to platelets, we developed a practice to dynamically lower the threshold to ~20 $\times$ 10^9/L. We describe our experience with patient-tailored platelet thresholds and related bleeding outcomes.

Materials and Methods: We retrospectively evaluated our platelet transfusion policy, bleeding complications and patient outcome in 229 ECMO-supported paediatric patients in our unit.

Results: We found that more than 97.4% of patients had a platelet count <100 $\times$ 10^9/L at some point during their ECMO course. Platelets were transfused only on 28.5% of ECMO days; and 19.2% of patients never required a platelet transfusion. The median lowest platelet count in children who had bleeding events was 25 $\times$ 10^9/L as compared to 33 $\times$ 10^9/L in children who did not bleed ($p < 0.001$). Our patients received fewer platelet transfusions and did not require more red blood cell transfusions, nor did they experience more haemorrhagic complications.

Conclusion: We have shown that a restrictive, 'patient-tailored' rather than 'goal-directed' platelet transfusion policy is feasible and safe, which can greatly reduce the use of platelet products. Although there was a difference in the lowest platelet counts in children who bled versus those who did not, the median counts were much lower than current recommendations.

Keywords

bleeding, extracorporeal membrane oxygenation, packed red blood cells, paediatric, platelets, transfusion

Highlights

- Thrombocytopaenia in paediatric patients on extracorporeal membrane oxygenation (ECMO) support is very common.
- Most centres employ a platelet transfusion threshold of 80–100 $\times$ 10^9/L for non-bleeding patients. In contrast, our practice has been to gradually decrease the platelet transfusion threshold to ~20 $\times$ 10^9/L, in non-bleeding paediatric patients.
- Our patient-tailored approach has resulted in decreased total volume of platelet transfusions over the entire ECMO course, without any increase in bleeding complications or red blood cell transfusions.

INTRODUCTION

Extracorporeal membrane oxygenation (ECMO) is a life-saving heart-lung bypass modality for patients with severe, reversible cardiac and/or respiratory failure. Although the exact mechanisms are still not fully clear, ECMO induces a reduction in the number of platelets and alteration in platelet function along with activation of complement and coagulation cascades [1–3]. Thrombocytopaenia is very common in ECMO-supported patients; observational studies in children have found that the platelet count drops by 26%–47% at the time of cannulation [4, 5]. Abnormal activation of platelets has also been observed in ECMO-supported patients [4].

In order to minimize the risk of bleeding, children on ECMO are transfused large volumes of platelets during their course of ECMO by multiple repeated transfusions. Recent studies have shown that platelets are transfused to children on two-thirds of each ECMO day, and each child on ECMO receives approximately 90 mL/kg of platelets throughout their ECMO course [6–11].

Paediatric ECMO-supported patients are commonly anticoagulated and often have surgical incisions, which further increases the risk of bleeding [12–14]. The challenge of maintaining a balanced haemostatic system has prompted guidelines for the transfusion of blood products on ECMO. The Association for the Advancement of Blood & Biotherapies (AABB), the Extracorporeal Life Support Organization (ELSO) and a recent consensus statement recommend transfusing platelets in a non-bleeding child on ECMO when the count is <80–100 $\times$ 10^9/L [15–17]. These guidelines are mainly based on expert opinion but there is a paucity of evidence-based data on the optimal threshold for platelet transfusions in paediatric ECMO patients. Despite the lack of prospective studies, many centres strictly follow these guidelines and set platelet transfusion goals at 80–100 $\times$ 10^9/L or higher [1, 6, 9, 11, 18–20].

We sought to evaluate clinical outcomes, including bleeding complications, in ECMO-supported paediatric patients who were transfused based on a protocol allowing for a gradual decrease in the transfusion threshold to 20 $\times$ 10^9/L. We hypothesized that the protocol would decrease exposure to platelet transfusions without any increase in the rate of bleeding.

MATERIALS AND METHODS

Study design

We conducted a retrospective cohort study of all children and young adults supported by ECMO in the cardiac intensive care unit (CICU) of a tertiary university-affiliated paediatric centre. The study was approved by the Institutional Review Board (Rabin Medical Center approval number 0676-18) who waived informed consent.

Study participants

We included all consecutive paediatric patients and young adults (birth to 28 years of age) with respiratory and/or cardiac failure who were supported by ECMO in our CICU from June 2010 to June 2020. There were no exclusion criteria.

Platelet transfusion practice

With the goal of reducing platelet product use and patient exposure, our practice for the past 10 years has been to individually tailor the platelet transfusion threshold for each patient and gradually lower the threshold from 100 $\times$ 10^9/L in the first ECMO day to ~20 $\times$ 10^9/L in non-bleeding patients, within 4–5 days of ECMO cannulation. The platelet transfusion threshold was determined during morning rounds for each patient according to age, illness severity and bleeding risk factors (as defined by coagulopathy, hepatic failure, recent surgery or the presence of active or recent bleeding). When bleeding occurred, the patient was transfused with blood products and/or haemostatic medications as needed. When the transfusion of platelets was required, per the pre-defined transfusion threshold, we used apheresis (single donor) platelets at a dose of 20 mL/kg.

Anticoagulation and transfusion management

The patients were anticoagulated with an infusion of unfractionated heparin to maintain an activated clotting time (ACT) of 180–220 s

(Hemochron® Response coagulation monitor, Werfen, Bedford, MA, USA, normal ACT 70–120 s). The heparin infusion was adjusted to a lower ACT (160–180 s) in bleeding patients and to 200–220 s when the ECMO flow was reduced. The complete blood count was assayed daily.

When the patients suffered a major bleeding event, fresh frozen plasma (FFP) was transfused if the ACT or activated partial thromboplastin time was higher than desired (usually >200 or >80 s, respectively), or when no response to tranexamic acid infusion was observed. If the patient was thrombocytopaenic (i.e., platelet count $<100 \times 10^9$/L) at the time of the bleeding, single-donor platelets were transfused. Antithrombin replacement was accomplished through the transfusion of plasma because antithrombin concentrate is not available in Israel. Cryoprecipitate was transfused prophylactically when the serum fibrinogen dropped below 100 mg/dL.

Definitions of endpoints and transfusion classification

Major bleeding events were classified as bleeding episodes that met at least one of the following criteria:

1. Intracranial haemorrhage (ICH; excluding intraventricular haemorrhage grade 1) as determined by ultrasound in infants, and CT or MRI imaging in older children.
2. Bleeding episodes that required any of the following: discontinuation of heparin infusion, transfusion of FFP, cryoprecipitate, packed red blood cells (PRBCs) or treatment with tranexamic acid or recombinant factor VIIa.
3. Re-operation for haemostatic control of surgical or cannulation site.

Bleeding events that did not meet these criteria were classified as minor.

Bleeding sites were recorded as the following: central nervous system, pulmonary, gastrointestinal, genitourinary, oropharyngeal, surgical site, cannulation site or post-procedural bleeding. A 'bleeding day' was defined as a day when a bleeding event occurred during the ECMO course, including days when the bleeding continued from a previous bleeding day (the same bleeding event). All bleeding events were reviewed and adjudicated by a senior paediatric cardiac intensivist.

Platelet transfusions were classified as prophylactic, that is, thrombocytopaenia in a non-bleeding patient; therapeutic, that is, active bleeding in a thrombocytopenic patient; minor bleeding in a volume-depleted patient; or pre-procedural. Active bleeding was defined as visually observed ongoing bleeding from mucus membranes, surgical or cannulation sites, gastrointestinal tract, airways and/or acute drop in haemoglobin levels with imaging evidence of ICH.

Survival was recorded at hospital discharge. Follow-up data were extracted only for patients who suffered from ICH while on ECMO.

ICH was screened by head sonography three times weekly. If bleeding was noted, sonography was repeated daily. Grades II–IV intraventricular haemorrhage, as previously classified, were included in ICH bleeding events [21]. Screening for ICH in older infants and children was based on daily neurological examinations during interruptions of sedation. This was achieved by temporary discontinuation of muscle relaxants and lightening of sedation regimen until movement of the head, limbs and evidence of respiratory effort. A neurological deficit in a physical examination prompted brain imaging.

Data collection and statistical analysis

Demographic, clinical and ECMO data were abstracted from the electronic health records. Data regarding transfusion of PRBCs, platelets, FFPs, cryoprecipitate and haemostatic medications were recorded, as well as daily platelet counts and bleeding events.

Statistical analysis was carried out using the SAS Software, version 9.4 (SAS Institute, Cary, NC). Continuous variables are presented as medians and interquartile ranges (IQR), and categorical variables by frequencies (percentages). The Mann–Whitney *U* test was used to compare the lowest platelet counts in the children who bled versus those who did not bleed. In addition, the Spearman rank correlation test was used to determine the relationship between the lowest platelet count and days of bleeding.

RESULTS

Patient characteristics

The analysis included 229 children who met the inclusion criteria; 55% were males and 53% were neonates (age ≤28 days). The majority (94%) were supported by venoarterial ECMO. Demographic, ECMO and outcome data are presented in Table 1. No patient required re-cannulation after disconnection from the ECMO circuit.

Platelet counts

During the study period, 3735 complete blood counts were assayed, averaging 1.5 counts/patient/ECMO day or 16.3 counts per patient. The overall median (IQR) platelet count was $72\ (44–111) \times 10^9$/L. The daily median, IQR and minimal platelet counts of the cohort during the first 28 days of ECMO support are shown in Figure 1. The distribution of the individual minimal platelet counts of the patients is presented in Figure 2. At some point during their ECMO run, 98.7% of the patients were in the thrombocytopaenic range and over half (56.3%) of the patients had a platelet count of $<30 \times 10^9$/L at least once during their ECMO run.

Blood product transfusions and haemostatic medications

During the ECMO course, 185 (81%) patients (95% confidence interval [CI]: 76%–86%) received 696 platelet units. The platelets were transfused for bleeding prophylaxis (59%, 95% CI: 55%–63%), ongoing bleeding (27%, 95% CI: 23%–30%), minor bleeding in a volume-depleted

TABLE 1 Demographic and ECMO data.

Characteristic	Study cohort
Age at cannulation, days, median (IQR)	20.9 (2.5–440.7)
Weight at cannulation, kg, median (IQR)	3.7 (3.0–10.0)
ECMO run duration, days, median (IQR)	6.8 (4.7–12.6)
Male sex, *N* (%)	125 (54.6)
Cannulation location, *N* (%)	
In house	131 (57.2)
Referring centre	98 (42.8)
Cannulation configuration, *N* (%)	
VA	214 (93.5)
VV	12 (5.2)
VVA	3 (1.3)
Cannulation during CPR (ECPR), *N* (%)	40 (17.5)
ECMO support outcome, *N* (%)	
Alive	153 (66.8)
Weaned off ECMO	150 (98)
Transition to durable VAD	3 (2)
In-hospital mortality	76 (33.2)
Withdrawal due to futility	54 (71.1)
Withdrawal due to irreversible brain damage	21 (27.6)
Cannula dislodgement	1 (1.3)
Survival to ICU discharge	138 (60.3)
Indication for ECMO support, *N* (%)	
Cardiac	114 (49.8)
Post-operative decompensation	56 (49.1)
Myocarditis	20 (17.5)
Inability to separate from CPB	15 (13.2)
Cardiomyopathy	7 (6.1)
Refractory arrhythmia	5 (4.4)
Diastolic dysfunction	2 (1.8)
Preoperative congenital heart disease	2 (1.8)
Poisoning	2 (1.8)
Acute mitral valve tear due to rheumatic fever	1 (0.9)
Neurogenic stunned myocardium	1 (0.9)
Pulmonary vein hypoplasia	1 (0.9)
Severe aortic stenosis, myocardial failure	1 (0.9)
Tachycardia-induced myocardial failure	1 (0.9)
Respiratory	98 (42.8)
Meconium aspiration syndrome	35 (35.7)
Congenital diaphragmatic hernia	32 (32.7)
Acute respiratory distress syndrome	15 (15.3)
Neonatal respiratory distress syndrome	5 (5.1)
Secondary pulmonary hypertension	4 (4.1)
Respiratory failure of unknown aetiology	1 (1.0)
Large airways obstruction	1 (1.0)
Pulmonary haemorrhage	1 (1.0)
Idiopathic pulmonary syndrome	1 (1.0)
Surfactant deficiency	1 (1.0)
Blood aspiration	1 (1.0)
Persistent pulmonary hypertension of the newborn	1 (1.0)
Other	17 (7.4)
Bacterial sepsis/infection	16 (94.1)
Haemorrhagic shock encephalopathy syndrome	1 (5.9)

Abbreviations: CPB, cardiopulmonary bypass; ECMO, extracorporeal membrane oxygenation; ECPR, extracorporeal cardiopulmonary resuscitation; ICU, intensive care unit, IQR, interquartile range; VA, veno-arterial; VAD, ventricular assist device; VV, veno-venous; VVA, veno-venous-arterial.

patient (14%, 95% CI: 11%–16%) or pre-procedural bleeding (0.4%). Platelets were transfused on 28.5% of the ECMO days. The average proportion (range) of the platelet transfusion days to the ECMO days was 26.0% (9.5%–39.1%) during the first 28 days of the ECMO course (Figure 3). Among the transfused patients, the median (IQR) daily platelet transfusion dose was 8.7 (4.4–13.3) mL/kg/ECMO day, and the median (IQR) platelet dose for the entire ECMO course was 70.0 (35.7–126.5) mL/kg/ECMO run. The median (IQR) daily transfusion dose per day transfused was 23.7 (17.4–30.9) mL/kg. For prophylactic platelet transfusion, the median (IQR) daily platelet transfusion dose was 6.2 (3.1–10.3) mL/kg/ECMO day, and the median (IQR) platelet dose for the entire ECMO course was 49.3 (25.3–89.5) mL/kg/ECMO run. The median (IQR) daily transfusion dose per day transfused was 23.0 (17.1–31.4) mL/kg.

During their ECMO course, 218 (95%) patients (95% CI: 92%–98%) received PRBCs; the median (IQR) dose was 100 (44.8–253.3) mL/kg/ECMO run, and the median (IQR) daily dose was 14.0 (7.1–24.6) mL/kg. FFP transfusions were received by 154 (67%) patients (95% CI: 61%–73%), mostly for antithrombin supplementation and not for bleeding treatment or prevention. Fifty-seven (25%) patients (95% CI: 19%–31%) received cryoprecipitate and 29 (13%, 95% CI: 8%–17%) were treated with tranexamic acid, either therapeutically or prophylactically. Recombinant factor VIIa was administered to 22 (10%) patients (95% CI: 6%–13%) for active bleeding.

Haemorrhagic complications and platelet counts

While supported by ECMO, 140 (61%) patients (95% CI: 55%–67%) experienced bleeding (Figure 4). Twenty (8.7%) patients, including 16 neonates, had ICH. Two patients underwent craniotomy for haematoma evacuation after weaning from ECMO support. On long-term follow-up, one infant developed paraplegia and a seizure

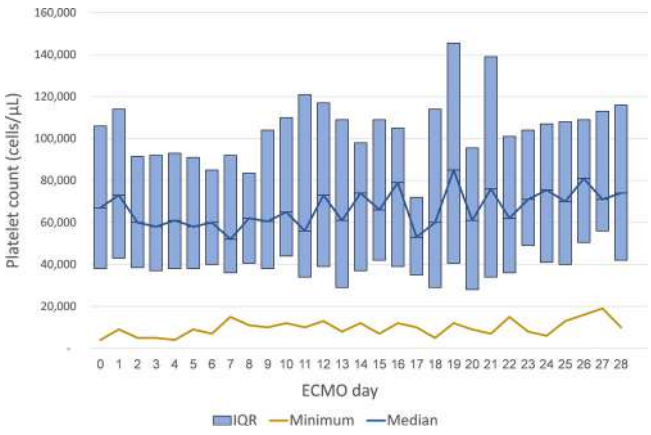

FIGURE 1 Median, interquartile range (IQR) and minimal platelet counts during the extracorporeal membrane oxygenation (ECMO) course.

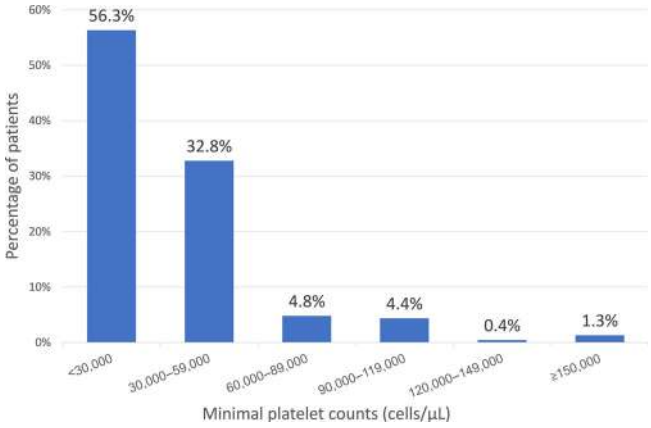

FIGURE 2 Distribution of the patients' minimal platelet counts.

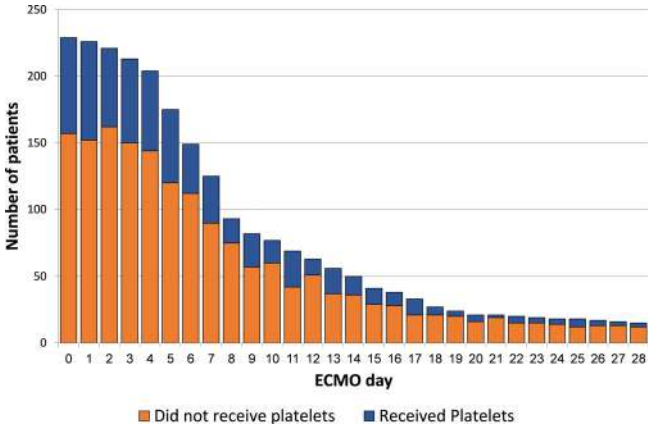

FIGURE 3 Proportion of patients who received platelet transfusions over the course of the (ECMO) run.

disorder, and the other was neurologically intact. Three patients died directly from cerebral bleeding. Procedural bleeding (i.e., following tracheostomy or chest drain insertion) occurred in seven (3.1%) patients.

FIGURE 4 Rates of bleeding days and haemorrhagic complications in our cohort (filled circles) and in the pertinent literature (empty circles). Reference numbers appear in parentheses. ECMO, extracorporeal membrane oxygenation; ICH, intracranial haemorrhage.

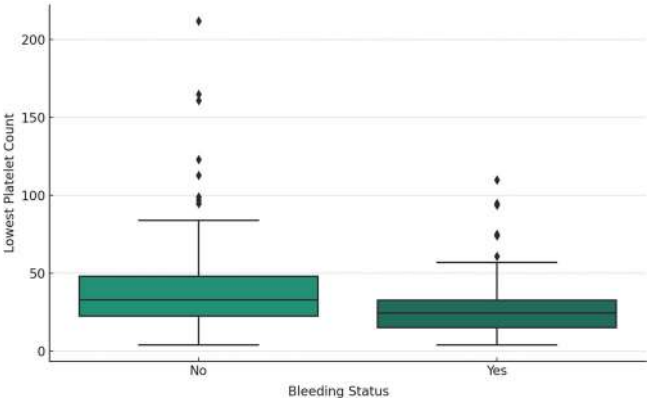

FIGURE 5 Boxplot of lowest platelets counts by bleeding status.

The median (IQR) lowest platelet count in children who had bleeding events was 25 (15–32) $\times$ 10^9/L. This was significantly different from the median (IQR) lowest platelet count in children who did not have bleeding events, which was 33 (23–48) $\times$ 10^9/L ($p < 0.001$), as shown in Figure 5.

The lowest platelet count of each patient had a weak, but significant negative correlation with the number of bleeding days, that is, the lower the platelet count, the higher the number of bleeding days. The Spearman rank correlation coefficient between the number of bleeding days and the lowest platelet count was −0.358 ($p < 0.001$), as seen in Figure S1.

DISCUSSION

In this retrospective analysis of 229 ECMO-supported paediatric patients, we present our dynamic, patient-tailored, restrictive platelet transfusion practice. As far as we know, this is the first large-scale

study that challenges the traditional paradigm of maintaining the platelet count >80–100 $\times$ 10^9/L in paediatric ECMO-supported patients. We found that while differences were seen in the lowest platelet counts of children who bled on ECMO versus those who did not, the overall median platelet counts were maintained much lower than current recommendations suggest.

Thrombocytopaenia on ECMO is a frequent, complex and multifaceted phenomenon resulting from platelet adhesion to the ECMO circuit, haemodilution with circuit priming, inflammatory response, medication effects, platelet consumption by circuit clots and infections [4, 10]. Historically, ECMO pioneers such as Bartlett and Heiss recommended platelet transfusion thresholds of 50–80 $\times$ 10^9/L [22, 23]. However, more recent guidelines recommend higher thresholds, without strong evidence [24]. In paediatric ECMO-supported patients, the AABB recommends a platelet transfusion threshold of 100 $\times$ 10^9/L, similar to the ELSO guidelines [15, 16].

Recently, a group of paediatric critical care and haematology experts (TAXI-CAB group) formulated recommendations for the transfusion of platelets and/or plasma in neonates and children undergoing cardiac surgery and/or supported by ECMO [17]. The group concluded that evidence to recommend a specific threshold for platelet transfusion is lacking. Specifically, prophylactic transfusion in the absence of clinically significant bleeding seemed unlikely to benefit ECMO-supported patients when the platelet count is >100 $\times$ 10^9/L. The guidelines state that most centres maintain platelet levels >100 $\times$ 10^9/L and emphasize that platelet transfusions may expose patients to risks without potential benefit and should be avoided when possible. These recommendations were incorporated in guidelines that address transfusion policies in critically ill paediatric patients [25]. Similarly, in a collaborative study that evaluated the epidemiology of haemostatic transfusions in 514 paediatric patients, the daily median platelet transfusion goal was 100 $\times$ 10^9/L, with some variability in subgroups of non-bleeding patients, veno-arterial ECMO and non-neonates [11]. In a recent survey among 108 medical centres, platelet transfusion thresholds in paediatric ECMO-supported patients were shown to vary by age and centre; 91–100 $\times$ 10^9/L was the most common threshold for platelet transfusion, and most of the centres without age-based thresholds maintained a threshold >61 $\times$ 10^9/L [20]. These findings were similar to a survey from 2013, which documented a median platelet transfusion threshold of 100 $\times$ 10^9/L among 187 ELSO centres [26]. There are, however, centres that have adopted a lower threshold than commonly recommended [6, 18, 19].

In a substantial proportion of our patients, the lowest platelet count was in the severe thrombocytopaenia range (<30 $\times$ 10^9/L), and almost all the patients were moderately thrombocytopaenic at some point. These counts are significantly lower than found in published data, in which the median platelet counts were close to 100 $\times$ 10^9/L during the ECMO course [9, 11]. Although we did demonstrate a difference in the lowest platelet counts in children who bled versus those who did not, all the median counts were much lower than current recommendations. These results provide further evidence that it is safe to set a lower threshold for platelet transfusions than current recommendations suggest; however, there is likely a lower limit below which is associated with a higher bleeding risk. This must be studied in larger, prospective cohorts.

The contribution of thrombocytopaenia to the risk of bleeding has long been debated. The presence and degree of thrombocytopaenia has been shown to have a poor association with bleeding, mortality or ICH in ECMO-supported patients or in those with ICH [11, 27–29]. Nellis et al. showed that a platelet count as low as ≤55 $\times$ 10^9/L for children on ECMO was not associated with increased chest tube output [30]. Furthermore, a large randomized trial in premature neonates found increased bleeding and/or mortality and worse 2-year neurological outcomes in those transfused at a higher threshold as compared to a lower threshold [31, 32].

In our series, platelets were transfused on only 28.5% of the ECMO days; the median daily platelet transfusion dose was 8.7 mL/kg, and the total median transfusion dose was 70 mL/kg for the entire ECMO run. A recent study investigating the epidemiology of platelet transfusion in the paediatric ECMO population showed that daily platelet transfusions were positively associated with chest tube output, bleeding requiring RBC transfusion, plasma transfusion and set platelet goal but not with platelet counts and that the haemostatic goals did not change according to the bleeding status of the patients. Compared to our study, values were higher for the proportion of platelet transfusion days (67.8%), and the daily median and total median transfusion doses (17.3 and 82 mL/kg/day, respectively) [11]. Significantly higher platelet transfusion volume was also observed in a study that investigated 2 anticoagulation protocols (25.4 and 20.1 mL/kg/day) [19]. Furthermore, an analysis of two large paediatric ECMO studies showed that platelet transfusion was given prophylactically to 79% of the patients compared to only 61% in our cohort [6]. Although ECMO-induced platelet dysfunction may persist, despite transfusion to a level of >100 $\times$ 10^9/L, platelet transfusions are associated with platelet refractoriness, organ dysfunction, sepsis, nosocomial infection, prolonged ICU stay, bleeding, thrombosis and mortality [8, 33–37]. Additionally, autologous and homologous platelets have been associated with immunomodulation and pro-inflammatory reactions, which may exacerbate the ECMO-induced inflammatory syndrome [38–41].

Practicing a more restrictive platelet transfusion approach did not result in greater PRBC exposure in our patients compared to patients in other studies. This is evident from our median daily PRBC dose of 14 mL/kg/day compared to 21.2–46.1 and 38.7 mL/kg/day previously reported [19, 42]. It should also be noted that PRBC transfusions in paediatric ECMO are frequently used in non-bleeding days and, on many occasions, when the lowest haematocrit was above the institutional transfusion threshold [42].

The proportion of our patients with any bleeding was 61.1%, which is lower than any previously published series (63.9%–76%), but higher than reported in the ELSO registry report (39%) [9, 14, 18, 43]. The major difficulty in comparing bleeding rates in paediatric ECMO-supported patients is the lack of a uniform definition of bleeding. Some studies identified bleeding events based on the need for transfusion, with the exception of ICH, without standardization of transfusion triggers, while ELSO has specific criteria to define major bleeding [14, 16].

In addition, while others, including ELSO, record bleeding from the same source that occurs over multiple days as one bleeding day, we considered each day of an ongoing haemorrhage as a separate bleeding day [14]. Likewise, oropharyngeal bleeding, which was common (25.8%) in our cohort, could have been regarded as minor bleeding and discarded in other series. None of our patients had significant genitourinary bleeding, in contrast to 6% of the patients in one series [14].

Intracranial haemorrhage was evident in 8.7% of our patients. This rate is within the reported range by other studies and by the ELSO registry (3.0%–17%) [6, 9, 13, 14, 16, 31, 37, 43–51]. Comparing ICH rates between studies is challenging because of the lack of standardization of the imaging modality, frequency of testing and severity. As ICH does not usually cause a decrease in haemoglobin level or necessitate blood transfusion, it may have been underdiagnosed in some series. Lastly, an analysis of the ELSO registry documented haemorrhagic deaths (defined as death after withdrawal of ECMO support due to intractable bleeding) in 3.4% of patients compared to none in our cohort [43].

Survival to ICU discharge was 60.3% in our cohort, well within the range of reported survival rates by other single- and multi-centre cohorts and the ELSO registry (46.4%–65%) [9, 11, 13, 18, 19, 44, 45].

The main limitation of our study is the retrospective design, which could have been subject to recall and misclassification biases. To mitigate these concerns, we examined all the daily nursing and medical reports of the patients for descriptions of bleeding events. A single-centre report may limit generalizability of our findings but ensures consistency of practices and management protocols, which may be lacking in multi-site studies. The dynamic-transfusion goal was not standardized or recorded in the EMR and hence is lacking from the data. Nonetheless, our platelet counts do demonstrate a lower transfusion threshold than other centres have demonstrated. Another limitation is the lack of timing of platelet transfusions in relation to bleeding or to platelet count measurements. We do not have access to data on donor exposure of the patients within our cohort. As with retrospective studies, we were not able to adjust for confounders when comparing our data to the pertinent literature, which may weakened our comparisons. Many other factors may impact bleeding outcomes, such as the parameters used for anticoagulation, and were not accounted for in the comparison of bleeding events. In addition, data regarding outcomes should be interpreted with caution, as some of our patients were transferred to the referring centre after decannulation, where long-term follow-up data was inaccessible.

In conclusion, this single-centre cohort study showed that a 'patient-tailored' rather than 'goal-directed' platelet transfusion policy is safe and feasible with no apparent excessive blood transfusion or increase in bleeding complications rates. Although our protocol challenges the existing convention, daily adjustment of the transfusion threshold based on a patient's general condition, bleeding tendency and risk factors may prove to better serve our patients. A prospective randomized controlled study examining outcomes of paediatric ECMO-supported patients allocated to different platelet transfusion thresholds is urgently needed.

ACKNOWLEDGEMENTS

E.S., O.S. and O.D. conceptualized and designed the study; O.S. wrote the first draft and analysed the data; G.P. performed the research and analysed the data; E.S., O.M.S., J.Y., G.F., G.A. and M.E.N. reviewed and edited the manuscript. All authors approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

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

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REFERENCES

1. MacLaren G, Monagle P. Platelet transfusion during extracorporeal membrane oxygenation: possible harm, ongoing uncertainty. Pediatr Crit Care Med. 2020;21:208–9.
2. Rauch A, Dupont A, Rosa M, Desvages M, Le Tanno C, Abdoul J, et al. Shear forces induced platelet clearance is a new mechanism of thrombocytopenia. Circ Res. 2023;133:826–41.
3. Van Den Helm S, Yaw HP, Letunica N, Barton R, Weaver A, Newall F, et al. Platelet phenotype and function changes with increasing duration of extracorporeal membrane oxygenation. Crit Care Med. 2022;50:1236–45.
4. Cheung PY, Sawicki G, Salas E, Etches PC, Schulz R, Radomski MW. The mechanisms of platelet dysfunction during extracorporeal membrane oxygenation in critically ill neonates. Crit Care Med. 2000;28:2584–90.
5. Robinson TM, Kickler TS, Walker LK, Ness P, Bell W. Effect of extracorporeal membrane oxygenation on platelets in newborns. Crit Care Med. 1993;21:1029–34.
6. Nellis ME, Saini A, Spinella PC, Davis PJ, Steiner ME, Tucci M, et al. Pediatric plasma and platelet transfusions on extracorporeal membrane oxygenation: a subgroup analysis of two large international point-prevalence studies and the role of local guidelines. Pediatr Crit Care Med. 2020;21:267–75.
7. Anderson HL, Cilley RE, Zwischenberger JB, Bartlett RH. Thrombocytopenia in neonates after extracorporeal membrane oxygenation. ASAIO Trans. 1986;32:534–7.
8. Cashen K, Dalton H, Reeder RW, Saini A, Zuppa AF, Shanley TP, et al. Platelet transfusion practice and related outcomes in pediatric extracorporeal membrane oxygenation. Pediatr Crit Care Med. 2020;21:178–85.
9. Santiago MJ, Gómez C, Magaña I, Muñoz V, Saiz P, Sánchez A, et al. Hematological complications in children subjected to extracorporeal membrane oxygenation. Med Intens. 2019;43:281–9.
10. Cashen K, Meert K, Dalton HJ. Platelet count and function during pediatric extracorporeal membrane oxygenation. Semin Thromb Hemost. 2020;46:357–65.
11. Karam O, Goel R, Dalton H, Nellis ME. Epidemiology of hemostatic transfusions in children supported by extracorporeal membrane oxygenation. Crit Care Med. 2020;48:e698–705.
12. Hamzah M, Jarden AM, Ezetendu C, Stewart R. Evaluation of bivalirudin as an alternative to heparin for systemic anticoagulation in pediatric extracorporeal membrane oxygenation. Pediatr Crit Care Med. 2020;21:827–34.

13. Dalton HJ, Garcia-Filion P, Holubkov R, Moler FW, Shanley T, Heidemann S, et al. Association of bleeding and thrombosis with outcome in extracorporeal life support. Pediatr Crit Care Med. 2015;16:167–74.
14. Dalton HJ, Reeder R, Garcia-Filion P, Holubkov R, Berg RA, Zuppa A, et al. Factors associated with bleeding and thrombosis in children receiving extracorporeal membrane oxygenation. Am J Respir Crit Care Med. 2017;196:762–71.
15. Josephson C. Neonatal and pediatric transfusion practice. In: Fung MK, editor. AABB technical manual. 18th ed. Bethesda, MD: AABB; 2014. p. 571–97.
16. Lequier L, Annich G, Al-Ibrahim O, Bembea M, Brodie D, Brogan T, et al. ELSO guidelines for cardiopulmonary extracorporeal Life support. ELSO anticoagulation guideline. Ann Arbor, MI: Extracorporeal Life Support Organization; 2014.
17. Cholette JM, Muszynski JA, Ibla JC, Emani S, Steiner ME, Vogel AM, et al. Plasma and platelet transfusions strategies in neonates and children undergoing cardiac surgery with cardiopulmonary bypass or neonates and children supported by extracorporeal membrane oxygenation: from the Transfusion and Anemia EXpertise Initiative-Control/Avoidance of Bleeding. Pediatr Crit Care Med. 2022;23:e25–36.
18. O'Halloran CP, Andren KG, Mecklosky J, Larsen S, Brediger S, Vitali S, et al. Mortality and factors associated with hemorrhage during pediatric extracorporeal membrane oxygenation. Pediatr Crit Care Med. 2020;21:75–81.
19. Northrop MS, Sidonio RF, Phillips SE, Smith AH, Daphne HC, Pietsch JB, et al. The use of an extracorporeal membrane oxygenation anticoagulation laboratory protocol is associated with decreased blood product use, decreased hemorrhagic complications, and increased circuit life. Pediatr Crit Care Med. 2015;16:66–74.
20. Ozment CP, Scott BL, Bembea MM, Spinella PC, Pediatric ECMO (PediECMO) Subgroup of the Pediatric Acute Lung Injury and Sepsis Investigators (PALISI) Network and the Extracorporeal Life Support Organization (ELSO). Anticoagulation and transfusion management during neonatal and pediatric extracorporeal membrane oxygenation: a survey of medical directors in the United States. Pediatr Crit Care Med. 2021;22:530–41.
21. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. J Pediatr. 1978;92:529–34.
22. Bartlett RH, Gazzaniga AB. Extracorporeal circulation for cardiopulmonary failure. Curr Probl Surg. 1978;15:1–96.
23. Heiss KF, Bartlett RH. Extracorporeal membrane oxygenation: an experimental protocol becomes a clinical service. Adv Pediatr. 1989;36:117–35.
24. Roseff SD, Luban NLC, Manno CS. Guidelines for assessing appropriateness of pediatric transfusion. Transfusion. 2002;42:1398–413.
25. Nellis ME, Karam O, Valentine SL, Bateman ST, Remy KE, Lacroix J, et al. Executive summary of recommendations and expert consensus for plasma and platelet transfusion practice in critically ill children: from the Transfusion and Anemia EXpertise Initiative-Control/Avoidance of Bleeding (TAXI-CAB). Pediatr Crit Care Med. 2022;23:34–51.
26. Bembea MM, Annich G, Rycus P, Oldenburg G, Berkowitz I, Pronovost P. Variability in anticoagulation management of patients on extracorporeal membrane oxygenation: an international survey. Pediatr Crit Care Med. 2013;14:e77–84.
27. Hardart GE, Fackler JC. Predictors of intracranial hemorrhage during neonatal extracorporeal membrane oxygenation. J Pediatr. 1999;134:156–9.
28. von Lindern JS, van den Bruele T, Lopriore E, Walther FJ. Thrombocytopenia in neonates and the risk of intraventricular hemorrhage: a retrospective cohort study. BMC Pediatr. 2011;11:16.
29. Muthukumar P, Venkatesh V, Curley A, Kahan BC, Choo L, Ballard S, et al. Severe thrombocytopenia and patterns of bleeding in neonates: results from a prospective observational study and implications for use of platelet transfusions. Transfus Med. 2012;22:338–43.
30. Nellis ME, Dalton H, Karam O, PediECMO Investigators. Quantifiable bleeding in children supported by extracorporeal membrane oxygenation and outcome. Crit Care Med. 2019;47:e886–92.
31. Curley A, Stanworth SJ, Willoughby K, Fustolo-Gunnink SF, Venkatesh V, Hudson C, et al. Randomized trial of platelet-transfusion thresholds in neonates. N Engl J Med. 2019;380:242–51.
32. Moore CM, D'Amore A, Fustolo-Gunnink S, Hudson C, Newton A, Santamaria BL, et al. Two-year outcomes following a randomised platelet transfusion trial in preterm infants. Arch Dis Child Fetal Neonatal Ed. 2023;108:452–7.
33. Hod E, Schwartz J. Platelet transfusion refractoriness. Br J Haematol. 2008;142:348–60.
34. Nellis ME, Karam O, Mauer E, Cushing MM, Davis PJ, Steiner ME, et al. Platelet transfusion practices in critically ill children. Crit Care Med. 2018;46:1309–17.
35. Keene SD, Patel RM, Stansfield BK, Davis J, Josephson CD, Winkler AM. Blood product transfusion and mortality in neonatal extracorporeal membrane oxygenation. Transfusion. 2020;60:262–8.
36. Du Pont-Thibodeau G, Tucci M, Robitaille N, Ducruet T, Lacroix J. Platelet transfusions in pediatric intensive care. Pediatr Crit Care Med. 2016;17:e420–9.
37. Kahn S, Chegondi M, Nellis ME, Karam O. Overview of plasma and platelet transfusions in critically ill children. Front Pediatr. 2020;8:601659.
38. Scherlinger M, Richez C, Tsokos GC, Boilard E, Blanco P. The role of platelets in immune-mediated inflammatory diseases. Nat Rev Immunol. 2023;23:495–510.
39. Thomas MR, Storey RF. The role of platelets in inflammation. Thromb Haemost. 2015;114:449–58.
40. Cholette JM, Henrichs KF, Alfieris GM, Powers KS, Phipps R, Spinelli SL, et al. Washing red blood cells and platelets transfused in cardiac surgery reduces postoperative inflammation and number of transfusions: results of a prospective, randomized, controlled clinical trial. Pediatr Crit Care Med. 2012;13:290–9.
41. Vlaar APJ, Hofstra JJ, Kulik W, van Lenthe H, Nieuwland R, Schultz MJ, et al. Supernatant of stored platelets causes lung inflammation and coagulopathy in a novel in vivo transfusion model. Blood. 2010;116:1360–8.
42. O'Halloran CP, Alexander PMA, Andren KG, Mecklosky J, Salvin JW, Larsen S, et al. RBC exposure in pediatric extracorporeal membrane oxygenation: epidemiology and factors associated with large blood transfusion volume. Pediatr Crit Care Med. 2018;19:767–74.
43. Werho DK, Pasquali SK, Yu S, Donohue J, Annich GM, Thiagarajan RR, et al. Hemorrhagic complications in pediatric cardiac patients on extracorporeal membrane oxygenation: an analysis of the Extracorporeal Life Support Organization Registry. Pediatr Crit Care Med. 2015;16:276–88.
44. Hervey-Jumper SL, Annich GM, Yancon AR, Garton HJL, Muraszko KM, Maher CO. Neurological complications of extracorporeal membrane oxygenation in children. J Neurosurg Pediatr. 2011;7:338–44.
45. Barbaro RP, Paden ML, Guner YS, Raman L, Ryerson LM, Alexander P, et al. Pediatric extracorporeal life support organization registry international report 2016. ASAIO J. 2017;63:456–63.
46. Tian F, Farhat A, Morriss MC, Tweed J, Li X, Huet B, et al. Cerebral hemodynamic profile in ischemic and hemorrhagic brain injury acquired during pediatric extracorporeal membrane oxygenation. Pediatr Crit Care Med. 2020;21:879–85.
47. Mehta A, Ibsen LM. Neurologic complications and neurodevelopmental outcome with extracorporeal life support. World J Crit Care Med. 2013;2:40–7.
48. Polito A, Barrett CS, Wypij D, Rycus PT, Netto R, Cogo PE, et al. Neurologic complications in neonates supported with extracorporeal

membrane oxygenation. An analysis of ELSO registry data. Intensive Care Med. 2013;39:1594–601.
49. Raets MMA, Dudink J, Ijsselstijn H, van Heijst AFJ, Lequin MH, Houmes RJ, et al. Brain injury associated with neonatal extracorporeal membrane oxygenation in the Netherlands: a nationwide evaluation spanning two decades. Pediatr Crit Care Med. 2013;14:884–92.
50. Biehl DA, Stewart DL, Forti NH, Cook LN. Timing of intracranial hemorrhage during extracorporeal life support. ASAIO J. 1996;42:938–41.
51. Cengiz P, Seidel K, Rycus PT, Brogan TV, Roberts JS. Central nervous system complications during pediatric extracorporeal life support: incidence and risk factors. Crit Care Med. 2005;33:2817–24.

SUPPORTING INFORMATION

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

How to cite this article: Schiller O, Pula G, Shostak E, Manor-Shulman O, Frenkel G, Amir G, et al. Patient-tailored platelet transfusion practices for children supported by extracorporeal membrane oxygenation. Vox Sang. 2024;119:326–34.

Received: 12 September 2023 | Revised: 5 December 2023 | Accepted: 3 January 2024

DOI: 10.1111/vox.13593

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Development, implementation and impact of an immunoglobulin stewardship programme in Saskatchewan, Canada

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Funding information
The authors received no specific funding for this work.

Abstract

Background and Objectives: Intravenous immunoglobulin (IVIG) is one of the most costly and limited-supply blood products. Judicious use of this therapy is important to ensure a continued supply is available for patients in need. The Saskatchewan IG Stewardship Program was initiated to monitor and reduce inappropriate IG use.

Materials and Methods: The Program was developed and implemented through the collaborative efforts of a multidisciplinary, inter-organizational team. Funding was provided from provincial organizations to create new positions within the Program and to support stakeholder engagement throughout the process of implementation. Data were collected from local and national databases regarding the amount of IVIG used and appropriateness of orders based on published criteria.

Results: Over 20 months, the Program helped to reduce unnecessary IVIG use from pre-intervention levels by more than 20%. Interventions from nurse navigators alone reduced inappropriate IVIG use by 2.6%. During the 20-month period following Program initiation, more than 4 million CAD less was spent on IVIG compared with the previous 20 months.

Conclusion: The Saskatchewan IG Stewardship Program has led to more appropriate IVIG use across the province, more effective preservation of this limited healthcare resource, and cost savings that more than cover the cost of administering the Program.

Keywords

immunoglobulin, implementation, programme development, stewardship

Highlights

- A multidisciplinary, inter-organizational collaborative effort helped create a successful provincial immunoglobulin stewardship programme.

- Dedicated funding, a communication plan, and a focus on education and awareness were key aspects of successfully achieving patient and provider engagement and reducing unnecessary immunoglobulin use.
- Improving the appropriateness of intravenous immunoglobulin use promotes better patient care and can reduce the healthcare costs associated with this therapy that is limited in supply.

INTRODUCTION

Intravenous immunoglobulin (IVIG), manufactured from the serum of 1000–15,000 donors per batch, is considered to be one of the most expensive and limited-supply blood products [1], with Canada being among the top three users of IVIG per capita in the world [2]. Within Canada, Saskatchewan has been one of the highest per capita IVIG users [3].

Due to its robust immunomodulatory, immunoregulatory and anti-inflammatory properties, IG is used for various indications across many therapeutic areas [1, 4–6]. Unfortunately, IG is also often used improperly, with variability in dosing regimens and prescribing patterns. In spite of evidence-based guidelines and criteria [7], studies report inappropriate and off-label use of IG with uncertain benefits [5, 8].

The widespread use of IG frequently results in ambiguous benefits for patients and significant costs for healthcare organizations [5]. This widespread use has raised national and international concerns regarding IG availability [9, 10]. To mitigate the risk of insufficient supply in Canada, provincial blood offices were challenged by Canadian Blood Services (CBS) in October 2020 to reduce IG usage.

In Saskatchewan, IG use has not been comprehensively tracked, with no patient/provider registry and no strategic oversight for initial orders and renewals. The Saskatchewan IG Stewardship Program was initiated to develop and implement a long-term stewardship plan in response to potential IG shortages and to curb inappropriate use with respect to indication, dose and/or duration of treatment. This manuscript describes the development, implementation and impact of the Saskatchewan IG Stewardship Program.

MATERIALS AND METHODS

Context

This study is a retrospective narrative analysis of the development and implementation of the Saskatchewan IG Stewardship Program and its impact on IVIG use. In Saskatchewan, Canada, healthcare is funded primarily through the Government of Saskatchewan, Ministry of Health (MoH). The primary delivery of healthcare is through the provincial Saskatchewan Health Authority (SHA), which serves a population of approximately 1.2 million people.

In Canada (excluding the province of Quebec), all blood products are supplied to hospital transfusion medicine laboratories by CBS. Orders are written by the patient's physician. The nursing unit/infusion clinic caring for the patient receives the order and sends a request form to their transfusion medicine laboratory for release of the product to the requesting infusion location.

Data collection and analysis

IVIG usage data were retrieved from the IG Stewardship Program patient registry (Microsoft Excel) from November 2021 through June 2023. Additional IG usage data were provided by CBS. For data and statistical analyses, QI Macros, R and Graphpad were used [11–13]. Statistical process control charts were used to identify changes and trends among time series data [14–16] as well as segmented regression analysis for total IVIG use.

RESULTS

Development of the Saskatchewan IG Stewardship Program

Following notice of the predicted IG shortage (October 2020), an interdisciplinary oversight team was created within the SHA. The oversight team included stakeholders from the SHA Clinical Excellence portfolio (i.e., Stewardship and Clinical Appropriateness Department, Choosing Wisely Saskatchewan), clinicians from different specialties (e.g., transfusion medicine specialists, haematologists, immunologists, neurologists, rheumatologists), transfusion medicine technologists and safety managers, and executive sponsorship from the MoH and SHA. Stakeholder roles included SHA staff and clinicians from the senior leadership level (e.g., Director of Clinical Excellence) to front-line staff involved in the IG order process. The oversight team held a series of meetings and agreed to establish the Saskatchewan IG Stewardship Program and follow the published tri-provincial criteria for approval of any IVIG order set [7]. The first edition of the criteria was used at the outset of the Program and the second edition was adopted when published in February 2022.

Initially, the Program received 1 year of temporary funding from the MoH by submitting a briefing note (February 2021) outlining the need for and objectives of the IG Stewardship Program. The requested 395,000 CAD for 1 year of funding was approved (March 2021) with ongoing funding subject to achieving performance measures, namely cost recovery. A request for additional

funding was submitted to the Physician Compensation Quality Improvement Program (operated by the MoH and Saskatchewan Medical Association), which was approved in May 2021. This funding included up to 356 h for up to 20 physicians to be remunerated for their participation in meetings and other sessions that were not already part of their contracts. In June 2021, four new positions (a project manager, data analyst and two part-time nurse navigators) were created to perform the core activities of the Program in collaboration with the larger oversight team described above. Total cost of the Program was approximately 495,000 CAD for 1 year of operation; however, this amount does not include in-kind support from some physicians, managers and quality improvement specialists provided by other SHA departments.

The IG Stewardship team initiated an environmental scan of other jurisdictions across Canada to learn about their IG Stewardship experiences and identify existing resources. The team also held meetings with IVIG prescribers to receive their input on order sets and processes. Based on this feedback, an order set for adults (Appendix S1) and processes were developed to screen IVIG orders for meeting the criteria for use.

The IG Stewardship team also engaged patient and family partners to better understand their concerns, experiences with IVIG therapy and adverse events they encountered. Their involvement provided insightful feedback and suggestions for improving processes, ultimately allowing for a programme better tailored to meet the needs and expectations of patients.

Prior to Program launch, a patient/provider database and 'interim virtual clinic' was created by the data analyst using Microsoft Excel (Appendix S2) to monitor IVIG usage. The intention was to use electronic medical record (EMR) software to house the virtual clinic; however, contract delays for EMR software overlapped the launch of the Program, requiring an interim solution.

Implementation of the Saskatchewan IG Stewardship Program

Prior to launch, a communication plan was implemented to raise awareness among prescribers of the upcoming changes. This included providing key messages and answers to frequently asked questions through specific communication tactics including memos, educational meetings and presentations, articles in the provincial SHA newsletter, and creation of supporting documents and patient education materials. For example, several virtual 'town hall' meetings were held to provide information to large audiences. One such meeting was recorded and made available on the saskblood.ca website for further dissemination. All physicians in the province were also emailed information about the Program along with some of the documents that had been developed to promote awareness and solicit further feedback.

The Program officially launched on 1 November 2021. At that point, all previous order sets for IVIG were retired and the new provincial order set was made available. All non-urgent, outpatient IVIG orders were reviewed by the nurse navigators for appropriateness based on indication, dose, dosing weight and frequency, as per the adopted criteria [7]. The nurse navigators did not review inpatient or urgent/after-hours outpatient orders; however, these were still captured in the database and assessed for appropriateness by transfusion medicine laboratory staff. Laboratory staff were trained by the nurse navigators to review IVIG orders for appropriateness based on the same criteria.

If every component of a reviewed order was correct, it was authorized and forwarded to the blood transfusion lab to be processed. If the dose was incorrect, the nurse navigator modified it in accordance with the criteria, in collaboration with the ordering physician. If the indication was not concordant with criteria, it was denied. At the outset of the Program, no formal appeal process existed for denied orders. Instead, orders not meeting indication criteria were discussed with the ordering physician prior to denial. Through this discussion, either the order was modified to become concordant with criteria, or an IG Stewardship Program physician, in collaboration with the ordering prescriber, would initiate a tapering regimen and a letter would be sent to the patient to inform them of the change in their therapy. Within weeks, the denial process was amended to invite the prescriber to directly appeal and discuss the order with the transfusion medicine physician on call.

Information from each order was collected by the nurse navigators in the virtual clinic database (Appendix S2). A formula was created that alerted the nurse navigators when an order was within 6 weeks of its expiry date. This allowed the nurse navigators to send letters to patients and prescribers to remind them that their order was about to expire and a follow-up appointment and renewal order would be necessary. In most cases, orders were approved for a maximum of 6 months. In cases where treatment efficacy was being trialled, orders may have been approved for only 3 months, whereas in cases where efficacy of treatment was well established, orders could be approved for longer than 6 months, at the discretion of a Program physician.

Throughout the IG Stewardship Program's operation, the Program team continued to meet biweekly to discuss project updates, obstacles and successes. A continuous idea board and other project management tools were used to identify and track lessons learned and implement future improvements. Examples of improvements included the addition of the IG patient consent form to the order set, adjustments to dosing language, including review periods for orders, expiry dates and the use of check boxes to reduce input errors. The Program team also met regularly with the MoH to receive input on the Program and report on progress. Ad hoc meetings were held with physicians, nurses and laboratory technologists to provide education, present data, highlight special cases and discuss instances of incorrect dosing. Valuable feedback on Program processes was also provided by these stakeholders: for example, one laboratory technologist proposed the inclusion of a QR code for the adjusted body weight calculator in the order set, enabling physicians to conveniently scan the code and use the calculator on their mobile devices when entering orders. Physicians were reimbursed for their time

spent at these meetings to encourage their attendance and participation.

Impact of the Saskatchewan IVIG Stewardship Program

The success of the IG Stewardship Program was measured by monitoring reductions in overall and inappropriate IVIG use. Between 1 November 2021 and 30 June 2023, the IG Stewardship team reviewed 1117 orders, 239 (21.4%) of which were deemed inappropriate. Following intervention by the nurse navigators, the total number of inappropriate IVIG orders (i.e., indications not meeting criteria or inappropriate dosing) that were fulfilled was only 33 of 1117 (3.0%); these represent emergency use orders on which they were unable to intervene (Table 1). The majority of IVIG orders, 1004 of 1117 (89.9%), were for appropriate indications (i.e., criteria were satisfied), followed by 104 of 1117 (9.3%) for indications that were concordant with criteria but with conditions, and 9 of 1117 (0.8%) that did not meet criteria for use (Table 2). Again, orders not meeting the criteria were for emergency use and were inadvertently missed by the technologist issuing the product. In one case, although the indication was deemed inappropriate at the time, the Program physician knew the updated criteria would reclassify the indication as 'appropriate with conditions' when published in early 2022. There was a transient downward shift observed in the proportion of inappropriate orders received each month between April 2022 and November 2022, but overall, there was no sustained reduction from the beginning of the IG Stewardship Program (Figure 1).

Between November 2021 and June 2023, without nurse navigator intervention, 267,708 g of IVIG would have been used; however, with nurse navigator intervention, the total was reduced to 260,659 g. Thus, nurse navigator interventions alone resulted in a 7049 g (2.6%) reduction in IVIG use, leading to a cost avoidance of 452,107 CAD (Table 3).

The total amount of IVIG used differed between the 20-month period post-IG Stewardship Program initiation and the preceding 20 months (Table 4). From March 2020 to October 2021, 292 g/1000 population of IVIG was used (344,338 g total), while from November 2021 to June 2023 only 232 g/1000 population was used (278,323 g total). This represents a statistically significant (Student's *t*-test,

TABLE 1 Number of appropriate/inappropriate IVIG orders reviewed by nurse navigators (November 2021–June 2023).

Order appropriateness	Before review, *n* (%)	After review, *n* (%)
Inappropriate	239 (21.4%)	33 (3.0%)
Appropriate	878 (78.6%)	1084 (97.0%)
Total	1117 (100%)	1117 (100%)

Note: 'Before Review' represents the number of orders that were received, and 'After Review' represents the number of orders that were fulfilled.
Abbreviation: IVIG, intravenous immunoglobulin.

TABLE 2 Number of appropriate/inappropriate IVIG orders based on the indication meeting criteria (November 2021–June 2023).

Concordance of indication with criteria	Number of orders, *n* (%)
Criteria met	1004 (89.9%)
Criteria met with conditions	104 (9.3%)
Criteria not met	9 (0.8%)
Total	1117 (100%)

Abbreviation: IVIG, intravenous immunoglobulin.

FIGURE 1 No sustained reduction in the proportion of inappropriate IG orders received after implementation of the Saskatchewan IG Stewardship Program. The X control chart shows inappropriate intravenous immunoglobulin (IVIG) orders between November 2021 and June 2023. A downward shift in the number of inappropriate IVIG orders is observed between April 2022 and November 2022 (red section of the data line). However, this shift was not maintained and there is no overall reduction in inappropriate orders observed since the initiation of the IG Stewardship Program. The centre line (CL) represents the average monthly number of inappropriate IVIG orders and the dashed line represents the upper confidence limit (UCL; 3-σ from the mean).

TABLE 3 IVIG use with and without nurse navigator review (November 2021–June 2023).

IVIG use without nurse navigator review (g)	267,708
IVIG use after nurse navigator review (g)	260,659
Absolute reduction due to nurse navigator review (g)	7049
Absolute reduction due to nurse navigator review (%)	2.6
Cost avoidance due to nurse navigator review (CAD)	452,107

Abbreviation: IVIG, intravenous immunoglobulin.

TABLE 4 IVIG use comparison between the 20-month post-IG Stewardship Program initiation period (November 2021–June 2023) and the preceding 20 months (March 2020–October 2021).

Time period	March 2020–October 2021	November 2021–June 2023
IVIG use (g/1000 population)	292	232
Absolute reduction (%)	-	20.5
t-test (*p*-value)	-	<0.001
Total IVIG use (g)	344,338	278,324
Absolute reduction (g)	-	66,014
Cost avoidance (CAD)	-	4,234,202

Abbreviation: IVIG, intravenous immunoglobulin.

$p < 0.001$) 20.5% decrease in IVIG use between these two time periods. The 66,015-g decrease in total IVIG use in the period after IG Stewardship Program initiation represented 4,234,202 CAD less spent on IVIG.

Average monthly IVIG use decreased from 14.6 g/1000 population/month prior to the initiation of the IG Stewardship Program (April 2020–October 2021, Figure 2) to 11.6 g/1000 population/month over the 20 months after its initiation (November 2021–June 2023, Figure 2). There was a statistically significant difference in the pre- and post-intervention averages (Student's *t*-test, $p < 0.0001$). The control chart in Figure 2 also shows a reduction in variability of monthly IVIG use as evidenced by the reduced spread between the upper and lower control limits (8.8 g/1000 population from April 2020 to October 2021 compared with 5.1 g/1000 population from November 2021 to May 2023).

National data collected by CBS also highlight the unique impact of the IG Stewardship Program in Saskatchewan compared with other provinces and territories across Canada. Saskatchewan was the only region with an estimated decrease in IVIG use when comparing fiscal years 2021/2022 to 2022/2023 (Figure 3a). Indeed, Saskatchewan was the only region where actual IVIG use decreased between these fiscal years (Figure 3b).

DISCUSSION

Medication stewardship is clearly established in the antimicrobial literature and similar ideas are now being applied to other therapies, particularly those that strongly rely on expert consensus [17–19]. In November 2021, the Saskatchewan IG Stewardship Program was initiated to ensure adherence to established consensus criteria, focusing on dosing regimens and indications for IVIG use. Since then, we have seen a reduction in overall IVIG use by ensuring adherence to those criteria.

Successes

Within the first 20 months of the initiation of the IG Stewardship Program, 206 of 239 orders with inappropriate doses were corrected by the nurse navigators, resulting in a reduction in unnecessary IVIG use. Our results reflect those of a previous study, which showed that using adjusted body weight instead of actual body weight for dose calculations resulted in saving 3880 g of IVIG across 262 inpatient cases in the United States [20].

When reviewing IVIG use over time, Figure 1 shows two reductions in both variability of IVIG use and the monthly average. The initial decrease in IVIG use (beginning April 2020) correlates with the beginning of the COVID-19 pandemic in Saskatchewan (first case identified in March 2020). This reduction was likely influenced by recommendations from CBS to switch to *subcutaneous* IG due to supply chain shortages and to allow patients to administer IG at home. After the initiation of the IG Stewardship Program in November 2021, another reduction in variability within the system and average monthly IVIG use was observed. The decrease in the difference between the upper and lower control limits provides evidence that the implementation of the IG Stewardship Program may have contributed to more controlled administration of IVIG.

Healthcare professionals provided valuable feedback for continuous improvement of the IG Stewardship Program. In the example provided above about including a QR code for the adjusted body weight calculator in the order set, this suggestion addressed a physician concern regarding the time-consuming process of using the calculator on their laptop while filling out the order set. Another important aspect of implementation was providing a reimbursement incentive for physicians to attend meetings. In our experience, allocating resources towards incentives such as this contributed to increased engagement from physicians in the development and implementation of the Program.

One success that is less obvious was the ability to taper the dose of some patients, resulting in small reductions in IVIG use that added up over time and across many patients. In fact, some patients were able to discontinue therapy altogether. Conversely, but also beneficial to patients, the nurse navigators were able to increase doses of IVIG for patients whose orders were inappropriately low. While there were challenges with reducing or discontinuing IVIG treatment for some patients (see below), the system benefits of sparing of a limited resource and reduced costs of unnecessary treatment required such an investment in education and awareness among prescribers and patients.

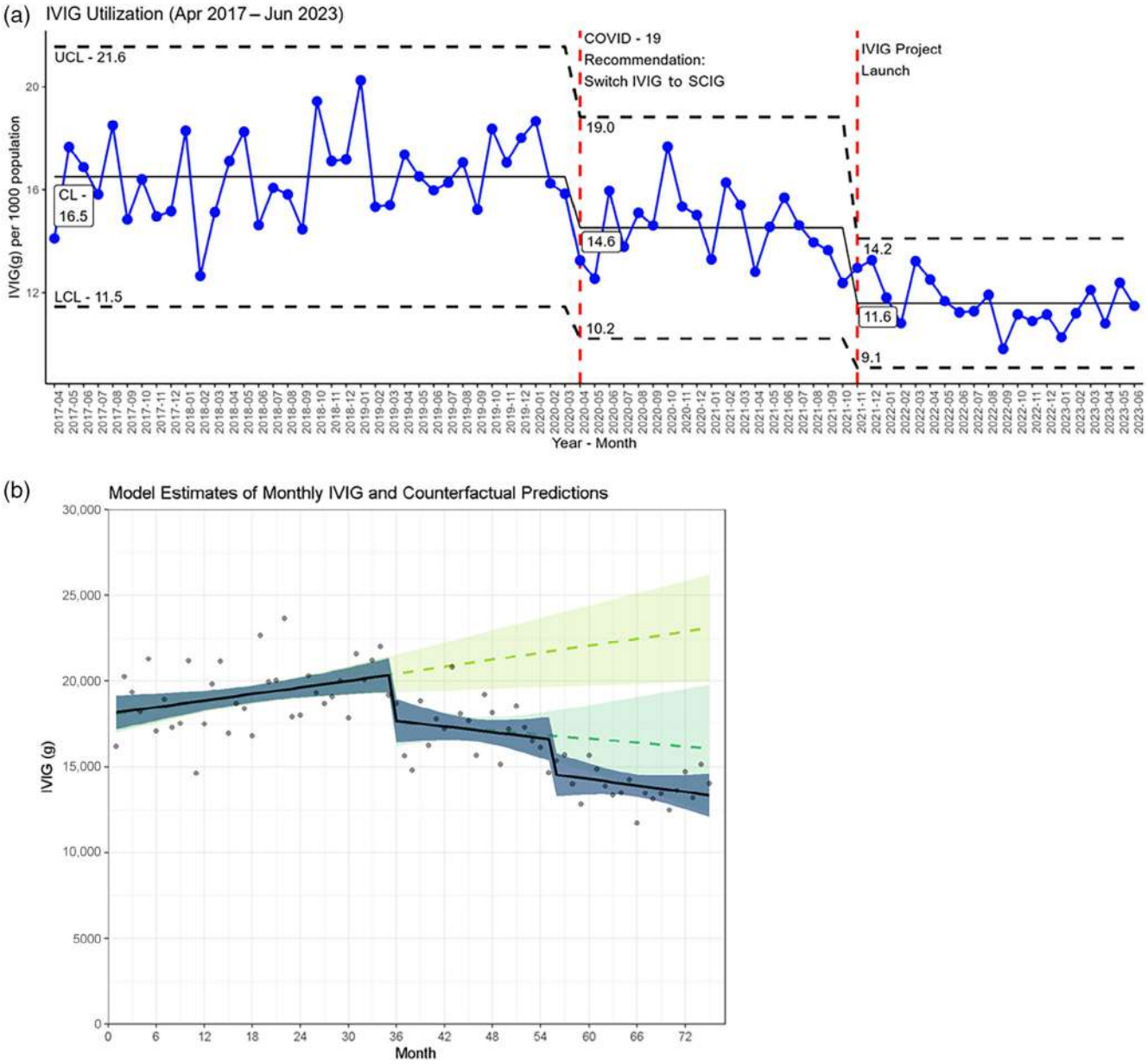

FIGURE 2 Reduction in intravenous immunoglobulin (IVIG) use following implementation of the Saskatchewan IG Stewardship Program. (a) The X control chart shows IVIG use between April 2017 and June 2023. Reductions in monthly IVIG use are observed over time, with two process changes corresponding to the beginning of the COVID-19 pandemic in Saskatchewan (March 2020) and the initiation of the IG Stewardship Program (November 2021). The initiation of the IG Stewardship Program is associated with a reduction in average monthly IVIG use and a reduction in overall variability in use, as shown by the reduced distance between the upper and lower confidence limits (UCL and LCL, respectively). The centre line (CL) represents the average monthly IVIG use for each time period and the dashed lines represent the UCL and LCL (3-σ from the mean). (b) A segmented regression analysis indicates changes in overall IVIG use between April 2017 and June 2023. The blue line represents the factual estimate and the green lines represent the counterfactuals (assumed estimate if the intervention had never taken place). The beginning of the COVID-19 pandemic in Saskatchewan (March 2020, month 36) is associated with a change in the slope and intercept of the line. The change is maintained following a further drop in use after the introduction of the IG Stewardship Program (November 2021, month 57).

Challenges

The IG Stewardship Program faced several challenges from clinicians, particularly during the first year of activity. Initially, much effort was required to educate clinicians on the criteria for IVIG use. Even after education was provided, there remained individuals who required continuous reminders of the criteria as they repeatedly submitted inappropriate IVIG orders. Additionally, when the IVIG order set was introduced, some clinicians objected that it was too long, which required one-on-one coaching and follow-up conversations to alleviate their concerns. Conversations included an explanation that the multi-step approval process for the order necessitated a longer order

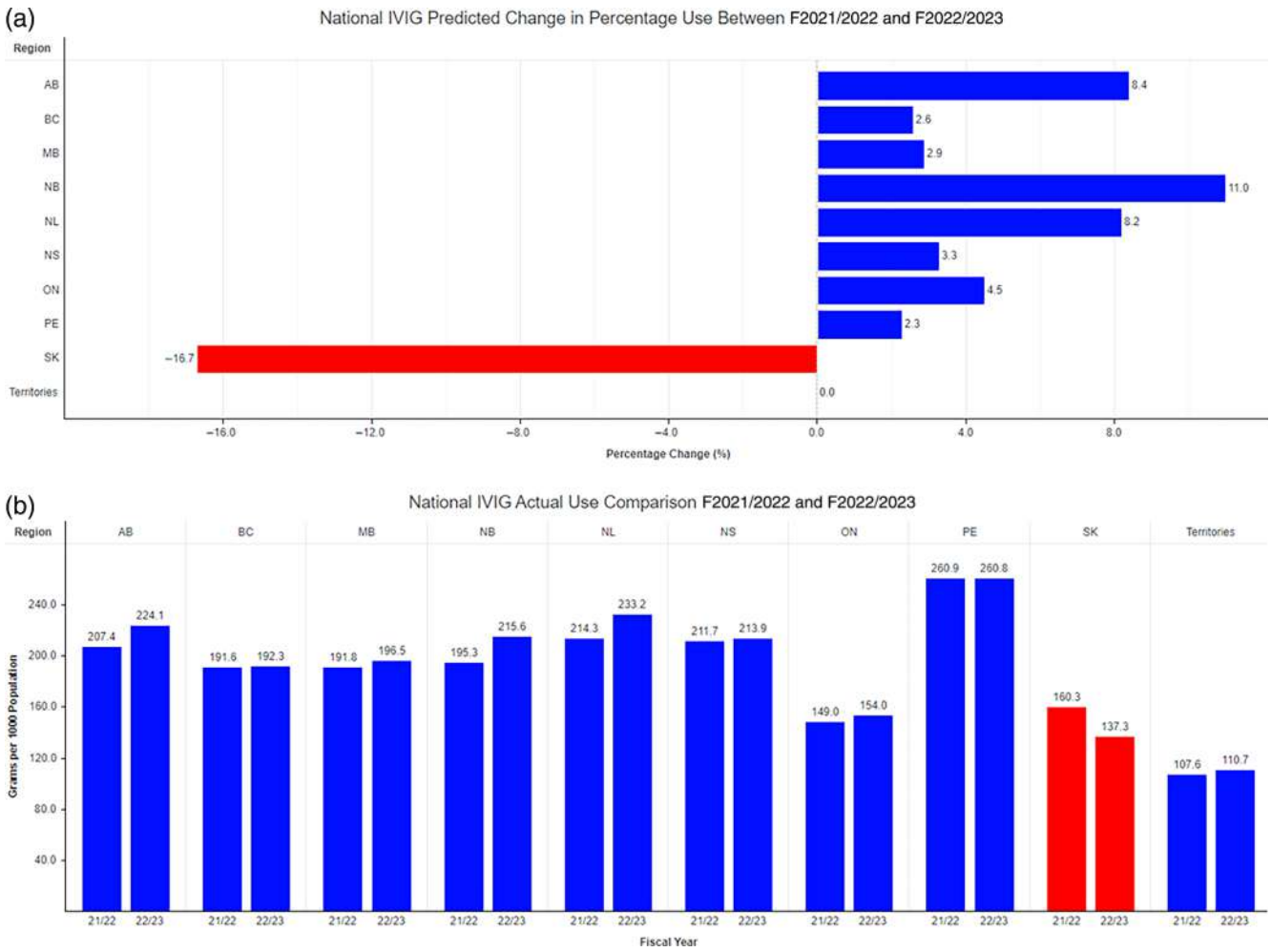

FIGURE 3 National data show a reduction in predicted and overall intravenous immunoglobulin (IVIG) use in Saskatchewan following implementation of the IG Stewardship Program. (a) National data provided by Canadian Blood Services indicate that Saskatchewan was the only region in Canada predicted to reduce IVIG use between 2021/2022 and 2022/2023 fiscal years. (b) Saskatchewan was also the only region to exhibit an actual decrease in IVIG use between the 2021/2022 and 2022/2023 fiscal years.

form, but also ensured that the IG Stewardship Program was able to maintain comprehensive oversight regarding adherence to criteria. The Program team also reviewed the order set after the first year and updated it based on feedback received from practitioners (Appendix S1).

One notable challenge shared by the on-call Program physicians was the need to manage a variety of controversial orders (i.e., those not meeting criteria for use) for patients with complex medical conditions. Due to their wealth of clinical experience, and with an additional neurologist available for consultation as needed, they were able to address these complicated orders.

The IG Stewardship Program has experienced high staff turnover rates. Due to annual funding, staff were hired on one-year contracts. Without long-term job security, foundational team members left their positions for permanent positions elsewhere, including a new hire in both nurse navigator positions within 1 year and the data analyst shortly after. This caused a lack of continuity in team members who were integral to the development and success of the Program. This resulted in both a loss of institutional knowledge and impeded the Program's efficiency as new staff had to be oriented to the Program and trained in relevant areas (e.g., learning the criteria, processes, database management, etc.).

Related to staff turnover within the Program, both frequent staff turnover and the large number of staff within the transfusion medicine laboratory also made it difficult to educate all lab personnel on IVIG order screening. Additionally, due to the large number of laboratory staff, not all personnel were able to screen an IVIG order within an appropriate timeframe after their initial training, which may have reduced their proficiency with this skill. Thus, IG Stewardship Program staff were required to re-screen all IVIG orders reviewed by laboratory staff to ensure their appropriateness. Due to lags in fax communication, this meant some inappropriate orders were already administered by the time an error was noted.

Managing patient consent forms and order expiry dates also proved to be complicated. In the SHA, signed consent forms for receipt of blood products are valid for 1 year; however, the IG Stewardship Program did not have a consent database within the first year of operation. At times, an order may have been approved without a

patient consent form, which meant the infusion clinic where the patient received their IVIG would have to consent the patient and store the form in the clinic. This, in turn, led to disorganization and unnecessary phone calls to physicians who raised concerns about being contacted too frequently by the Program about their IVIG orders. To resolve this, a series of meetings were held with a variety of stakeholders and consent forms are now required from the ordering physician prior to an order being approved. Related to form management, as there was no way of tracking if a fax was actually received by a department, missed faxes led to some delays in treatment. In response, some infusion clinics requested that the approved order set be sent via email, instead. Lastly, each individual infusion clinic tracked order expiry dates using their own method. Implementation of a standardized order expiration tracking method (i.e., 6 months after the completion of the first dose) required many hours of education and planning with all stakeholders.

Extensive use of a Microsoft Excel file as a virtual clinic quickly resulted in a cumbersome database open to potential data input errors. An actual EMR system would ensure that patient's renewal orders are not missed and data capture may be less prone to error. The Program also lacked baseline data required to establish measurement and evaluation criteria. Baseline data are important in any healthcare stewardship programme as they provide a starting point for measuring progress, help to identify areas for improvement, allow for tracking of changes over time and inform the development of more effective future interventions. While data for overall IG use were available through CBS, we had no way of assessing the levels of appropriate/inappropriate IVIG use before the launch of the Program. Additionally, the national data available through CBS are based on hospital-reported IVIG use, which creates a limitation for the accuracy of this data set. The aggregation of these data also limits detailed statistical comparison between regions.

Finally, the 'personal attachment' that some patients develop towards their IVIG therapy posed a challenge for Program implementation. Patients advocating for their continued treatment (e.g., after their therapy was deemed to not meet criteria for use and almost certainly unnecessary for patient well-being) resulted in the Program receiving letters from individuals who were concerned about the Program potentially overriding their prescribers' orders. Addressing this challenge required a significant educational effort and resources directed towards patients, aiming to clarify the origin of IVIG and the reasons behind its limited availability as a resource.

Future directions

Future work for the IG Stewardship Program includes seeking stable, long-term funding for Program staff and acquiring a suitable EMR software for a virtual clinic/database. A review of orders for additional blood products will be considered for incorporation under the IG Stewardship Program. Future research efforts may also include a review of which components of the implementation of this Program had the most impact on behavioural changes among prescribers.

In conclusion, due to the limited supply and significant costs associated with IVIG use, the Saskatchewan IG Stewardship Program works to ensure that IVIG therapy is only recommended for appropriate indications where there is a known benefit. Utilization review, appropriate dose monitoring, interdisciplinary collaboration, education and commitment from senior leadership and government have contributed to the success of the IG Stewardship Program. The Program has led to more appropriate IVIG use, more effective preservation of this limited healthcare resource and cost savings that more than cover the cost of administering the Program. Continued evaluation and refinement of the Program can ensure ongoing success in optimizing IVIG use and improving patient care.

ACKNOWLEDGEMENTS

K.S. wrote the first draft of the manuscript, and all authors contributed to and approved the final manuscript. J.R.V. conceived the study. O.A., K.S., P.G., W.E.B., M.L.D. and J.R.V. acquired and analysed the data. The authors would like to thank the members of the Saskatchewan Immunoglobulin Stewardship Program and our collaborators who contributed to the success of this work.

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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REFERENCES

1. Jolles S, Sewell WA, Misbah SA. Clinical uses of intravenous immunoglobulin. Clin Exp Immunol. 2005;142:1–11.
2. Government of Canada. Protecting access to immune globulins for Canadians: final report of the expert panel on immune globulin product supply and related impacts in Canada. 2018. Available from: https://publications.gc.ca/site/eng/9.854785/publication.html. Last accessed 12 Nov 2023.
3. Sask Blood. Saskatchewan immune globulin Stewardship Program. SaskBlood. 2015. Available from: https://saskblood.ca/programs/sk-ivig-program/. Last accessed 29 Jul 2023.
4. Leong H, Stachnik J, Bonk ME, Matuszewski KA. Unlabeled uses of intravenous immune globulin. Am J Health-Syst Pharm. 2008;65:1815–24.
5. Feasby TE, Quan H, Tubman M, Pi D, Tinmouth A, So L, et al. Appropriateness of the use of intravenous immune globulin before and after the introduction of a utilization control program. Open Med Peer-Rev Indep Open-Access J. 2012;6:e28–34.
6. NIH Consensus Conference. Intravenous immunoglobulin: prevention and treatment of disease. JAMA. 1990;264:3189–93.
7. Prairie Collaborative Immune Globulin Utilization Management Framework Project. Alberta Ministry of Health, Shared Health Manitoba, and Saskatchewan Ministry of Health. Prairie Ig final guideline. Available from: https://www.ihe.ca/public/uploaded/Prairie%20Ig%20Final%20Guideline%2009.02.22.pdf. Last accessed 23 Aug 2023.

8. Hanna K, Poulin-Costello M, Preston M, Maresky N. Intravenous immune globulin use in Canada. Can J Clin Pharmacol. 2003;10:11–6.
9. NAC. The national plan for management of shortages of immunoglobulin products (Ig) interim guidance. 2020. Available from: https://nacblood.ca/sites/default/files/2021-09/The%20National%20Plan%20for%20Management%20of%20Shortages%20of%20Immunoglobulin%20Products%20%28Ig%29%20%20Interim%20Guidance_July%2027%202020.Published.pdf. Last accessed 29 Jul 2023.
10. FDA. CBER-regulated products: current shortages. FDA; 2022. Available from: https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/cber-regulated-products-current-shortages. Last accessed 9 Jan 2024.
11. The R Foundation. R: the R project for statistical computing. Available from: https://www.r-project.org/. Last accessed 24 Aug 2023.
12. QI Macros. SPC software for Excel|Six Sigma Software|QI Macros Excel Add-in. Available from: https://www.qimacros.com/. Last accessed 24 Aug 2023.
13. GraphPad Software. T test calculator. Available from: https://www.graphpad.com/quickcalcs/ttest1.cfm. Last accessed 24 Aug 2023.
14. Pimentel L, Barrueto F. Statistical process control: separating signal from noise in emergency department operations. J Emerg Med. 2015;48:628–38.
15. Vetter TR, Morrice D. Statistical process control: no hits, no runs, no errors? Anesth Analg. 2019;128:374–82.
16. Williams E. Understanding variation–part 2: the control chart. Curr Probl Pediatr Adolesc Health Care. 2018;48:202–5.
17. Blum RM, Stevens CA, Carter DM, Hussey AP, Marquis KA, Torbic H, et al. Implementation of a dexmedetomidine stewardship program at a tertiary academic medical center. Ann Pharmacother. 2013;47:1400–5.
18. Güerri-Fernández R, Villar-García J, Herrera-Fernández S, Trenchs-Rodríguez M, Fernández-Morato J, Moro L, et al. An antimicrobial stewardship program reduces antimicrobial therapy duration and hospital stay in surgical wards. Rev Esp Quimioter. 2016;29:119–21.
19. Ashfaq A, Zhu A, Iyengar A, Wu H, Humphries R, McKinnell JA, et al. Impact of an institutional antimicrobial stewardship program on bacteriology of surgical site infections in cardiac surgery. J Card Surg. 2016;31:367–72.
20. Rocchio MA, Hussey AP, Southard RA, Szumita PM. Impact of ideal body weight dosing for all inpatient i.v. immune globulin indications. Am J Health-Syst Pharm. 2013;70:751–2.

SUPPORTING INFORMATION

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

How to cite this article: Sarker K, Vanstone JR, Adigun O, Boutilier B, Comeau J, Degelman ML, et al. Development, implementation and impact of an immunoglobulin stewardship programme in Saskatchewan, Canada. Vox Sang. 2024;119:335–43.

Received: 17 October 2023 | Revised: 6 December 2023 | Accepted: 17 December 2023

DOI: 10.1111/vox.13584

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Development of multiplexed flow cytometry-based red blood cell antibody screen and identification assays

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Funding information
The authors received no specific funding for this work.

Abstract

Background and Objectives: The purpose of this study was to develop a high-throughput method of performing red blood cell antibody screens and identification by utilizing flow cytometry and intracellular dyes to allow a multiplexed assay where three-cell screens can be performed in a single test well and 11-cell panels in three test wells.

Materials and Methods: Reagent red blood cells were labelled using Violet Proliferation Dye 450 (V450) and Oregon Green fluorescent dyes, which bind intracellular proteins to allow up to four cells to be interrogated in a single test well. Sixteen 3-cell screen panels and ten 11-cell identification panels were tested using sera with known antibody specificity. Antibody binding was detected using secondary anti-immunoglobulin G and anti-immunoglobulin M fluorescently labelled antibodies.

Results: Intracellular dyes allowed clear separation of the different screen and identification panel test cells. Three distinct populations of V450+, Oregon Green+ and negative for both stains were demonstrated in the screening panel and an additional double positive for V450 and Oregon Green was utilized to include a fourth cell in the identification panel testing to increase throughput. A total of 158 screen or identification panel RBC/serum combinations were tested against different known antibodies, and expected results were obtained with 100% concordance.

Conclusion: This study demonstrates the successful development of a high-throughput multiplexed flow cytometry-based red cell antibody screen and identification panel assays. This method could be implemented in clinical laboratories to complement existing antibody detection methods. The multiplexing enabled via intracellular staining could be utilized to further augment other flow cytometry-based transfusion assays.

Keywords

antibody identification panel, antibody screen, flow cytometry, irregular blood group antibody, transfusion

Highlights

- Intracellular staining was used to create multiplexed flow cytometry-based antibody screen and identification panel assays.
- The antibody screen and identification panels achieved 100% concordance with expected results.
- Flow cytometry-based screens and identification panels could be utilized clinically as antibody detection methods.

INTRODUCTION

Flow cytometry is a widely utilized tool in both clinical and research laboratories [1]. It has extensive use in clinical laboratories in neoplastic hematopathology for evaluation of neoplastic cell populations and has many other uses including in the evaluation of immunodeficiency, enumeration of non-neoplastic cellular populations such as in stem cell enumeration and lymphocyte crossmatching for the purposes of histocompatibility testing in solid organ transplantation [2]. Its use has also been described in transfusion medicine including in the assessment of ABO antigen expression, Rh D antigen expression, quantification of foetal-maternal haemorrhage and, more recently, as a technique to allow high-throughput red blood cell (RBC) phenotyping [3–7].

Flow cytometry uses lasers to assess cells as they move singularly through an aperture. These lasers evaluate cellular characteristics such as size, shape and can also detect the presence of specific antigens via the use of fluorescently labelled antibodies [8]. This antibody–antigen interaction can be used to directly detect the presence of an antigen of interest when using fluorescently conjugated primary antibodies; however, antigens and/or antibodies of interest can also be evaluated indirectly via the use of fluorescently conjugated secondary antibodies directed against immunoglobulin G (IgG) or immunoglobulin M (IgM), as in a lymphocyte crossmatch [2] and in the RBC phenotyping method developed and validated by Liwski et al. [7].

Flow cytometry has many potential advantages as a technique to evaluate the presence of antigens or antibodies on RBCs when compared with existing agglutination-based methods. It requires greatly reduced amounts of both reagents and donor RBCs or plasma and can be high-throughput via the use of 96-well or 384-well microplates on modern flow cytometers. Results are quantitative, in that fluorescence can be measured and recorded, and analysis can be automated using median fluorescence intensity (MFI) cutoffs to define positive or negative reactions as is performed in a lymphocyte crossmatch assay [7]. Importantly, analysis by flow cytometry is specific with regard to detection of antibodies as non-antibody-mediated causes of agglutination, which can lead to false positive reactions in agglutination-based assays, do not interfere with flow cytometry. In addition, flow cytometry-based testing could be largely automated via the use of liquid handlers. Finally, a unique benefit is that cells are individually interrogated, which allows potential labelling and multiplexing of assays.

Presently, prior to RBC transfusion, patients must undergo an antibody screen to evaluate for the presence of allogeneic antibodies directed against clinically significant donor antigens [9]. This is done by incubating recipient plasma with commercially acquired reagent RBCs with known antigen expression and adding anti-human globulin directed at IgG to cause visibly detectable agglutination in the presence of IgG antibodies. Detection of visible agglutination is indicative of the presence of recipient antibodies directed towards RBC antigens and recipients would typically then undergo testing against an extended identification panel of RBCs with known antigen expression to determine antibody specificity.

Currently, three test platforms are commonly used to perform RBC antibody screens and identification panels: test tube, gel-card and solid-phase assay [10, 11]. All these platforms rely on visible detection of agglutination, and thus require large amounts of reagents and provide only semi-quantitative results. In addition, because cells cannot be individually interrogated, there is no capacity for multiplexing, and each test requires an individual test reaction (i.e. tube or test well) which limits throughput while increasing the cost.

Intercellular stains such as carboxyfluorescein succinimidyl ester, Violet Proliferation Dye 450 (V450) and CellTrace Oregon Green are non-toxic dyes often used as cell tracers and in proliferation studies [12]. These dyes are initially non-fluorescent and passively enter cells where they are cleaved by intracellular esterases to yield a fluorescent product which covalently binds to intracellular protein amine groups. The dye–protein conjugates are stable and well-retained, preventing transfer to unstained adjacent cells. Staining is specific for viable cells. Fluorescently stained cells are detected by flow cytometry and dyes are available in a multitude of colours, which facilitates sample multiplexing. Importantly, intercellular staining does not interfere with subsequent cell phenotyping, allowing multiplex testing of RBC antibody screen panel and identification panel reactions.

Given the limitations of existing agglutination assays, and considering the possible benefits of flow cytometry, we sought to develop flow cytometry-based antibody screen panel and identification panel assays that uses intracellular dye-labelled screen and identification panel reagent RBCs to allow multiplexing of these assays such that an antibody screen can be performed in a single well on a 96-well microplate and a complete identification panel can be performed using 3 microplate wells instead of 12.

STUDY DESIGN AND METHODS

Reagents

Screening panel RBCs (0.8% suspension) (See Table 1) and identification panel RBCs (0.8% suspension) were purchased from Ortho

TABLE 1 Red blood cell screen cell antigen expression.

Screen cell	D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s
1	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+
2	+	0	+	+	0	+	+	+	+	+	+	+	0	+	0
3	0	0	0	+	+	0	+	+	0	+	0	+	0	+	+

Note: + denotes positive expression of antigen in column. 0 denotes negative expression of antigen in column.

TABLE 2 Results of red blood cell antibody screen testing.

Antibody specificity	Cell 1 anti-IgG MFI	Cell 2 anti-IgG MFI	Cell 3 anti-IgG MFI	Cell 1 anti-IgM MFI	Cell 2 anti-IgM MFI	Cell 3 anti-IgM MFI
D	5197	13,671	−65	−2.8	−7	−216
E	−2.2	574	−50	−17	−22	−97
E	−3.2	649	−58	−7.8	−27	−82
E	−5.2	507	−45	−8.8	−15	−75
c	−7.2	2892	6097	−9.8	−31	−46
c	2.8	348	1126	−7.8	−36	−141
C	−27	−26	−53	3813	−26	−141
K	−8	4350	−0.25	−5.8	−27	−118
K	−11	4318	−57	−9.8	−23	−120
K and Fy^a	−18	6038	3846	−5.8	−26	−196
Jk^a	−12	1765	3681	−4.8	−11	33
Fy^a	−12	1901	4118	−6.8	−22	−193
Fy^b	4122	3527	85	−1.8	−25	−128
Fy^b	3827	3371	71	12.2	−18	−82
s	60,278	−42	3091	−12.8	−28	−139
M	0.8	182	154	−4.8	1359	804

Note: Antibody specificity is the known specificity of the antibody contained in the 16 test sera. Results are expressed in MFI relative to negative controls and values graded as positive are coloured in red.
Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M; MFI, median fluorescence intensity.

Clinical Diagnostics. Sixteen proficiency testing (PT) sera, 10 from the College of American Pathologists (CAP) PT and 6 Immucor Tech Check sera (Immucor, Norcross, GA) with known antibody to RBC reactivity were used in this study (see Table 2 for specificity). Six red cell antibody-negative fresh frozen plasma samples were used as negative controls. Allophycocyanin (APC)-conjugated polyclonal goat anti-human IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) and phycoerythrin (PE)-conjugated polyclonal donkey anti-human IgM (One Lambda, Canoga Park, CA) secondary detection antibodies were used to detect human IgG and IgM, respectively, in the flow cytometry RBC antibody screening and identification panel procedures. Diluent 2 (Ortho Clinical Diagnostics, Markham, ON) was used to resuspend screening and identification panel reagent RBC prior to antibody testing procedure. Flow wash buffer (FWB) composed of Dulbecco's phosphate buffered saline (DPBS; Life Technologies Inc.) and 2% (v/v) foetal calf serum (Life Technologies Inc.) was used for all washes during the red blood cell antibody screening and identification panel testing. Intracellular staining of screening panel RBCs was performed using cell permanent dyes CellTrace Oregon Green 488 carboxylic acid diacetate succinimidyl ester (Thermo Fisher Scientific, Waltham, MA) at a final concentration of 5 μM and V450 (BD Biosciences, Mississauga, ON) at a final concentration of 1 μM. Final concentrations for panel cell staining were 1 μM for Oregon Green and 0.2 μM for V450. Quenching buffer composed of DPBS and 10% (v/v) foetal calf serum was used for all washes in the intracellular staining procedure.

RBC intracellular staining

For the RBC screening panel procedure, 75 μL of three separate 0.8% suspensions of screening panel RBCs (screen cell 1, 2 and 3) were added separately to individual wells of a 96-well U-bottom Falcon microplate and washed twice in 200 μL of DPBS at 1800×*g* for 30 s. RBCs were resuspended in 200 μL of DPBS (Screening panel RBC 1), 200 μL of V450 intracellular staining solution (Screening panel RBC 2) or 200 μL of Oregon Green intracellular staining solution (Screening panel RBC 3) and incubated for 10 min at room temperature (RT). RBCs were washed twice in 200 μL of quenching buffer at 1800×*g* for 30 s and resuspended in 75 μL of Diluent 2. Finally, 75 μL of each

pre-stained RBC suspension (screen panel cell 1, 2 and 3) was combined into a single RBC mixture for antibody screen testing.

For RBC identification panel testing, 75 µL of 0.8% identification panel RBCs (cell 1–11) and screening panel RBC (cell 2) suspension were added to individual wells of a 96 well U-bottom Falcon microplate and washed twice in 200 µL of DPBS at 1800×*g* for 30 s. RBCs were resuspended in 200 µL of DPBS (identification panel RBC 1, 5 and 9), 200 µL of V450 intracellular staining solution (identification panel RBC 2, 6 and 10), 200 µL of Oregon Green intracellular staining solution (identification panel RBC 3, 7 and 11) or 200 µL of V450 and Oregon Green intracellular staining solution (identification panel RBC 4 and 8 and screening panel RBC 2) and incubated for 10 min at RT. RBCs were washed twice in 200 µL of quenching buffer at 1800×*g* for 30 s and resuspended in 75 µL of Diluent 2. Finally, 75 µL of each RBC suspension was combined into three separate wells to create three RBC mixtures as follows: identification panel RBC 1–4, identification panel RBC 5–8 and identification panel RBC 9–11 + screening panel RBC 2.

Antibody screen preparation

The combined screening panel RBC 1–3 mixture measuring 7.5 µL was added to the appropriate wells of a 96 well U-bottom Falcon microplate. Fifteen microlitres of serum was added to each well containing RBCs and incubated for 15 min at RT then manually washed three times in 200 µL of FWB at 1800×*g* for 30 s. Twenty microlitres of the secondary antibody cocktail (anti-IgM–PE, 1:100, and anti-IgG–APC, 1:200, in DPBS) were added to each well, mixed by vortexing and incubated at RT for 5 min. Cells were manually washed once in 200 µL FWB at 1800×*g* for 30 s and resuspended in 150 µL of FWB before acquisition by flow cytometry.

Antibody panel preparation

Ten microlitres of the combined identification panel RBC 1–4, 5–8 or 9–11+ screening panel RBC two mixtures was added to the appropriate wells of a 96-well U-bottom Falcon microplate. Twenty microlitres of serum was added to each well containing RBCs and incubated for 15 min at RT, then manually washed three times in 200 µL of FWB at 1800×*g* for 30 s. Twenty microlitres of the secondary antibody cocktail (anti-IgM–PE, 1:100, and anti-IgG–APC, 1:200, in DPBS) was added to each well, mixed by vortexing and incubated at RT for 5 min. Cells were manually washed once in 200 µL FWB at 1800×*g* for 30 s and resuspended in 150 µL of FWB before acquisition by flow cytometry.

Flow cytometry acquisition and analysis

RBC events were acquired on the FACSLyric flow cytometers (BD Biosciences, Mississauga, ON) and analysed with BD FACSuite™ software (BD Biosciences, Mississauga, ON) using the 5-log MFI scale. Initial gating on RBC events was performed using the side and forward scatter dot-plots. Subsequent gating on specific RBC populations included in the RBC mixtures was performed using the V450 by Oregon Green fluorescence dot-plots (Figure 1 and Figure 2). For anti-IgG–APC and anti-IgM–PE fluorescence data, MFI values were normalized to the lowest MFI for the particular reagent RBC on each run. Positive cutoffs to assign positive antibody reactions were established using the mean +/− 4 SD of the MFI values obtained with the six RBC allo-antibody-negative plasma cell samples tested against each screening or identification panel RBC.

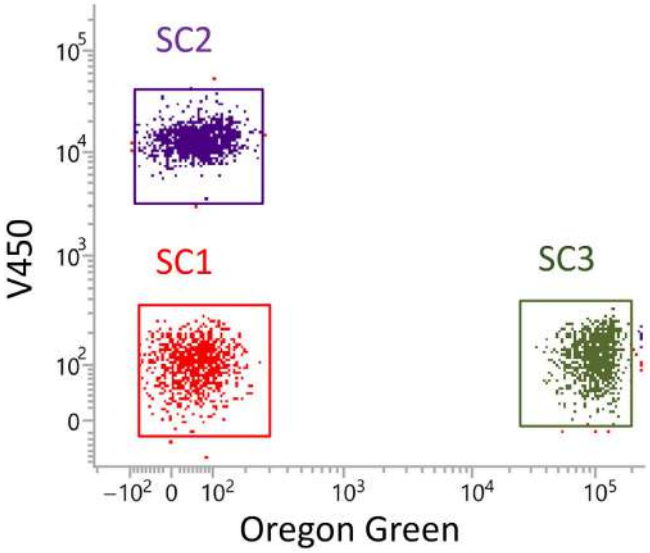


FIGURE 1 An example showing the staining results used for the red blood cell screen assay and resultant gating strategy. Median fluorescence intensity (MFI) is on the *x* and *y* axis and shows the results of staining which shows a population positive for Violet Proliferation Dye 450 (V450) (SC2 = screen panel cell 2), positive for Oregon Green (SC3 = screen panel cell 3) and negative for both (SC1 = screen panel cell 1).

RESULTS

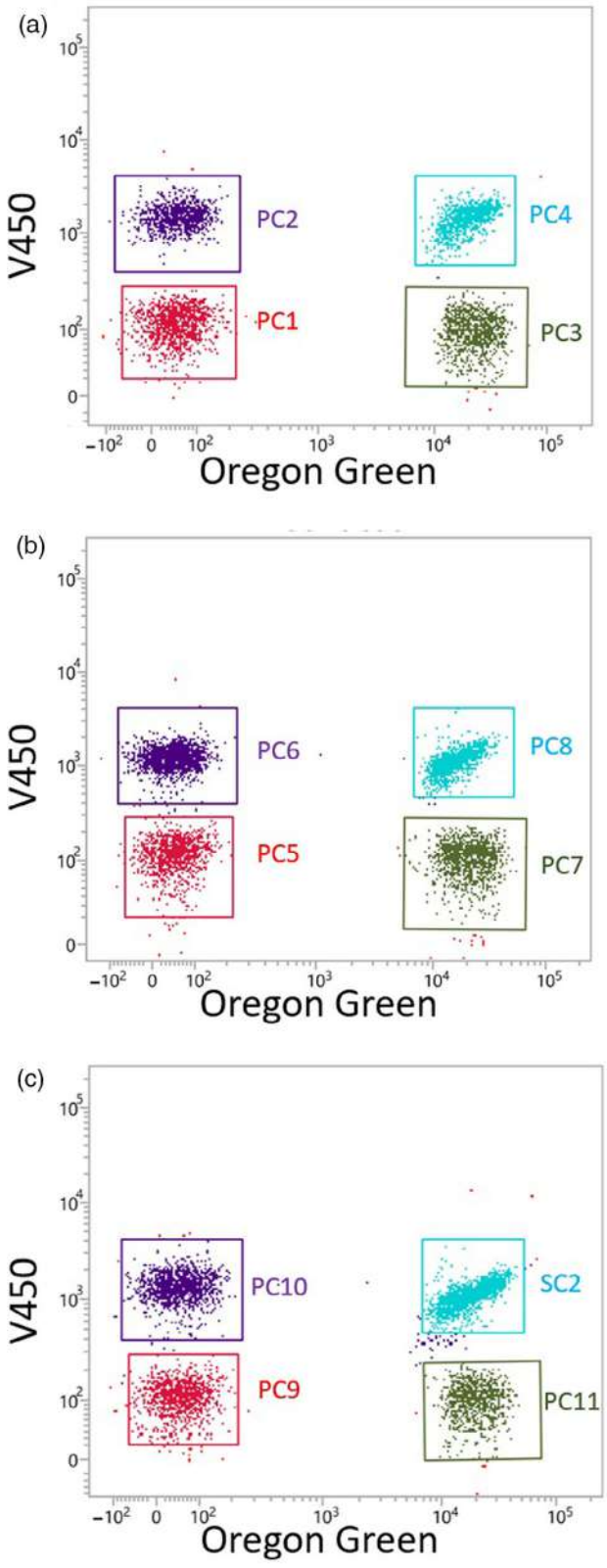
An illustrative example of a three-cell reagent RBC mixture staining results for the antibody screen is shown in Figure 1. Figure 2 shows the results of staining for the three different cell mixtures used in the antibody identification panel testing. As shown in Figure 2, V450 and Oregon Green staining was uniform among the cells, and all cells could be easily distinguished using the V450 by Oregon Green gating strategy depicted by rectangles around cellular populations shown in Figures 1 and 2, including the double stained (V450 and Oregon Green) RBCs.

Sixteen antibody screens were performed using sera with known RBC allo-antibody specificities obtained from CAP proficiency testing and Tech Check and confirmed at our institution via the standard gel card-based screen assay. Antigen expression of our three-cell reagent RBC screen panel cells are shown in Table 1. Positive cutoffs for

anti-IgG–APC with screening RBC panel cells 1, 2 and 3 were MFI exceeding 20, 68 and 112, respectively, and 20, 92 and 256 MFI for screening RBC panel cells 1, 2 and 3 with anti-IgM–PE relative to mean background fluorescence of the negative controls. Expected results were obtained for all 16 RBC antibody screens performed, representing 100% concordance for all 48 screen cell/serum combinations (Table 2). Figure 3 shows representative histograms of the antibody screen assay using a negative control serum (Figure 3a–c) and a test serum containing anti-Fy^a-specific antibody (Figure 3b,d). As shown, anti-Fy^a serum reactions with Fy^a-positive screening panel RBC 2 and 3 are clearly detected with anti-IgG–APC (Figure 3b), but not with anti-IgM–PE (Figure 3d) secondary antibody.

An additional 10 antibody identification panels were performed using the same sera used for the antibody screen assay. Positivity thresholds for cells stained with Oregon Green, V450, Oregon Green and V450 or no stain were MFI of 69, 88, 69 and 159 for anti-IgG–APC, respectively, and 79, 45, 23 and 33 for anti-IgM–PE, respectively. A total of 110 antibody identification panel RBC/serum combinations were tested and expected results (i.e., reactivity against antigen-positive cells) were obtained in 100% of samples defined as expected reactivity with either anti-IgG–APC or anti-IgM–PE. In one instance, an anti-C antibody was detected only via anti-IgM–PE. In addition, the anti-M antibody showed reactivity with both anti-IgG–APC and anti-IgM–PE. Results are shown in Table 3. In addition, representative histograms of anti-D and anti-C antibody reactions in antibody identification panel RBC testing are shown in Figure 4. Anti-D reactivity with panel RBC 9 and screening RBC 2 is detected with anti-IgG–APC (Figure 4a) but not with anti-IgM–PE (Figure 4b) secondary antibody. In contrast, anti-C reactivity with antibody identification panel RBC 2 and 3 is detected only with anti-IgM–PE (Figure 4d) but not with anti-IgG–APC (Figure 4c) secondary antibody.

Seven of the sera were titrated to compare the detection limit of flow cytometry versus our existing gel-based technique. In six of the seven sera, the antibodies were detected at a 2–32-fold lower titre in flow cytometry than in the gel-based technique. A single serum with anti-C specificity showed an equal titre in both methods.



DISCUSSION

This article successfully demonstrates the use of intracellular stains to pre-label commercial antibody screen and identification panel RBCs to create a multiplexed flow cytometry-based antibody screen and panel assay. Proficiency testing samples with known reactivity were

FIGURE 2 An example showing the staining results used for the red blood cell antibody identification panel assay and resultant gating strategy. Median fluorescence intensity is on the *x* and *y* axis and shows the results of staining which shows a population positive for Violet Proliferation Dye 450 (V450) (purple), positive for Oregon Green (green), positive for both (teal) and negative for both (red). Panel A shows identification panel RBCs (PC) 1–4, panel B shows identification panel RBCs 5–8 and panel C shows identification panel RBCs 9–11 plus a repeat of screen panel cell 2.

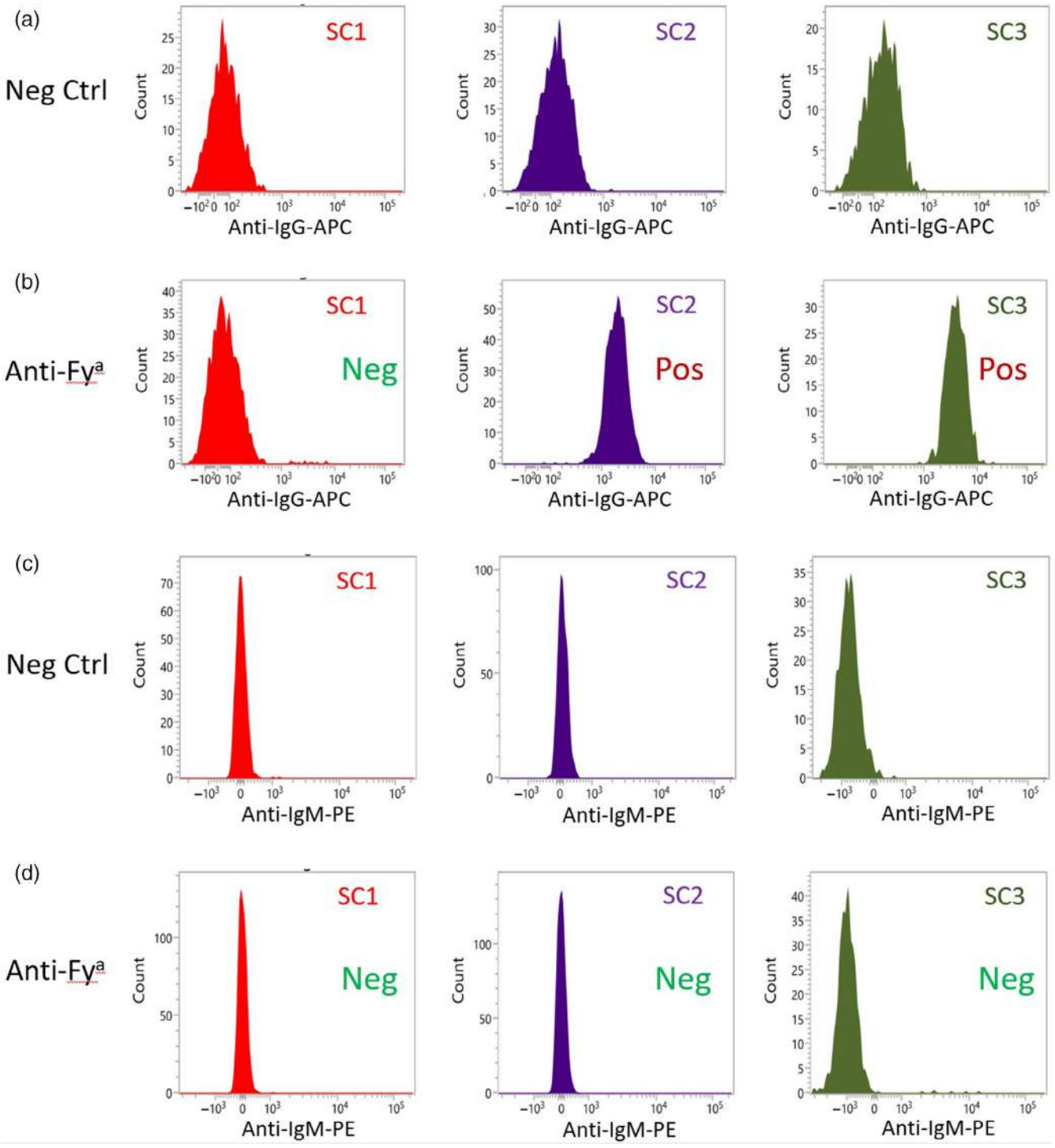


FIGURE 3 Representative results of red blood cell (RBC) antibody screen. An example of histograms showing median fluorescence intensity (MFI) for anti-immunoglobulin G (IgG)–allophycocyanin (APC) of the negative control (neg ctrl) is shown in panel A and an anti-Fy^a antibody is shown in panel B. MFI for anti-immunoglobulin M (IgM)-phycoerythrin (PE) for the negative control (panel C) and anti-Fy^a (panel D) are also shown. Neg refers to reactions graded as negative and pos refers to reactions graded as positive. SC1, 2 and 3 refer to screening panel RBC cell 1, 2 and 3, respectively.

used as the gold standard for testing of these assays as discrepancies between methods of antibody detection such as tube and gel card are well known [10, 11]. We were able to obtain the expected results across 16 antibody screen panels and 10 identification panels, representing a total 158 unique RBC and serum combinations. An extensive clinical validation has yet to be performed as we do not have flow cytometers on site in our transfusion laboratory and thus cannot implement this test clinically in our blood transfusion services

TABLE 3 Results of red blood cell antibody panel testing.

Antibody specificity	Antigen-positive anti-IgG MFI (hetero/homo)		Antigen-negative anti-IgG MFI	Antigen-positive anti-IgM MFI (hetero/homo)		Antigen-negative anti-IgM MFI
D	4444		−28	−28		−22
E	135	222	−24	−14	−56	−28
c	2734	4084	−6	−69	−41	−41
C	−65	−42	−34	6487	3986	−30
K	4315	NT	−25	−2.5	NT	−27
Jk[a]	846	2038	−56	−4.6	−9.6	−9.8
Fy[a]	1601	2499	−20	−25	−19	−28
Fy[b]	3007	4303	−46	−12	−19	−14
s	2222	4782	−35	−30	−34	−29
M	45[a]	396	−45	1235	2357	4.4

Note: Antibody specificity is the known specificity of the antibody contained in the 10 test sera. Antigen positive MFI corresponds to average MFI measurement of panel cells which were known to positively express the antigen corresponding to the antibody specificity in column 1. Values are provided for both hetero and homo cells when relevant. Values graded as positive are coloured in red. NT is not tested due to absence of homozygous Kell cells.
Abbreviations: Hetero, heterozygous; Homo, homozygous; IgG, immunoglobulin G; IgM, immunoglobulin M; MFI, median fluorescence intensity.
[a]Average MFI is below positivity cutoff as one hetero cell was negative for anti-IgG.

at this time. Our current flow cytometry phenotyping work is performed in our histocompatibility laboratory.

Some interesting results were noted in testing. Although anti-IgM–PE was added to target specificity of antibodies commonly known to have an IgM component such as anti-M, one anti-C-specific antibody was identified exclusively via anti-IgM–PE. The anti-M antibody showed both an IgM and IgG component, although in the identification panel, one heterozygous cell was non-reactive with anti-IgG–APC. Agglutination was observed in some testing particularly in samples with IgM antibodies (the anti-C and anti-M), and this would manifest as uneven event counts between the different panel cells during acquisition by flow cytometry. The negative controls showed essentially a 1:1:1 ratio of events among the three screening cells during acquisition, although this ratio could be skewed in the antibody cases tested. This was resolved by vortexing the samples after washing steps and before sample analysis on the flow cytometer. In addition, by setting the flow cytometer to count a minimum of 1000 events per reagent RBC cell type, we were able to ensure sufficient events for each cell type were acquired.

We have discussed many benefits of flow cytometry-based testing in transfusion medicine in both the introduction and our previous article outlining a phenotyping method we previously described and validated [7]. With regards to phenotyping, this method allowed high-throughput testing with extremely low volumes of costly reagents. Reagent screen and identification panel RBCs are relatively cheap compared with anti-sera used for phenotyping, and thus the benefit is diminished when comparing our phenotyping assay to the antibody detection assays described in this article. Likewise, high degrees of automation and relatively high-throughput instruments already exist and allow for automated antibody screen and identification panels. The primary benefit of the methodology described in this article of pre-staining RBCs with V450 and Oregon Green intracellular dyes is that it allows multiplexing of many reactions in a single test well, which improves potential throughput, while reducing costly consumables such as test tubes or gel cards. In this article, we have shown the use of pre-staining three RBC screen panel cells which would allow 96 antibody screens to be performed in a single flow cytometry microtitre plate and labelling of 4 identification panel RBCs which would allow 32 antibody identification panels to be performed on a single microtitre plate. Using the dual-staining approach, the number of antibody screen or identification panels that can be performed could be increased in an exponential manner, as adding a single additional stain theoretically doubles the number of RBC labelling combinations that can be achieved. However, we did not attempt to use any additional stains in this case. Nevertheless, the desired throughput can be tailored by either increasing or decreasing the number of stains used. In addition, titration studies showed flowed cytometry was able to detect lower levels of antibody in most sera when compared with our gel method. Further studies are required to fully characterize the relative limit of detection of flow cytometry-based antibody testing.

There are three main limitations to the methods described within. First, commercial solutions for this assay do not exist, and although expertise with flow cytometry is common in clinical laboratories, it is typically outside the operations of blood transfusion services, and thus the lack of commercially available solutions including purpose-built liquid handlers, pre-stained reagent cells, etc., are a limitation to the utilization of flow cytometry-based techniques in transfusion medicine. Second, regarding the screen, a commercial, flow cytometry-based solution for ABO typing does not exist, and thus ABO typing would have to be separated and performed using a traditional agglutination-based assay. Finally, for small to medium-sized laboratories, the overhead required to implement flow cytometry-based transfusion testing would be difficult to justify based on its benefits with regard to cost per test or throughput when compared with traditional agglutination assays, although these economic and volume considerations are

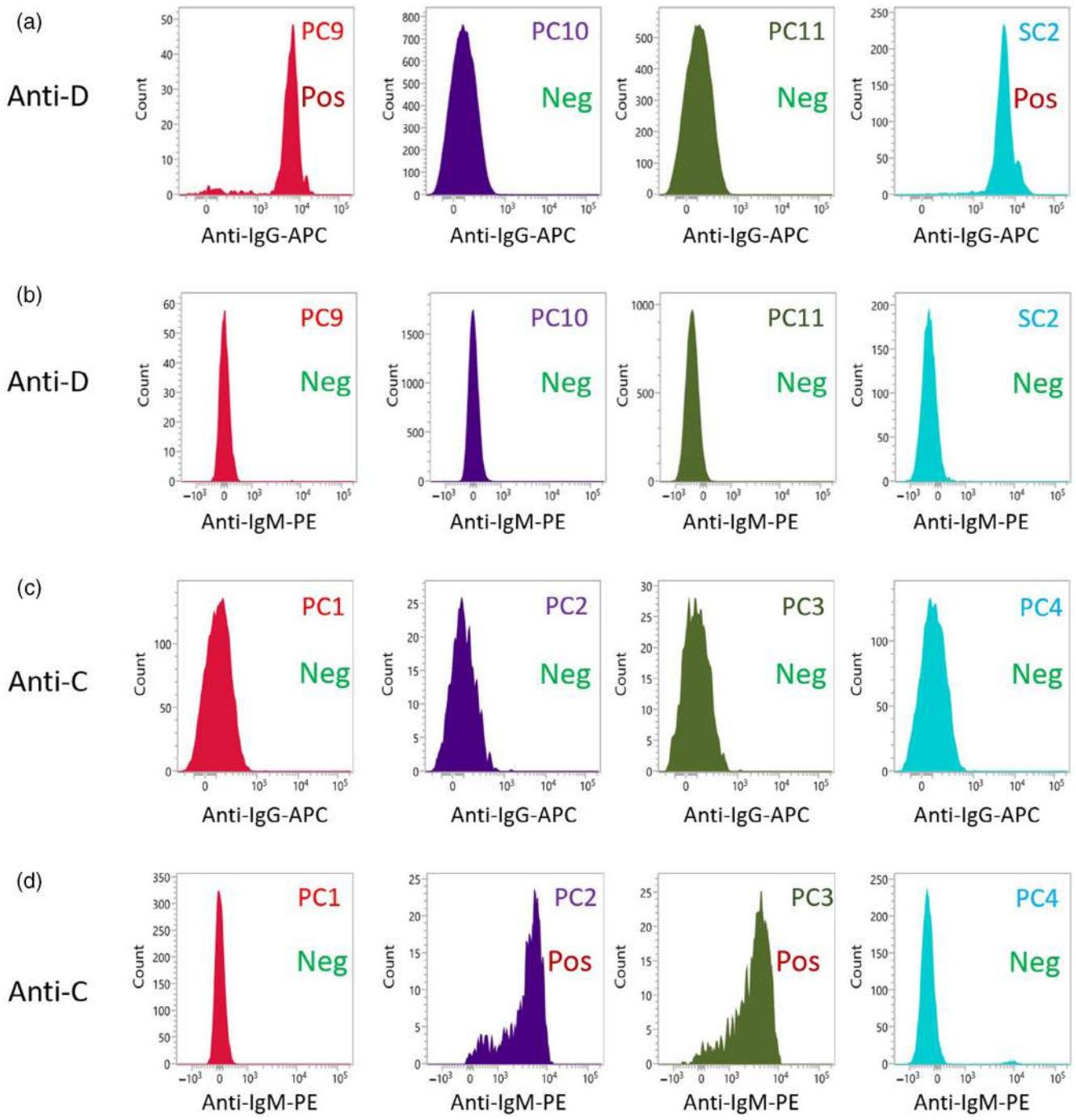


FIGURE 4 Representative results of red blood cell (RBC) antibody identification panel. An example of histograms showing median fluorescence intensity (MFI) for anti-immunoglobulin G (IgG)–allophycocyanin (APC) (panel A) and anti-immunoglobulin M (IgM)–phycoerythrin (PE) (panel B) for an anti-D antibody is shown. Histograms showing MFI for anti-IgG–APC (panel C) and anti-IgM–PE (panel D) for an anti-C antibody is also shown. Neg refers to reactions graded as negative and pos refers to reactions graded as positive. PC refers to 'identification panel' RBC cells 1–11 and SC2 refers to 'screening panel RBC cell 2'.

laboratory-specific, influenced by many factors and are beyond the scope of this discussion. That said, we have demonstrated the ability to perform high-volume phenotyping [7], antibody screens and identification panels via flow cytometry, and given the increasing amount of transfusion testing that can be performed via flow cytometry, this may make it a more viable consideration for some laboratories.

Future directions for this work are to increase the throughput via increased number of stains used to label RBCs. Similarly, we have validated similar approach utilizing intracellular stains to dramatically increase the throughput of our flow cytometry-based RBC phenotyping method which will be reported separately. In the current study, RBC panel cells were stained with intracellular dyes

immediately before testing. Future studies will evaluate the stability and viability of pre-stained screen and identification panel RBCs to determine if pre-stained RBC cell mixes could be stored for prolonged periods of time prior to testing to further improve efficiency. In this study, incubation was performed at room temperature and all expected reactivity was obtained, although future studies will evaluate if different incubation temperatures can further optimize the assay. Finally, the development of flow cytometry-based ABO typing that are suitable for clinical use would allow flow cytometry to be a complete transfusion solution for laboratories in the future.

ACKNOWLEDGEMENTS

J.Q. was involved in design, experimental work, analysis of data and writing of the article. R.L. was involved in design, experimental work, data acquisition, analysis and article writing. C.C. was involved in some aspects of analysis and article review. I.G. was involved in data acquisition, data analysis and article preparation. A.G. was involved in some aspects of design, experimental work and article review.

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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REFERENCES

1. Brestoff JR. Full spectrum flow cytometry in the clinical laboratory. Int J Lab Hematol. 2023;45:44–9.
2. Liwski RS, Greenshields AL, Conrad DM, Murphey C, Bray RA, Neumann J, et al. Rapid optimized flow cytometric crossmatch (FCXM) assays: the Halifax and Halifaster protocols. Hum Immunol. 2018;79:28–38.
3. Farias MG, Dal Bó S, Castro SM, da Silva AR, Bonazzoni J, Scotti L, et al. Flow cytometry in detection of fetal red blood cells and maternal F cells to identify fetomaternal hemorrhage. Fetal Pediatr Pathol. 2016;35:385–91.
4. Davis BH, Davis KT. Laboratory assessment of fetomaternal hemorrhage is improved using flow cytometry; ortho (United States). Lab Med. 2007;38:365–71.
5. Arnoni CP, Muniz JG, Vendrame TAP, Medeiros R, Cortez AJP, Latini FRM, et al. Correlation among automated scores of agglutination, antigen density by flow cytometry and genetics of D variants. Transfus Apher Sci. 2019;58:680–4.
6. Arndt PA, Garratty G. A critical review of published methods for analysis of red cell antigen-antibody reactions by flow cytometry, and approaches for resolving problems with red cell agglutination. Transfus Med Rev. 2010;24:172–94.
7. Liwski R, Clarke G, Cheng C, Abidi SSR, Abidi SR, Quinn JG. Validation of a flow-cytometry-based red blood cell antigen phenotyping method. Vox Sang. 2023;118:207–16.
8. McKinnon KM. Flow cytometry: an overview. Curr Protoc Immunol. 2018;120:5.1.1–5.1.11.
9. British Committee for Standards in Haematology, Milkins C, Berryman J, Cantwell C, Elliott C, Haggas R, et al. Guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories. British Committee for Standards in Haematology. Transfus Med. 2013;23:3–35.
10. Novaretti MC, Silveira EJ, Filho EC, Dorlhiac-Llacer PE, Chamone DAF. Comparison of tube and gel techniques for antibody identification. Immunohematology. 2000;16:138–41.
11. Schmidt A, Bendix BJ, Jacob EK, Bryant SC, Stubbs JR. Single-center comparison of gel microcolumn and solid-phase methods for antibody screening. Immunohematology. 2013;29:101–4.
12. Wallace PK, Tario JD, Fisher JL, Wallace SS, Ernstoff MS, Muirhead KA. Tracking antigen-driven responses by flow cytometry: monitoring proliferation by dye dilution. Cytometry A. 2008;73:1019–34.

How to cite this article: Liwski R, Greenshields A, Grace I, Cheng C, Quinn JG. Development of multiplexed flow cytometry-based red blood cell antibody screen and identification assays. Vox Sang. 2024;119:344–52.

Received: 8 September 2023 | Revised: 12 December 2023 | Accepted: 18 December 2023

DOI: 10.1111/vox.13585

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Clinical significance of decreased or loss of ABO blood group expression in acute myeloid leukaemia: A single-centre retrospective study

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Funding information
The authors received no specific funding for this work.

Abstract

Background and Objectives: Decreased or loss of ABO blood group antigen expression has been observed in acute myeloid leukaemia (AML) patients. We studied the clinical significance of this group in AML patients.

Materials and Methods: This was a retrospective, single-centre cohort study in which the data were retrieved from April 2009 to December 2019. A total of 1592 AML patients with normal ABO blood group antigen (Group I) and 65 patients of decreased or loss of ABO blood group antigen (Group II) group were enrolled. Data were collected at the time of initial admission for pathological diagnosis. To interrogate the underlying mechanism, publicly available The Cancer Genome Atlas AML data were downloaded.

Results: Group II consisted of 3.9% (65/1657) of AML patients. The 90-day survival (D90) probability was higher for Group II with a mean survival of 86.4 days compared to 80.6 days for Group I ($p = 0.047$). Group II had higher haematocrit (28.6 vs. 27.4%) and lower D-dimer, fibrinogen degradation production and C-reactive protein. Publicly available data revealed that among 11 CpG methylation sites within the *ABO* gene, 4 sites with elevated methylation level were associated with improved D90 survival probability and demonstrated an inverse correlation with *ABO* gene expression. Lower expression of the *ABO* gene showed improved survival trends for D90 ($p = 0.058$) and 180-day survival ($p = 0.072$).

Conclusion: AML with decreased expression or loss of ABO blood group showed better early survival during D90. Transfusion support for this subgroup of AML patients should be meticulously performed considering serum typing.

Keywords

ABO blood group antigen, acute myeloid leukaemia, decreased ABO blood group antigen expression, *ELF5*, *GATA1*, TCGA

Highlights

- ABO blood group antigen expression levels are not infrequently decreased in acute myeloid leukaemia (AML).

- AML patients with decreased ABO blood group expression had a higher 90-day survival probability as well as higher haematocrit levels and lower D-dimer, fibrinogen degradation product and C-reactive protein levels.
- Blood group determination should be meticulously performed for safe transfusion support in this patient group.

INTRODUCTION

The ABO blood group comprises carbohydrate molecules found in red blood cells, endothelial cells and other organs [1, 2]. The determination of the ABO blood group antigen expression is reported to be governed by the glycosyltransferase encoded by the *ABO* gene. This enzyme attaches *N*-acetyl galactosamine to the H antigen for the A blood group or galactose to the H antigen for the B Blood group [3, 4]. The *ABO* gene expression is influenced by enhancer, promotor and methylation state in the promotor region and genetic sequence in the coding or non-coding region that interact with transcription factors [5–16]. Phenotypic variants are caused by the interaction between the *ABO* gene and these factors [2].

Acute myeloid leukaemia (AML) is a clonal haematopoietic malignancy, and a subset of the AML group was reported to be associated with a decreased or loss of the ABO blood group antigen expression [3, 17]. These aberrant ABO blood group antigens in red blood cells are caused by genetic changes occurring in malignant progenitor cells [3]. The ABO blood group is also important in AML patients because a large amount of transfusion is required during chemotherapy and until haematopoietic stem cell transplantation is performed [18]. In addition, matching of the ABO group in haematopoietic stem cell transplantation is associated with favourable prognosis [19].

Genetic alterations including hypermethylation in the ABO promotor are reported to be associated with a decrease or loss of A,B antigen expression in AML patients. The intronic sequence at which the transcription factor *GATA1*, *ELF5* or *RUNX1* adheres has been reported to be altered in decreased ABO phenotypic expression [2, 4].

The genetic alteration in clonal leukaemic cells is thought to be reflected in red blood cells. We hypothesized that decreased ABO phenotypic expression might have clinical significance, which is undetermined so far. In this study, the clinical significance of the aberrant, which is mostly the decreased or loss of ABO blood group antigen expression, in AML patients was studied. In addition, publicly available The Cancer Genome Atlas (TCGA) AML data were analysed to possibly elucidate the causative source of the decreased ABO blood group antigen.

MATERIALS AND METHODS

This study was approved by the institutional review board of Seoul St. Mary's Hospital. This study was a retrospective, single-centre study, and the AML patient data were collected from April 2009 to December 2019. A total of 1830 AML patients (age >18) and non-acute promyelocytic leukaemia patients who were newly diagnosed at the time of admission were collected for study. All data were gathered at the time of initial admission before the commencement of chemotherapy treatment or transplantation. Diagnosis of AML was based on the 2017 WHO criteria and the ELN classification for risk assessment [20, 21].

From the data, those of 1657 patients with tested ABO blood group were collected for analysis. AML patients with normal ABO blood group antigen test were 1592, and these patients were regarded as a Group I (control group). The clinical and laboratory data were collected on the day of the bone marrow biopsy. Sixty-five patients were with aberrant ABO blood group phenotype or showed abnormally decreased A or B antigen phenotype (Group II) (Table 1). ABO blood groups were determined through the manual acrylic tile and manual tube methods, employing murine monoclonal anti-A and anti-B (Shin-yang Diagnostics, Seoul, Korea). In instances of ABO discrepancies, additional tests were conducted using anti-A1 and anti-H (Diagast, Loos, France). Serum typing was performed using A1 and B cells (MIRR SciTech Corp., Seoul, Korea) [23, 24]. Since 2018, automated Ortho Vision (Ortho Clinical Diagnostics, Raritan, NJ, USA) is being used for ABO blood group antigen typing using EDTA as an anticoagulant. Whenever a decreased or loss of blood group antigen is found, manual methods are used [23]. The antibody screening test was performed using the LISS/Coombs card (Bio-Rad, Cressier-sur-Morat, Switzerland). ID-DiaCell I, II were incubated with the patient's serum. Antibody identification test was performed using ID-DiaPanel (Bio-Rad) for suitable cases. Determination of decreased or loss of blood group antigen was based on the AABB Technical manual [23]. Assessed expression level of the ABO antigen was interpreted based on the following criteria: one solid agglutination, 4+; several large agglutinates, 3+; various small agglutinates with background not entirely clear, 2+; very small agglutinates, 1+; very small agglutinates requiring a brief period (less than 2 min) for agglutination, ±; and absence of agglutinates, (−) [4].

For transfusion support, the blood was irradiated by either a gamma or an x-ray irradiator. Leukocyte reduction was performed using a filter for packed red cells, or a pre-storage leukocyte-filtered packed red cell (FRBC) component was used for transfusion. In the case of platelets, most patients received apheresis platelets, and if apheresis platelets were unavailable, platelet concentrate was regarded as one-sixth of apheresis platelets in this study [18].

Public dataset analysis

To interrogate the cause of the aberrant ABO gene expression, the Genomic Data Commons (GDC) TCGA AML dataset from the National

TABLE 1 Baseline characteristics of normal and decreased group of ABO blood group antigen expression.

Variables	Group I $N = 1592$ (%)	Group II $N = 65$ (%)	*p*-value
Age	51.4 ± 15.6	45.0 ± 14.5	0.001
Sex, female/male	726 (45.6)/866 (54.5)	24 (36.9)/59 (63.1)	0.221
Diagnosis			<0.001
CBFB-MYH11/CEBPA	62 (3.9)/60 (3.8)	0 (0.0)/9 (13.8)	
RUNX1-RUNXT1/NPM1	174 (10.9)/127 (8.0)	0 (0.0)/0 (0.0)	
NPM1, FLT3/KMT2A-MLL3	40 (2.5)/29 (1.8)	0 (0.0)/0 (0.0)	
NOS	565 (35.5)	29 (44.6)	
DEK-NUP/KMT2A	8 (0.5)/32 (2.0)	0 (0.0)/0 (0.0)	
MECOM/FLT3	13 (0.8)/154 (9.7)	1 (1.5)/9 (13.8)	
Complex, monosomal[a]	124 (7.8)	5 (7.7)	
5 or del(5q) or −7	33 (2.1)	1 (1.5)	
MRC/2ndary AML	125 (7.9)/40 (2.5)	10 (15.4)/1 (1.5)	
CEBPA mutation[b]			<0.001
No/yes	435 (54.7)/60 (7.5)	7 (43.7)/9 (56.2)	
Karyotyping			
Normal	625	31	
One abnormality	373	14	
Two abnormalities	151	6	
Complex karyotype	276	6	
ABO blood group typing			NA
A/B/O/AB	567/422/452/151	0/0/0/0	
Anti-A (3+), anti-B (−)[c]	0	19	
Anti-A (2+), anti-B (−)[c]	0	11	
Anti-A (1+), anti-B (−)[c]	0	4	
Anti-A (±), anti-B (−)[c]	0	4	
Anti-A (−), anti-B (3+)[d]	0	4	
Anti-A (−), anti-B (2+)[d]	0	5	
Anti-A (−), anti-B (±)[d]	0	1	
Anti-A (3+), anti-B (4+)[e]	0	5	
Anti-A (±), anti-B (4+)[e]	0	1	
Anti-A (3+), anti-B (3+)[e]	0	6	
Anti-A (2+), anti-B (3+)[e]	0	1	
Anti-A (±), anti-B (3+)[e]	0	1	
Anti-A (4+), anti-B (2+)[e]	0	0	
Anti-A (2+), anti-B (2+)[e]	0	2	
Anti-A (±), anti-B (±)[e]	0	1	

Abbreviation: AML, acute myeloid leukaemia.
[a]Karyotyping results. Data without karyotyping for Group I ($n = 167$) and Group II ($n = 8$).
[b]*CEBPA* biallelic mutation study ($n = 511$); NA, not applicable.
[c]Decreased or loss of A blood typing, confirmed through serum A blood group typing.
[d]Decreased or loss of B blood typing, confirmed through serum B blood group typing.
[e]Decreased or loss of AB blood typing, confirmed through serum AB blood group typing.

Cancer Institute was downloaded from the Xena Brower platform from the University of Santa Cruz (https://xenabrower.net) [24]. Primary tumours with gene expression data were selected, followed by the deletion of duplicated cases. Survival analysis of the gene expression of interest was performed for the *ABO* gene. In addition, transcription factors associated with the *ABO* gene, namely *ELF5*, *GATA1*, *RUNX1* and *SP1*, were analysed. The Kaplan–Meier method and the log-rank method were used for the

comparison of the high-expression and low-expression groups. High or low expression was determined by the receiver operating characteristic curve for days 30, 60, 90 and 180 (D30, D60, D90, D180) analysis. In addition, a quantitative methylation dataset was downloaded and the methylation CpG site related to the *ABO* gene was analysed. Survival status for the hyper- or hypo-methylation group for D90 survival probability was analysed.

Statistical analysis

All continuous variables were tested using Student's *t*-test, and categorical variables were tested using the Chi-squared test or Fisher's exact test. Survival analysis was performed using the Kaplan–Meyer test, and Group I and Group II were tested by the log rank test. For correlation analysis, the Pearson method was used. All analyses were performed using the R program [25].

RESULTS

Baseline characteristics of the patient data showed that AML with *CEBPA* biallelic mutation was 3.8% (65/1592) and 13.8% (9/65) for Group I and Group II, respectively. Decreased or loss of A, B or AB blood group antigens, confirmed through serum A, B or AB blood group typing, was identified in 58.5% (38/65), 15.3% (10/65) and 26.2% (17/65) of cases, respectively (Table 1).

Laboratory data indicated that Group II had elevated haematocrit and mean cell haemoglobin concentration. Group II showed better hematological laboratory parameters, including less prolonged prothrombin time (PT) or activated partial thromboplastin time (APTT). Fibrinogen degradation product (FDP) and D-dimer were lower for Group II (Table 2). Within Group II, encompassing nine cases of AML with *CEBPA* biallelic mutation, seven had a diminished A blood group phenotype, while two had a decreased A and B phenotype (Table S1). Decreased serum typing was observed in three patients, and all of

TABLE 2 Laboratory data for Group I (control group) and Group II (decreased or loss of ABO blood group antigen expression).

	N	Group I (*N* = 1592)	Group II (*N* = 65)	*p*-value
WBC ($\times 10^9$)	1675	39.2 ± 67.9	45.2 ± 77.1	0.487
RBC ($\times 10^{12}$)	1675	2.8 ± 0.6	2.9 ± 0.7	0.230
Haemoglobin (g/dL)	1675	9.2 ± 1.9	9.3 ± 1.9	0.310
Haematocrit (%)	1675	27.4 ± 5.7	28.6 ± 5.9	0.003*
MCV (fL)	1675	93.3 ± 7.0	96.9 ± 8.4	<0.001*
MCH (pg)	1675	31.5 ± 2.5	31.3 ± 2.5	0.571
MCHC (%)	1675	33.8 ± 1.4	32.5 ± 1.4	<0.001*
Platelet ($\times 10^9$)	1675	71.2 ± 69.9	58.1 ± 67.7	0.139
Neutrophil (%)	1675	18.4 ± 19.2	21.0 ± 21.6	0.293
Lymphocyte (%)	1675	32.6 ± 27.0	24.2 ± 22.1	0.004*
Monocyte (%)	1675	10.7 ± 17.2	8.2 ± 10.9	0.084
Eosinophil (%)	1675	0.6 ± 1.6	1.7 ± 6.5	0.164
Basophil (%)	1675	0.2 ± 0.9	0.2 ± 0.5	0.224
ANC ($\times 10^9$)	1675	3.1 ± 6.9	4.2 ± 6.4	0.209
RDW (fL)	976	16.2 ± 1.9	17.4 ± 2.3	<0.001*
PT (s)	1596	13.5 ± 2.0	12.9 ± 1.8	0.016*
APTT (s)	1596	30.6 ± 6.6	27.7 ± 5.4	<0.001*
FDP (μg/mL)	1278	14.2 ± 19.9	9.7 ± 12.6	0.006*
D-dimer (nmol/L)	1463	29.0 ± 8.3	45.5 ± 32.8	0.028*
Fibrinogen (g/L)	1314	3.5 ± 1.0	3.3 ± 0.7	0.848
CRP (mg/L)	1641	47 ± 66	30 ± 52	0.014*
Antibody screening	1659			0.100
Negative		1542 (96.5)	61 (93.8)	
Positive		35 (2.1)	3 (4.6)	
NA		15 (0.9)	1 (1.5)	

Abbreviations: ANC, absolute neutrophil count; APTT, activated partial thromboplastine time; CRP, C-reactive protein; FDP, fibrinogen degradation product; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; MCV, mean corpuscular volume; NA, not available; PT, prothrombin time; RBC, red blood cell; RDW, red cell distribution width; WBC, white blood cell.
*$p < 0.05$.

them showed decreased cell typing, which was as follows: anti-A (2+), anti-B (±) with A cell (−) and B cell (±); anti-A (2+), anti-B (−) with A cell (−) and B cell (3+); anti-A (−), anti-B (±) with A cell (4+) and B cell (3+).

Furthermore, survival analysis based on the ABO group revealed comparable overall survival probabilities (Figure S1). The mean survival days (standard error) at D30 for blood groups A, B, O and AB were 29.1 (0.1), 29.1 (0.1), 28.6 (0.2) and 29.4 (0.2), respectively. At D60, the mean survival days (standard error) for blood groups A, B, O and AB were 55.6 (0.5), 55.2 (0.5), 55.1 (0.6), and 57.4 (0.7), respectively. At D90, the mean survival days (standard error) for blood groups A, B, O and AB were 81.0 (0.9), 79.9 (1.1), 80.3 (1.1) and 84.7 (1.4), respectively. These findings collectively indicate the absence of a notable survival advantage associated with a specific ABO blood group type.

Survival analysis showed that Group II had better early survival ranging from D30 to D90. D90 survival curve showed that Group II had favourable D90 survival with a mean survival duration of 86.4 days compared to 80.6 days for Group I with statistical significance ($p = 0.047$) (Figure 1; Table S2).

Transfusion support during initial admission was analysed. Groups I and II were transfused with 0.2 ± 1.4 and 0.3 ± 1.5 of packed red cells, respectively ($p = 0.504$); with 4.3 ± 5.7 and 5.9 ± 5.7 of prestorage filtered red cells, respectively ($p = 0.024$); and with 11.5 ± 11.5 and 15.2 ± 11.5 of apheresis platelets, respectively ($p = 0.011$). In the case of platelet concentrate, 0.9 ± 2.5 and 0.9 ± 2.7 units were administered for Groups I and II, respectively ($p = 0.757$).

Public dataset analysis

To investigate the cause of survival benefit shown by Group II, TCGA AML data were downloaded from the Xena browser, and the genes of interest were studied. There were 161 samples with gene expression and survival data. The higher *ABO* gene expression group, compared to the low *ABO* gene expression group, was not associated with D90, D180 and D30, D60 survival (Figures 2 and S2). Among transcription factors, the high gene expression group of *ELF5*, *GATA1* and *SP1*

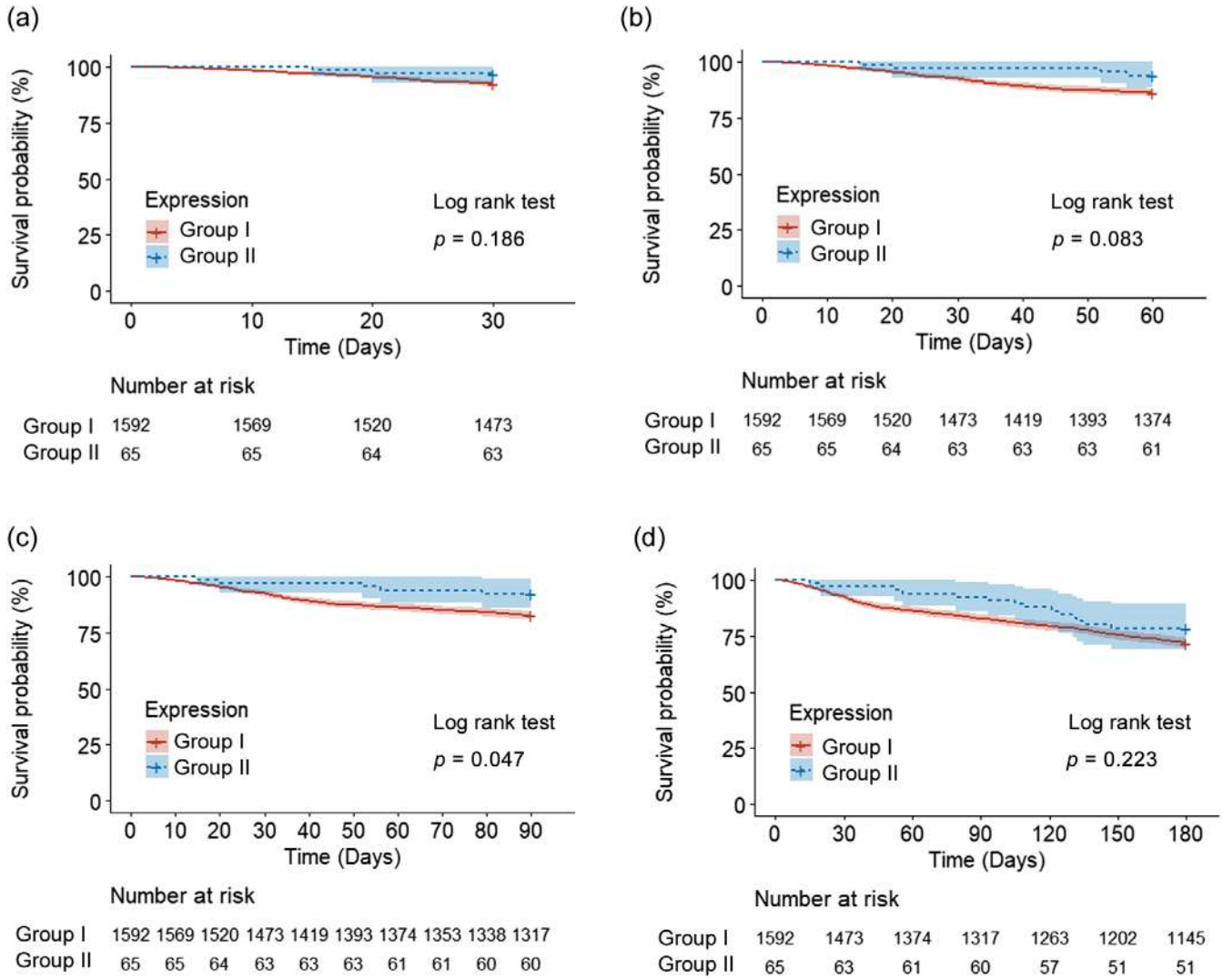

FIGURE 1 Survival analysis of control group with normal expression of ABO blood group (red) and decreased ABO expression group (blue) for (a) day-30, (b) day-60, (c) day-90 and (d) day-180 survival analysis.

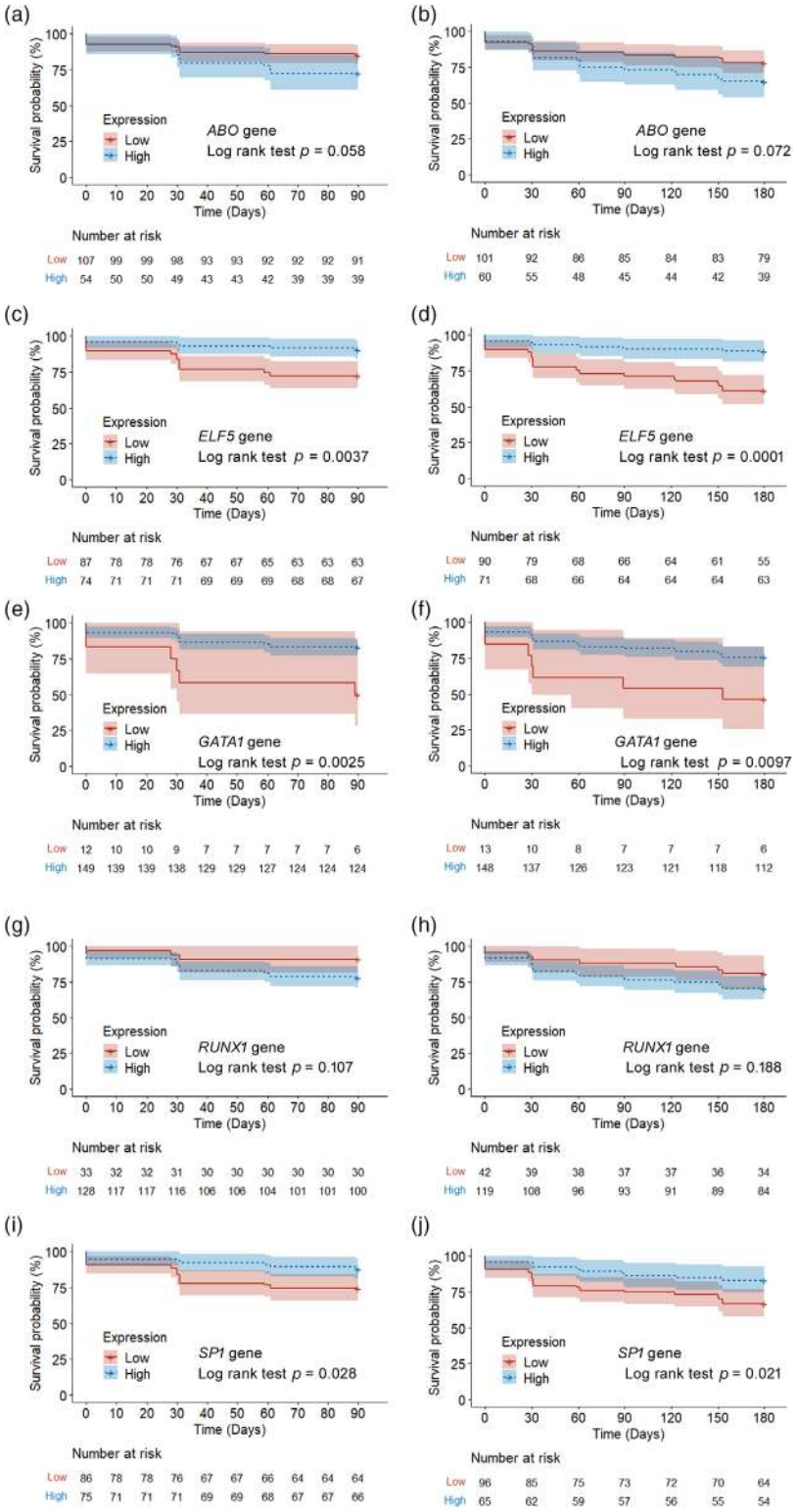

FIGURE 2 Day-90 and day-180 survival analysis of gene expression data from TCGA AML data for (a, b) *ABO* gene, (c, d) *ELF5* gene, (e, f) *GATA1* gene, (g, h) *RUNX1* gene and (i, j) *SP1* gene. AML, acute myeloid leukaemia; TCGA, The Cancer Genome Atlas.

genes showed favourable D90 and D180 survival (Figure 2). In addition, only high expression of *ELF5* and *GATA1* genes showed favourable D90 survival (Figure S2). Correlation analysis showed that only *GATA1* gene expression was positively correlated with *ABO* gene expression, with a correlation coefficient of 0.370 ($p < 0.001$).

DNA methylation status related to the *ABO* gene was also analysed from 99 downloaded samples. Among them, 11 CpG sites were

analysed of which 4 were inversely correlated with the *ABO* gene expression (Table S3). The correlation coefficients (*p*-values) for the DNA methylation level in the four CpG sites were as follows: cg22535403, −0.497 ($p < 0.001$); cg24267699, −0.488 ($p < 0.001$); cg07241568, −0.401 ($p < 0.001$); and cg13506600, −0.333 ($p < 0.001$) (Figure S3). Among 99 samples, 83 samples had survival data, and Kaplan–Meier survival analysis was performed. Except for cg07241568, all the CpG sites with higher expression showed favourable D90 survival compared to the lower expression group, with statistical significance (Figure 3). The mean survival days and standard error for hyper- or hypo-methylation group for these CpG sites were as follows: cg22535403, 85.7 (2.1) days, 64.7 (7.0) days; cg24267699, 80.5 (2.8) days, 63.8 (12.8) days; cg13506600, 83.5 (3.3) days, 73.7 (4.8) days.

DISCUSSION

ABO blood group expression is important for transfusion support in hematological malignancies and patients undergoing haematopoietic stem cell or solid organ transplantation [19, 26, 27]. AML is regarded as an emergent condition that requires treatment including cytoreduction, supportive care and induction chemotherapy [21]. Early mortality has been reported to be higher in AML compared to other hematological malignancies [28, 29]. Although early mortality has decreased from 18.7% to 5.8% during the last four decades, it continues to be high [28, 29]. Therefore, early survival benefit in decreased or loss of ABO blood group antigen among AML patient might enhance the probability to receive treatment including transfusion and clinical trials. In this study, Group II received more transfusion components, including pre-stored filtered red cells and apheresis platelets, because Group II survived longer and could receive continuous transfusion support [18, 22].

Aside from survival benefit, a decreased or loss of the ABO blood group hinders transfusion support in patients with haematopoietic diseases including AML. Transfusion of the RBC product with lesser antigen expression based on phenotype could be a practical solution. Therefore, transfusion of O blood group could be a universal choice for a patient with a decreased or loss of blood group A or B.

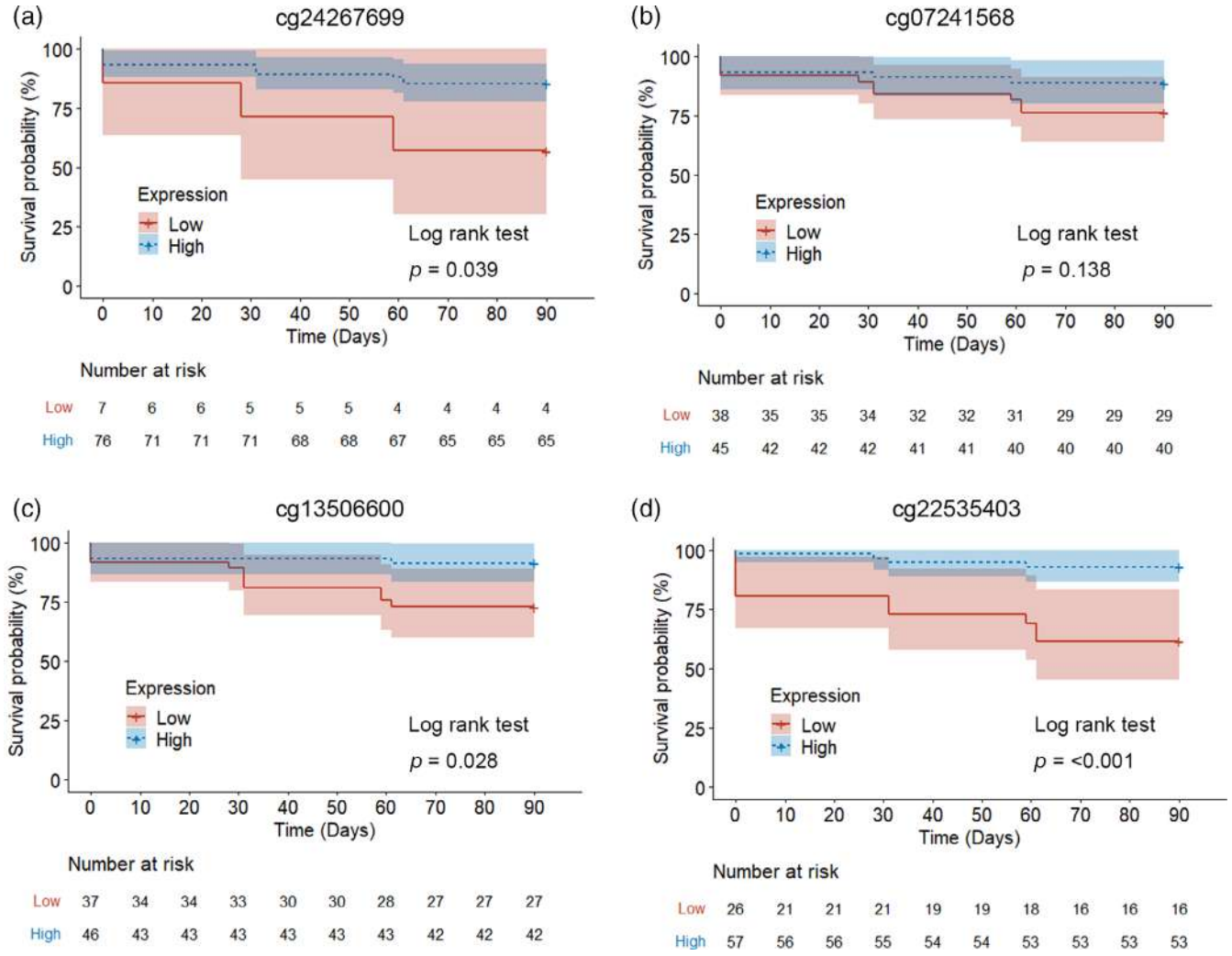

FIGURE 3 Day-90 survival analysis of ABO promotor region CpG methylation site the from TCGA AML data: (a) cg24267699, (b) cg07241566, (c) cg13506600, and (d) cg22535403. AML, acute myeloid leukaemia; TCGA, The Cancer Genome Atlas.

However, could there be other solutions for providing blood products for these patients?

This study showed that serum typing provided definite results except for 4.1% (3/65) cases. Definite serum typing result of A or B with decreased or loss of blood group antigen in red blood cells such as anti-A (3+) to anti-A (±) or anti-B (3+) to anti-B (±) could be regarded either as a blood group A or as B based on serum typing. For transfusion support, A or B blood group might be more suitable than O. Continuous transfusion of the O blood group would result in attenuation of the blood group A or B with blurring of serum typing. This might lead to blood group O as the sole blood component of choice.

The selection of blood group in these cases should be meticulously considered. Therefore, we suggest that serum typing results should get more consideration and be emphasized in the determination of blood group for transfusion support for Group II. One plausible explanation is the anticipated attenuation of immunological responses among AML patients. Moreover, the successive administration of a cytoreductive agent or chemotherapeutic regimen induces a further decrease in immune cells, and consequently results in diminished immunological reactions. Conversely, the transfusion of A or B blood group products to immunocompetent individuals with decreased or loss of ABO blood group expression may elicit alloimmunization, giving rise to the production of anti-A or anti-B antibodies.

In this study, only three cases of serum typing were not determinant for a definite conclusion. Transfusion support for these three cases was as follows. Patients with anti-A (2+), anti-B (±) with A cell (−) and B cell (±) were transfused with blood group O red cell component or AB blood group PLT or FFP. Patients with anti-A (2+), anti-B (−) with A cell (−) and B cell (3+) were transfused with blood group A red blood cells, PLT or FFP component. Patients with anti-A (−), anti-B (±) with A cell (4+) and B cell (3+) were transfused with blood group O packed red cells with AB blood group platelets or fresh frozen plasma. No adverse effect of the transfusion support related to the ABO was evident or caused a clinical significance [22].

Decreased or loss of the ABO blood group can be seen in leukaemia patients, especially in patients with AML. Loss of A, B, H genes or diminished A or B phenotypic expression is frequently reported [3]. The suggested mechanism for loss or decreased *ABO* gene expression is the genetic alteration in stem cells, which results in AML. These genetic or epigenetic alterations might have affected ABO blood group antigen expression. ABO blood group expression was determined by the methylation status of the promotor CpG site or single nucleotide variation in the site where transcription factor or enhancer is attached [13, 14].

As the ABO blood group is an inherited trait, blood group expression levels are constant throughout the life of an individual. Decrease or loss of blood group antigens is infrequently seen in normal populations, except for the ABO subgroup including individuals with the *Cis-AB* gene. The *Cis-AB* gene possesses both glycosyl transferase activity by attaching N-acetyl galactosamine (blood group A) and galactose (blood group B). *Cis-AB* gene paired with the O blood group gene is typical, which results in an A_2B_3 phenotype along with the presence of anti-B [30].

We wondered if AML with decreased *ABO* gene expression could be an independent subgroup of AML. To satisfy this condition, the causative source should be elucidated: whether it is a mutation, the presence of fusion gene or epigenetic changes along with prognostic significance or difference in the treatment option. As early survival during D90 was better for Group II than Group I, there is a possibility that Group II could be a phenotypic characteristic of an AML subgroup with related genetic alterations. The genetic alteration that caused decreased *ABO* gene expression might have caused leukemogenes. Decreased *ABO* gene might be affected by the collateral effect of orderly or random methylation that was associated with leukaemia. Or, there could be similar features as *NPM1* gene mutation or *CEBPA* gene biallelic mutation, which was associated with favourable prognosis [31].

To study the genetic alteration of the *ABO* gene expression in AML, TCGA AML data were downloaded from the XENA browser and analysed [24]. Decreased *ABO* gene expression showed a tendency for better survival but statistical significance was not reached. On the contrary, increased expression of the transcription factors, including the *ELF5*, *GATA1* and *SP1* genes, is associated with better survival probability at D90 and D180. In addition, high expression of the *ELF5* gene was associated with better D30 and D60 survival. The *GATA1* gene was also required for the terminal differentiation of erythrocytes, megakaryocytes, eosinophils and mast cells, and the *GATA1* gene activates beta-globulin expression during erythropoiesis. Decreased *ABO* gene expression was inversely correlated with the hypermethylation status of the *ABO* gene promotor region. The hypermethylation status at the CpG sites, cg24267699, cg13506600 and cg22535403, showed higher D90 survival probability. It could be hypothesized that the decrease or loss of ABO was caused by promotor hypermethylation status, which resulted in favourable D90 survival probability. We deduced that hypermethylation directly related to leukemogeneis incidentally decreased ABO phenotypic expression. The common cause of leukemogenesis or proliferation of leukaemic cells is expected to affect the *ABO* gene expression. The *ABO* gene is thought to be a mere innocent bystander, which reflects underlying leukaemia with favourable D90 prognosis among AML patients.

In this study, the data showed that Group II had better haematological laboratory data. Haematocrit was increased in Group II, which was in line with the fact that the *ELF5*, *GATA1* gene expression group showed higher survival probability. On the other hand, FDP was lower and PT and APTT were shorter compared to those of Group I. Therefore, we thought that these transcription factors might be engaged in haematopoiesis, but not in leukaemic cell proliferation.

In a recent study, blood donors with blood group B were found to exhibit elevated haemoglobin content and haematocrit in comparison to those with blood group A. Among the precursors of blood group B, increased miRNA-215-5p and miRNA-182-5p were identified, exerting suppressive effects on their target transcription factors *RUNX1* and *HES-1* [32]. In contrast to the preceding study, the haemoglobin levels (standard deviation) in g/dL, stratified by ABO blood group in this study, were as follows: A, 8.8 (1.6); B, 8.9 (1.7); O, 8.9 (1.8); AB, 8.8 (1.6). Correspondingly, the haematocrit levels (standard deviation)

in percentage, categorized by ABO blood group, were as follows: 26.6 (12.2); 26.4 (5.2); 26.5 (5.3); 25.9 (4.8). Additionally, survival analysis based on the ABO group revealed comparable overall survival probability (Figure S1). Collectively, these data indicate the absence of a discernible survival advantage based on the ABO blood group type in AML patients.

The limitation of the study is that *ABO* gene sequencing data were not available. Such data of the *ABO* gene including intron might have revealed genetic alternations. As next-generation sequencing data were not available, associated genetic mutation or mutation as a causative source for decreased ABO gene expression was unavailable. Fresh frozen plasma data were not collected in this cohort. Quantifying the anti-A or anti-B titres could provide a resolution to the ABO blood group discrepancies, which was omitted in this study. Commercialized monoclonal anti-A antisera with titres below 256 and anti-A,B with titers below 128 proved inadequate in identifying weak A blood group antigen expression. Conversely, titres equal to or exceeding 2048 for anti-A showed superior reactivity [33].

In conclusion, AML with a decreased or loss of the *ABO* blood group phenotypic expression, or Group II, showed better early survival, especially during early D90. Haematological laboratory data indicated a favourable prognosis for Group II. Transfusion support for this subgroup of AML patients should be meticulously performed considering serum typing.

ACKNOWLEDGEMENTS

J.H.H. performed data analysis, designed the study and wrote initial manuscript. H.L., J.K.K. and J.Y. acquired and analysed the data. K.P. performed laboratory research. D.W.J. designed the study and wrote the draft of the manuscript. Y.K. supervised the research and edited the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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REFERENCES

1. Yamamoto F. Molecular genetics of ABO. Vox Sang. 2000;78:91–103.
2. Kominato Y, Sano R, Takahashi Y, Hayakawa A, Ogasawara K. Human ABO gene transcriptional regulation. Transfusion. 2020;60:860–9.
3. Bianco T, Farmer BJ, Sage RE, Dobrovic A. Loss of red cell A, B, and H antigens is frequent in myeloid malignancies. Blood. 2001;97:3633–9.
4. AABB. Technical manual: methods and appendices. 20th ed. Available from: https://www.aabb.org/docs/default-source/default-document-library/publications/technical-manual-20th-edition-methods-and-appendices.docx. Last accessed 21 Jan 2023.
5. Seltsam A, Wagner FF, Grüger D, Gupta CD, Bade-Doeding C, Blasczyk R. Weak blood group B phenotypes may be caused by variations in the CCAAT-binding factor/NF-Y enhancer region of the ABO gene. Transfusion. 2007;47:2330–5.
6. Sano R, Fukuda H, Kubo R, Oishi T, Miyabe-Nishiwaki T, Kaneko A, et al. Emergence of an erythroid cell-specific regulatory region in ABO intron 1 attributable to A- or B-antigen expression on erythrocytes in Hominoidea. Sci Rep. 2023;13:4947.
7. Sano R, Nakajima T, Takahashi Y, Kubo R, Kobayashi M, Takahashi K, et al. Epithelial expression of human ABO blood group genes is dependent upon a downstream regulatory element functioning through an epithelial cell-specific transcription factor, Elf5. J Biol Chem. 2016;291:22594–606.
8. Wu PC, Lee YQ, Möller M, Storry JR, Olsson ML. Elucidation of the low-expressing erythroid CR1 phenotype by bioinformatic mining of the GATA1-driven blood-group regulome. Nat Commun. 2023;14:5001.
9. Oda A, Isa K, Ogasawara K, Kameyama K, Okuda K, Hirashima M, et al. A novel mutation of the GATA site in the erythroid cell-specific regulatory element of the ABO gene in a blood donor with the Am B phenotype. Vox Sang. 2015;108:425–7.
10. Nakajima T, Sano R, Takahashi Y, Kubo R, Takahashi K, Kominato Y, et al. Mutation of the GATA site in the erythroid cell-specific regulatory element of the ABO gene in a Bm subgroup individual. Transfusion. 2013;53:2917–27.
11. Liu F, Li G, Li J, Gui R, Luo Y, Zhou M. A novel mutation eliminates GATA-1 and RUNX1-mediated promoter activity in galactosyltransferase gene. Transfus Med Hemother. 2022;49:331–7.
12. Hayakawa A, Sano R, Takahashi Y, Kubo R, Harada M, Omata M, et al. RUNX1 mutation in a patient with myelodysplastic syndrome and decreased erythrocyte expression of blood group A antigen. Transfusion. 2020;60:184–96.
13. Kominato Y, Hata Y, Takizawa H, Tsuchiya T, Tsukada J, Yamamoto F. Expression of human histo-blood group ABO genes is dependent upon DNA methylation of the promoter region. J Biol Chem. 1999;274:37240–50.
14. Bianco-Miotto T, Hussey DJ, Day TK, O'Keefe DS, Dobrovic A. DNA methylation of the ABO promoter underlies loss of ABO allelic expression in a significant proportion of leukemic patients. PLoS One. 2009;4:e4788.
15. Takahashi Y, Isa K, Sano R, Nakajima T, Kubo R, Takahashi K, et al. Deletion of the RUNX1 binding site in the erythroid cell-specific regulatory element of the ABO gene in two individuals with the Am phenotype. Vox Sang. 2014;106:167–75.
16. Sano R, Nakajima T, Takahashi K, Kubo R, Kominato Y, Tsukada J, et al. Expression of ABO blood-group genes is dependent upon an erythroid cell-specific regulatory element that is deleted in persons with the B(m) phenotype. Blood. 2012;119:5301–10.
17. Zhang W, Liu J, Zhang W, Zhuang Y. The potential association of the transcription levels of the ABO gene with the disease phases in AML patients. Transfus Apher Sci. 2017;56:719–22.
18. Jekarl DW, Kim JK, Han JH, Lee H, Yoo J, Lim J, et al. Transfusion support in hematopoietic stem cell transplantation. Blood Res. 2023;58:S1–7.
19. Guru Murthy GS, Logan BR, Bo-Subait S, Beitinjaneh A, Devine S, Farhadfar N, et al. Association of ABO mismatch with the outcomes of allogeneic hematopoietic cell transplantation for acute leukemia. Am J Hematol. 2023;98:608–19.
20. Jung J, Cho BS, Kim HJ, Han E, Jang W, Han K, et al. Reclassification of acute myeloid leukemia according to the 2016 WHO classification. Ann Lab Med. 2019;39:311–6.
21. Lee H, Park S, Yoon JH, Cho BS, Kim HJ, Lee S, et al. The factors influencing clinical outcomes after leukapheresis in acute leukaemia. Sci Rep. 2021;11:6426.
22. Yoo J, Yu H, Choi H, Lee GW, Song YS, Lee S, et al. Evaluation of the automated immunohematology analyzer DAYMATE M. Lab Med Online. 2017;7:163–9.

23. Lee SJ, Chae S, Yoo J, Jekarl DW, Lee S, Kim M, et al. Various ABO genotyping-phenotyping results for ABO blood group discrepancy: a retrospective study. Lab Med Online. 2023;23:97–102.
24. Goldman MJ, Craft B, Hastie M, Repečka K, McDade F, Kamath A, et al. Visualizing and interpreting cancer genomics data via the Xena platform. Nat Biotechnol. 2020;38:675–8.
25. R Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2021. Available from: https://www.R-project.org/. Last accessed 22 Aug 2023.
26. Storry JR, Olsson ML. The ABO blood group system revisited: a review and update. Immunohematology. 2009;25:48–59.
27. Ciurea SO, Al Malki MM, Kongtim P, Fuchs EJ, Luznik L, Huang XJ, et al. The European Society for Blood and Marrow Transplantation (EBMT) consensus recommendations for donor selection in haploidentical hematopoietic cell transplantation. Bone Marrow Transplant. 2020;55:12–24.
28. Ferrara F. Prediction of early death in acute myeloid leukemia. J Clin Oncol. 2012;30:462–3.
29. Percival ME, Tao L, Medeiros BC, Clarke CA. Improvements in the early death rate among 9380 patients with acute myeloid leukemia after initial therapy: a SEER database analysis. Cancer. 2015;121:2004–12.
30. Chun S, Choi S, Yu H, Cho D. Cis-AB, the blood group of many faces, is a conundrum to the novice eye. Ann Lab Med. 2019;39:115–20.
31. Kim HS, Han E, Jang W, Kim M, Kim Y, Han K, et al. Germline CEBPA mutations in Korean patients with acute myeloid leukemia. Leuk Res. 2019;76:84–6.
32. Kronstein-Wiedermann R, Blecher S, Teichert M, Schmidt L, Thiel J, Muller MM, et al. Novel evidence that the ABO blood group shapes erythropoiesis and results in higher hematocrit for blood group B carriers. Leukemia. 2023;37:1126–37.
33. Miola MP, Colombo TE, Fachini RM, Ricci-Junior O, de Mattos CCB, de Mattos LC. Anti-A and anti-A,B monoclonal antisera with high titers favor the detection of A weak phenotypes. Transfus Apher Sci. 2020;59:102865.

SUPPORTING INFORMATION

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

How to cite this article: Han JH, Lee H, Kim JK, Yoo J, Park K, Jekarl DW, et al. Clinical significance of decreased or loss of ABO blood group expression in acute myeloid leukaemia: A single-centre retrospective study. Vox Sang. 2024;119:353–62.

Received: 5 October 2023 | Revised: 9 December 2023 | Accepted: 17 December 2023

DOI: 10.1111/vox.13589

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Incidence of formation of anti-D between patients with and without a history of solid organ transplant

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Funding information
The authors received no specific funding for this work.

Abstract

Background and Objectives: Solid organ transplant surgeries including liver transplants constitute a substantial risk of bleeding complications and given frequent national blood shortages, supporting D-negative transplant recipients with D-negative red blood cell products perioperatively can be difficult for the transfusion services. This study was designed to compare the incidence of alloimmunization after D-mismatched red cell transfusions between patients with and without a history of solid organ transplant at a single tertiary care hospital. The patients undergoing solid organ transplants are on strong immunosuppressive regimens perioperatively to help reduce the risk of rejection. We hypothesized that the use of these immunosuppressive agents makes these patients very less likely to mount an immune response and form anti-D antibodies when exposed to the D-positive red blood cell products perioperatively.

Study Design and Methods: At our center, D-negative patients who received ≥1 unit of D-positive red blood cell products were identified using historical transfusion records. Antibody testing results were examined to determine the incidence of the formation of anti-D and any other red cell alloantibodies after transfusion and these results were compared between patients with and without a history of solid organ transplant.

Results: We were able to identify a total of 22 patients over 10 years with D-negative phenotype who had undergone a solid organ transplant and had received D-positive red blood cell products during the transplant surgeries. We also identified a second group of 54 patients with D-negative phenotype who had received D-positive red blood cell products for other indications including medical and surgical.

A comparison of the data showed no new anti-D formation among patients with a history of D mismatched transfusion during solid organ transplant surgeries.

Conclusion: Among our limited study population, we observed a very low likelihood of D alloimmunization among solid organ transplant recipients. A larger, prospective study could help further evaluate the need for prophylactic D matching for red cell transfusions during solid organ transplant surgeries.

Keywords

anti-D alloimmunization, anti-D in transplant recipients, blood bank inventory, patient blood management, RhD alloimmunization, transfusion support in transplantation

Highlights

- D-negative phenotype transplant recipients are at very low risk of anti-D formation perioperatively when transfused with D-positive red blood cell products.
- Prophylactic D matching might not be strictly indicated for D-negative phenotype transplant recipients perioperatively.
- A larger prospective study could help further clarify this important consideration.

INTRODUCTION

The D antigen (RhD antigen) expressed on red cells is historically known to be highly immunogenic [1]. Hence, a prophylactic D matching is routinely performed to prevent alloimmunization against D antigen in D-negative recipients. This is particularly significant in females of childbearing age, as D alloimmunization can cause haemolytic disease of the foetus and newborn [2].

As only 15% of the donor population is D-negative, D-positive red blood cell products are sometimes used for D-negative male or postmenopausal female patients who do not have pre-formed anti-D, especially during inventory shortages or massive transfusion needs [3].

Based on earlier studies on healthy volunteers, the rate of D alloimmunization after exposure to D-positive red blood cell products was estimated to be as high as 80% [1]. However, contemporary clinical studies have shown a much lower rate of anti-D formation in hospitalized patients approaching as low as 20% in part related to the immunosuppressed status of these individuals in the setting of acute stress or illness [4, 5].

Transplant surgeries, particularly liver transplants, constitute a particularly high risk of bleeding complications, and O-negative inventory in the blood center could particularly be difficult to manage effectively. With the advances in transplant medicine and the increasing rate of transplant surgeries in patients with end-organ disease, hospital transfusion services soon are going to have an increasing need for transfusion support for this diverse group of patients.

The patients undergoing solid organ transplants are unique in terms of the requirement for the use of multiple immunosuppressive agents to help reduce the risk of transplant rejection. Immunosuppressive agents including an induction regimen with anti-thymocyte globulin/Baciliximab followed by maintenance immunosuppression with combination agents including Cyclosporin, Mycophenolate, Tacrolimus, Azathioprine and steroids all help suppress the immune system including B cell function and antibody formation [6].

Supporting the D antigen-negative transplant recipients with D-positive red blood cell products should not lead to a significant alloimmunization risk in the setting of robust immunosuppression. This is an important patient consideration, if supported intra-operatively with D-negative red cells only, O-negative red blood cell products may have to be used when ABO type-specific D-negative red cell products are depleted, potentially causing a significant impact on the inventory of this universal red cell product that is frequently in shortage [7].

In our study, we analysed the incidence of D alloimmunization in the solid organ transplant recipients with D-negative phenotype who were given D-positive red blood cell products perioperatively. These results were compared with the D-negative phenotype patients who received D-positive red blood cell products for non-transplant surgeries and other medical indications.

METHODS

This study was approved by the Stanford University Institutional Review Board (protocol 66118). All D-negative patients who received ≥1 unit of D-positive red cells between 1 January 2011 and 31 December 2020 were initially identified from analysis of historical transfusion records. Prophylactic D matching is routinely performed for all red cell transfusions at our center, although D-negative patients may be switched to D-positive red blood cell products during significant inventory shortages. Patients who had a positive red cell antibody screen within 14 days before the first D-positive red cell transfusion were excluded from the study. Historical red cell antibody testing results between 1 January 2011 and 31 December 2021 were analysed, to capture up to 1 year of passive serological surveillance data after D-positive red cell transfusion. Patient's gender, age at the time of D-positive transfusion, solid organ transplant history and red cell transfusion indication were obtained by manual review of historical electronic medical records. Antibody detection testing included a screen using an automated solid phase red cell adherence assay on three screening cells, with low ionic strength saline (LISS) as a potentiator. Antibody identification testing for positive screens was performed using a manual tube method with polyethylene glycol (PEG) as a potentiator. Pan-reactivity observed with the tube method using PEG as potentiator was repeated with the tube method using LISS as potentiator; if reactivity persisted and/or not all clinically significant alloantibodies were ruled out, an auto-adsorption or differential adsorption was performed if the patient had a history of red cell transfusion within the last 3 months. Direct antiglobulin test (DAT) was also performed for specimens demonstrating pan reactivity, followed by an elution if the DAT was positive at the IgG phase.

The results analysed included the formation of anti-D and any other red cell alloantibodies between patients with and without a history of transplant. Statistical testing was performed using Fisher's exact test, and statistical significance was defined using a two-sided p-value of <0.05. Effect sizes were reported using odds ratio (OR) with the corresponding 95% confidence interval (CI).

RESULTS

A total of 76 D-negative phenotype patients who fulfilled eligibility criteria were identified and included in the study, of which 22 patients (29.9%) had a history of solid organ transplant. A total of 705 D-positive red blood cell products were used for these 76 patients (mean = 9.3 units, median = 6 units, range = 1–65 units). Additional patient characteristics are summarized in Table 1. New red cell antibodies were identified in 15 of the 76 total patients (19.7%) after transfusion with red blood cell products. None of the transplant patients developed new anti-D; in contrast, 12 of the non-transplant patients developed new anti-D (OR = 0; 95% CI, 0.00–0.78; $p = 0.01$) (Table 2). When the results were generalized to include all new red cell antibodies formation, only one transplant patient developed a new anti-E while 14 non-transplant patients developed new alloantibodies (OR = 0.14; 95% CI, 0.00–1.03; $p = 0.05$) (Table 2). Although this study was specifically designed to detect alloimmunization events in D-negative patients transfused with D-positive red cells, it should be noted that not all new

TABLE 2 Comparison of alloimmunization rates between transplant and non-transplant patients.

Outcomes	Transplant patients ($n = 22$)	Non-transplant patients ($n = 54$)
Primary outcome: Anti-D		
Anti-D detected	0	12
No anti-D detected	22	42
Secondary Outcome: Any new alloantibodies		
New alloantibody	1	14
No new alloantibody	21	40

TABLE 1 Characteristics of patients included in the analysis, defined based on history of transplantation.

Patient characteristics		Transplant patients ($n = 22$)	Non-transplant patients ($n = 54$)
Number of D+ units transfused	Median (range)	6.0 (1–44)	4.5 (1–65)
Age (years)	Median (range)	58 (28–73)	69 (10–87)
Gender	Female, *n* (%)	6 (27.3)	15 (27.8)
	Male, *n* (%)	16 (72.7)	39 (72.2)
Serological follow up (days)[a]	Median (range)	262.0 (14–2141)	34.5 (8–2979)
Number of antibody screens	Median (range)	10 (2–46)	5 (2–24)
Duration of D+ transfusion (days)	Median (range)	1 (1–16)	1 (1–33)
All D+ RBC transfused within 24 h	Yes, *n* (%)	19 (86.4)	38 (70.4)
Indication for D+ transfusion	Surgery	22 (100.0)	41 (75.9)
	Medical	0 (0.0)	10 (18.5)
	Trauma	0 (0.0)	3 (5.6)
Organ transplanted	Liver (%)	12 (54.5)	Not applicable
	Heart (%)	3 (13.6)	
	Lung (%)	1 (4.6)	
	Multi-organ[b] (%)	6 (27.3)	
D matching in organ transplanted	No (%)	10 (45.5)	Not applicable
	Yes (%)	1 (4.5)	
	Unknown (%)	11 (50.0)	
Induction immunosuppression	Anti-thymocyte globulin (%)	19 (86.4)	Not applicable
	Basiliximab (%)	2 (9.1)	
	None (%)	1 (4.5)	
Maintenance immunosuppression	Tacrolimus	17 (77.3)	Not applicable
	Mycophenolate mofetil	15 (68.2)	
	Prednisone	14 (63.6)	
	Cyclosporine	5 (22.7)	
	Azathioprine	1 (4.5)	
Number of maintenance immunosuppressive agents	3	12 (54.5)	Not applicable
	2	7 (31.8)	
	1	3 (13.6)	

[a]Serological follow-up was defined as the number of days between the first D+ RBC transfusion and the last antibody screen.
[b]Multi-organ transplants included kidney/liver ($n = 3$), heart/liver ($n = 1$), heart/kidney ($n = 1$) and heart/kidney/lung ($n = 1$).

TABLE 3 Individual patients with new red cell alloantibodies detected after D-positive RBC transfusion.

Gender	Age at transfusion (years)	Antibodies detected	Time to first detected antibody (days)	Transplant history	Transfusion indication	Transfused D-positive red cells (units)
Female	69	D, E	5	No	Trauma	2
Female	62	D, Kpa	162	No	Medical	2
Female	78	Jka	4	No	Surgery	8
Female	67	D, Jka	58	No	Surgery	1
Female	57	E	36	Yes	Surgery	13
Male	63	D	58	No	Surgery	12
Female	62	D	1554	No	Surgery	16
Male	74	C, D, E, panagglutinin	111	No	Surgery	10
Male	76	D	2660	No	Surgery	4
Female	69	E, K, pan-agglutinin	182	No	Surgery	8
Female	70	D, C	2128	No	Surgery	1
Male	72	D	7	No	Surgery	3
Male	56	D	32	No	Surgery	6
Male	19	D, C, panagglutinin	148	No	Trauma	1
Male	61	D, C, E, K, panagglutinin	233	No	Trauma	6

alloimmunization events were due to the D antigen—three patients had new antibodies that were either pan reactive or directed against other antigens (including E, K and Jka) without formation of anti-D (Table 3). Furthermore, seven patients developed multiple new antibodies in addition to anti-D; these other antibodies were frequently directed against other antigens or were pan agglutinins suggestive of new warm autoantibodies. Additional characteristics of all new alloimmunization events are described in Table 3.

DISCUSSION

In our study, we analysed and compared the incidence of D alloimmunization in D-negative recipients who received transfusion with D-positive red blood cell products for transplant surgeries and other indications. We found that patients who received D-positive red blood cell products in the perioperative period of solid organ transplant had a much lower likelihood of alloimmunization. The inclusion of other types of solid organ transplantation in our analyses indicates that this significantly reduced risk of anti-D formation may be generalizable across patients on post-transplant immunosuppression and suggests that prophylactic D matching for red cell transfusions may not be strictly indicated for transplant patients. Our results are similar to findings from two previous similar studies, which found no anti-D formation among D-negative liver transplant recipients who received D-positive red cells during their transplant surgeries [8–10]. These results may be particularly helpful when planning intra-operative transfusion support during transplant surgeries, especially for patients with high bleeding risk.

The alloimmunization rate for D based on our overall study population is approximately 16%, which is comparable to that reported in other published studies [11, 12]. In addition, among the non-transplant patients who formed anti-D, we observed that approximately half of these patients also formed antibodies against other red cell antigens, frequently against other Rh antigens. This is expected since Rh antigens are in linkage disequilibrium, D-negative individuals most commonly carry the rr haplotype (dce/dce) and transfusion with D-positive red cells can lead to the formation of anti-C and/or anti-E as well (due to the high association of E and C antigen with D instead of e and c). Notably, we also observed that four of the patients with multiple new alloantibodies also had pan agglutinins, suggestive of new warm autoantibodies. Development of warm autoantibodies in alloimmunized patients has been described in other studies, and while the exact mechanism is unclear, it may represent the predisposition of these individuals to form antibodies [13].

We would like to highlight a few limitations of the study including a small study population in part due to the low prevalence of D-negative phenotype and a high percentage of prophylactic D matching that is currently routinely performed at our institution even among male and post-menopausal female patients, with switching to D-positive red blood cell products only during times of significant inventory shortage. In addition, since our study relied on a historical data review of transfusion service and inpatient medical records, there was no uniform follow-up period to detect new alloantibodies post-transfusion. Two patients also had very early detection of new antibodies—although a negative antibody screen within 2 weeks before the first D-positive red cell transfusion was one of our inclusion criteria, such rapid antibody detection raised the possibility of an amnestic response and may have overestimated the rate of new alloimmunization events. Also, a small percentage of patients who type as D negative on the routine testing might have weak D or Ddel variants that are not capable of making anti-D even when exposed to D-positive red blood cell products [14, 15]. Since blood product recipients in general also did not undergo genotyping there is a small likelihood of missing these individuals.

As the individuals in the non-transplant groups did not need as close follow-ups and laboratory testing after being discharged, the serologic follow-up period for the group was comparatively shorter (median of 34.5 days as compared to a median of 262 days for the second group). This could potentially have been missing a few individuals who might have had rhesus alloimmunization after this time. Due to the retrospective nature of the study, we are not able to confirm the history of any transfusions in the patients who developed anti-D in the second group before their current presentation to the hospital.

Transfusion of D mismatched platelet products could also lead to rhesus alloimmunization; however, our study was only designed to account for red blood cell transfusion [16].

As the lifespan of the transfused red blood cells is up to 4 months, there might be a risk of delayed haemolysis in the patients who receive D-positive red blood cell products and later develop anti-D. A review of the medical records in our patient cohort who developed anti-D revealed that only two of the 15 patients had DAT testing performed in the subsequent 4-month period post-transfusion and had positive DAT for IgG component. However, other laboratory parameters including serum haptoglobin and serum LDH levels were not consistently checked for each patient largely related to low concern for hemolysis.

In conclusion, in addition to gender and age, immunosuppression history should be an important factor when estimating the risk of anti-D formation and determining indications for prophylactic D matching. Transfusion services can implement this by prospectively discussing bleeding risk and transfusion plans with the clinical team before transplant surgeries for D-negative patients. This could be pivotal for D-negative inventory management in the setting of frequent national blood shortages.

ACKNOWLEDGEMENTS

M.Y. designed the research study and was actively involved in the writing of the manuscript. J.W. conducted the research and wrote the draft of the manuscript. M.A. and A.N. procured the laboratory data and provided their expertise for the laboratory technical aspects. H.S. and S.P. were pivotal in creating the study design and reviewing the draft during different stages of the study.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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

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REFERENCES

1. Urbaniak SJ, Robertson AE. A successful program of immunizing Rh-negative male volunteers for anti-D production using frozen/thawed blood. Transfusion. 1981;21:64–9.
2. Gedik Özköse Z, Oğlak SC. The combined effect of anti-D and non-D Rh antibodies in maternal alloimmunization. Turk J Obstet Gynecol. 2021;18:181–9.
3. Selleng K, Jenichen G, Denker K, Selleng S, Müllejans B, Greinacher A. Emergency transfusion of patients with unknown blood type with blood group O rhesus D positive red blood cell concentrates: a prospective, single-centre, observational study. Lancet Haematol. 2017;4:e218–24.
4. Ji Y, Luo G, Fu Y. Incidence of anti-D alloimmunization in D-negative individuals receiving D-positive red blood cell transfusion: a systematic review and meta-analysis. Vox Sang. 2022;117:633–40.
5. Pandey P, Setya D, Singh MK. Anti-D alloimmunization after RhD positive red cell transfusion to selected RhD negative patients. Indian J Hematol Blood Transfus. 2022;38:577–84.
6. Taylor AL, Watson CJ, Bradley JA. Immunosuppressive agents in solid organ transplantation: mechanisms of action and therapeutic efficacy. Crit Rev Oncol Hematol. 2005;56:23–46.
7. Nieto-Calvache AJ, Quintero-Santacruz M, Macia-Mejía C, López-Girón MC, Vergara-Galliadi LM, Ariza F. Dangerous shortage of blood banks as an indirect effect of SARS-CoV-2: an obstetrics perspective. Int J Gynaecol Obstet. 2020;151:424–30.
8. Ramsey G, Hahn LF, Cornell FW, Boczkowski DJ, Staschak S, Clark R, et al. Low rate of rhesus alloimmunization from Rh-incompatible blood transfusion during liver and heart transplant surgery. Transplantation. 1989;47:993–5.
9. Yuan S, Davis R, Lu Q, Goldfinger D, Ziman AF. Low risk of alloimmunization to the D antigen in D−orthotopic liver transplant recipients receiving D+ RBCs perioperatively. Transfusion. 2008;48:2653–5.
10. Vijayanarayanan A, Wlosinski L, El-Bashir J, Galusca D, Nagai S, Yoshida A, et al. Lack of alloimmunization to the D antigen in D-negative orthotopic liver transplant recipients receiving D-positive red blood cells perioperatively. Vox Sang. 2022;117:1043–7.
11. Luyten U, Peeraer S, Pirlet C, Khaouch Y, Streel C, Deneys V. O-negative blood shortage management in a university hospital: impact of transfusing RhD-positive red blood cells to RhD-negative patients. Transfus Clin Biol. 2023;30:402–9.
12. Williams LA 3rd, Sikora J, Aldrees R, Pham HP, Marques MB. Anti-Rh alloimmunization after trauma resuscitation. Transfus Apher Sci. 2019;58:102652.
13. Zumberg MS, Procter JL, Lottenberg R, Kitchens CS, Klein HG. Autoantibody formation in the alloimmunized red blood cell recipient: clinical and laboratory implications. Arch Intern Med. 2001;161:285–90.
14. Ramsey G, Park YA, Eder AF, Bobr A, Karafin MS, Karp JK, et al. Obstetric and newborn weak D-phenotype RBC testing and Rh immune globulin management recommendations: lessons from a blinded specimen-testing survey of 81 transfusion services. Arch Pathol Lab Med. 2023;147:71–8.
15. Kwon DH, Sandler SG, Flegel WA. DEL phenotype. Immunohematology. 2017;33:125–32.
16. Dunbar NM. Does ABO and RhD matching matter for platelet transfusion? Hematology Am Soc Hematol Educ Program. 2020;2020:512–7.

How to cite this article: Wali JA, Abdelmonem M, Nguyen A, Shan H, Pandey S, Yunce M. Incidence of formation of anti-D between patients with and without a history of solid organ transplant. Vox Sang. 2024;119:363–7.

Received: 23 August 2023 | Revised: 8 January 2024 | Accepted: 15 January 2024

DOI: 10.1111/vox.13594

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Extensive red blood cell matching considering patient alloimmunization risk

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Funding information
Stichting Sanquin Bloedvoorziening, Grant/Award Number: PPOC20-05/2490; NIHR Artificial Intelligence in Health and Care Award, Grant/Award Number: 02331; NIHR BTRU in Donor Health and Behaviour, Grant/Award Number: NIHR203337; NIHR Cambridge BRC, Grant/Award Numbers: NIHR203312, BRC-1215-20014

Abstract

Background and Objectives: Red blood cell (RBC) transfusions pose a risk of alloantibody development in patients. For patients with increased alloimmunization risk, extended preventive matching is advised, encompassing not only the ABO-D blood groups but also the most clinically relevant minor antigens: C, c, E, e, K, Fy^a, Fy^b, Jk^a, Jk^b, S and s. This study incorporates patient-specific data and the clinical consequences of mismatching into the allocation process.

Materials and Methods: We have redefined the MINimize Relative Alloimmunization Risks (MINRAR) model to include patient group preferences in selecting RBC units from a finite supply. A linear optimization approach was employed, considering both antigen immunogenicity and the clinical impact of mismatches for specific patient groups. We also explore the advantages of informing the blood bank about scheduled transfusions, allowing for a more strategic blood distribution. The model is evaluated using historical data from two Dutch hospitals, measuring shortages and minor antigen mismatches.

Results: The updated model, emphasizing patient group-specific considerations, achieves a similar number of mismatches as the original, yet shifts mismatches among patient groups and antigens, reducing expected alloimmunization consequences. Simultaneous matching for multiple hospitals at the distribution centre level, considering scheduled demands, led to a 30% decrease in mismatches and a 92% reduction in shortages.

Conclusion: The reduction of expected alloimmunization consequences by incorporating patient group preferences demonstrates our strategy's effectiveness for patient health. Substantial reductions in mismatches and shortages with multi-hospital collaboration highlights the importance of sharing information in the blood supply chain.

Keywords

extensive antigen typing, linear optimization, RBC matching, supply chain

Highlights

- More than 300 red blood cell (RBC) antigens are known, and antibodies against 11 of these are considered to be of great clinical importance.
- Patient group-directed extensive RBC matching can significantly reduce expected alloimmunizations for high-risk patient groups.
- Sharing information on elective transfusions within the blood supply chain reduces both shortages and alloimmunization risk.

INTRODUCTION

Red blood cell (RBC) units are the most commonly transfused blood products. In the Netherlands, there is one organization responsible for the blood supply to 113 hospitals serving a population of 17.6 million inhabitants. There are about 380,000 whole blood donors, and yearly, about 400,000 RBC units are distributed [1]. The blood supply service distributes RBC units to the hospitals daily. These orders are partially based on specific scheduled transfusions, but mainly on order-up-to levels on the 'major' RBC antigens (ABO and D). At the hospitals, products from their inventory are assigned to patients in need of a transfusion, which is generally done by issuing units compatible on these major blood groups. However, more than 300 RBC antigens are known to exist [2]. RBC alloantibodies against 11 of these 'minor' RBC antigens are considered to be of such clinical importance that, for a selection of patient groups, preventive matching on specific subsets of these antigens is part of transfusion guidelines [3]. In general, upon an ABO-D matched transfusion, about 1%–5% of recipients become alloimmunized [4–6], which may increase up to 8%–15% after multiple transfusions [7, 8]. In subsequent transfusion episodes with products containing such antigens, the newly formed antibodies might destroy the transfused RBCs [8–10]. Therefore, before a blood transfusion is administered, the recipient is screened for the presence of alloantibodies to select compatible blood. Because of the transient character of antibodies, over time 70% of alloantibodies will not be detected anymore while still being capable of inducing delayed transfusion reactions [11]. Therefore, in the Netherlands, all identified alloantibodies are registered in a database, which is accessible by all hospitals [12].

Patients who developed RBC alloantibodies (Allo) will in subsequent transfusion episodes receive units that are, next to D, also matched for CcEe and K and compatible for the implicated antigen. For blood recipients for whom an increased risk of alloantibody formation is expected, the Dutch Blood Transfusion Guideline summarizes advice for preventive extended matching and for patients who do not have alloantibodies [3]. This concerns patients with autoimmune haemolytic anaemia (AIHA), sickle cell anaemia (SCD) or thalassemia (Thal) and patients with myelodysplastic syndrome (MDS). The latter three patient categories are in need of repeated blood transfusions, which increases the exposure to foreign blood group antigens and the risk of developing alloantibodies [8]. In the Netherlands, extended matching for these patient groups includes matching on, in addition to the three major antigens A, B and D, a selection of 11 clinically relevant minor antigens: C, c, E, e, K, Fy^a, Fy^b, Jk^a, Jk^b, S and s. Furthermore, RBCs for women aged below 45 years (Wu45) are matched for c, E and K to prevent alloimmunization that may lead to haemolytic disease of the foetus or newborn in future pregnancies. Patients who do not belong to any of these groups (Other) receive RBC units matched for the major antigens only. This group mainly includes elective transfusions, for example, for surgery.

Recently, high throughput genotyping technology has facilitated prediction of the full RBC phenotype of both patients and donors. Using molecular techniques, the presence of hundreds of antigens can be determined in a single assay [13–16]. Although the combinations of 14 antigens proposed theoretically constitute 6566 different blood group combinations, van Sambeeck et al. have shown that most (>95%) of transfusion-induced alloimmunization events can be prevented when both donors and recipients are comprehensively typed and matched [17]. Despite this impressive result, it is impossible to provide fully compatible products to all patients, meaning the challenge will remain how to manage the risk of alloimmunization for individual patients. This is all but a trivial task, not only because of the huge number of possible antigen combinations considered in large-scale extensive matching but also because the risks associated with mismatching differ for each antigen and in particular for each patient group. We therefore propose an issuing strategy that differentiates and prioritizes matching requirements in alignment with the patient groups, as mentioned above.

Aiming to make extensive matching possible in practice, Van de Weem et al. have proposed the 'MINRAR' model (short for MINimize Relative Alloimmunization Risks) to quantify the quality of a match and to determine optimal issuing strategies such that the number of clinically relevant mismatches is minimized [18]. The study at hand shows that, with a straightforward extension of the MINRAR model, offering extensively matched products to all patients does not increase the alloimmunization rate in high-risk groups. In addition, it will demonstrate to what extent the quality of in-hospital matching of RBC products may be improved when hospitals inform the blood bank about future transfusions, which allows for improving the distribution of blood products to the hospitals.

MATERIALS AND METHODS

Constructing the matching model

We propose a modified version of the MINRAR model, as presented in Van de Weem et al. [18], to match extensively typed RBC units to

TABLE 1 Classification of the perceived clinical consequences of incompatible matching for all patient groups considered for different antigens.

	C	c	E	e	K	Fy^a	Fy^b	Jk^a	Jk^b	S	s
Allo	×	×	×	×	×	1	1	1	1	2	3
SCD	×	×	×	×	×	×	1	×	×	1	2
Thal	×	×	×	×	×	2	2	2	2	3	3
MDS	×	×	×	×	×	2	2	2	2	3	3
AIHA	×	×	×	×	×	1	1	1	1	2	2
Wu45	2	×	×	2	×	3	3	3	3	3	3
Other	2	2	2	2	2	3	3	3	3	3	3

Note: Four levels of compatibility are distinguished: ×: mismatching is not allowed; 1: compatible matching is important; 2: compatible matching is preferred; 3: compatible matching has low priority.
Abbreviations: AIHA, autoimmune haemolytic anaemia; MDS, myelodysplastic syndrome; SCD, sickle cell anaemia; Thal, thalassemia; Wu45, women aged below 45 years.

extensively typed patients, while minimizing alloimmunization risk. In the MINRAR model, all products compatible on the major blood group (ABO-D) are considered a valid match for any patient, meaning a shortage is incurred whenever there are insufficient ABO-D compatible products available in the hospital inventory for the patient. The MINRAR model uses antigen immunogenicity (Table S1) to calculate the weight for mismatching on a particular antigen. We extend this approach by also considering the perceived clinical consequences of certain alloantibodies, as estimated by the immunohaematology expert authors (JL, MDH, EvdS and RN). The clinical consequences, as perceived by our experts, are a mixture of pathogenicity and anticipated health implications and are shown in Table 1. When we incorporate this patient group differentiation into our model, we redefine a shortage as a lack of sufficient inventory products available for a patient, all of which must be compatible on the antigens denoted by a × in Table 1 for the corresponding patient group. For a detailed description on how the other preferences are transformed to numerical mismatch weights to be used by the MINRAR model, the reader is referred to Supplement A.

The model is designed to match RBC products to requests for a single day, prioritizing the minimization of shortages. This means it focuses on providing all patients with their requested number of products, all of these being compatible on the antigens marked with a × in Table 1. Once the minimum number of shortages is established, four other objectives are minimized simultaneously:

1. mismatches on the minor antigens for which mismatching is allowed, marked with a number in Table 1;
2. antigen substitution: issuing an antigen-negative product to a patient who is positive for that antigen;
3. remaining shelf life of issued products: older products are preferred over fresh products; and
4. major antigen usability of issued products: low usability (e.g., *AB*+) is preferred over high usability (e.g., *O*−).

A full description of the MINRAR model can be found in Supplement E and in the original paper by van de Weem et al. [18]. The mixed-integer linear programming (MILP) model [19] is programmed in Python (version 3.9) [20] and solved using Gurobi Optimization software (version 9.1) [21]. The simulation code is available from https://github.com/Sanquin/blood_matching.

Data on patients and RBC products supplied

The model is designed to be able to match real patients to real products in inventory. For the purpose of model testing, however, data were generated both for demand and for supply. In this section, we elaborate on the considerations for generating the data on patients and supplied products.

When generating patient request data, the distribution of patient groups among all patients needs to be taken into account. Data from two hospitals were available to represent the two distinct hospital types. The Amsterdam UMC, location AMC is chosen to represent university hospitals, and the OLVG, location East to represent regional hospitals. Table S5 in Supplement B shows the distribution of units requested by each patient group in these hospitals.

Considering the antigen profile of simulated patients, phenotypes of all simulated patients are based on antigen frequencies within the Caucasian population, with the exception of the SCD patients. For simulating these patients, we used antigen prevalences for individuals of African ancestry, since SCD mainly occurs in that population. Antigen prevalences for both populations are provided in Supplement F, Table S7.

Transfusions are frequently scheduled in advance, allowing for product requests to be anticipated. The immunohaematology expert authors (JL, MdH, EvdS and RN) estimated lead times for all patient groups, that is, the number of days between the request becoming known and the transfusion. For MDS, SCD and Thal patients, simulated requests become known 1 week before the transfusion. Lead times for the Allo and Other patient groups are uniformly distributed between 0 and 6 days. For Wu45 patients, we assume that the requests are known either on the day of transfusion or 1 day before, each with a 50% probability. Requests for AIHA are not known in advance and only become available on the day of transfusion.

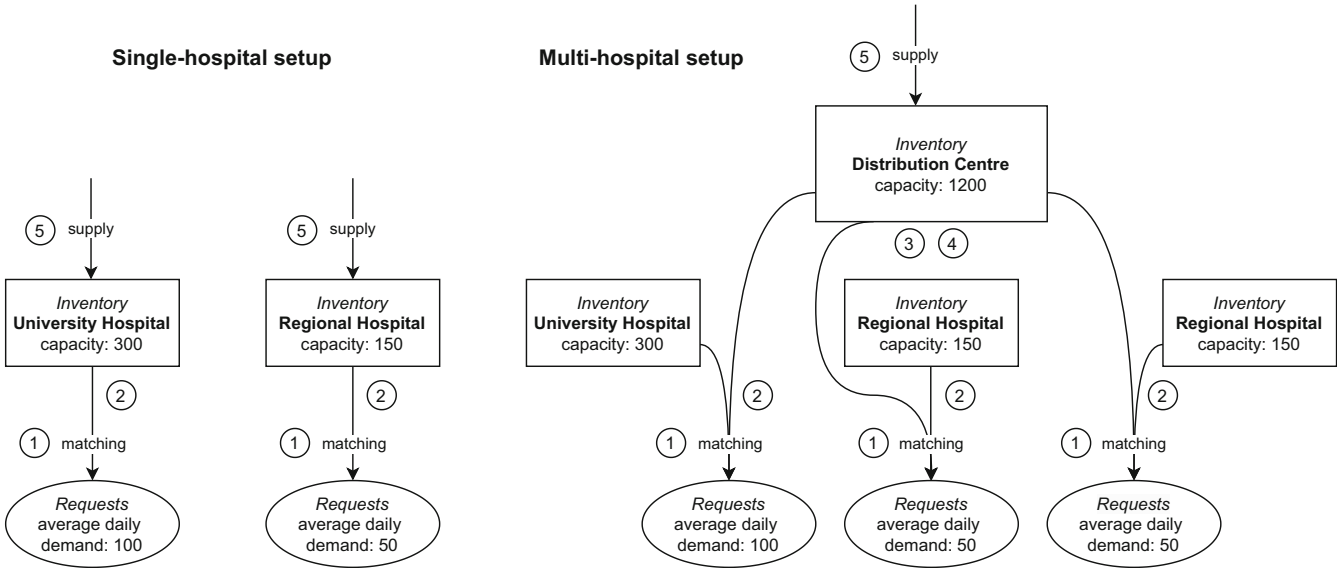

FIGURE 1 Daily tasks performed in both the single- and multi-hospital simulations. (1) Requests are matched from hospital inventory. (2) All products assigned to today's requests are issued and removed from the hospital's inventory. (3) Products assigned to hospital requests to be satisfied tomorrow are shipped to the designated hospital. (4) Supply hospitals to restore target inventory levels. (5) Replenish inventory with random red blood cell units from the donor population.

The number of units requested per patient is sampled from a historical in-hospital distribution using Dutch Transfusion Datawarehouse data, comprising 438,260 transfusions from January 2012 to December 2019 in six Dutch hospitals [22]. For patient groups Allo, Wu45 and Other, the assumed unit requirement ranges from one to four, sampled based on the distribution shown in Supplement B, Figure S1. The remaining groups are assumed to always need two units per patient, the most common request. The phenotypes of the patients are generated at random based on the prevalence of different blood groups among Caucasians (Supplement F, Table S7). Furthermore, we did not model recurring requests for individual patients.

The major antigen profile of the supplied units is sampled in accordance with its prevalence in the donor population, as depicted in Supplement C, Figure S2. As donors are only invited based on their major blood groups, we assume that the minor antigen prevalence in donors mirrors the general population. Minor antigens are therefore sampled based on Caucasian phenotype prevalence for each blood group system (Supplement F, Table S7).

Simulation setup

The performance of the proposed model was assessed through multiple 1-year simulations, where for each separate day an optimal set of products was selected from inventory by the model. A 70-day initialization phase to stabilize inventory levels was applied. Figure 1 illustrates two experimental configurations for the simulations, with numbers 1–5 representing the steps to be executed on each day of the simulation. In the single-hospital setup, matching is performed by the proposed model, and products allocated to today's requests are removed from inventory. At the end of each day, any expired products are removed as well, and the hospital's inventory is replenished with fresh RBC units, as detailed in Section 2.2.

The multi-hospital setup involves three hospitals (two regional, one university) and a distribution centre providing RBC units. The first two steps each day resemble those of the single-hospital setup; however, requests may be matched to products from either the hospital's or the distribution centre's inventory. However, we do assume that requests for today's transfusions can only be satisfied from the hospital's own inventory, as emergency deliveries from the distribution centre are expensive and should be avoided. Next, expired products are removed from all inventories, and products matched from the distribution centre to requests to be satisfied the next day are shipped to the hospitals, along with supplementary products to ensure each hospital's inventory is replenished up to its normal capacity. The selection of products for replenishment is done by preferring older products over younger products and preferring products with low usability on the minor antigens over products with high usability. Finally, the distribution centre's inventory is resupplied with fresh RBC units as discussed in Section 2.2.

RESULTS

In this section, we assess the performance of the modified MINRAR model (as described in Section 2.1.) in matching extensively typed RBC units to extensively typed patients while minimizing alloimmunization risk. First, we will investigate the impact of using patient group-specific weights on the minor antigen mismatches for

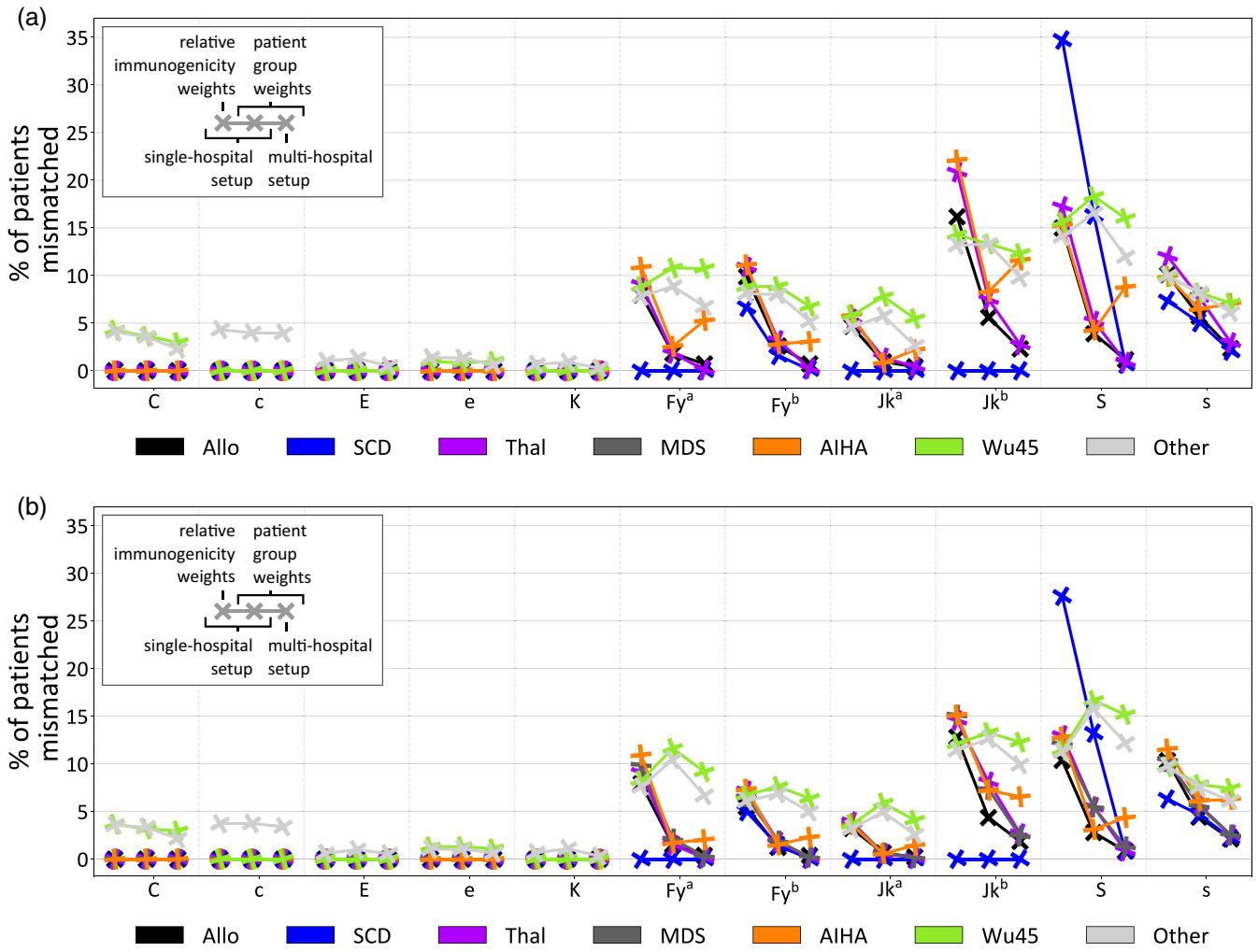

FIGURE 2 Proportion of patients per patient group who received a mismatched red blood cell product. Matching is performed using relative immunogenicity weights (Supplement A, Table S1) or patient group-specific weights (Supplement A, Table S4), either in a single- or multi-hospital setup. In all three scenarios depicted, mismatching was not permitted for matches marked with a × in Table 1. (a) Regional hospitals. (b) University hospitals. AIHA, autoimmune haemolytic anaemia; MDS, myelodysplastic syndrome; SCD, sickle cell anaemia; Thal, thalassemia; Wu45, women aged below 45 years.

each group and then examine the benefits of integrating a distribution centre in the multi-hospital simulation setup.

Impact of patient group-specific weights in the single-hospital scenario

Figure 2 shows the proportion of patients per patient group that is mismatched on each of the minor antigens considered, displayed in sets of three data points. The first data point is the original MINRAR model in a single-hospital setup, and the other two data points represent the new model with patient group-specific mismatch weights, in a single-hospital (second point) or multiple-hospital (third point) setup. First, the effects of employing patient group-specific mismatch weights are compared in the single-hospital setup.

Figure 2 reveals a substantial number of mismatches for antigens Fy^a, Fy^b, Jk^a, Jk^b, S and s when only applying relative immunogenicity weights. Upon implementing patient group-specific weights, mismatches for patient groups Allo, SCD, Thal, MDS and AIHA are reduced by 65% and 62% for the regional and university hospital, respectively, reducing the number of expected alloimmunizations in these groups by 75% in both hospitals. However, an inverse effect is noted for the larger patient groups Wu45 and Other, which together constitute 94% and 75% of the regional and university hospital patient population, respectively. Application of patient group-specific weights led to a 4% and 17% increase in mismatches and consequently 14% and 35% increase in the number of expected alloimmunizations, in the regional and university hospitals, respectively. This increase is a direct result of the lower patient group-specific weights assigned to these two groups, reflecting their lower risk of mismatching in terms of both antibody formation and consequences of alloimmunization. Changes in both the number of mismatches and expected alloimmunizations as an effect of patient group directed matching, relative to matching based on only relative immunogenicity, are

TABLE 2 Changes (%) in the total number of mismatches and expected alloimmunizations per patient group and hospital type when comparing outcomes from patient group-specific weights to outcomes from relative immunogenicity weights.

		Allo	SCD	Thal	MDS	AIHA	Wu45	Other	Total
Regional	Mismatches	−67.69	−52.83	−64.56	-	−66.34	+4.59	+4.00	−0.13
	Alloimmunizations	−76.51	−56.67	−74.60	-	−78.04	+17.06	+14.26	+11.22
University	Mismatches	−70.15	−50.23	−59.97	−60.16	−67.00	+18.80	+16.32	−1.35
	Alloimmunizations	−78.34	−54.94	−76.98	−74.18	−81.16	+34.58	+35.17	+18.77

Abbreviations: AIHA, autoimmune haemolytic anaemia; MDS, myelodysplastic syndrome; SCD, sickle cell anaemia; Thal, thalassemia; Wu45, women aged below 45 years.

TABLE 3 Changes (%) in the total number of mismatches and expected alloimmunizations per patient group and hospital type when comparing outcomes from where the distribution centres are informed about elective transfusions in each of the connected hospitals included in the multi-hospital setup to outcomes from the single-hospital setup.

		Allo	SCD	Thal	MDS	AIHA	Wu45	Other	Total
Regional	Mismatches	−63.32	−87.50	−72.46	-	+46.14	−14.34	−31.50	−30.76
	Alloimmunizations	−62.91	−95.51	−79.69	-	+94.72	−16.74	−42.39	−41.29
University	Mismatches	−61.09	−84.39	−71.90	−69.63	+13.32	−13.12	−27.69	−29.01
	Alloimmunizations	−69.31	−94.60	−83.94	−78.50	+54.11	−20.51	−40.52	−39.48

Abbreviations: AIHA, autoimmune haemolytic anaemia; MDS, myelodysplastic syndrome; SCD, sickle cell anaemia; Thal, thalassemia; Wu45, women aged below 45 years.

displayed in Table 2. For both Table 2 and Table 3, the number of expected alloimmunizations was estimated by multiplying the number of mismatches by the probability of alloimmunization after receiving two mismatching units [8].

Impact of integrating a distribution centre: The multi-hospital scenario

In Figure 2, the third data point within each set represents the relative number of mismatches in the multi-hospital setup, still using patient group-specific weights for mismatching. Comparing these values to the second data point, representing patient group directed matching in the single-hospital setup, highlights the benefits of informing a distribution centre about blood to be reserved for elective transfusions.

As shown in Table 3, the decrease in the number of mismatches is 61%–87% in the patient groups with highest risk (Allo, SCD, Thal and MDS), leading to an expected decrease of alloimmunizations by 70%–95%. The only increase in mismatches occurs for AIHA patients, being a direct result of all requests for this group becoming known on the day of transfusion. Nevertheless, the overall number of mismatches decreases by 29%–31% and the subsequent number of expected alloimmunizations by 39%–41%. In Supplement H, the change in mismatches between the three scenarios is visualized. Changes in the absolute number of mismatches per year are shown in Supplement H, Figure S5.

Table 4 shows a few simulation characteristics for all patient groups considered in single- and multi-hospital scenarios. It demonstrates that shortages for nearly all patient groups decline significantly when the individual hospitals propagate patient requests to the distribution centre, underlining the benefit of informing distribution centres about the blood required for elective transfusions.

For SCD patients, the proportion of shortages in single-hospital simulations is relatively high compared with other patient groups for both university and regional hospitals. Given that for SCD patients mismatching is not allowed for most minor antigens (Table 1), finding compatible products in a single hospital's inventory proves to be challenging. Consequently, forwarding these requests to the distribution centre is a successful strategy for preventing shortages.

The AIHA patient group suffers a slight increase in the number of shortages for both hospital types. This is likely due to the assumption that these patients' requests cannot be planned in advance and a compatible product can only be found if already in stock. As the distribution centre aims to reserve products with high usability for allocation to hospitals in need, fewer antigen-negative units are stored in hospital inventories, increasing the number of shortages for requests that become known on the day of use. A similar effect can be observed for the Wu45 patient group, as a result of our assumption that 50% of these transfusions are non-elective.

DISCUSSION

In this study, we investigated how the MINRAR model, as proposed by Van de Weem et al. [18], can be extended by applying patient group-specific mismatch weights to minimize the risk of alloimmunization for groups requiring extensively matched RBC products. We used empirical data on the relative immunogenicity, combined with a classification of antigen and patient group importance, to establish patient group-specific mismatch weights. To assess the effectiveness of the

TABLE 4 Number of shortages and units reserved at the distribution centre for various patient groups, as a percentage of the total number of requests for patients of that group (95% CI).

	Regional hospital				University hospital			
	Shortages in single-hospital simulations		Shortages in multi-hospital simulations	Units reserved at distribution centre	Shortages in single-hospital simulations		Shortages in multi-hospital simulations	Units reserved at distribution centre
Allo	1.17	(0.85–1.49)	0.52 (0.16–0.89)	85.72 (84.17–87.27)	0.25	(0.00–0.54)	0.06 (0.00–0.13)	84.52 (83.66–85.39)
SCD	14.34	(10.62–18.05)	0.12 (0.00–0.38)	99.19 (98.48–99.91)	13.19	(12.02–14.35)	0.15 (0.02–0.28)	99.05 (98.63–99.46)
Thal	1.78	(0.00–3.87)	0.00 (0.00–0.00)	99.07 (98.45–99.69)	0.42	(0.13–0.71)	0.00 (0.00–0.00)	99.05 (98.8–99.30)
MDS	-	-	- -	- -	0.36	(0.06–0.67)	0.02 (0.00–0.08)	98.75 (98.64–98.86)
AIHA	2.16	(0.17–4.15)	2.51 (1.74–3.27)	0.00 (0.00–0.00)	0.43	(0.02–0.84)	0.75 (0.44–1.07)	0.00 (0.00–0.00)
Wu45	0.11	(0.00–0.32)	0.46 (0.21–0.70)	47.15 (45.15–49.16)	0.00	(0.00–0.00)	0.07 (0.04–0.11)	46.31 (45.38–47.24)
Other	0.14	(0.00–0.29)	0.00 (0.00–0.00)	81.41 (81.03–81.79)	0.00	(0.00–0.00)	0.00 (0.00–0.00)	79.71 (79.15–80.27)
Total	0.33	(0.19–0.48)	0.08 (0.06–0.10)	78.79 (78.3–79.27)	0.83	(0.78–0.89)	0.04 (0.03–0.05)	77.73 (77.39–78.08)

Note: A shortage occurs whenever there is no product available that is compatible on the major antigens A, B and D or on any of the antigens marked with a × in Table 1, for at least one of a patient's requested units. Units that were reserved at distribution centre indicate that for some patient, a better match was available at the distribution centre than in the hospital's inventory, and the product was therefore reserved to be transported to that hospital.

Abbreviations: AIHA, autoimmune haemolytic anaemia; MDS, myelodysplastic syndrome; SCD, sickle cell anaemia; Thal, thalassemia; Wu45, women aged below 45 years.

proposed weights in reducing mismatches for the selected patient groups, multiple 1-year simulations were conducted. The outcomes of these simulations should not be considered practical advice or a prediction of future RBC matching in the blood supply chain. Instead, our objective is to gain insights and illustrate opportunities for RBC matching in a scenario where both donors and patients are extensively typed for clinically relevant minor antigens.

For the single-hospital simulation setup, our method demonstrated an effective reduction in the number of mismatches for all high-risk patient groups considered, mainly on the antigens that either come with a high probability of alloimmunization or where alloimmunization has the most severe consequences. As a result of implementing a patient group directed approach, expected alloimmunization can be reduced by 75% for these groups. However, this benefit leads to an increase of alloimmunization incidence by 14%–35% for patients for whom the effects of mismatching are assumed to be less impactful, being the patient groups Wu45 (women aged below 45 year) and Other (patients not belonging to any of the specific patient groups).

We investigated to what extent the matching quality improves for various patient groups when information on requested products is propagated to the distribution centre and incorporated into supply decisions for specific hospitals. For the majority of patient groups, this results in an increased number of antigens that could be matched compatibly. Additionally, the number of shortages can be significantly reduced, in particular for the SCD patient group, as most minor antigens considered are not allowed to be mismatched for these patients. Note that in reality, a shortage does not imply that a patient will receive either an incompatible product or no product at all; hospital staff will make sure to obtain a compatible product, by requesting an emergency delivery of a compatible product from the distribution centre. Nonetheless, it is highly desirable to avoid such measures to provide patients with appropriate blood products. The observed effects of information sharing on the reduction of both mismatches and shortages can be used by blood supply services to drive changes in information sharing within the supply chain design.

It is crucial to emphasize that the results and improvements as demonstrated by our simulations are confined within the specific parameters and assumptions decided for this study. As each of these decisions stems from simulations being an approximate representation of reality, both necessary and unavoidable simplifications were incorporated in the simulations. These assumptions impact the way model outputs should be interpreted with respect to the real world, rather than their potential for practical applicability. First, the model optimizes matching over a day, as if all requests for a given day were known and could be optimized together. In reality, orders may come in one after the other, and units already given are not available anymore. Second, the data used to represent patients and supplied RBC products are limited. Data on patient group distributions were based only on two hospitals, and actual lead times for requests likely exhibit greater variation than included in our simulations. In addition, for patient groups SCD, Thal, AIHA and MDS, we assumed a demand of two units for each request, whereas in reality, there is more variation in the number of units requested. Finally, we did not account for units

that are requested for transfusion but are returned to hospital stock, which is quite common in practice. Particularly for patients undergoing surgery, a surplus of blood products is typically reserved for use only in cases of excessive blood loss. Unused units will return to inventory and a foreseen mismatching unit will in fact not result in a mismatch, indicating that the estimated number of mismatches is an overestimation. However, the risk of outdating will increase when issued units are (repeatedly) not transfused. Neither of these effects is accounted for in our model and will be a topic for future research.

In this study, we considered patient group-specific mismatch weights, which are calculated as the product of three elements: relative immunogenicity, antigen weight and patient group weight (as discussed in Supplement A). It is important to acknowledge that only one of these elements (immunogenicity) is obtained from empirical experiments and that the other two rely on expert opinion. Future research might aim to investigate and improve these estimates (and weights). Moreover, the model's second objective function balances the number of mismatches, minor antigen substitution, the age of the issued products and their usability. The overall matching quality is likely to benefit from more research on how to best balance these objectives.

More research is necessary to support steps towards potential implementation of (automated) extended matching in hospital practice. In an ideal world, information regarding RBCs is included in the planning for the distribution of all RBCs throughout the supply chain, as soon as it becomes available. In a future where all donors have been typed on all clinically relevant antigens and information on RBC requests is shared immediately with the blood bank, it may even become possible to guide donor invitations to provide the best quality blood products to all patients.

ACKNOWLEDGEMENTS

The work presented in this paper is inspired by the work performed by R.H.G.W. for his master's degree in Computing Science at Utrecht University (the Netherlands) under supervision of H.H. and M.P.J. This work was established as part of the Stichting Sanquin Bloedvoorziening grant PPOC20-05/L2490. The work of F.B.O. was funded by the NIHR under an Artificial Intelligence in Health and Care Award (AI Award 02331) and through the NIHR BTRU in Donor Health and Behaviour (NIHR203337) and the NIHR Cambridge BRC (BRC-1215-20014; NIHR203312).

M.L.W. wrote the initial draft for this article in collaboration with M.P.J; data on donor and patient distributions were obtained by J.S.L. All authors contributed to, revised and approved the final manuscript. The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The simulation code is available from https://github.com/Sanquin/blood_matching.

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REFERENCES

1. Stichting Sanquin Bloedvoorziening. Stichting Sanquin Bloedvoorziening Jaarverslag 2021. [cited 23 Aug 2023]. Available from: https://www.sanquin.nl/binaries/content/assets/sanquinnl/over-sanquin/pers--actueel/jaarverslagen/stichting-sanquinbloedvoorziening_jaarverslag-2021.pdf
2. International Society of Blood Transfusion Working Party on Red Cell Immunogenetics and Blood Group Terminology. Classifications in Blood Group Terminology: Systems, Collections, 700 Series and 901 Series. [cited 23 Aug 2023]. Available from: https://www.isbtweb.org/isbt-working-parties/rcibgt/blood-group-terminology.html
3. Federatie Medisch Specialisten. Bloedtransfusiebeleid. [cited 23 Aug 2023]. Available from: https://richtlijnendatabase.nl/richtlijn/bloedtransfusiebeleid/
4. van de Watering L, Hermans J, Witvliet M, Versteegh M, Brand A. HLA and RBC immunization after filtered and buffy coat-depleted blood transfusion in cardiac surgery: a randomized controlled trial. Transfusion. 2003;43:765–71.
5. Schonewille H, Honohan Á, van der Watering LMG, Hudig F, te Boekhorst PA, Koopman-van Gemert AWMM, et al. Incidence of alloantibody formation after ABO-D or extended matched red blood cell transfusions: a randomized trial (MATCH study). Transfusion. 2016;56:311–20.
6. Zalpuri S, Zwaginga JJ, Le Cessie S, Elshuis J, Schonewille H, van der Bom JG. Red-blood-cell alloimmunization and number of red-blood-cell transfusions. Vox Sang. 2012;102:144–9.
7. Higgins JM, Sloan SR. Stochastic modeling of human RBC alloimmunization: evidence for a distinct population of immunologic responders. Blood. 2008;112:2546–53.
8. Evers D, Middelburg RA, de Haas M, Zalpuri S, de Vooght KMK, van de Kerkhof D, et al. Red-blood-cell alloimmunisation in relation to antigens' exposure and their immunogenicity: a cohort study. Lancet Haematol. 2016;3:e284–92.
9. Giblett ER. A critique of the theoretical hazard of inter vs. intra-racial transfusion. Transfusion. 1961;1:233–8.
10. Tormey CA, Stack G. Immunogenicity of blood group antigens: a mathematical model corrected for antibody evanescence with exclusion of naturally occurring and pregnancy-related antibodies. Blood. 2009;114:4279–82.
11. Tormey CA, Hendrickson JE. Transfusion-related red blood cell alloantibodies: induction and consequences. Blood. 2019;133:1821–30.
12. van Gammeren AJ, van den Bos AG, Som N, Veldhoven C, Vossen RCRM, Folman CC. A national transfusion register of irregular antibodies and cross (X)-match problems: TRIX, a 10-year analysis. Transfusion. 2019;59:2559–66.
13. Gleadall NS, Veldhuisen B, Gollub J, Butterworth AS, Ord J, Penkett CJ, et al. Development and validation of a universal blood donor genotyping platform: a multinational prospective study. Blood Adv. 2020;4:3495–506.

14. Sullivan JKC, Gleadall N, Lane WJ. Blood group genotyping. Adv Mol Pathol. 2021;4:127–43.
15. Lane WJ, Westhoff CM, Gleadall NS, Aguad M, Smeland-Wagman R, Vege S, et al. Automated typing of red blood cell and platelet antigens: a whole-genome sequencing study. Lancet Haematol. 2018;5:e241–51.
16. Lane WJ, Vege S, Mah HH, Lomas-Francis C, Aguad M, Smeland-Wagman R, et al. Automated typing of red blood cell and platelet antigens from whole exome sequences. Transfusion. 2019;59:3253–63.
17. van Sambeeck JH, van der Schoot CE, van Dijk NM, Schonewille H, Janssen MP. Extended red blood cell matching for all transfusion recipients is feasible. Transfus Med. 2022;32:221–8.
18. van de Weem RH, Wemelsfelder ML, Luken JS, de Haas M, Niessen RW, van der Schoot CE, et al. Preventing alloimmunization using a new model for matching extensively typed red blood cells. Vox Sang. 2021;117:580–6.
19. Schrijver A. Theory of linear and integer programming. Chichester, England: John Wiley & Sons; 1998.
20. Python Core Team. Python: A dynamic, open source programming language. Python version 3.9.13. Python Software Foundation. [cited 2023 Aug 23]. Available from: https://www.python.org/
21. Gurobi Optimization, LLC. Gurobi optimizer reference manual. Gurobi version 10.0.2. [cited 23 Aug 2023]. Available from: https://www.gurobi.com/documentation/current/refman/detailed_release_notes_10.html
22. van Hoeven LR, Hooftman BH, Janssen MP, de Bruijne MC, de Vooght KMK, Kemper P, et al. Protocol for a national blood transfusion data warehouse from donor to recipient. BMJ Open. 2016;6:e010962.

SUPPORTING INFORMATION

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

How to cite this article: Wemelsfelder ML, van de Weem RHG, Luken JS, de Haas M, Niessen RWLM, van der Schoot CE, et al. Extensive red blood cell matching considering patient alloimmunization risk. Vox Sang. 2024;119:368–76.

Received: 25 August 2023 | Revised: 5 December 2023 | Accepted: 7 December 2023

DOI: 10.1111/vox.13580

SHORT REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

Novel regulatory variant in *ABO* intronic RUNX1 binding site inducing A_3 phenotype

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Abstract

Background and Objectives: Mixed-field agglutination in ABO phenotyping (A_3, B_3) has been linked to genetically different blood cell populations such as in chimerism, or to rare variants in either *ABO* exon 7 or regulatory regions. Clarification of such cases is challenging and would greatly benefit from sequencing technologies that allow resolving full-gene haplotypes at high resolution.

Materials and Methods: We used long-read sequencing by Oxford Nanopore Technologies to sequence the entire *ABO* gene, amplified in two overlapping long-range PCR fragments, in a blood donor presented with A_3B phenotype. Confirmation analyses were carried out by Sanger sequencing and included samples from other family members.

Results: Our data revealed a novel heterozygous g.10924C>A variant on the *ABO*A* allele located in the transcription factor binding site for RUNX1 in intron 1 (+5.8 kb site). Inheritance was shown by the results of the donor's mother, who shared the novel variant and the anti-A specific mixed-field agglutination.

Conclusion: We discovered a regulatory variant in the 8-bp RUNX1 motif of *ABO*, which extends current knowledge of three other variants affecting the same motif and also leading to A_3 or B_3 phenotypes. Overall, long-range PCR combined with nanopore sequencing proved powerful and showed great potential as an emerging strategy for resolving cases with cryptic ABO phenotypes.

Keywords

A_3 phenotype, ABO blood group, Oxford nanopore sequencing, regulatory variant, RUNX1 transcription factor

Highlights

- Novel *ABO* intronic variant in the regulatory +5.8 kb region explains the cryptic blood donor phenotype.
- Additional evidence is obtained that disrupting the RUNX1 binding motif in this region is predictive for mixed-field A_3 and B_3 phenotypes.
- Nanopore long-read sequencing simplifies detection of regulatory variants as covering the gene's intronic and flanking regions.

INTRODUCTION

Reduced activity of the ABO glycosyltransferase protein is in the vast majority of cases caused by single nucleotide variants (SNVs) in exonic and splice site regions of the *ABO* gene [1]. However, a particular complex phenotype of reduced activity, the so-called A_3 or B_3 phenotype, which presents as a mixed-field agglutination with anti-A or anti-B, can also be caused by variation in *ABO* regulatory elements located in flanking or intronic regions of the gene [2]. Standard diagnostic workflows often fail to detect these variants because sequencing is typically limited to exons and exon–intron boundary regions. Correctly characterizing A_3 and B_3 phenotypes is made even more challenging because mixed-field agglutination can also be caused by either chimerism (i.e., the presence of allogenic blood cells as a consequence of twin pregnancy [3], recent transfusion or stem cell transplantation) or haematopoietic mosaicism [4]. To unambiguously resolve such cases, additional laborious methods such as digital PCR (dPCR) or sequencing to a high read-depth using short- or long-read sequencing are needed.

Latest third-generation long-read sequencing by Oxford Nanopore Technologies (ONT) has the potential to resolve these complex weak ABO phenotypes. In combination with long-range PCR [5], this method enables the generation of fully resolved haplotypes of the entire gene including flanking regions. Additionally, high read-depth obtained by amplicon sequencing allows identifying cases of chimerism or mosaicism by facilitating variant calling in subclonal cell populations. Here, we describe the case of a 23-year-old female blood donor presenting with an A_3B phenotype. By sequencing the entire *ABO* gene with ONT, we found an unknown SNV affecting the RUNX1 motif in the regulatory 5.8 kb site in *ABO* intron 1. Causality for the observed phenotype was corroborated by additional analyses in the index case as well as other family members.

MATERIALS AND METHODS

The ABO phenotype of the blood donor was determined by standard serological methods for ABH including gel cards (Grifols, Spain) and tube tests (Bio-Rad, Switzerland), as well as by anti-A_1 and anti-H specific agglutination (Bio-Rad Seraclone, Switzerland). Standard genotyping of main *ABO* variants was carried out by commercially available kits (Inno-train, Germany) based on PCR with sequence-specific primers (PCR-SSP). Presence of A- and B-antigens on erythrocytes was quantified on a FACSCanto II flow cytometer (BD Biosciences, Switzerland) using monoclonal IgM antibodies (anti-A and anti-B from clones BIRMA-1 and LB-2, respectively) in combination with secondary antibodies conjugated to fluorescent compound V500 (BD Biosciences, Switzerland). Potential chimerism was investigated by analysing the allelic distribution of 24 genetic variants across the genome using dPCR (Stilla, France). The entire *ABO* gene including flanking regions was amplified by two overlapping long-range PCRs (~16 and ~13 kb, respectively) with a PrimeSTAR GXL DNA

TABLE 1 Serological and genetic results of the index case, mother and brother.

	Serology/forward phenotyping					Reverse phenotyping			Genetics			Phenotype
	Anti-A	Anti-B	Anti-AB	Anti-H	Anti-A_1	A1	A2	B	O	PCR-SSP	Sequencing (ONT)	Sequencing (Sanger)	
Index case	mf	4+[a]	4+	1+	0	0	0	0	0	*A1/B*[b]	*A1*+g.10924C>A|*B.01*[c]	g.10924C/A	A_3B
Mother	mf	0	mf	2+	mf	0	0	4+	0	*A1/O.01*	n/a	g.10924C/A	A_3
Brother	0	0	0	n/a	n/a	4+	4+	4+	0	*O.01/O.01*	n/a	g.10924C/C	O

Abbreviations: mf, mixed-field; PCR-SSP, PCR with sequence-specific primers.
[a]1–4+ refers to agglutination strength.
[b]*ABO* allelic category is abbreviated, e.g. *A1* is short for *ABO*A1*.
[c]g.10924 coordinate refers to *ABO* reference sequence NG_006669.2.

polymerase (TaKaRa Bio, France). Primer sequences and detailed PCR conditions are given elsewhere [5]. The amplicons were sequenced on part of a MinION R9.4 flowcell (ONT). Both ONT sequencing and bioinformatic analyses were carried out as described recently [5]. Nanopore sequencing results were verified by Sanger sequencing of all seven *ABO* exons as well as the identified candidate region containing the RUNX1 motif in intron 1. The position frequency matrix of that motif was interrogated with JASPAR, a manually curated database for transcription factor binding site (TFBS) profiles [6]. To elucidate a somatic versus germline origin of the identified candidate variant, we extended phenotypic and genetic analyses to the donor's mother and brother, but limited Sanger sequencing to the candidate region in intron 1. All three family members gave written consent for molecular blood group analyses, which are not subject to ethical authorization in Switzerland.

RESULTS

Genotyped as *ABO*A1* and *ABO*B* by PCR-SSP, the proband's forward phenotyping showed a dual population in the agglutination with anti-A and strong reactions with anti-B and anti-AB (Table 1). Agglutination with anti-H and anti-A_1 was weak and absent, respectively. All observations were in agreement with an A_3B phenotype. Flow cytometry revealed that ~80% of erythrocytes lacked the A-antigen (Figure 1). No sign of chimerism was identified by dPCR.

To resolve the genetic basis of the observed A_3B phenotype, we sequenced the entire *ABO* gene. Read depth of nanopore sequencing exceeded 10,000x for both PCR amplicons, allowing variant calling in subclonal cell populations. Genetic variation in the overlapping region of PCR amplicons enabled read-based phasing of all genetic variants across the entire gene. As expected, one of the two *ABO* haplotypes corresponded to an *ABO*B* allele, comprising all seven *ABO*B.01* defining exonic SNVs. They were all heterozygous. The second haplotype was identified as an *ABO*A1* allele. No exonic variant was found that would explain the mixed-field agglutination. However, we discovered on this haplotype a novel C>A variant at NG_006669.2:g.10924 (alternatively, NM_020469.3:c.28+5871C>A) located in a known TFBS for RUNX1 in the +5.8 kb site of intron 1 (Figure 2) [7]. In JASPAR, the affected C nucleotide and the adjacent downstream C were the most conserved positions within the RUNX1 motif.

To further exclude the non-inherited phenomena of chimerism and mosaicism as a possible explanation for the A_3B phenotype, we also analysed the proband's mother and brother. The mother was genotyped *ABO*A1/ABO*O.01* by PCR-SSP, who also showed a mixed-field agglutination with anti-A (Table 1). Compared to her daughter, she had higher proportion of erythrocytes presenting A-antigen (~35%, Figure 1). This is in line with previous observations that the *in-trans* allele impacts the expression level of the weak allele [8, 9]. Sanger sequencing confirmed the heterozygous presence of the novel variant NG_006669.2:g.10924C>A in the RUNX1 binding site. As expected, the brother, who was genotyped as *ABO*O.01/ABO*O.01*, did not show any variation in the RUNX1 motif (Table 1).

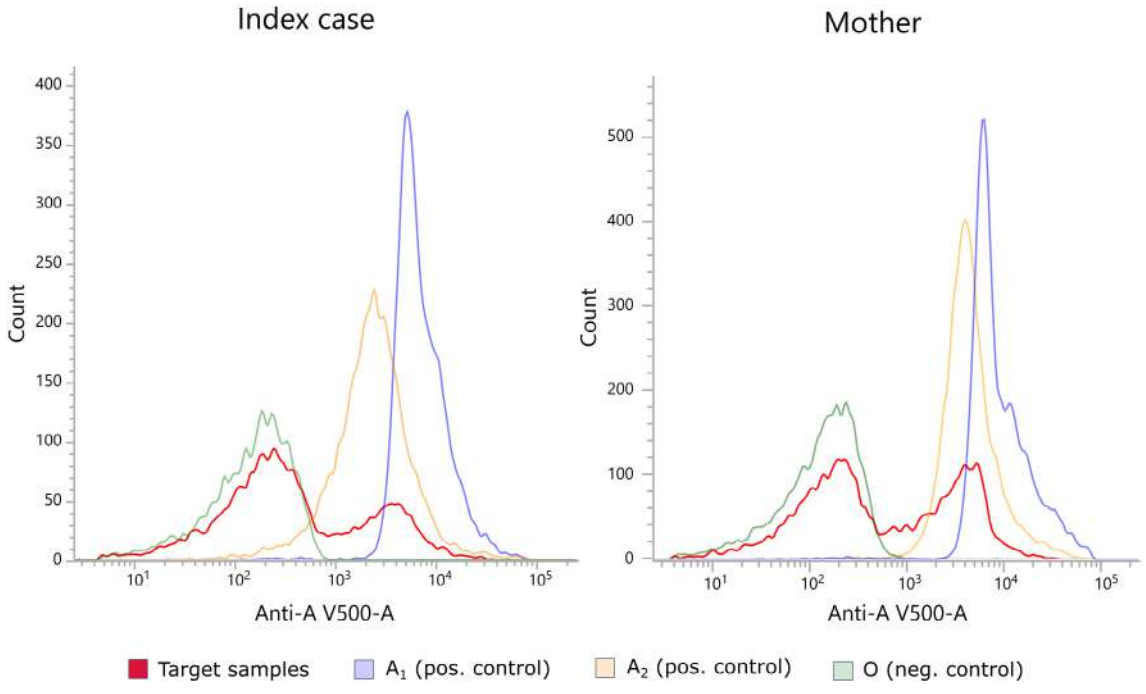

FIGURE 1 Cell counts by flow cytometry according to presence of A-antigen. Histograms show counts of red blood cells with anti-A V500-derived fluorescence on a logarithmic scale. The red line refers to the index case (left) and her mother (right). Erythrocytes from blood group A_1 (blue), A_2 (orange) and O (green) serve as positive and negative controls, respectively. The dual population of cells point to ~80% of cells lacking the A-antigen for the proband and ~65% for her mother.

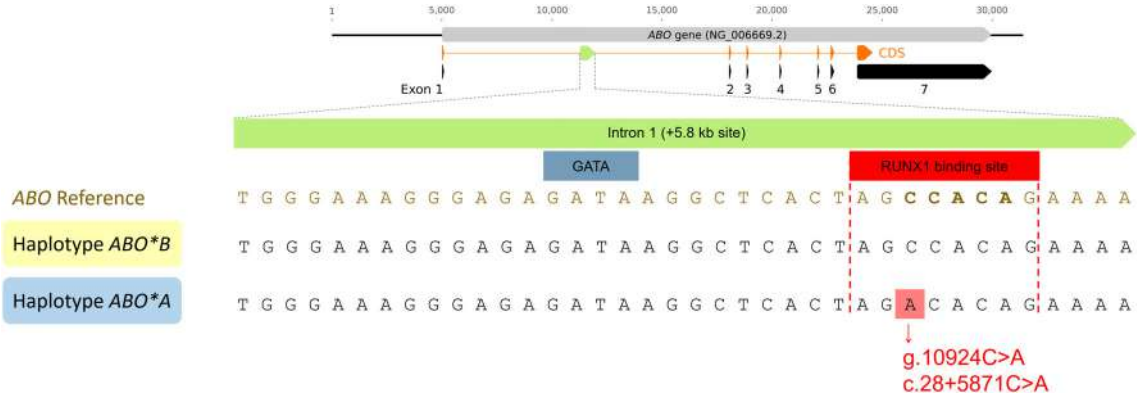

FIGURE 2 Alignment of *ABO* reference sequence (NG_006669.2) and nanopore-based maternal (blue) and paternal (yellow) haplotype sequences of index case over the regulatory 5.8 kb site in *ABO* intron 1. Overall genetic structure of *ABO* is provided above the alignment. The coding DNA sequence (CDS) is shown in orange. The novel regulatory variant located in the RUNX1 binding site (NG_006669.2:g.10924C>A; NM_020469.3:c.28+5871C>A) on the *ABO*A1* haplotype is highlighted with a red box. Nucleotides at the core motif for RUNX1 binding [7] (CCACA) are highlighted in bold on the reference sequence.

TABLE 2 Overview of reported functional consequences of genetic variation within and in close proximity to the *ABO* RUNX1-binding site.

RUNX1 motif position[a]	Gene position[b]	Variation	Phenotypic consequence	*N*[c] (ethnicity)	Reference
(a) Within RUNX1-binding site					
Pos. 1	g.10922A	-	-	-	-
Pos. 2	g.10923G	-	-	-	-
Pos. 3*	g.10924C	C>A	A_3	2 (European)	Current study
Pos. 4*	g.10925C	C>T	B_3 or B_w	3 (Chinese)	Ying et al. [11]
				7 (Koreans)	Yu et al. [12]
Pos. 5*	g.10926A	A>C	B_3	1 (n/a)	Hult et al. [9]
Pos. 6*	g.10927C	-	-	-	-
Pos. 7*	g.10928A	A>G	B_3	4 (n/a)	Hult et al. [9]
Pos. 8	g.10929G	G>A	n/a	1 (n/a)	dbSNP: rs1834858399
Pos. 1–8	Entire motif absent	23 bp-del	A_m	2 (Japanese)	Takahashi et al. [15]
		3 kb-del	B_m	1 (Japanese)	Sano et al. [14]
		5.8 kb-del	B_m	111 (Japanese)	Sano et al. [10]
		5.9 kb-del	A_x	1 (Taiwanese)	Wu et al. [16]
(b) In proximity to RUNX1-binding site					
	g.10930A	A>G	A_3	3 (Japanese)	Takahashi et al. [13]
		delAAAA	A_3	3 (Thai)	Hult et al. [9]

Note: The RUNX1 transcription factor binding site covers positions 10,922–10,929 of the *ABO* reference sequence NG_006669.2. For a complete overview, all positions within the motif are listed, regardless of whether genetic variation has been described. All single nucleotide variants listed seem to preferentially cause phenotypes of mixed-field agglutination (A_3, B_3). Deletion of the entire motif results in A_x, A_m or B_m phenotypes, in which none of the respective antigens is detected on erythrocytes.

[a]All positions of the *ABO* RUNX1 binding site are listed. Nucleotides at the core consensus sequence [7] (CCACA) are highlighted by asterisks (*).

[b]Gene position refers to NG_006669.2.

[c]Number of reported cases.

DISCUSSION

We resolved the genetic basis of an elusive A_3 phenotype using latest third-generation long-read sequencing. In light of the diverse sources for phenotypes of mixed-field agglutination, including genetic variants in exonic regions, regulatory elements and subclonal cell populations, we employed nanopore sequencing as our method of choice. In combination with long-range PCR, this sequencing technology allowed us to comprehensively investigate the complete *ABO* gene at high read-depth as well as constructing entire *ABO* haplotypes. Our approach revealed a novel regulatory variant in the RUNX1 binding site of the *ABO* intron 1 underlying the observed A_3 phenotype.

Typically, assessing causality for an expression-linked variant would require additional experiments such as reporter gene assays or electrophoretic mobility shift assays [10]. However, in this particular case, the available evidence strongly supports the causal association. First, the presence of genetically different cell populations was excluded by dPCR and by the observed heritability of the cryptic phenotype. Consequently, additional analyses such as identifying variants in subclonal cell populations were no longer necessary. Second, besides the *ABO*B.01* defining SNVs, no variants were detected in the coding or splice site regions of the *ABO* gene. Third, the identified intronic C>A variant is exceedingly rare based on its absence in large databases like dbSNP, lied on the haplotype representing an *ABO*A1* allele and was inherited along with the A_3 phenotype from the mother. Finally, and most convincingly, three SNVs targeting the core consensus sequence [7] (5′-CCACA-3′, Figure 2) of the 8-bp RUNX1 binding site had already been reported previously and had experimentally been shown to be causative for A_3 and B_3 phenotypes (Table 2a) [9, 11, 12]. Although our understanding of the mutational consequences at positions outside the core element remains limited (Table 2a), it is noteworthy that variants in the direct vicinity have been reported to cause the same phenotype (Table 2b) [9, 13]. Interestingly, the deletion of the entire RUNX1 motif results in stronger suppression of ABO glycosyltransferase activity because induced A_x, A_m or B_m phenotypes (Table 2a) [10, 14–16] are characterized by (nearly) complete absence of the respective A or B-antigen on erythrocytes. Other cell types, however, are not affected, as this regulatory motif acts in an erythroid-specific manner. Accordingly, there is usually no formation of isoagglutinins in these phenotypes.

Overall, long-read sequencing greatly simplified the discovery of the causal variant of the observed A_3 phenotype because it enabled the efficient investigation of the complete *ABO* gene, including regulatory sites in introns and flanking regions. Such regulatory regions may often be either unknown or overlooked, and consequently not covered by standard approaches such as Sanger sequencing. Of note, variants affecting the RUNX1 motif in the *ABO* gene had so far almost exclusively been reported from East Asian populations (Table 2) and were never transferred to public databases for blood group alleles [1] or for clinically relevant variant data (e.g., ClinVar). Lack of submission to appropriate databases unfortunately hampers knowledge dissemination and may foster incomprehensive investigation of such weak or cryptic phenotypes.

In summary, we detected a novel regulatory variant in the *ABO* RUNX1 binding site in intron 1 by ONT sequencing. The resulting A_3 phenotype adds to the growing knowledge on mutational consequences in this regulatory element. More broadly, long-range PCR combined with long-read sequencing represents a promising strategy to generally resolve cases with cryptic ABO phenotypes.

ACKNOWLEDGEMENTS

We thank the proband's mother and brother for their willingness to donate blood for research purpose. This work was financially supported by the Blood Transfusion Service Zurich SRC (Switzerland).

S.M. and M.P.M.-G. designed the study, supervised the data analysis and reviewed and edited the manuscript. G.A.T. and M.G. performed the experiments, analysed the data and wrote the manuscript. S.S., E.M, E.G., L.S., Y.M., N.T. and K.N. contributed to experiments. C.E. and B.M.F. contributed to the design of the study and facilitated sample collection. All authors read and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The identified variant NG_006669.2:g.10924C>A, causative for the observed ABO blood group phenotype A3, has been submitted to ClinVar (accession number VCV001707607.1). A 402 bp sequence region around this variant in the +5.8 kb site of ABO intron 1 has been submitted to NCBI GenBank (accession number OP479978). The entire haplotype sequence of the ABO*A3 allele is also available from NCBI GenBank (accession number OR905580).

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REFERENCES

1. Red Cell Immunogenetics and Blood Group Terminology (ISBT). 2023. Available from: http://www.isbtweb.org/isbt-working-parties/rcibgt/blood-group-allele-tables.html. Last accessed 22 Dec 2023.
2. Kominato Y, Sano R, Takahashi Y, Hayakawa A, Ogasawara K. Human ABO gene transcriptional regulation. Transfusion. 2020;60:860–9.
3. van Dijk BA, Boomsma DI, de Man AJ. Blood group chimerism in human multiple births is not rare. Am J Med Genet. 1996;61:264–8.
4. Borley R, Hsu TC, Sawitsky A, Johnson CL, Marsh WL. Mosaicism of red cell ABO type without recognizable cause. Rev Fr Transfus Immunohematol. 1980;23:299–304.
5. Gueuning M, Thun GA, Wittig M, Galati AL, Meyer S, Trost N, et al. Haplotype sequence collection of ABO blood group alleles by long-read sequencing reveals putative A1-diagnostic variants. Blood Adv. 2023;7:878–92.
6. Rauluseviciute I, Riudavets-Puig R, Blanc-Mathieu R, Castro-Mondragon JA, Ferenc K, Kumar V, et al. JASPAR 2024: 20th anniversary of the open-access database of transcription factor binding profiles. Nucleic Acids Res. 2023;gkad1059. https://doi.org/10.1093/nar/gkad1059
7. Bowers SR, Calero-Nieto FJ, Valeaux S, Fernandez-Fuentes N, Cockerill PN. Runx1 binds as a dimeric complex to overlapping Runx1 sites within a palindromic element in the human GM-CSF enhancer. Nucleic Acids Res. 2010;38:6124–34.
8. Hult AK, Olsson ML. Many genetically defined ABO subgroups exhibit characteristic flow cytometric patterns. Transfusion. 2010;50:308–23.
9. Hult AK, Hellberg A, Hosseini-Maaf B, Pisacka M, Hustinx H, Lejon-Crottet S, et al. Disrupted RUNX1 motifs in the ABO gene explain samples with A3 and B3 phenotypes. Vox Sang. 2020;115(S1):15.
10. Sano R, Nakajima T, Takahashi K, Kubo R, Kominato Y, Tsukada J, et al. Expression of ABO blood-group genes is dependent upon an erythroid cell-specific regulatory element that is deleted in persons with the B(m) phenotype. Blood. 2012;119:5301–10.

11. Ying Y, Hong X, Xu X, Ma K, He J, Zhu F. A novel mutation +5904 C>T of RUNX1 site in the erythroid cell-specific regulatory element decreases the ABO antigen expression in Chinese population. Vox Sang. 2018;113:594–600.
12. Yu H, Kim TY, Moon SJ, Chung YN, Yoo HJ, Kim JH, et al. Sequence variants in the proximal promoter and +5.8-kb site of ABO in Koreans with weak B phenotypes. Vox Sang. 2022;117:442–6.
13. Takahashi Y, Isa K, Sano R, Nakajima T, Kubo R, Takahashi K, et al. Presence of nucleotide substitutions in transcriptional regulatory elements such as the erythroid cell-specific enhancer-like element and the ABO promoter in individuals with phenotypes A3 and B3, respectively. Vox Sang. 2014;107:171–80.
14. Sano R, Nogawa M, Nakajima T, Takahashi Y, Takahashi K, Kubo R, et al. Blood group B gene is barely expressed in in vitro erythroid culture of Bm-derived CD34+ cells without an erythroid cell-specific regulatory element. Vox Sang. 2015;108:302–9.
15. Takahashi Y, Isa K, Sano R, Nakajima T, Kubo R, Takahashi K, et al. Deletion of the RUNX1 binding site in the erythroid cell-specific regulatory element of the ABO gene in two individuals with the Am phenotype. Vox Sang. 2014;106:167–75.
16. Wu PC, Lin YH, Tsai LF, Chen MH, Chen PL, Pai SC. ABO genotyping with next-generation sequencing to resolve heterogeneity in donors with serology discrepancies. Transfusion. 2018;58:2232–42.

How to cite this article: Thun GA, Gueuning M, Sigurdardottir S, Meyer E, Gourri E, Schneider L, et al. Novel regulatory variant in *ABO* intronic RUNX1 binding site inducing A_3 phenotype. Vox Sang. 2024;119:377–82.

Received: 5 December 2023 | Revised: 31 December 2023 | Accepted: 2 January 2024

DOI: 10.1111/vox.13591

SHORT REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

Novel missense mutation c.797T>C (p.Met266Thr) gives rise to the rare B(A) phenotype in a Chinese family

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Funding information
The authors received no specific funding for this work.

Abstract

Background and Objectives: B(A) phenotype is usually formed by nucleotide mutations in the *ABO*B.01* allele, with their products exhibiting glycosyltransferases (GTs) A and B overlapping functionality. We herein report a *B(A)* allele found in a Chinese family.

Materials and Methods: The entire ABO genes of the probands, including flanking regulatory regions, were sequenced through PacBio third-generation long-read single-molecule real-time sequencing. 3D molecular models of the wild-type and mutant GTB were generated using the DynaMut web server. The effect of the mutation on the enzyme function was predicted by PROVEAN and PolyPhen2. The predictions of stability changes were performed using DynaMut and SNPeffect.

Results: Based on serological and sequencing features, we concluded the two probands as possible cases of the B(A) phenotype. Crystallization analysis showed that Thr266 substitution does not disrupt the hydrogen bonds. However, some changes in interatomic contacts, such as loss of ionic interactions and hydrophobic contacts, and addition of weak hydrogen bonds, may have affected protein stability to some extent. This mutation was predicted to have a benign effect on enzyme function and slightly reduce protein stability.

Conclusion: The probands had the same novel *B(A)* allele with a c.797T>C (p.Met266Thr) mutation on the *ABO*B.01* backbone.

Keywords

allele, B(A), mutation, sequencing

Highlights

- The B(A) phenotype is usually caused by nucleotide mutations in the *ABO*B.01* allele, resulting in their products having glycosyltransferase A and B overlapping functionality.
- The probands have the same *B(A)* allele with a mutation of c.797T>C (p.Met266Thr) on the *ABO*B.01* backbone.
- This allele at position 266 did not belong to glycosyltransferase A or B.

INTRODUCTION

The ABO blood group plays a vital role in blood transfusion and organ transplantation. The ABO blood group gene, located on chromosome 9, encodes different glycosyltransferases (GTs). These GTs bind N-acetylgalactosamine or D-galactose to the end of substance H, thereby forming the A or B antigen. The variations between GTA and GTB are Arg176Gly, Gly235Ser, Leu266Met and Gly268Ala. Positions

TABLE 1 Amino acid substitutions associated with the B(A) phenotype.

	Amino acid position					
Alleles	**176**	**214**	**234**	**235**	**266**	**268**
*ABO*A1.01*	**Arg**	Met	Pro	**Gly**	**Leu**	**Gly**
*ABO*B.01*	**Gly**	Met	Pro	**Ser**	**Met**	**Ala**
*ABO*BA.01/BA.03*	**Gly**	Met	Pro	**Gly**	**Met**	**Ala**
*ABO*BA.02*	**Gly**	Met	Ala	**Ser**	**Met**	**Ala**
*ABO*BA.04*	**Gly**	Val	Pro	**Ser**	**Met**	**Ala**
*ABO*BA.05*	**Gly**	Thr	Pro	**Ser**	**Met**	**Ala**
*ABO*BA.06*	**Gly**	Met	Pro	**Ser**	**Met**	**Gly**
*ABO*BA* allele in this study	**Gly**	Met	Pro	**Ser**	**Thr**	**Ala**

Note: Bold indicates amino acid substitutions at the four Positions (176, 235, 266, 268).

266 and 268 are particularly crucial in differentiating sugar specificity [1].

B(A) is a rare ABO subgroup. *B(A)* alleles are typically formed because of nucleotide mutations in the *ABO*B.01* allele, which results in their products with GTA and GTB overlapping functionality [2]. In serological tests, B(A) is easily confused with cisAB or other AB subgroups. Nevertheless, A antigen is stronger than B antigen in the cisAB phenotype, whereas B(A) is characterized by the expression of weak A antigen on type B red blood cells (RBCs) and detection of anti-A antibodies in the serum [3]. The frequency of B(A) is low among Caucasians in Europe, ranging from approximately 1 in 170,000–580,000, but it is relatively high in China (approximately 1 in 50,000–100,000) [4]. Moreover, the frequency of B(A) gradually increases from north to south China [5]. Nowadays, six *B(A)* alleles, namely *ABO*BA.01–06*, have been documented on the ISBT website (https://www.isbtweb.org/resource/001aboalleles.html, accessed 15 Nov 2023). Most common *B(A)* alleles in China are *ABO*BA.02* and *ABO*BA.04*, the latter predominately reported from north China [4].

Molecular genetic analysis revealed that at least six amino acid substitution positions are responsible for the B(A) phenotype. In addition to the four key positions (176, 235, 266, 268) corresponding to the characteristic A and B, there are two positions 214 and 234 (Table 1). The purpose of this work was to report a novel *B(A)* allele with c.797T>C (p.Met266Thr) on the *ABO*B.01* backbone in a father and his daughter belonging to a northern Chinese family and assess the possible impact of this novel mutation.

MATERIALS AND METHODS

Samples

A father (age: 57 years) with his daughter (age: 32 years) visited our blood group reference laboratory of Dalian Blood Center and requested for ABO group tests, because they were found to have an inconclusive blood typing results during their previous hospital visits. After they provided informed consent, EDTA anticoagulant blood samples were collected.

Serology for ABO grouping

ABO forward and reverse typing was performed using the saline tube method. In the forward typing test, anti-A, anti-B, anti-A,B and anti-H (monoclonal anti-A and anti-B: Changchun Brother Biotech Co, Ltd.; anti-A,B: DIAGAST; anti-H: Shanghai Hemo-pharmaceutical Biological Company) were used to detect the A, B and H antigens, respectively, on the RBCs. Reverse typing was determined with A1, B and O cells (Shanghai Hemo-pharmaceutical Biological Company) with a tube test by trained staff according to the manufacturer's instructions. Adsorption and elution were performed to confirm the presence of A antigens on RBCs with monoclonal anti-A antibody according to the standard protocol. Elution was performed using heat elution procedure.

PacBio long-read single-molecule real-time sequencing

Genomic DNA was extracted from peripheral whole blood samples using a commercially available HiPure Blood DNA Mini Kit according to the manufacturer's instructions. The entire ABO gene, including flanking regulatory regions in the three overlapping fragments (Figure S1), was amplified. The fragments overlapped more than 1 kb. Long-range PCR reaction mixtures were composed of 5 μL of 5× PrimeSTAR GXL buffer, 2 μL of dNTP mixture (2.5 mM each), 0.5 μL of PrimeSTAR GXL DNA polymerase, 0.26 μL of each PCR primer mix (100 μM), 30 ng DNA templates and DNase/RNase-free deionized water in a final reaction volume of 25 μL. The cycling program was 94°C for 2 min, followed by 26 cycles of 98°C for 12 s, 68°C for 12 min (starting from the 11th cycle, increase each cycle by 30 s) and a final extension step at 68°C for 10 min. The PCR products were used for preparing the library for PacBio sequencing.

In silico analysis

3D molecular models of the wild-type (PDB ID, 1LZ7) and mutant GTB were generated using the DynaMut web server (https://biosig.lab.uq.edu.au/dynamut/). The effect of the mutation on the enzyme function was predicted by PROVEAN v1.1.3. (http://provean.jcvi.org/index.php) and PolyPhen2 v2.2.3r406 (http://genetics.bwh.harvard.edu/pph2/). The predictions of stability changes were performed using DynaMut and SNPeffect (https://snpeffect.switchlab.org/). These tools provided the difference in the Gibbs free energy between the ΔG mutant and the ΔG wild-type protein, $\Delta\Delta G = \Delta Gm - \Delta Gw$, implying the impact of substituted mutation on the stability of a protein.

RESULTS

Serological results

Two probands were identified to have an ABO phenotype with similar unusual features. Although both A and B antigens were present, A antigen exhibited weak reactivity during agglutination tests. B antigen exhibited normal reactivity, and anti-A antibodies were noted in their serum (Table 2). According to serological characteristics, we concluded that the two probands were possible cases of the B(A) phenotype.

PacBio sequencing results

Based on the PacBio sequencing results, the ABO allele haplotype sequences of each proband were determined. In addition to the *ABO*O.01.01* and *ABO*B.01* alleles present in the father and daughter, respectively, they also carried an *ABO*B.01* allele with a missense mutation at c.797C>T. The c.797C>T mutation causes replacement of methionine by threonine at key position 266. Interestingly, threonine at this position is not associated with GTA or GTB (Table 1).

In silico analysis

In order to assess the possible impact of this novel mutation, the DynaMut platform was used to generate the mutant's structure using 3D molecular modelling [6]. The crystallization of the wild-type and mutant GTB was analysed. Figure 1b depicts the difference in vibrational entropy and interatomic interactions between the wild type and mutant. As determined by ENCoM, the Δvibrational entropy energy between the mutant and wild type was 0.398 kcal mol^{-1} K^{-1}, which indicated an increase in molecular flexibility. Differences in interatomic interactions such as hydrogen bonds and ionic interactions of the wild type and mutant are depicted in Figure 1c,d. Thr266 substitution does not disrupt the hydrogen bonds (red dotted line). However, some changes in interatomic contacts, such as loss of ionic interactions (yellow dotted line) and hydrophobic contacts (green dotted lines), and addition of weak hydrogen bonds (orange dotted lines), can affect the stability of proteins. The algorithms of PROVEAN and PolyPhen2 predicted the effect of the mutation on the enzyme function. The PROVEAN score of mutation was −0.213, which indicates that the mutation was 'neutral'. Using the PolyPhen-2 analysis, this variant was predicted to be 'benign' based on both HumDiv and HumVar datasets. DynaMut and SNPeffect were employed to calculate the effect of the mutation on protein stability. According to the predicted DynaMut ΔΔG value (−0.454 kcal mol), the mutant destabilized the protein compared with the wild type. Moreover, the mutation at position 266 resulted in a ΔΔG of 0.89 kcal mol, as estimated by the SNP effect. This indicates that the mutation slightly reduced protein stability.

DISCUSSION

GTA and GTB are very similar and have overlapping functions. GTB can synthesize blood group A antigen using the same donor and acceptor substrates as used by GTA [7]. The B(A) blood group is a rare subtype. RBCs of an individual are described as B(A) when, despite them coming from a B individual who lacks the A gene, they are weakly agglutinated by potent anti-A monoclonals. There is also anti-A in their serum that reacts with both A1 and A2 RBCs. Although the B(A) phenotype is inherited in a *cis* manner, it can be classified separately from *cis*AB phenotype due to the presence of anti-A in the serum [8]. The amount of B antigen on B(A) subtype RBCs is lower than that on normal B RBCs [9]. This may cause the H antigen intensity of subtype B(A) to be higher than that of the normal B type.

In the present study, the full-length haplotype sequences of the ABO gene in the probands with the B(A) phenotype were obtained through third-generation long-read single-molecule real-time sequencing. Two probands harboured the novel *B(A)* allele, which was different from the normal *ABO*B.01* allele only by one nucleotide substitution: c.797T>C (p.Met266Thr). This allele was neither recorded on the ISBT website nor found in the dbSNP. The daughter carried the *B* allele in *trans*, whereas the father carried *O* allele in *trans*, explaining the observed increased agglutination reactivity with anti-H (Table 2).

Different amino acids may play varied roles in the protein structure. Modelling 3D molecules and analysing variant changes in the protein structure are crucial for gaining a deeper understanding of the potential mechanisms underlying protein changes. Our 3D structural analysis revealed that the threonine-introducing mutation does not disrupt hydrogen bonds and some changes in interatomic contacts may reduce protein stability. The residue 266 occupies a position in the GTB's active site that can interact with donor-sugar residues. Met266 and Ala268 residues in GTB are both bulkier with respect to GTA, leaving GTB with a smaller and more conformationally restricted active site favouring UDP-Gal over UDP-GalNAc [10]. In this study, a hydrophobic and non-polar molecule methionine in residue 266 in the wild-type GTB was replaced by a hydrophilic and polar molecule

TABLE 2 Results of serological grouping and *ABO* gene analysis.

	RBC grouping					Serum grouping			Genotype	
Proband	-A	-B	-A_1	-AB	-H	A_1 cells	B cells	O cells	Allele 1	Allele 2
Father	1+	4+	-	4+	4+	1+	-	-	*ABO*O.01.01*	*ABO*B.01* with c.797C>T
Daughter	1+	4+	-	4+	w+	1+	-	-	*ABO*B.01*	*ABO*B.01* with c.797C>T

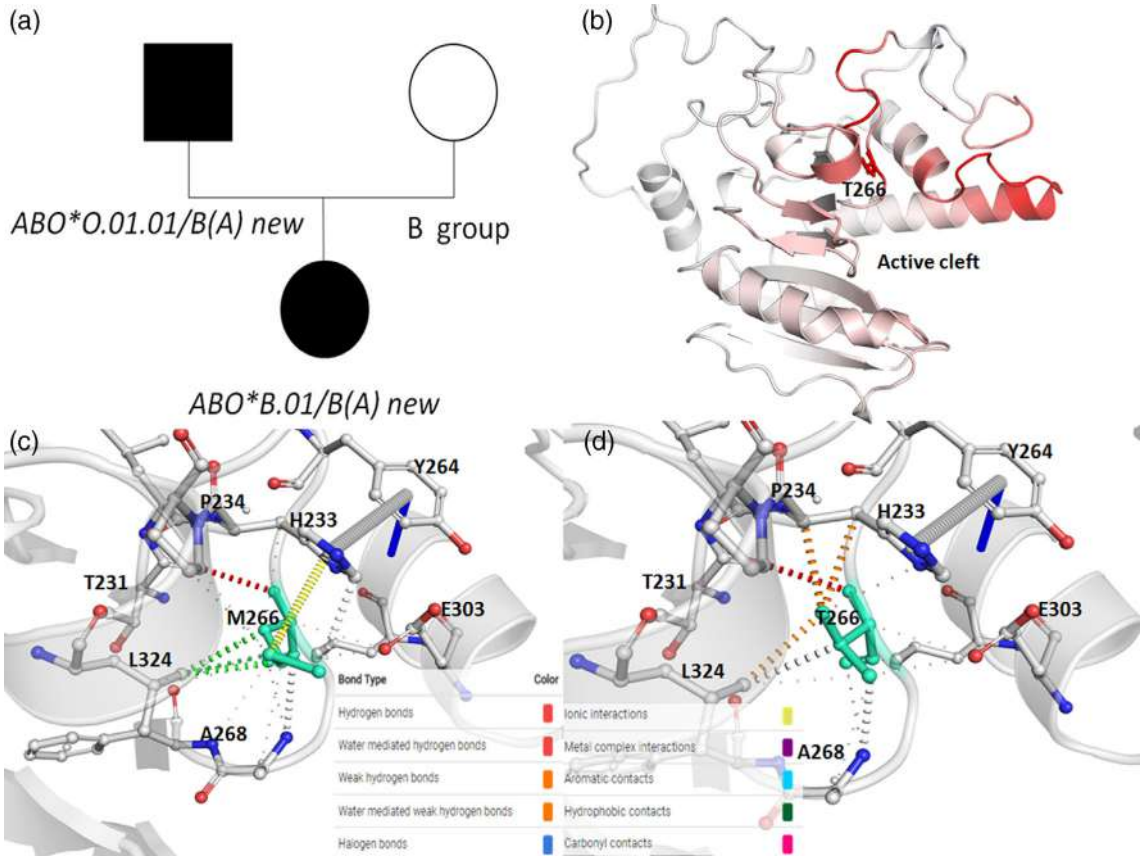

FIGURE 1 (a) Schematic diagram of the inheritance of one Chinese family. Black fill indicates the probands. (b) The structure of 3D molecular mutant and visual representation of protein flexible conformation based on the vibrational entropy difference. Amino acids coloured according to the vibrational entropy change upon mutation. Red represents a gain in flexibility of the structure. The interatomic interaction between wild-type (c) and mutant structures on GTB structure (d). Wild-type and mutant residues are depicted as light green sticks alongside the surrounding residues that are involved in any form of interaction. Thr266 substitution does not disrupt the hydrogen bonds (red dotted line). However, some changes in interatomic contacts.

threonine. Moreover, threonine is slimmer than the corresponding residue methionine in the wild type. We inferred that the substitution possibly alters the spatial structure and causes changes in the sugar donor specificity at that site to GTA, thereby giving rise to an overlapping functional transferase, as suggested by the serological findings of this study.

Another study involving the 3D spatial structure analysis of GTA and GTB confirmed that position 176 had no physical interactions with the substrates, but position 235 was in proximity, and positions 266 and 268 exhibited direct contact [10]. The mutation found in this study was at the key position 266. It causes GTB to synthesize small amounts of A antigen. Interestingly, the *ABO*B.01*, *ABO*cisAB.02* (which was first discovered in a Vietnamese family [11] and rare in the Chinese population [12], is caused by c.796A>C), and the novel *B(A)* allele found in this study (c.797T>C) only differed at position 266 (Met/Leu/Thr). However, they exhibited the phenotypes of normal B, AB_{weak} and $A_{weak}B$, respectively. This further demonstrates that the enzyme's specificity was associated with the crucial position 266. The residue 266 is positioned to contact the characteristic acetamido/hydroxyl groups and so distinguishes between UDP-GalNAc and UDP-Gal [10].

In summary, we here defined a novel *B(A)* allele in a Chinese family. c.797T>C (p.Met266Thr) mutation on the *ABO*B.01* backbone in the subjects exhibited an $A_{weak}B$ phenotype with anti-A antibodies in their serum.

ACKNOWLEDGEMENTS

S.H.Z. and X.H.L. conceptualized the study, L.N.S. conducted experiments, Y.C.Y. and Y.X.X. analysed the data, C.X.L. provided resources and L.N.S. wrote the draft.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no competing interests regarding the publication of this article.

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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REFERENCES

1. Chen Q, Li J, Xiao J, Du L, Li M, Yao G. Molecular genetic analysis and structure model of a rare B(A)02 subgroup of the ABO blood group system. Transfus Apher Sci. 2014;51:203–8.
2. Yamamoto F. A historical overview of advances in molecular genetic/genomic studies of the ABO blood group system. Glycoconj J. 2022;39:207–18.
3. Jiao LX, Zhang JY, Chen L, Yu XL, Han Y, Yang F, et al. Gene identification of rare B(A) blood group. Transfus Apher Sci. 2017;56:855–7.
4. Wang J, Wang Y, Tan J, Tan B. Serological and molecular analysis of the B(A) subtype in China – a systematic review. Transfus Clin Biol. 2023;30:443–8.
5. Liu F, Gu L. Serology and gene sequence analysis of the B(A) subtype of patients in Hunan. Transfus Apher Sci. 2022;61:103449.
6. Rodrigues CH, Pires DE, Ascher DB. DynaMut: predicting the impact of mutations on protein conformation, flexibility and stability. Nucleic Acids Res. 2018;46:W350–5.
7. Watkins WM. Monoclonal antibodies as tools in genetic studies on carbohydrate blood group antigens. J Immunogenet. 1990;17:259–76.
8. Yamamoto F, McNeill PD, Yamamoto M, Hakomori S, Harris T. Molecular genetic analysis of the ABO blood group system: 3. A(X) and B(A) alleles. Vox Sang. 1993;64:171–4.
9. Yu LC, Lee HL, Chan YS, Lin M. The molecular basis for the B(A) allele: an amino acid alteration in the human histoblood group B alpha-(1,3)-galactosyltransferase increases its intrinsic alpha-(1,3)-N-acetylgalactosaminyltransferase activity. Biochem Biophys Res Commun. 1999;262:487–93.
10. Patenaude SI, Seto NO, Borisova SN, Szpacenko A, Marcus SL, Palcic MM, et al. The structural basis for specificity in human ABO(H) blood group biosynthesis. Nat Struct Biol. 2002;9:685–90.
11. Mifsud NA, Watt JM, Condon JA, Haddad AP, Sparrow RL. A novel cis-AB variant allele arising from a nucleotide substitution A796C in the B transferase gene. Transfusion. 2000;40:1276–7.
12. Jin S, Cai X, Liu X, Wang J, Lu Q, Shen W, et al. Study on cisAB and B(A) subgroups in Shanghai blood donors, China. Chin J Blood Transfus. 2013;26:1198–201. (in Chinese).

SUPPORTING INFORMATION

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

How to cite this article: Shao L-N, Yang Y-C, Xia Y-X, Li C-X, Zhou S-H, Liang X-H. Novel missense mutation c.797T>C (p.Met266Thr) gives rise to the rare B(A) phenotype in a Chinese family. Vox Sang. 2024;119:383–7.

Received: 1 September 2023 | Revised: 7 December 2023 | Accepted: 21 December 2023

DOI: 10.1111/vox.13587

REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

End of selection criteria based on sexual orientation: An international symposium on alternatives to donation deferral

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Funding information
Canadian Blood Services MSM Research Grant Program; Federal Government (Health Canada); Provincial and Territorial Ministries of Health

Abstract

Background and Objectives: Until recently, gay, bisexual and other men who have sex with men (MSM) were deferred from donating blood for 3–12 months since the last male-to-male sexual contact. This MSM deferral has been discontinued by several high-income countries (HIC) that now perform gender-neutral donor selection.

Materials and Methods: An international symposium (held on 20-04-2023) gathered experts from seven HICs to (1) discuss how this paradigm shift might affect the mitigation strategies for transfusion-transmitted infections and (2) address the challenges related to gender-neutral donor selection.

Results: Most countries employed a similar approach for implementing a gender-neutral donor selection policy: key stakeholders were consulted; the transition was bridged by time-limited deferrals; donor compliance was monitored; and questions or remarks on anal sex and the number and/or type of sexual partners were often added. Many countries have now adopted a gender-neutral approach in which questions on pre- and post-exposure prophylaxis for human immunodeficiency virus (HIV) have been added (or retained, when already in place). Other countries used mitigation strategies, such as plasma quarantine or pathogen reduction technologies for plasma and/or platelets.

Conclusion: The experience with gender-neutral donor selection has been largely positive among the countries covered herein and seems to be acceptable to stakeholders, donors and staff. The post-implementation surveillance data collected so far appear reassuring with regards to safety, although longer observation periods are necessary. The putative risks associated with HIV antiretrovirals should be further investigated.

Highlights

- The experience with gender-neutral donor selection has been largely positive among the countries covered in this symposium: gender-neutral donor selection appears to be acceptable to stakeholders, donors and staff; and post-implementation surveillance data (while preliminary) appear reassuring regarding safety.
- Although a large proportion of men who have sex with men may remain ineligible, gender-neutral donor selection is a significant step forward in improving the inclusiveness of blood donation.
- The remaining concerns over infectious risks (e.g., residual risks of known transfusion-transmissible pathogens, new and emerging pathogens) can be addressed by combining an adequate and up-to-date donor history questionnaire, current screening, pathogen reduction technologies and active surveillance of emerging infectious agents.

INTRODUCTION

In the 1980s, the human immunodeficiency virus (HIV) epidemic severely disrupted the blood supply system. A significant number of blood recipients inadvertently acquired HIV, thus severely undermining the credibility of blood services [1]. In the absence of adequate testing, most middle- to high-income countries initially responded by permanently deferring candidate male donors who had had sex with another man since 1977 [2], because this group was disproportionately affected by HIV.

Although likely effective at the time, this deferral had a number of caveats. To begin with, all gay, bisexual and other men who have sex with men (MSM) were deferred from donating blood even though, when considered individually, many likely had a risk profile similar to that of other eligible donors in the general population. The deferral, which relied on open and accurate disclosure, was perceived as exclusionary and discriminatory by many MSM and other community groups, causing social harms (e.g., erosion of trust in the health care system) and potentially increasing the risk of non-compliance [3]. Moreover, the deferral may also have limited recipient access to safe blood products amid a dwindling blood supply, which has been further compounded by the COVID-19 pandemic [4–8]. Importantly, the introduction of sensitive serological and nucleic acid tests (NATs) for HIV has all but eliminated the risk of HIV transfusion transmission, at least outside the (now) very short window period of infection [9, 10].

To (partially) address these limitations, in the early 2010s, several countries implemented time-limited MSM deferrals policies that were progressively shortened. The risks associated with transfusion-transmitted infections (TTIs) remained stable in these countries, whether a permanent, 5-year, 12-month or 3-month deferral was in place [11–16]. This positive experience laid the foundation for more actions to improve the inclusiveness of blood donation and expand the donor base.

In 2021, the United Kingdom (UK) fully replaced the MSM time-based deferral with an evidence-based, gender-neutral donor selection policy [12, 17]. This move ensued from the For the Assessment of Individualized Risks (FAIR) review, which recommended that the MSM deferral be replaced with a new policy based on an assessment of sexual behaviours and predictors of each donor [12]. Since then, many countries have followed suit or are reassessing their own selection criteria for MSM based on FAIR.

Given this paradigm shift in blood donor selection, Héma-Québec (HQ) and Canadian Blood Services (CBS) recently convened international experts for a symposium, held on 20 April 2023, on the deferral criteria for HIV-related sexual behaviours. This event—and previous research that allowed for the progressive removal of the Canadian MSM deferral—was funded by the Public Health Agency of Canada.

The first part of the symposium discussed the experience of different countries that either implemented gender-neutral donor selection or are considering it; the second part explored the challenges associated with HIV pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP) in blood donation; and the third part discussed the strategies to alleviate the risk of TTIs amid the removal of the MSM deferral. The present article provides an overview of the presentations and discussions that took place during this event. The symposium has been recorded and is available at https://www.hema-quebec.qc.ca/diverse-et-inclusion/don-de-sang-et-diversite-sexuelle/symposium.en.html.

PART 1: INTERNATIONAL EXPERIENCE IN RELAXING THE MSM DEFERRAL

UK experience

The UK experience was described by Katy Davison. The UK pioneered the transition to a gender-neutral donor selection policy, in the wake of the recommendations that came from the FAIR review in 2020 (funded by the UK Forum) [12].

Many factors supported the FAIR review of the MSM policy in the UK. At the time, the country had an estimated rate of undetected TTIs below 1 in 1 million donations, mostly due to the very low incidence of HIV, hepatitis C virus (HCV) and hepatitis B virus (HBV) in the donor population. Moreover, the prevalence of HIV, HCV and HBV was very low in both the blood donor and the general population, and so was the number of individuals with undiagnosed HIV (even though MSM remained disproportionately affected). Although the rate of syphilis infection had increased among blood donors since 2020, this increase was less pronounced than that observed in men and women in the general population. Furthermore, the change from a 12-month to a 3-month MSM deferral had no impact on blood safety (Table 1) [12]. Lastly, community stakeholders continued lobbying in favour of more inclusive blood donation policies despite the progressive lifting of restrictions.

The FAIR review was guided by stakeholder consultations and scientific evidence. Donors, recipients, blood service staff, charities and communication staff were consulted throughout. The review synthesized and assessed evidence on the current epidemiology of blood-borne pathogens in the UK, the behaviours and predictors associated with these infections, and the psychosocial evidence around the best way to ask donors about these behaviours while maintaining a safe supply.

FAIR found strong evidence linking sex with multiple partners, anal sex, chemsex (i.e., use of drugs, such as methamphetamine to enhance a sexual experience) and history of bacterial sexually transmitted infections (STI; i.e., syphilis or gonorrhoea) with the acquisition of HIV or STI. These behaviours were shown to be reliably and accurately reported by donors, tended to cluster together. Their inclusion in the donor questionnaire was also acceptable to MSM, staff, donors and recipients. FAIR therefore recommended that all donors be asked about these behaviours irrespective of their or their partner's gender instead of asking about male-to-male sex [12]. The FAIR review considered asking donors about condom use, as there was good evidence that inconsistent condom use was associated with the acquisition of HIV and STIs. However, this possibility was discounted over concerns about the reliability and acceptability of this question to donors and staff.

The new questions, which were aligned with the FAIR recommendations, have generally been well received by the donors and staff since their implementation across the four UK blood services in June 2021 (Table 2 for the specific questions). There have been very few complaints about the new questions. Of note, the blood services emphasize the temporary nature of the deferrals (i.e., 3 months) to reassure some people who were previously eligible and are now deferred under FAIR.

While some data suggest donor deferrals have not been impacted by the policy change, the exact number of donors who are newly deferred under FAIR is unclear. However, the number of new MSM donors has clearly increased, while recently acquired infections and risky sexual behaviours among positive donors have not. There was a modest increase in donors with previously undiagnosed HBV and syphilis infections among first-time donors, but this trend started before the implementation of FAIR. Lastly, no post-transfusion viral transmissions have been reported after the implementation of FAIR.

The UK continues to assess the impact of FAIR. This assessment includes determining whether the new deferrals are understood and applied correctly. A survey will measure non-compliance among people who have donated under FAIR, and further research will examine whether the deferrals are applied appropriately, including polyamorous individuals.

The UK also plans to reassess their recommendation to remove the deferral of individuals who have partners with HIV and have undetectable viral loads while on treatment, as HIV virtually cannot be sexually transmitted by a partner with sustained undetectable viral loads [18]. However, whether this third-party information can or should be relied upon in the blood donation setting needs further consideration given that people with HIV-positive partners are deferred.

The Canadian experience

Epidemiology of HIV in Canada

Gilles Lambert presented updated data (February 2017 to February 2023) from the ENGAGE study, which informed on the epidemiology of HIV in Canada. ENGAGE is a closed prospective cohort study that enrolled 2449 sexually active MSM, including 2008 who were

TABLE 1 Timeline of changes in the blood donor selection policies of six high-income countries for gay, bisexual and other men who have sex with men.

Country	Donor selection policy	Year of implementation
United Kingdom[a]	Permanent MSM deferral	Until 2011
	12-Month MSM deferral	2011
	3-Month MSM deferral	2017
	Gender-neutral donor selection policy	2021
Canada	Permanent MSM deferral	Until 2013
	5-Year MSM deferral	2013
	12-Month MSM deferral	2016
	3-Month MSM deferral	2019
	MSM plasma quarantine programme[b]	2021
	Gender-neutral donor selection policy[c]	2022
The Netherlands	Permanent MSM deferral	Until 2015
	12-Month MSM deferral	2015
	4-Month MSM deferral	2019
	4-Month MSM deferral, except for those in a long-term mutually exclusive relationship[d]	2021
	Gender-neutral donor selection policy	2024 (planned)
France	Permanent MSM deferral	Until 2016
	12-Month MSM deferral	2016
	MSM plasma quarantine programme	2016
	4-Month MSM deferral	2020
	Removal of the MSM deferral[e]	2022
Israel	Permanent MSM deferral	Until 2016
	12-Month MSM deferral	2017
	Frozen MSM plasma quarantine program	2018
	Individualized donor risk assessment	2021
	Immediate use of donations made by MSM donors	2021
Republic of Ireland	Permanent MSM deferral	Until 2017
	12-Month MSM deferral	2017
	4-Month MSM deferral	2022
	Gender-neutral donor selection policy	2022

Abbreviation: MSM, gay, bisexual and other men who have sex with men.
[a]Of the four countries in the United Kingdom, Northern Ireland changed from a lifetime deferral to a 12-month deferral in 2016, and to a 3-month deferral in 2020. All United Kingdom transitioned to gender-neutral selection in 2021.
[b]In Canada, plasma quarantine was implemented by Canadian Blood Services as a pilot programme in two source plasma collection sites and stopped.
[c]In Quebec, the implementation included two steps: October 2021 for source plasma and December 2022 for all donors.
[d]Defined as an exclusive relationship lasting ≥12 months.
[e]France fully lifted its MSM deferral in 2022, relying on pre-existing criteria (which have been enforced for over 30 years) that defer donors with more than one sexual partner in the last 4 months and donors who are aware that their sexual partner has had one or more other sexual partner in the last 4 months.

HIV-negative. All participants lived in Toronto, Montreal or Vancouver (i.e., the three largest Canadian cities) and were recruited through respondent-driven sampling [19]. At baseline, 96.8%–99.8% (across the three cities) of participants with a confirmed HIV infection were aware of their status. Among HIV-negative participants, 76.8%–83.6% (across study cities) did not report a high-risk sexual activity with ≥1 of their last five sexual partners in the last 6 months—defined as engaging in condomless anal sex while not on PrEP with a partner of unknown HIV status or with a partner living with HIV with detectable or unknown viral loads. Among ENGAGE participants, the rate of HIV seroconversion was 0.4 per 100 person-years [19]. This rate is significantly lower than those reported in older studies of Canadian MSM, suggesting the residual risk of HIV associated with blood donations from MSM is likely decreasing.

ENGAGE explored potential blood donation deferral criteria, including some proposed by blood services operating outside Canada.

TABLE 2 Additional questions related to sexual risk behaviours in the context of individualized donor risk assessment, and associated outcomes depending on the donor's responses.

Country	Question(s) retained	Answer → outcome
Canada	• In the last 3 months, have you had a new sexual partner?	• If 'yes' → ask question on anal sex (below) • If 'no' → eligible
	• In the last 3 months, have you had more than one sexual partner?	• If 'yes' → ask question on anal sex (below) • If 'no' → eligible
	• In the last 3 months, have you had anal sex? *(only asked to those with a new partner or multiple sexual partners in the last 3 months)*	• If 'yes' → defer for 3 months • If 'no' → eligible
France	• In the last 4 months, have you or your partner had more than one sexual partner?[a]	• If 'yes' → defer for 4 months • If 'no' → eligible
Ireland	• In the last 4 months, have you had a new sexual partner or more than one sexual partner?	• If 'yes' → ask question on anal sex (below) • If 'no' → eligible
	• In the last 4 months, have you had anal sex? (*only asked to those with a new partner or multiple sexual partners in the last 4 months*)	• If 'yes' → defer for 4 months • If 'no' → eligible
Israel	NA[b]	NA[b]
The Netherlands	NA[c]	NA[c]
United Kingdom	• In the last 3 months, have you had more than one or a new sexual partner?	• If 'yes' → ask question on anal sex (below) • If 'no' → eligible
	• In the last 3 months, have you had anal sex? (*only asked to those with a new partner or multiple sexual partners in the last 3 months*)	• If 'yes' → defer for 3 months • If 'no' → eligible

Abbreviation: NA, not applicable.
[a]This criterion has been enforced in France for over 30 years (and has, therefore, not been implemented as part of individualized donor risk assessment).
[b]The questionnaire provides a list of deferral criteria, and the donors are asked not to donate if they engaged in a 'high-risk sexual relation' in the last 3 months, defined as anal sex, sex under the influence of drugs, or sex with new partner or multiple partners.
[c]The country plans to implement individualized risk assessment but has yet to decide the questions and algorithm that will be used.

The following combination resulted in a 100.0% sensitivity and a 100.0% negative predictive value (with respect to HIV seroconversion) and would defer 57% of ENGAGE participants: injection drug use OR ≥1 new anal sex partner OR ≥2 anal sex partners (in the past 6 months). The deferral rate increased to 60% after adding a criterion on PrEP use to this combination.

Deferral policies in Canada

Mindy Goldman, Sheila O'Brien and Antoine Lewin described the process, rationale and outcomes following the move from a permanent to a time-limited deferral. Like many other countries, Canada progressively relaxed its donor selection policy through increasingly shorter time-limited deferrals (Table 1). These changes had seemingly no impact on the prevalence, incidence and residual risk of HIV in the blood donor population [14]. Donor non-compliance also remained extremely low, at ∼0.2%–0.3%, as assessed by anonymous donor surveys.

In 2021, the MSM deferral was further relaxed when CBS implemented a criterion change at two source plasma collection sites. Donors registered as male were still asked whether they had had sex with another man in the last 3 months. If they responded affirmatively, they were asked whether they were in a mutually exclusive relationship and had not had a new sexual partner in the last 3 months, in which case they were eligible to donate source plasma. Their donation was quarantined until a subsequent donation tested negative. At Héma-Québec, in late 2022, a gender-neutral donor selection policy (described below) was first introduced among source plasma donors, thus allowing some MSM to donate without a quarantine scheme. This change was informed by the results of a stochastic model based on Bayesian networks and Monte Carlo simulations [20]. Specifically, the model estimated the residual risk of banking an HIV-infected source plasma donation under the assumption that (1) donations were treated with pathogen reduction technologies (PRT) and (2) the 3-month MSM deferral was withdrawn [20]. In the most likely scenario, none of the 300,000 simulated plasma pools (i.e., ∼2 billion donations) would contain a full RNA copy capable of causing an infection [20]. Source plasma donations from MSM were thus considered to pose minimal risks for the safety of the blood supply.

In 2022, the MSM deferral was fully lifted for all donations and replaced with a gender-neutral donor selection policy. This change was informed by another modelling study, in which current deferral criteria (and self-deferral) were assumed to reduce the risk of incident HIV donations by 98% [21]. Depending on the assumed HIV risk reduction (95%–99%), the residual risk of HIV with the new donation policy would range between 1 in 4.95 million and 1 in 26.5 million, and the confidence intervals (CIs) overlapped with those of the current risk (i.e., 1 in 40.2 million) [21]. The excess risk (if any) associated with a gender-neutral selection policy thus appeared minimal.

In Canada, two selection criteria have been implemented in the new gender-neutral questionnaire: (1) having a new sexual partner or

more than one sexual partner in the last 3 months and (2) having anal sex (Table 2 for the specific questions and algorithm). The optimal phrasing of the question on sexual partners was empirically validated among regular plasma donors and cis-MSM donors. Two scenarios were tested: one in which a compound question was asked, and one in which separate questions were asked (see the full publication for specific wording) [22]. Among regular plasma donors, the two scenarios had comparable (and near-perfect) sensitivities and specificities based on validation questions that included more specific questions on the type of partners and sexual activity. However, among cis-MSM, the scenario 1 question had a much lower (and unacceptable) sensitivity of 88.6%, compared with 100.0% for the scenario 2 questions [22], which were therefore implemented.

Surveillance data, donor perception and donor base

Surveillance data on the new donation policy remain preliminary, but only one HIV-positive donation has been identified at CBS since September 2022 and April 2023 (and none at HQ). CBS and HQ continue their post-implementation surveillance by monitoring and investigating the risk factors for HIV, the frequency of PEP and PrEP use, and NAT- and serology-positive donations.

The new criteria seem to be well received by the donors. The personnel have been trained, and most donors are comfortable answering the new questions. Although the question on anal sex was associated with a higher degree of discomfort, it appears as a secondary question, so that only donors with a new sexual partner or more than one sexual partner need to answer it. CBS and HQ have received few complaints since implementing these new criteria. However, the questions on PEP and PrEP use have been less well received by MSM, in part due to the apparent discordance between the donor criteria (i.e., donors on PrEP or PEP are deferred during and for 4 months after treatment) and public health messaging about the utility of these regimens in reducing the risk of HIV transmission.

Moreover, gender-neutral selection appears to have expanded the donor base, especially among MSM. The deferral rate for a new sexual partner, more than one sexual partner and anal sex has been much lower than anticipated based on the results of a large donor survey performed at blood drives. This discrepancy may be because of self-deferral, donor self-assessment, or because donors thought more carefully while completing the questionnaire.

The Dutch experience

Thijs van de Laar described the Dutch experience dealing with the MSM deferral.

In the Netherlands, the HIV epidemic is largely confined to MSM, with an estimated 70% of newly diagnosed infections occurring in this group [23]. Most newly diagnosed HIV infections in heterosexuals are imported from countries with a higher HIV prevalence [23]. Since 2008, the absolute number of newly diagnosed HIV infections in the Netherlands has gradually declined, from >1250 in 2008 to <450 in 2021 [23]. In 2021, both the proportion of MSM among persons with an incident HIV infection (59% vs. 70%) and the proportion of newly diagnosed MSM who acquired an HIV infection within the past 12 months (27% vs. 37%) had decreased relative to previous years [23].

The Netherlands also used time-limited deferrals (Table 1). The deferral was further relaxed in 2021 to allow MSM in a long-term (i.e., ≥12 months) mutually exclusive relationship to donate.

These changes did not appear to compromise blood safety. The residual risks of HIV and HBV were low with a permanent deferral of MSM and have further declined since then, mirroring the trend in the general population. Moreover, HCV incidence among Dutch donors has been (near) zero over the past 20 years. However, the rate of syphilis has increased, especially in first-time donors but also in repeat donors, since the country moved to a time-limited MSM deferral of 4 months in 2019. This might be due partly to a marked increase of syphilis infections in the general population (as observed in other countries), but there is also a clear association with an increasing proportion of syphilis-positive donors reporting male-to-male sex.

Before moving to a more individualized assessment of donor risks, Sanquin conducted a study (TO DO) to assess the degree of self-awareness among MSM [24]. Candidate MSM donors were asked three simple questions dealing with sexual risk behaviour, including (1) time since last sexual intercourse, (2) whether they were in a mutually exclusive sexual relationship and (3) whether they consistently used a condom during anal sex with a casual partner [24]. In addition, 10 serologic markers were evaluated to compute a risk score (termed 'infection pressure') that reflected their true risk. The donors' self-reported sexual risk was generally consistent with the infection pressure [24], thus indicating that individualized donor selection criteria could eventually be used among MSM.

The Netherlands initially planned to implement an individualized donor risk assessment approach, but only for MSM [25]. The rationale for this decision was that the gender-neutral approach was considered to be unethical because (1) introducing a question about anal sex would be akin to one on sexual orientation, as such a question would be intended to target MSM given the HIV epidemiology in the Netherlands; (2) some heterosexual donors who previously qualified and proved to be perfectly safe would suddenly become ineligible; (3) eligible donors may be lost because they feel uncomfortable answering the new more intimate questions; and (4) two countries (i.e., Italy and Spain) do not have any MSM deferral, and their rates of HIV detection among blood donors appear higher than expected based on the local epidemiology of HIV [26].

However, Sanquin faced a number of hurdles that forced the blood service to reconsider its decision. To begin, all relaxation measures had to be put on hold after the emergence of mpox which, like HIV, disproportionally affected MSM. Secondly, a clear-cut and easy to understand question on consistent condom use proved difficult to formulate. Moreover, multiple stakeholders urged to reconsider the chosen approach: recipients were concerned about the risk of window-period infections, mpox and PrEP use (although they

remained open to a more inclusive blood donation policy); and the LGBTQI+ community advocated for a more inclusive policy, arguing that the distinction between MSM and non-MSM (even with less stringent MSM deferral criteria in place) continues to lead to bad experiences at Sanquin for members of their community.

While reassessing its approach, Sanquin considered quantitative data collected by an independent research institute in 2021–2023 to investigate the attitude, knowledge and intentions of MSM to donate blood. A longitudinal study revealed that approximately half of the 214 MSM included in the study reported being in a non-monogamous relationship, but the answers were often inconsistent depending on the chosen phrasing. The same study found that, while MSM seemed interested in donating blood, few actually do (<0.5%). Moreover, a cross-sectional survey via social media showed a low intention to donate among MSM who were in a long-term monogamous relationship. Therefore, the new blood donation policy would need to be accompanied by initiatives to motivate MSM to donate.

Five recommendations emerged from the stakeholder focus group meetings. First, Sanquin staff must be trained to offer a better experience to MSM donors. Second, improvements in procedures must be monitored given the growing number of MSM donors. Third, more modern donor selection methods (e.g., online) should be used to prevent on-site donor deferral. Fourth, the limitations of laboratory procedures, in particular the risk of false-negative HIV test results during the window-period or when taking PrEP, should be better communicated to address the many misconceptions circulating in the MSM community. Lastly, donor recruitment campaigns are needed to raise awareness of blood donation in the MSM community.

In the end, all stakeholders (except recipients) were in favour of a gender-neutral selection policy. This approach was also recommended by Sanquin's medical advisory board, and its implementation is planned for January 2024.

The French experience

The French experience was described by Pierre Tiberghien.

Like many other countries, France moved from a permanent deferral to progressively shorter time-limited deferrals, but the country additionally implemented a quarantine programme that allowed MSM to donate apheresis plasma under the same criteria as other donors (Table 1). In this programme, new test-negative plasma donations were released only after a second donation made ≥2 months later also tested negative. In 2020, France further relaxed the MSM deferral to 4 months for all types of donations—including apheresis plasma. As a result, plasma donations from MSM no longer had to be quarantined if the last male-to-male sexual contact had occurred >4 months ago.

Donor compliance was assessed among blood donors in 2017, after France transitioned to a 12-month MSM deferral [27]. Although compliance was generally high, the questions on the number of sexual partners were associated with higher rates of non-compliance, particularly among MSM. Further reducing (or eliminating) the MSM deferral criteria might improve the inclusiveness of blood donation, so that MSM are more inclined to disclose relevant sexual behaviours.

The change to a 4-month deferral was guided by a modelling study in which two relaxation measures were considered: a 4-month deferral for all MSM (scenario 1) and a 4-month deferral that would only apply to MSM who had more than one sexual partner in the last 4 months (scenario 2) [28]. The model suggested that the residual risk of HIV would be unchanged with scenario 1 (vs. the current 12-month deferral) and might modestly increase with scenario 2. Hence, scenario 1 was retained, and the 4-month MSM deferral was implemented in 2020.

Although the quarantine plasma programme was hindered by a low donor accrual rate, it enabled France to collect data on the risk profile of sexually active MSM donors [29]. In this programme, the deferral rate related to sexual behaviours was much higher in MSM than in other donors (12.1% vs. 1.0%) [29]. Most of these deferrals were due to the number of sexual partners of the donor or the donor's partner [29]. The rates of HIV and syphilis positivity were significantly higher among MSM than other donors [29]. However, the findings regarding HIV should be considered cautiously in view of the very low number of occurrences.

The progressive relaxation of the time-limited MSM deferral did not appear to impact the safety of the blood supply. The rates of HIV-, HCV-, HBV- and syphilis-positive donations remained overall stable despite these changes, both in first-time and repeat donors. Likewise, the residual risk of HIV did not increase—and might in fact have decreased.

In light of these reassuring results, France fully lifted the MSM deferral in 2022. Notably, over the last 30 years, the country has been deferring donors with more than one sexual partner in the last 4 months. The only set of new deferral criteria introduced amid this change pertained to PEP and PrEP.

Following this change, France witnessed a modest uptick in the incidence of syphilis (in repeat and first-time donors) and HBV (in first-time donors) positivity. While seemingly worrisome, similar trends have been observed in the Netherlands and the UK (increase in previously undiagnosed infections in new donors started before FAIR). The data are also too preliminary to determine whether these increases ensue from changes in the donor selection criteria, reflect an increase in the general population, or both. Moreover, the number of HIV-NAT-positive and antibody-negative donations remained stable.

The Israeli experience

Israel's experience was described by Eilat Shinar.

From 1986 to 2016, the country permanently deferred MSM from donating blood (Table 1). In 2017, a 12-month MSM deferral was implemented but was severely criticized by LGBTQI+ rights advocates. Israel responded to these critics in 2018 by implementing a frozen plasma quarantine programme (FPQP), which allowed the release of whole-blood-derived plasma from MSM donors if two test-negative donations had been made ≥4 months apart. The red blood cells (RBCs) from these donations were discarded with donors' consent. Since 2021, following the introduction of a gender-neutral donor

selection, the donations made by MSM are authorized for immediate use. The donors who are not eligible according to the new criteria are either deferred for 3 months or can participate in the FPQP.

These policy changes were informed by anonymous donor compliance surveys. The survey conducted before the transition to a 12-month deferral found that as many as 76% of MSM had donated blood at least once despite the permanent deferral [30]. Relaxing the MSM deferral was viewed as a way of lowering this high rate of non-compliance, as it might improve the acceptability of the selection criteria by the MSM community. The high rate of non-compliance additionally prompted the national blood service to improve its NAT testing sensitivities. Another concerning finding was the high proportion of donors (64%) who were unaware that HIV was associated with male-to-male sex, suggesting donor education initiatives were needed [30]. Reassuringly, however, social solidarity (and not test seeking) was the predominant motivation for donating blood among MSM, and most MSM were willing to comply with a time-limited deferral [30].

Another donor survey was conducted after implementing the 12-month deferral and the FPQP. The non-compliance rate with the 12-month deferral policy was high (i.e., 13%) [31], albeit much lower than that observed in the previous survey under the permanent deferral (i.e., 76%). Sixty-five percent of the donors supported the FPQP and would consider donating as part of this programme [31]. The proportion of MSM who were inclined to disclose their sexual orientation was 85% in the context of the FPQP [31], compared with only 8.5% under the 12-month deferral policy.

These relaxation measures (i.e., FPQP and 12-month deferral) seemingly had no impact on the safety of the blood supply. Indeed, the rates of HIV, HCV and HBV positivity all decreased among blood donors when comparing the periods of 2012–2017 and 2018–2021.

Given these reassuring compliance and surveillance data, Israel replaced its MSM deferral policy with a gender-neutral donor selection policy in 2021. Although the retained criteria were inspired from FAIR (in the United Kingdom), the Israeli questionnaire differs in that it does not include separate, direct questions for each behavioural risk factor. Instead, the questionnaire provides a list of deferral criteria, and the donors are asked not to donate if they engaged in a 'high-risk sexual relation' in the last 3 months, defined as anal sex, sex under the influence of drugs, or sex with a new partner or multiple partners.

Recent surveillance data suggest the new policy has not adversely affected the safety of the blood supply. In fact, the rates of HIV, HCV and HBV positivity have further declined up until the end of 2022, although the rate of HIV positivity might have increased since the beginning of 2023. Deferral rates have also remained stable, possibly due to donor self-deferral.

The Republic of Ireland's experience

Ireland's experience was described by Tor Hervig and Stephen Field.

In the Republic of Ireland, the rate of incident HIV infections has substantially increased since 2011 among individuals born outside the country, but not among those born in Ireland. In 2018, individuals born outside the country accounted for 71% of incident HIV infections, those born in Ireland accounted for 21% and those without information on their country of birth accounted for 8%. Moreover, the number of HIV infections is rising among MSM, but is stable in other groups.

The donor selection policy changes implemented in Ireland (summarized in Table 1) seemingly had no impact on the rates of infectious disease markers among blood donors. The rates of HIV, HCV and HBV positivity remained stable from 2013 to 2020. Based on a modelling analysis (similar to that of Caffrey et al. in Canada [21]), the theoretical risk of releasing an infected product would be very low under a gender-neutral donor selection policy, ranging from 1 in 2.87 million donations to 1 in 3.76 million donations depending on the assumed HIV risk reduction factor.

In light of these reassuring results, the country moved forward with a gender-neutral donor selection policy in late 2022. Under this new policy, donors are asked questions on anal sex, recent STIs, chemsex, PEP and PrEP, their partner's HIV status, and the number (i.e., one vs. multiple) and type (i.e., long term vs. new) of sexual partner (Table 2 for the specific questions). These questions were selected because: (1) the associated risk factors were moderately or strongly supported by the literature; (2) donors were expected to reliably answer them; and (3) they minimized donor loss (according to an anonymous donor survey). The Irish Blood Transfusion Service (IBTS) initially contemplated using an electronic questionnaire, but later revised its plan due to technical limitations. Nonetheless, more donors would have been willing to donate with an electronic questionnaire.

The impact of the new policy has thus far been largely positive. Only two donors have tested positive for syphilis, and both complied with the selection criteria in place; no donors have tested positive for HIV, HCV and HBV. The deferral rate associated with the new questions is also very low, at only 0.23% of all deferrals. Furthermore, donor recruitment campaigns have restarted in universities and colleges after student boycotts in protest of what was perceived as a discriminatory blood donation policy. Furthermore, the IBTS has only received one complaint regarding the questionnaire during the first 6 months.

Similarities and differences across countries

Similarities

Most countries employed a similar approach to implementing gender-neutral donor selection. Several stakeholder groups, including recipients, donors and the LGBTQI+ community, were consulted. Moreover, the transition from a permanent MSM deferral to a gender-neutral selection was bridged by time-limited deferrals, which were progressively relaxed (i.e., shortened) as new and reassuring surveillance data became available. Most countries also monitored donor compliance throughout these policy changes.

Despite their lower acceptability, the criteria on anal sex and the number and/or type of sexual partners in the last 3–4 months were

often retained for gender-neutral selection. These criteria are supported by data from the Canadian ENGAGE study (summarized above) [19].

Moreover, these changes did not appear to compromise blood safety. The residual risks of HIV and HBV for deferred MSM have declined and followed the trend in the general population of high-income countries (HIC).

Conversely, a marked increase of syphilis infections in the general population of HICs has been observed in recent years. Several [32–37] but not all [38, 39] studies have reported an association of syphilis with HIV infection, although the literature on this topic is outdated. Therefore, syphilis has historically been used as a proxy for the risk of HIV sexual transmission, but recent trends in syphilis and HIV infections are decoupled: syphilis infections are surging, while HIV incidence is declining. This suggests syphilis might no longer be an appropriate proxy for HIV. Additionally, given the excellent sensitivity of current HIV screening tests, the usefulness of syphilis as a proxy for HIV could be questioned. However, from a public health perspective, syphilis testing might remain a relevant marker of STIs in the general population.

Overall, the experience with gender-neutral selection has been largely positive among the countries covered in this symposium. Although preliminary, the surveillance data collected after the implementation of gender-neutral selection appear reassuring. One exception may be the prevalence of syphilis, which appears to be increasing in the donor populations of many countries. This trend may ensue from changes in the donor selection criteria, a rising incidence in the general population, or both. Lastly, the new selection criteria appear to be well perceived by donors, with few complaints.

Differences

Only Israel, France and Canada leveraged a plasma quarantine programme to help implement a gender-neutral donor selection policy. Although the programme was relatively inefficient (largely due to a low donor accrual), its impact has been largely positive in France and Israel. In France, such a programme was a unique opportunity to collect data on the risks associated with donations from MSM. In Israel, the FPQP enabled some MSM donors to eventually become regular plasma donors (with the policy change in 2021). In Canada, it was implemented as a pilot programme but was halted as few donors were recruited through this programme.

Moreover, some of the novel or revised donor selection criteria that were proposed by blood services during their respective review processes were retained by some countries, but not others (Table 2). For example, unlike Ireland and the United Kingdom, Canada chose not to ask donors about chemsex, reasoning that the risk stems from having a new sexual partner (or high-risk partner) and not drug use per se. France is also the only country that abandoned the MSM deferral by relying on its pre-existing gender-neutral selection that excludes donors with multiple sexual partners in the last 4 months, without any questions added on anal sex.

The formulations of the questions also differ across countries. In Canada (including Québec), donors are asked whether they have had a new sexual partner in the last 3 months, and those who respond affirmatively are additionally asked—in a separate question—if they have had anal sex in the last 3 months. Similarly, donors are asked whether they have had more than one sexual partner in the last 3 months, and those who respond affirmatively are asked about anal sex in a separate question. A similar two-step question is in effect in the UK and Ireland.

In Canada, this formulation was facilitated by an electronic, self-completed donor questionnaire, which asks additional questions when the donor answers affirmatively to a first question. A paper questionnaire is in use in England, Scotland and Ireland, whereas an electronic questionnaire is used in Wales. In Israel, donors are provided with a list of deferral criteria and must indicate whether any apply to them. The rationale for such an approach is the objection of some MSM to mandatorily disclose their sexual identity and to be included in an 'MSM list' in the National Blood Services database. Although many participants of a 2019 survey provided positive responses regarding the disclosure of sexual identity, many MSM hide their sexual identity in fear of being ostracized for religious reasons. Therefore, many MSM donors might choose to self-defer from donating blood if they were asked direct questions about their sexual identity.

PART 2: THE CHALLENGE POSED BY PRE-EXPOSURE AND POST-EXPOSURE PROPHYLAXIS

Darrell Tan introduced the audience to the principles and evidence underlying the use of PEP and PrEP for HIV prevention.

PEP aims to prevent an HIV infection by initiating an antiretroviral regimen early after a possible exposure. The in-human effectiveness of PEP is supported by a single case–control study published in 2001, which found that health care workers exposed to HIV had 81%–86% lower odds of infection if they received zidovudine shortly after exposure [40]. However, the contemporary effectiveness of PEP is believed to be much higher with modern antiretroviral regimens. To be effective, a PEP regimen must be maintained ≥28 days and initiated within 72 h after exposure, beyond which HIV has disseminated in the bloodstream and the infection becomes chronic. The evidence supporting this timing stems from animal studies. In macaques infected with simian immunodeficiency virus (SIV), the antiretroviral PMPA fully protected against SIV when initiated ≤24 h post-exposure, was partially effective when initiated 24–72 h post-exposure, but was not effective when initiated beyond 72 h [41]. Likewise, some animals were infected with SIV when the regimen was maintained for less than 28 days (despite being initiated within 24 h post-exposure) [41].

PrEP aims to prevent an HIV infection by maintaining protective levels of antiretrovirals in mucosal tissues before and after an HIV exposure. PrEP may be offered as a daily oral regimen, a long-acting injectable (LAI) regimen or an 'on-demand' regimen (in MSM only), i.e., taken before and after a planned at-risk activity. The efficacy of

daily oral PrEP and on-demand PrEP is supported by several trials in which various PrEP regimens reduced the risk of HIV infection by 44%–86% [42–46]. However, PrEP efficacy is strongly influenced by medication adherence: It approaches 100% in fully or moderately adherent MSM patients (i.e., ≥4 pills per week for daily oral PrEP) but significantly declines with poorer adherence [47]. In the HPTN 083 trial, cabotegravir (an LAI PrEP administered every 8 weeks) was 66% more efficacious than daily oral PrEP among MSM and transgender women at high risk of HIV [48], likely because of improved adherence associated with this more convenient dosing schedule.

The PrEP breakthrough infections that ensue from poor adherence are not a major concern for blood safety, as they are likely to be detected by current tests. However, the rare cases of PrEP breakthrough HIV infections that arise in adherent patients might not be detected. Approximately 10 such cases have been documented worldwide, and the reasons underlying this phenomenon (e.g., altered viral kinetics, drug metabolism) are unclear.

Impact of pre- and post-exposure prophylaxis on blood safety

The blood safety risks associated with PEP and PrEP were explained by Brian Custer.

Blood services are concerned about PrEP breakthrough infections because PrEP could suppress HIV viral loads and mask an active HIV infection [49]. In clinical trials of PrEP, breakthrough infections occurred among users of LAI PrEP and daily oral PrEP [50]. Notably, among LAI PrEP users, the test results associated with these breakthrough infections fluctuated over time and across assays: some antibody/antigen tests switched from positive to negative, some confirmatory antibody tests switched from indeterminate to negative (i.e., seroreversion), and some qualitative NATs switched from positive to negative [50]. These results suggest breakthrough infections may be more difficult to identify in patients using LAI PrEP than daily oral PrEP, raising concerns about the implications of LAI PrEP for blood safety.

However, before the implementation of gender-neutral selection, PrEP use appeared relatively infrequent in the overall donor population. In a 2018–2019 study conducted in the United States, 0.6% of first-time, HIV-negative male donors (who lived in cities with larger MSM populations) had tenofovir (TDF) and emtricitabine (FTC)—a PrEP drug combination—in their donation [51]. Among these TDF/FTC users, 66% were inferred to have taken their last dose of PrEP ≥2 days ago based on the drug concentration detected in plasma [51], which could reflect the use of on-demand PrEP or poor adherence to daily oral PrEP. In a 2021–2022 study conducted in the Netherlands, 0.02% of ~10,000 donations from male donors in the five major cities contained a PrEP medication.

Some data suggest that PEP and PrEP use might be associated with a higher risk of STI. In one UK study (2018–2019), TDF and FTC were detected in 6.5% of donations from syphilis-positive and HIV-negative donors [52]. In a further UK study (2020–2021), TDF and FTC were detected in 5.6% of plasma donations from syphilis-positive donors [53]. In the Netherlands, PrEP medication was detected in 1.5% of syphilis-positive donors and 2.6% of male, syphilis-positive donors.

Nevertheless, many questions remain regarding the impact of PEP and PrEP on blood safety. The sensitivity of current tests to detect breakthrough infections is unknown but will be investigated in the Recipient Epidemiology and Donor Evaluation (REDS-IV-P) study. Moreover, the infectivity of the blood of individuals with a PrEP breakthrough infection is unclear. In a group discussion session, Brian Custer suggested that the lack of reported cases of transfusion transmission associated with a PrEP breakthrough infection is reassuring, but not definitive. Steven J. Drews additionally proposed that in vitro infectivity assays and animal experiments be carried out to determine whether the low viral titres found in PrEP breakthrough infections are infectious.

Dosage of antiretrovirals to monitor the use of pre- and post-exposure prophylaxis in blood donors

Steven Drews described how CBS plans to study PEP and PrEP use.

Several aspects of PEP and PrEP use need further clarification. The characteristics of PEP and PrEP users are poorly defined. Moreover, the reasons for donating while on PEP or PrEP are not fully understood. Furthermore, the best strategy to address the challenge of HIV detection in PrEP users is unclear, but two options may be considered: The sensitivity of HIV screening tests might be improved (e.g., by deconvoluting NAT minipools to individual donor NAT), and PRT might be implemented for blood products when available.

CBS seeks to address these knowledge gaps through the conduct of a prospective observational study among donors at high risk of HIV. The study will enrol blood donors falling in one of three groups: (1) syphilis- or HIV-positive donors; (2) donors who return after a PEP or PrEP deferral; and (3) selected first-time male donors in two major Canadian cities. The donations will be tested for TDF (quantitatively) and FTC (qualitatively).

In a group discussion session, Brian Custer expressed doubts about the need for systematically testing donors for antiretroviral use, especially given the characteristics of the tests that are currently available (i.e., high-throughput assays unavailable, and most tests assess a single drug).

Donor selection criteria based on the use of pre- and post-exposure prophylaxis

Blood donors are asked to disclose PEP or PrEP use in the last 3–4 months. This 3- to 4-month deferral is justified by the antiretroviral washout period and the subsequent time to viremia rebound (which should enable HIV detection by NAT).

In the United States, donors are asked 'In the past 3 months, have you taken any medication to prevent an HIV infection?' Relatively

few donors were deferred for PrEP use in the United States in 2020, with the highest deferral rates occurring among first-time or lapsed male donors (range in these groups: 23.5 to 62.2 per 100,000 interviews at Vitalant and the American Red Cross). The US questionnaire will soon be adapted to account for the approval of cabotegravir as an LAI PrEP and for the adoption of gender-neutral questions similar to those in Canada. Until more data become available, users of LAI cabotegravir (for PrEP) will conservatively be deferred for 2 years.

In Canada and in the Netherlands, donors are asked 'In the last 4 months, have you taken any medication to prevent an HIV infection, such as PrEP or PEP?'. Few donors have been deferred for PEP or PrEP use since 2019, but their number appears to be increasing. Of note, CBS estimates that the question on general medication use in the last 3 days would have missed more than half of PEP and PrEP users [54], hence the need for a specific question. The UK applies a 3-month deferral for PrEP or PEP use.

PART 3: STRATEGIES TO ALLEVIATE THE RISK OF TTIs AMID THE REMOVAL OF THE MSM DEFERRAL

Pathogen reduction technology

Overview of PRT

The rationale and principles underlying the use of PRT in addition to blood screening were described by Marion Lanteri.

Advances in blood screening and donor selection criteria have substantially reduced the risk of TTI but have inherent limitations. Blood screening cannot detect an HIV infection during the window period, when viral loads and antibody levels are below the limit of detection of the screening assays. The HIV window period refers to the time between an HIV infection and when a test can detect the virus in the blood. After an HIV infection, viral loads increase above the limit of detection of NATs before seroconversion occurs. Therefore, the window period is shorter with NAT than with serological screening. If an HIV treatment is successful, the viral loads are suppressed to levels below the limit of detection of NAT, and the infection can only be detected using serological screening.

Individuals at risk of HIV are targeted by public health campaigns to end the HIV epidemic. Specifically, the goal is (1) to test as many people as possible, so that more are aware of their HIV status and can be treated early, and (2) to prevent HIV transmission through PrEP and PEP use, with new LAI antiretrovirals for PrEP.

These interventions can impact HIV blood screening. In individuals with a known HIV infection who are treated with antiretrovirals, viral loads may fall below the limit of detection of NATs, but HIV antibodies should be detectable by serological screening. However, with early antiretroviral treatment, reduced viral loads may delay or suppress seroconversion in addition to extending the window period of NATs, thus raising concerns over the detection of an infection [55]. In individuals infected while on PrEP, PEP or LAI antiretrovirals (breakthrough infections), the window period may be longer if the viral loads rise and antibodies become detectable before seroreversion [49].

Donors may also be non-compliant with the selection criteria for various reasons. They may, for example, misinterpret the concept that undetectable HIV viral loads cannot lead to disease transmission, which applies to sexual transmission but not transfusion transmission [18, 56, 57]. Moreover, they may not accurately report their HIV infectious status, antiretroviral drug intake or recent sexual risk behaviours.

These limitations may be addressed through the addition of PRT for the ex vivo treatment of blood components. Various PRT systems have been developed to prevent the replication of contaminating pathogens. They rely on ultraviolet illumination, with or without the addition of photosensitizers or intercalating agents. Three PRTs are commercially available to treat plasma and platelets or whole blood, and their efficacy in reducing infectious pathogen titres varies. Others are still in development or undergoing clinical evaluation to treat RBCs. The only FDA-approved PRT treats plasma and platelets with amotosalen (an intercalating agent) and UVA illumination. This system is used in many countries, including the United States, and has been recently implemented in Canada to treat platelets. It is highly efficient at inactivating HIV, HCV and HBV, reducing infectious titres by five or more logs [58]. While PRT may mitigate the risks associated with extended window periods and suppressed viral loads or antibody levels, NAT is important to identify donations containing high viral loads above the PRT inactivation capacity. Combining NAT and PRT would eliminate the risk associated with HIV window-period donations and obviate the need for serologic testing once PRT for RBC becomes available.

PRT may also offer advantages beyond mitigating the risk of HIV, HCV and HBV. The technology is effective against a wide range of pathogens—including viruses, bacteria and parasites—and should reduce the unknown risks associated with emerging pathogens [58–60]. Moreover, the combined use of PRT and NAT will address the risk associated with donor non-compliance, thus allowing for the removal of some donor selection criteria and expanding the donor base. Although the technology is not currently available for RBCs, ineligible RBC donors may be redirected to other types of donations (e.g., plasma or platelets) that can be treated with PRT and for which the donor's risk profile would be acceptable.

Impact of PRT on deferrals for HIV, HCV and HBV sexual risk behaviours

Marie-Pier Domingue presented the results of a model (developed in collaboration with HQ) that explored the possibility of removing sexual risk behaviour deferrals when using PRT.

Specifically, the model assessed the risk of releasing an HIV-, HCV- or HBV-contaminated product in Canada using an approach that combined Bayesian network and Monte Carlo simulations. For each scenario (e.g., baseline, most likely), the model simulated 1 billion

donors and their donations, and processed them as follows: Test-negative donations were pooled (in preparation for PRT), PRT was applied and the products were released. A released blood product was considered contaminated if it contained more than three RNA or DNA copies per unit. The model considered several variables that might influence a donation's RNAemia or DNAemia, including donor demographics, behavioural risk factors (e.g., MSM), donor viral loads (i.e., pre-PRT), time since infection and the type of donation.

The results indicated that PRT (alone, with no deferral) would substantially reduce the risk of releasing an HIV-, HCV- or HBV-contaminated product compared with a hypothetical scenario in which neither PRT nor donor deferral would be used. Similar results were obtained when current donor selection criteria (i.e., gender-neutral selection) were used as comparator, although the difference was less pronounced. In absolute terms, the residual risk of HIV and HBV was very low after PRT. However, the residual risk of HCV was higher, reflecting the high viral loads of this virus that translate into higher post-PRT titres.

Therefore, these results support the notion that, if PRT was implemented, at least some donor selection criteria related to sexual risk behaviours could be removed. During a group discussion session, some speakers suggested that the approval of PRT for RBCs (based on amustaline and glutathione) might allow for the withdrawal of selection criteria related to the use of antiretrovirals, PrEP and PEP. This possibility could be explored with the model described in this presentation.

Viral safety of plasma derivatives and the role of molecular testing

Denis Klochkov described the perspective of a plasma fractionator on the need for molecular testing of plasma derivatives for viral markers amid the transition to a gender-neutral selection policy.

The relative importance of the measures that ensure plasma safety has substantially evolved in recent years. Donor selection, which once was a key safety pillar, now plays a more secondary role given the high performance of NAT and the stringency of manufacturing processes. In fact, plasma manufacturing processes now account for the bulk of the risk reduction (i.e., 10^6- to 10^{12}-fold), owing to the addition of dedicated virus reduction steps (in addition to the purification steps). NAT also plays a more predominant role in plasma safety (i.e., 10^3- to 10^6-fold reduction), and the industry standard has evolved from a '3 NAT' panel (i.e., HIV, HCV, HBV) to a '5 NAT' panel (i.e., with the addition of hepatitis A virus [HAV] and parvovirus B19). Of note, 5 NAT is recommended even in countries where HAV vaccination is mandatory.

HAV exhibits several characteristics that justify its testing by NAT. Outbreaks of HAV have recently occurred in North America, Europe and Australia. Notably, some have affected MSM, and the virus may have spilled over into the general population. HAV is also transfusion transmissible and is associated with very high titres that may reach 10^9 IU/mL (even in asymptomatic donors) [61].

Although a wealth of reassuring data is becoming available, the impact of gender-neutral selection on TTI among MSM is not fully understood. Its impact on the risk of HIV (if any) thus far appears negligible, and systematic donor testing in combination with the virus reduction steps included in the manufacturing processes further safeguards the supply of plasma-derived medicinal products against this virus. However, an impact on the risk of HAV cannot be ruled out, as outbreaks of HAV have been linked to MSM. This dearth of evidence, combined with the possibility of donor non-compliance, supports maintaining 5 NAT testing amid the transition to a gender-neutral selection policy.

CONCLUSION

The experience with gender-neutral selection has been largely positive among the countries covered in this symposium. Furthermore, post-implementation surveillance data (while preliminary) appear reassuring. Nonetheless, the putative risks associated with antiretrovirals for HIV treatment, PrEP and PEP warrant further research. Although the vast majority of MSM in long-term monogamous relationships should be eligible, a large proportion of MSM will likely remain ineligible. Albeit imperfect, gender-neutral selection is a significant step forward to improving the inclusiveness of blood donor for increased blood availability while maintaining blood safety. Further improvements may be facilitated by technological advances, such as PRT, which might alleviate current concerns related to antiretroviral drug use and other TTI risks beyond HIV.

ACKNOWLEDGEMENTS

The symposium was funded by the Canadian Blood Services MSM Research Grant Program, funded by the Federal Government (Health Canada) and the Provincial and Territorial Ministries of Health.

A.L., S.F.O., C.R., G.L., Mi.G. and Ma.G. organized the symposium, Mi.G., M.B., Ma.G., A.L., K.D., T.L., P.T., E.S., S.F.O., G.L., S.F., T.H., D.H.S.T., B.C., S.J.D., M.C.L., D.K., E.W. and M-P.D. presented during the symposium. All authors have read, commented and revised the manuscript. Medical writing assistance was provided by Samuel Rochette, an employee of Héma-Québec.

CONFLICT OF INTEREST STATEMENT

Eleonora Widmer and Denis Klochkov are employees of CSL Behring. Marion L. Lanteri was an employee and shareholder of Cerus Corporation, which commercializes the INTERCEPT Blood Systems for platelets and plasma.

DATA AVAILABILITY STATEMENT

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

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REFERENCES

1. Donegan E, Stuart M, Niland JC, Sacks HS, Azen SP, Dietrich SL, et al. Infection with human immunodeficiency virus type 1 (HIV-1) among recipients of antibody-positive blood donations. Ann Intern Med. 1990;113:733–9.
2. Benjamin RJ, Bianco C, Goldman M, Seed CR, Yang H, Lee J, et al. Deferral of males who had sex with other males. Vox Sang. 2011; 101:339–67.
3. Lewin A, Grégoire Y, Delage G, Thibeault C, Viau C, Milot C, et al. Reported non-compliance with pre-donation screening among blood donors in Québec, Canada: a focus on the 3-month deferral for men who have sex with men. Vox Sang. 2022;117:966–70.
4. Ellingson KD, Sapiano MR, Haass KA, Savinkina AA, Baker ML, Chung K-W, et al. Continued decline in blood collection and transfusion in the United States–2015. Transfusion. 2017;57:1588–98.
5. Jones JM, Sapiano MR, Mowla S, Bota D, Berger JJ, Basavaraju SV. Has the trend of declining blood transfusions in the United States ended? Findings of the 2019 National Blood Collection and Utilization Survey. Transfusion. 2021;61:S1–S10.
6. Klein HG, Hrouda JC, Epstein JS. Crisis in the sustainability of the US blood system. N Engl J Med. 2017;377:1485–8.
7. American Red Cross. Red Cross Declares First-ever Blood Crisis amid Omicron Surge. Available from: https://www.redcross.org/about-us/news-and-events/press-release/2022/blood-donors-needed-now-as-omicron-intensifies.html#:~:text=The%20Red%20Cross%20has%20experienced%20low%20donor%20turnout%20ever%20since,reverse%20this%20national%20blood%20crisis. Last accessed 14 Jul 2023.
8. NHS Blood and Transplant. Extra Safety Measures after Coronavirus Uncertainty Causes Drop in Donations. Available from: https://www.blood.co.uk/news-and-campaigns/news-and-statements/extra-safety-measures-after-coronavirus-uncertainty-causes-drop-in-donations/. Last accessed 14 Jul 2023.
9. Pillonel J, Laperche S. Trends in residual risk of transfusion-transmitted viral infections (HIV, HCV, HBV) in France between 1992 and 2002 and impact of viral genome screening (Nucleic Acid Testing). Transfus Clin Biol. 2004;11:81–6.
10. Dodd R. Managing the microbiological safety of blood for transfusion: a US perspective. Future Microbiol. 2009;4:807–18.
11. Steele WR, Dodd RY, Notari EP, Haynes J, Anderson SA, Williams AE, et al. HIV, HCV, and HBV incidence and residual risk in US blood donors before and after implementation of the 12-month deferral policy for men who have sex with men. Transfusion. 2021; 61:839–50.
12. For the Assessment of Individualised Risk (FAIR) group. Can donor selection policy move from a population-based donor selection policy to one based on a more individualised risk assessment? Conclusions from the For the Assessment of Individualised Risk (FAIR) group. 2020. Available from: https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/21001/fair_sabto_20201211.pdf. Last accessed 8 Jan 2024.
13. Goldman M, W-Y Shih A, O'Brien SF, Devine D. Donor deferral policies for men who have sex with men: past, present and future. Vox Sang. 2018;113:95–103.
14. Caffrey N, Goldman M, Osmond L, Yi Q-L, Fan W, O'Brien SF. HIV incidence and compliance with deferral criteria over three progressively shorter time deferrals for men who have sex with men in Canada. Transfusion. 2022;62:125–34.
15. Vassallo RR, Bravo MD, Kamel H. Pandemic blood donor demographics–do changes impact blood safety? Transfusion. 2021; 61:1389–93.
16. US Food and Drug Administration. Revised Recommendations for Reducing the Risk of Human Immunodeficiency Virus Transmission by Blood and Blood Products. Available from: https://www.fda.gov/media/92490/download. Last accessed 14 Jul 2023.
17. NHS Blood and Transplant. UK to Change Eligibility to Give Blood on World Blood Donor Day with Launch of New Donor Safety Assessment. Available from: https://www.nhsbt.nhs.uk/news/uk-to-change-eligibility-to-give-blood-on-world-blood-donor-day-with-launch-of-new-donor-safety-assessment/#:~:text=Donors%20will%20no%20longer%20be,giving%20blood%20will%20not%20change. Last accessed 14 Jul 2023.
18. Broyles LN, Luo R, Boeras D, Vojnov L. The risk of sexual transmission of HIV in individuals with low-level HIV viraemia: a systematic review. Lancet. 2023;402:464–71.
19. Lambert G, Cox J, Fourmigue A, Dvorakova M, Apelian H, Moodie EE, et al. HIV incidence and related risks among gay, bisexual, and other men who have sex with men in Montreal, Toronto, and Vancouver: informing blood donor selection criteria in Canada. Transfusion. 2022;62:2555–67.
20. Aubé E, Lewin A, O'Brien SF, Grégoire Y, Pillonel J, Steele WR, et al. HIV residual risk in Canada for apheresis source plasma donation without deferral for men who have sex with men. Vox Sang. 2022;117:201–7.
21. Caffrey N, Goldman M, Lewin A, Grégoire Y, Yi QL, O'Brien SF. Removing the men who have sex with men blood donation deferral: informing risk models using Canadian public health surveillance data. Transfus Clin Biol. 2022;29:198–204.
22. Lewin A, Renaud C, Germain M, Boivin A, Thibeault C, Caruso J, et al. Validation of new, gender-neutral questions on recent sexual behaviors among plasma donors and men who have sex with men. Transfusion. 2022;62:2464–9.
23. Stitching HIV Monitoring. HIV Monitoring Report 2022. 2023. Available from: https://www.hiv-monitoring.nl/application/files/1816/6851/5357/NL_HIV_MONITORING_REPORT_2022.pdf. Last accessed 8 Jan 2024.
24. Van Bilsen WP, Zaaijer HL, Matser A, Van Den Hurk K, Slot E, Schim van der Loeff MF, et al. Infection pressure in men who have sex with men and their suitability to donate blood. Clin Infect Dis. 2019;68:1001–8.
25. Pierik R, Verweij M, van de Laar T, Zaaijer H. Facing difficult but unavoidable choices: donor blood safety and the deferral of men who have sex with men. Bioethics. 2022;36:840–8.
26. Germain M, Grégoire Y, Custer BS, Goldman M, Bravo M, Kamel H, et al. An international comparison of HIV prevalence and incidence in blood donors and general population: a BEST collaborative study. Vox Sang. 2021;116:1084–93.
27. Sauvage C, Charpentier F, Garrabé É, Pelat C, Spinardi R, Danic B, et al. Noncompliance to blood donor selection criteria by men who have sex with men–Complidon 2017, France. Vox Sang. 2020;115: 628–36.
28. Pillonel J, Pelat C, Tiberghien P, Sauvage C, Danic B, Martinaud C, et al. The evolving blood donor deferral policy for men who have sex with men: impact on the risk of HIV transmission by transfusion in France. Transfusion. 2020;60:525–34.
29. Tiberghien P, Lecam S, Huet J, Malard L, Tavenard T, Pillonel J, et al. Evolving deferral criteria for blood donation in France: plasma donation by men who have sex with men. Vox Sang. 2023;118:440–6.
30. Levy I, Olmer L, Livnat Y, Yanko A, Shinar E. Attitudes, perceptions and knowledge among men who have sex with men towards the blood donation deferral policy in Israel. PloS One. 2017;12:e0170364.
31. Levy I, Olmer L, Livnat Y, Shalhavi R, Hizki O, Shinar E. Attitudes and perceptions among men having sex with men towards a new non-deferral blood donation policy in Israel. Vox Sang. 2019;114:310–6.
32. Darrow WW, Echenberg DF, Jaffe HW, O'Malley PM, Byers RH, Getchell JP, et al. Risk factors for human immunodeficiency virus (HIV) infections in homosexual men. Am J Public Health. 1987;77: 479–83.

33. Hutchinson CM, Rompalo AM, Reichart CA, Hook EW. Characteristics of patients with syphilis attending Baltimore STD clinics: multiple high-risk subgroups and interactions with human immunodeficiency virus infection. Arch Intern Med. 1991;151:511–6.
34. Kreiss JK, Koech D, Plummer FA, Holmes KK, Lightfoote M, Piot P, et al. AIDS virus infection in Nairobi prostitutes. N Engl J Med. 1986; 314:414–8.
35. Quinn TC, Classer D, Cannon RO, Matuszak DL, Dunning RW, Kline RL, et al. Human immunodeficiency virus infection among patients attending clinics for sexually transmitted diseases. N Engl J Med. 1988;318:197–203.
36. Ramsey G, Soltis F, Bowman R, McNamee J, Hahn LF, Dixon BW. Syphilis serology in blood donors: a possible surrogate marker for human immunodeficiency virus risk. Vox Sang. 1991;60:165–8.
37. Stamm WE, Handsfield HH, Rompalo AM, Ashley RL, Roberts PL, Corey L. The association between genital ulcer disease and acquisition of HIV infection in homosexual men. JAMA. 1988;260: 1429–33.
38. Goldberg MA, Laycock KA, Kinard S, Wang H, Pepose JS. Poor correlation between reactive syphilis serology and human immunodeficiency virus testing among potential cornea donors. Am J Ophthalmol. 1995;119:1–6.
39. Petersen LR, Doll LS, White CR, Johnson E, Williams A, Group HBDS. Heterosexually acquired human immunodeficiency virus infection and the United States blood supply: considerations for screening of potential blood donors. Transfusion. 1993;33:552–7.
40. Cardo DM, Culver DH, Ciesielski CA, Srivastava PU, Marcus R, Abiteboul D, et al. A case–control study of HIV seroconversion in health care workers after percutaneous exposure. N Engl J Med. 1997;337:1485–90.
41. Tsai C-C, Emau P, Follis KE, Beck TW, Benveniste RE, Bischofberger N, et al. Effectiveness of postinoculation (R)-9-(2-phosphonylmethoxypropyl) adenine treatment for prevention of persistent simian immunodeficiency virus SIVmne infection depends critically on timing of initiation and duration of treatment. J Virol. 1998;72:4265–73.
42. Baeten JM, Donnell D, Ndase P, Mugo NR, Campbell JD, Wangisi J, et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. N Engl J Med. 2012;367:399–410.
43. Choopanya K, Martin M, Suntharasamai P, Sangkum U, Mock PA, Leethochawalit M, et al. Antiretroviral prophylaxis for HIV infection in injecting drug users in Bangkok, Thailand (the Bangkok Tenofovir Study): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2013;381:2083–90.
44. Grant RM, Lama JR, Anderson PL, McMahan V, Liu AY, Vargas L, et al. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. N Engl J Med. 2010;363:2587–99.
45. McCormack S, Dunn DT, Desai M, Dolling DI, Gafos M, Gilson R, et al. Pre-exposure prophylaxis to prevent the acquisition of HIV-1 infection (PROUD): effectiveness results from the pilot phase of a pragmatic open-label randomised trial. Lancet. 2016;387:53–60.
46. Thigpen MC, Kebaabetswe PM, Paxton LA, Smith DK, Rose CE, Segolodi TM, et al. Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. N Engl J Med. 2012;367: 423–34.
47. Grant RM, Anderson PL, McMahan V, Liu A, Amico KR, Mehrotra M, et al. Uptake of pre-exposure prophylaxis, sexual practices, and HIV incidence in men and transgender women who have sex with men: a cohort study. Lancet Infect Dis. 2014;14:820–9.
48. Landovitz RJ, Donnell D, Clement ME, Hanscom B, Cottle L, Coelho L, et al. Cabotegravir for HIV prevention in cisgender men and transgender women. N Engl J Med. 2021;385:595–608.
49. Elliott T, Sanders EJ, Doherty M, Ndung'u T, Cohen M, Patel P, et al. Challenges of HIV diagnosis and management in the context of pre-exposure prophylaxis (PrEP), post-exposure prophylaxis (PEP), test and start and acute HIV infection: a scoping review. J Int AIDS Soc. 2019;22:e25419.
50. Marzinke MA, Grinsztejn B, Fogel JM, Piwowar-Manning E, Li M, Weng L, et al. Characterization of human immunodeficiency virus (HIV) infection in cisgender men and transgender women who have sex with men receiving injectable cabotegravir for HIV prevention: HPTN 083. J Infect Dis. 2021;224:1581–92.
51. Custer B, Quiner C, Haaland R, Martin A, Stone M, Reik R, et al. HIV antiretroviral therapy and prevention use in US blood donors: a new blood safety concern. Blood. 2020;136:1351–8.
52. Harvala H, Reynolds C, Ijaz S, Maddox V, Penchala SD, Amara A, et al. Evidence of HIV pre-exposure or post-exposure prophylaxis (PrEP/PEP) among blood donors: a pilot study, England June 2018 to July 2019. Sex Transm Infect. 2022;98:132–5.
53. Maddox V, Reynolds C, Amara A, Else L, Brailsford SR, Khoo S, et al. Undeclared pre-exposure or post-exposure prophylaxis (PrEP/PEP) use among syphilis-positive blood donors, England, 2020 to 2021. Eurosurveillance. 2023;28:2300135.
54. Saeed S, Goldman M, Uzicanin S, O'Brien SF. Evaluation of a pre-exposure prophylaxis (PrEP)/post-exposure prophylaxis (PEP) deferral policy among blood donors. Transfusion. 2021;61:1684–9.
55. de Souza MS, Pinyakorn S, Akapirat S, Pattanachaiwit S, Fletcher JLK, Chomchey N, et al. Initiation of antiretroviral therapy during acute HIV-1 infection leads to a high rate of nonreactive HIV serology. Clin Infect Dis. 2016;63:555–61.
56. Baggaley RF, Boily M-C, White RG, Alary M. Risk of HIV-1 transmission for parenteral exposure and blood transfusion: a systematic review and meta-analysis. AIDS. 2006;20:805–12.
57. Patel P, Borkowf CB, Brooks JT, Lasry A, Lansky A, Mermin J. Estimating per-act HIV transmission risk: a systematic review. AIDS. 2014;28:1509–19.
58. Lanteri MC, Santa-Maria F, Laughhunn A, Girard YA, Picard-Maureau M, Payrat J-M, et al. Inactivation of a broad spectrum of viruses and parasites by photochemical treatment of plasma and platelets using amotosalen and ultraviolet a light. Transfusion. 2020; 60:1319–31.
59. Irsch J, Lin L. Pathogen inactivation of platelet and plasma blood components for transfusion using the INTERCEPT blood system™. Transfus Med Hemother. 2011;38:19–31.
60. Stramer SL, Lanteri MC, Brodsky JP, Foster GA, Krysztof DE, Groves JA, et al. Mitigating the risk of transfusion-transmitted infections with vector-borne agents solely by means of pathogen reduction. Transfusion. 2022;62:1388–98.
61. Schoch S, Wälti M, Schemmerer M, Alexander R, Keiner B, Kralicek C, et al. Hepatitis a virus incidence rates and biomarker dynamics for plasma donors, United States. Emerg Infect Dis. 2021; 27:2718–824.

How to cite this article: Lewin A, Goldman M, Busch MP, Davison K, van de Laar T, Tiberghien P, et al. End of selection criteria based on sexual orientation: An international symposium on alternatives to donation deferral. Vox Sang. 2024;119:388–401.

Received: 15 March 2024 | Accepted: 16 March 2024

DOI: 10.1111/vox.13624

ANNOUNCEMENT

Vox Sanguinis ISBT International Society of Blood Transfusion

Vox Sanguinis Best Paper 2023

The Editor-in-Chief is delighted to announce that the Jean Julliard Prize Committee has awarded the *Vox Sanguinis* Best Paper Prize 2023 to Dr. Rocío Trueba-Gómez et al. from Mexico City, Mexico, for their paper entitled, '*Use of computational biology to compare the theoretical tertiary structures of the most common forms of RhCE and RhD*'. This prize is awarded each year for the best original paper published in *Vox Sanguinis* in the previous calendar year and consists of a certificate and a cash payment of 5000 Euros. The prize will be presented to the authors during the opening ceremony of the 38th International Congress of the International Society of Blood Transfusion that will be held between 23 and 27 June in Barcelona. We congratulate the authors on this success.

DOI: 10.1111/vox.13635

EVENTS

See also https://www.isbtweb.org/events.html	
16–17 April 2024	International Plasma Protein Congress 2024. https://www.pptaglobal.org/events/international-plasma-protein-congress
18–20 April 2024	NATA24. https://nataonline.com/annual-symposium/
14–15 May 2024	5th Iran International Congress of Transfusion Medicine. https://ibtc5.com/
15–16 May 2024	IPFA/PEI 30th International Workshop on Surveillance and Screening of Blood-borne Pathogens. https://ipfa.nl/events/ipfa-pei-30th-international-workshop-on-surveillance-and-screening-of-blood-borne-pathogens/
29 May–1 June 2024	ISCT Vancouver. https://www.isctglobal.org/isct2024/registration
23–27 June 2024	38th International ISBT Congress, Barcelona, Spain. https://www.isbtweb.org/events/isbt-barcelona-2024.html
11–13 September	DGTI & DGI 2024. https://immungenetik.de/index.php/veranstaltungen/dgi-jahrestagungen/jahrestagung-2024
27–29 September 2024	ESPGI 2024—Platelet and Granulocyte Immunobiology. https://sanquinacademy.nl/en/offers/espgi-2024/

