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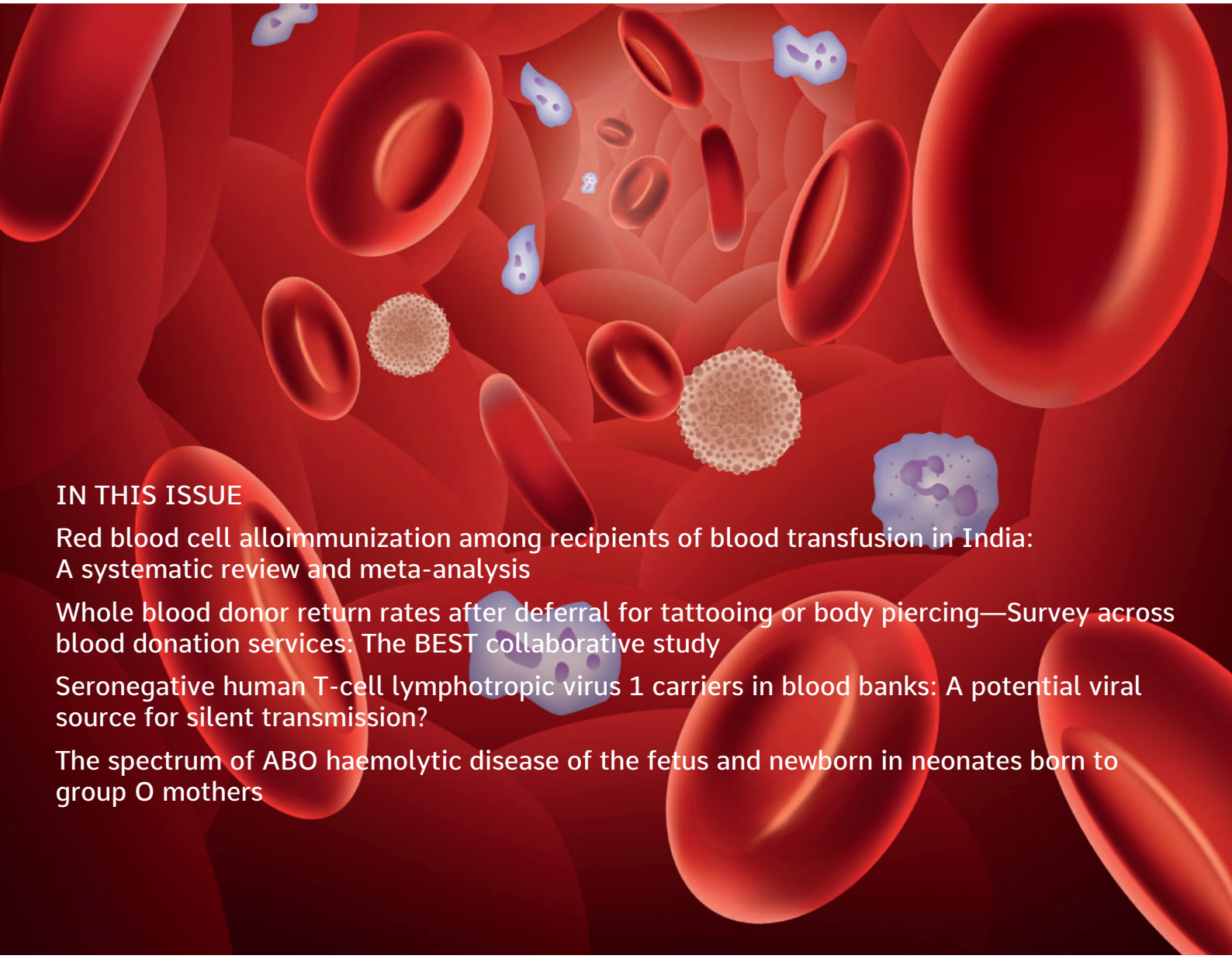

IN THIS ISSUE

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ISBT International Society of Blood Transfusion

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International Journal of Blood Transfusion

Official Journal of the International Society of Blood Transfusion

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Vox Sanguinis

International Journal of Blood Transfusion

Aims and Scope

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

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

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

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117/9/2022

Contents

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REVIEW

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Red blood cell alloimmunization among recipients of blood transfusion in India: A systematic review and meta-analysis

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Abstract

Background and Objectives: There is a varied prevalence of red cell alloimmunization being reported from different parts of India. This study aimed to estimate the overall prevalence of alloimmunization in India by performing a systematic review of the literature and to establish the most suitable antigen-matching strategy to reduce the red blood cell (RBC) alloimmunization rate among transfusion recipients.

Materials and Methods: A systematic search of all the original articles published in English on RBC alloimmunization among transfusion recipients from India in MEDLINE, SCOPUS, CINAHL and Google Scholar bibliographic databases was conducted. After screening the articles as per inclusion/exclusion criteria, data extraction was done independently by two sets of investigators. Meta-analysis was performed by the binary random-effects model using the restricted maximum likelihood method.

Results: A total of 44 studies on RBC alloimmunization, with a cumulative sample size of 309,986 patients, were grouped into hospital-based and multiply-transfused patients, which yielded a prevalence of 0.5 (95% confidence interval; 0.3–0.8) and 4.8 (95% confidence interval; 3.9–5.7) per 100 patients, respectively. As many as 1992 alloantibodies were identified among the 1846 alloimmunized patients. The most common antibody identified was anti-E (127; 31.99%), followed by anti-c (75; 18.89%) in multiply-transfused patients.

Conclusion: The rate of alloimmunization was 0.5 per 100 patients tested for antibodies and 4.8 per 100 patients receiving transfusion. Considering E- and c-antigen-matched red cells along with ABO and RhD matching may significantly reduce the overall occurrence of alloimmunization among Indian population who are transfusion-dependent.

KEYWORDS
alloimmunization, antibodies, extended phenotyping, red blood cells, transfusion

Highlights

- The rate of alloimmunization was higher in multiply transfused patients (4.8 per 100 patients) than the hospital-based studies (0.5 per 100 patients) in India. Anti-E and anti-c were the most common red cell antibodies identified in multiply transfused patients.

- Considering E and c antigen matched red cell units for patients requiring chronic transfusion may reduce the overall rate of alloimmunization in this group.

BACKGROUND

Alloimmunization to red blood cell (RBC) antigens is caused by exposure to the exogenous red cell antigens either through transfusion or pregnancy. Naturally occurring antibodies are an exception in which the antibodies may be produced in the absence of exposure to the foreign RBCs. Clinically significant alloantibodies can lead to serious transfusion reactions or haemolytic disease of the newborn. These antibodies are capable of reducing the life span of the RBCs possessing the corresponding antigen. Pre-transfusion testing involves ABO typing and antibody screening. In developing countries, there is no recommendation exists on the transfusion of red cells that are matched for minor blood group antigens for multiply-transfused patients such as those with sickle cell anaemia or for the general patient population.

Blood group systems are inherently polymorphic and show great variability across populations. To date, 39 blood group systems containing more than 300 blood group antigens have been reported. India comprises of more than one billion people and is a land of vast human diversity. The National Estimation of Blood Requirement in India, a project report by the National AIDS control organization, India, shows that the demand for the whole blood and RBCs is 6.3 and 6.6 million units per annum, respectively [1]. Broadly, ethnicity can be classified as people united based on some common physical or socio-cultural attributes like race, culture, language, religion, region, nationality and heritage, or as a segment of a larger population which is seen by others to be different in some combination of the following characteristics—language, religion, race and ancestral homeland with its related culture [2, 3]. India has more than 2000 ethnic groups with various religions, multiple languages, caste and so on [4]. Considering the population diversity in India, one may expect a considerably high rate of alloimmunization among recipients of blood transfusion. The existing literature on RBC alloimmunization has mostly reported the prevalence of antibodies among a specific group of patients and restricted to single centres. There is limited knowledge on the most prevalent RBC antibodies in the Indian population. This knowledge gap is taken as the main review question to study the commonly found red cell antibodies among Indians receiving RBC transfusion. Based on the results, the secondary objective of the study was to substantiate whether the currently existing recommendation on partial red cell antigen matching for Rh and Kell blood group system to reduce alloimmunization is applicable to the Indian population.

METHODOLOGY

The review was planned and conducted following PRISMA guidelines [5]. The protocol of the systematic review comprising the study objective, inclusion and exclusion criteria and the primary and secondary outcome was registered with PROSPERO with the registration number PROSPERO 2020 CRD42020161098 and is available through the following link: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020161098.

Search strategy and study selection

We searched the MEDLINE, SCOPUS, CINAHL and Google Scholar bibliographic databases with no restriction in search dates using key words 'erythrocytes' OR 'red' AND 'blood' AND 'cell' OR 'red blood cell' AND alloimmunization AND 'blood transfusion' OR 'blood' AND 'transfusion' AND 'India' on 31 May 2020. The software Covidence was used for the identification and refining of duplicates of the studies retrieved using the search strategy. Papers included at the full-text screening stage were subjected to forward and backward citation analysis. All the original articles in the English language on alloimmunization among recipients of blood transfusion conducted on the Indian population were included for the review. Studies conducted on both paediatric and adult age groups were considered. Studies on alloimmunization among antenatal cases without a history of blood transfusion, as well as animal studies, were excluded.

Data extraction

For data extraction, the titles and abstracts of studies obtained after removal of duplicates and studies retrieved from additional sources were screened independently by two review authors (S.S. and D.C.) for relevance according to the inclusion criteria outlined above. The full texts of the selected studies were retrieved and independently assessed for eligibility for review by two teams of investigators (S.S./S.D. and D.C./A.B.). Any disagreement was resolved through discussion with the reviewer (R.K.C.). A standardized data extraction form was used to extract data from the studies included for evidence synthesis. Extracted information included the study setting, study population, participant demographic and baseline characteristics, study methodology and outcomes. The methodological quality of the included studies was assessed independently by two study authors (S.S. and D.C.) adopting the critical appraisal checklist for studies on reporting prevalence data [6]. The risk of bias was assessed using parameters adopted from JBI critical appraisal checklist for studies reporting prevalence data. All the studies were assessed for whether the sample frame was appropriate to address the target population, whether the subjects and setting were described in detail, whether data analysis was conducted with sufficient coverage of samples, whether valid methods were used for identification of the condition, whether the condition was measured in a standard and reliable way for all participants and whether appropriate statistical analysis was performed to describe the rate of alloimmunization.

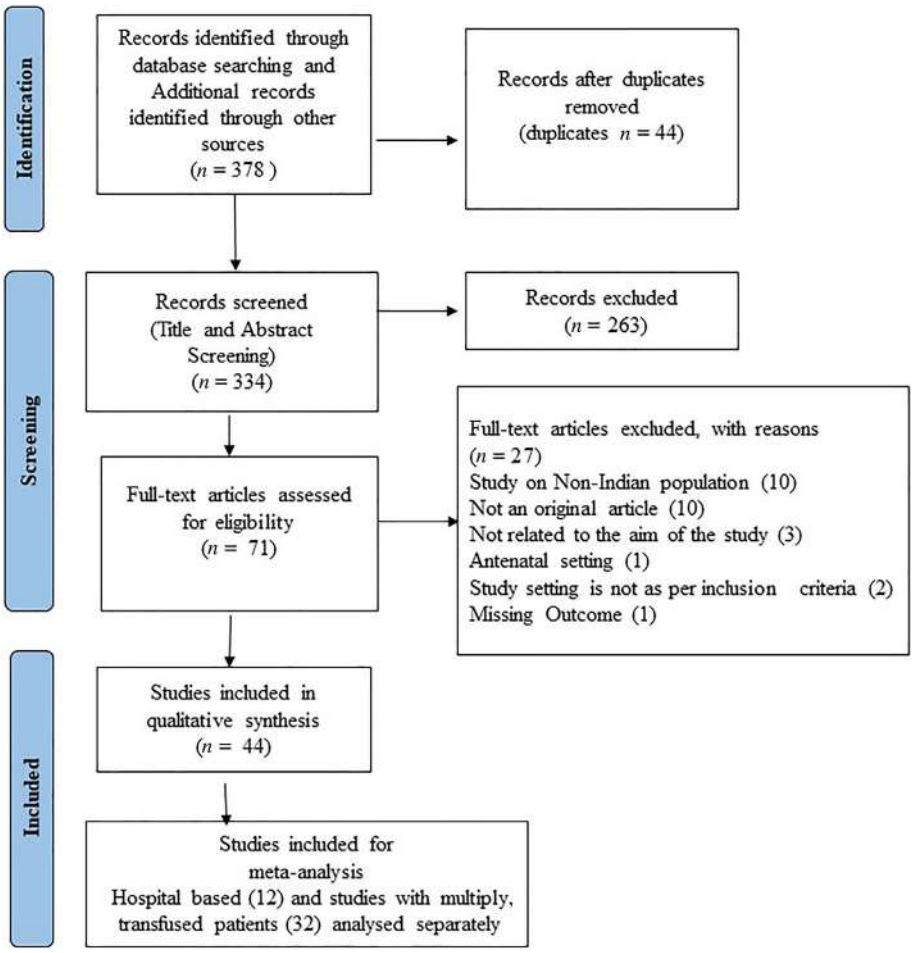

FIGURE 1 PRISMA chart showing the summary of data extraction history

Data synthesis and statistical analysis

Studies with similar characteristics were categorized as hospital-based and those that included multiply-transfused patients and were subjected to meta-analysis separately. Data were analysed using the software Open Meta Analyst. Meta-analysis was performed by the binary random-effects model using the restricted maximum likelihood method. Heterogeneity in results across the studies was assessed using the I^2 statistic [7]. Funnel plots were used to visually check for the possible existence of publication bias, performed by the regression test Fail-safe N Calculation Using the Rosenthal Approach. Subgroup analysis was performed for categories such as the zone of state in both categories. Meta-regression was performed to evaluate effect of the categories on the prevalence of alloimmunization.

RESULTS

Search results

A total of 378 articles were identified in the search process. After the removal of duplicates, 334 articles remained. After title and abstract screening, 263 studies were excluded from full-text screening, which was done for 71 articles. Of these, 44 met the inclusion criteria. Three-quarters of the studies included were conducted on groups of patients who were multiply-transfused (32; 72.72%); the remaining were hospital-based studies that included all patients requiring transfusion during the study period. The PRISMA flow diagram is shown in Figure 1. The articles included in the review were published between 1999 and 2020 [8–51]. Studies in the multiply-transfused group mostly comprised patients with thalassemia, sickle cell anaemia, oncology cases and renal failure. The majority of the studies were conducted in the north zone 21 (47.72%), followed by 13 (29.55%), 5 (11.36%), 4 (9.1%) and 1 (2.27%) from the west, east, south and central zones, respectively. The sample size per study ranged from 32 to 74,214 patients. Table 1 describes the general characteristics of the included studies.

Proportion of RBC alloantibodies

The serological work-up for red cell antibody screening and identification was performed using different techniques, with most of them

TABLE 1 General characteristics and outcome of the eligible studies

S no.	First author (year publication)	Zone	Type of study	Study population—all patients/mention category	Multiply-transfused (yes/no)	Method of detection	Total number of patients	Total no of alloimmunized patients	Rate of alloimmunization (%)	Number of patients with multiple alloantibodies
1	Thakral et al. (2008)	North	Cross-sectional study	All multiply-transfused patients	Yes	CTT	531	18	3.39	0
2	Patel et al. (2009)	West	Prospective	All multiply-transfused patients	Yes	Not mentioned	504	39	7.74	Antibody specificity not mentioned
3	Pahuja et al. (2010)	North	Cross-sectional study	Thalassemics	Yes	CAT	211	8	3.79	2
4	Gupta et al. (2011)	North	Cross-sectional study	Thalassemics	Yes	CAT	116	11	9.48	1
5	Pathak et al. (2011)	North	Cross-sectional study	All patients needing transfusion	No	CAT	45,373	59	0.13	7
6	Makroo et al. (2013)	North	Cross-sectional study	Thalassemics	Yes	SPRCA	462	19	4.11	2
7	Sood et al. (2013)	North	Prospective	Chronically transfusion-dependent patients	Yes	CAT, SPRCA	306	13	4.25	6
8	Elhence et al. (2014)	North	Retrospective	Thalassemics	Yes	CAT	280	24	8.57	4
9	Makroo et al. (2014)	North	Cross-sectional study	All patients	No	SPRCA	49,077	239	0.49	54
10	Philip et al. (2014)	West	Retrospective	All multiply-transfused patients	Yes	CAT	200	11	5.50	0
11	Zaman et al. (2014)	North	Retrospective	All patients	No	Not mentioned	11,235	157	1.40	11
12	Jain et al. (2014)	West	Prospective	Thalassemics	Yes	Not mentioned	96	5	5.21	0
13	Ket al. (2014)	North	Cross-sectional study	Thalassemics	Yes	CAT	319	18	5.64	3
14	Datta et al. (2015)	East	Cross-sectional study	Thalassemics	Yes	CAT	500	28	5.60	7
15	Saleem et al. (2015)	South	Cross-sectional study	Thalassemics	Yes	CAT	55	1	1.82	0
16	Gamit et al. (2015)	West	Cross-sectional study	Patients with chronic renal failure	Yes	CTT	50	5	10.00	Antibody specificity not mentioned
17	Ambuja et al. (2015)	South	Retrospective	All patients	No	CAT	60,518	332	0.55	42
18	Dogra et al. (2015)	North	Cross-sectional study	Thalassemics	Yes	CAT	70	6	8.57	0

(Continues)

TABLE 1 (Continued)

S no.	First author (year publication)	Zone	Type of study	Study population—all patients/mention category	Multiply-transfused (yes/no)	Method of detection	Total number of patients	Total no of alloimmunized patients	Rate of alloimmunization (%)	Number of patients with multiple alloantibodies
19	Sharma et al. (2016)	Central	Retrospective	Thalassemics	Yes	CAT	120	4	3.33	1
20	Patel et al. (2016)	West	Cross-sectional study	Thalassemics	Yes	CTT	50	4	8.00	Antibody specificity not mentioned
21	Shah et al. (2016)	West	Cross-sectional study	Thalassemics	Yes	CTT	185	10	5.41	0
22	Agrawal et al. (2016)	North	Cross-sectional study	All multiply-transfused patients	Yes	EMA	258	7	2.71	2
23	Bajpai et al. (2016)	North	Retrospective	Patients with liver disease	Yes	CAT	842	44	5.23	10
24	Bhuva et al. (2017)	West	Cross-sectional study	All multiply-transfused patients	Yes	CAT	300	9	3.00	0
25	Varshney et al. (2017)	West	Cross-sectional study	All patients	No	CAT and CTT	2258	11	0.49	0
26	Chauhan et al. (2015)	South	Retrospective	All patients	No	Not mentioned	1912	7	0.37	0
27	Makroo et al. (2017)	North	Retrospective	Liver transplant recipients	Yes	SPRCA	1433	22	1.54	Number of patients with multiple antibodies is not mentioned
28	Garg S et al. (2017)	West	Cross-sectional study	All multiply-transfused patients	Yes	CAT	150	16	10.67	0
29	Kaur et al. (2017)	North	Retrospective	All patients	No	CAT	6136	1	0.02	0
30	Makroo et al. (2017)	North	Prospective	Patients undergoing cardiac surgery	Yes	SPRCA	250	1	0.40	0
31	Kulkarni et al. (2018)	West	Cross-sectional study	Thalassemics	Yes	CTT	200	15	7.50	0
32	Sawhney et al. (2018)	North	Cross-sectional study	Chronic renal failure patients	Yes	CTT	96	12	12.50	4
33	Gupta et al. (2018)	North	Cross-sectional study	All patients	No	CAT	350	3	0.86	0
34	Bhattacharya et al. (2018)	East	Cross-sectional study	All patients showing incompatible crossmatch only	No	CAT	14,387	16	0.11	6
35	Mahapatra et al. (2019)	East	Cross-sectional study	All multiply-transfused patients	Yes	CAT	816	33	4.04	4

(Continues)

TABLE 1 (Continued)

S no.	First author (year publication)	Zone	Type of study	Study population—all patients/mention category	Multiply-transfused (yes/no)	Method of detection	Total number of patients	Total no of alloimmunized patients	Rate of alloimmunization (%)	Number of patients with multiple alloantibodies
36	Poornima et al. (2019)	South	Cross-sectional study	All multiply-transfused patients	Yes	CAT	63	4	5.88	0
37	Gupta et al. (2019)	West	Cross-sectional study	All patients	No	EMA, CAT	74,214	510	0.69	Number of patients with multiple antibodies is not mentioned
38	Jariwala et al. (2019)	West	Retrospective	Thalassemics and sickle cell anaemia patients	Yes	CAT	471	19	4.03	0
39	Nagrath et al. (2020)	East	Retrospective	Thalassemia, myeloproliferative disorder, haematological disorder, end-stagerenal failure	Yes	CAT and SPRCA	343	11	3.21	3
40	Dhar et al. (2015)	East	Retrospective	HSCT recipients and surgical oncology recipients	Yes	CAT	550	14	2.55	2
41	Tiwari et al. (2014)	North	Cross-sectional study	All patients	No	CAT	32,560	40	0.12	0
42	Chaudhary et al. (2011)	North	Cross-sectional study	All patients	Not mentioned	CAT	2026	26	1.28	0
43	Chaudhari et al. (2010)	West	Cross-sectional study	Thalassemics	Yes	CAT	32	6	18.75	1
44	Shukla et al. (1999)	North	Retrospective	Chronic renal failure	Yes	Not mentioned	81	8	9.88	1

Abbreviations: CAT, column agglutination technique; CTT, conventional tube technique; EMA, electromagnetic bead assay; SPRCA, solid-phase red cell adherence assay.
[Correction added on 20 Jun 2022 after first online publication: Table 1 was corrected in this version.]

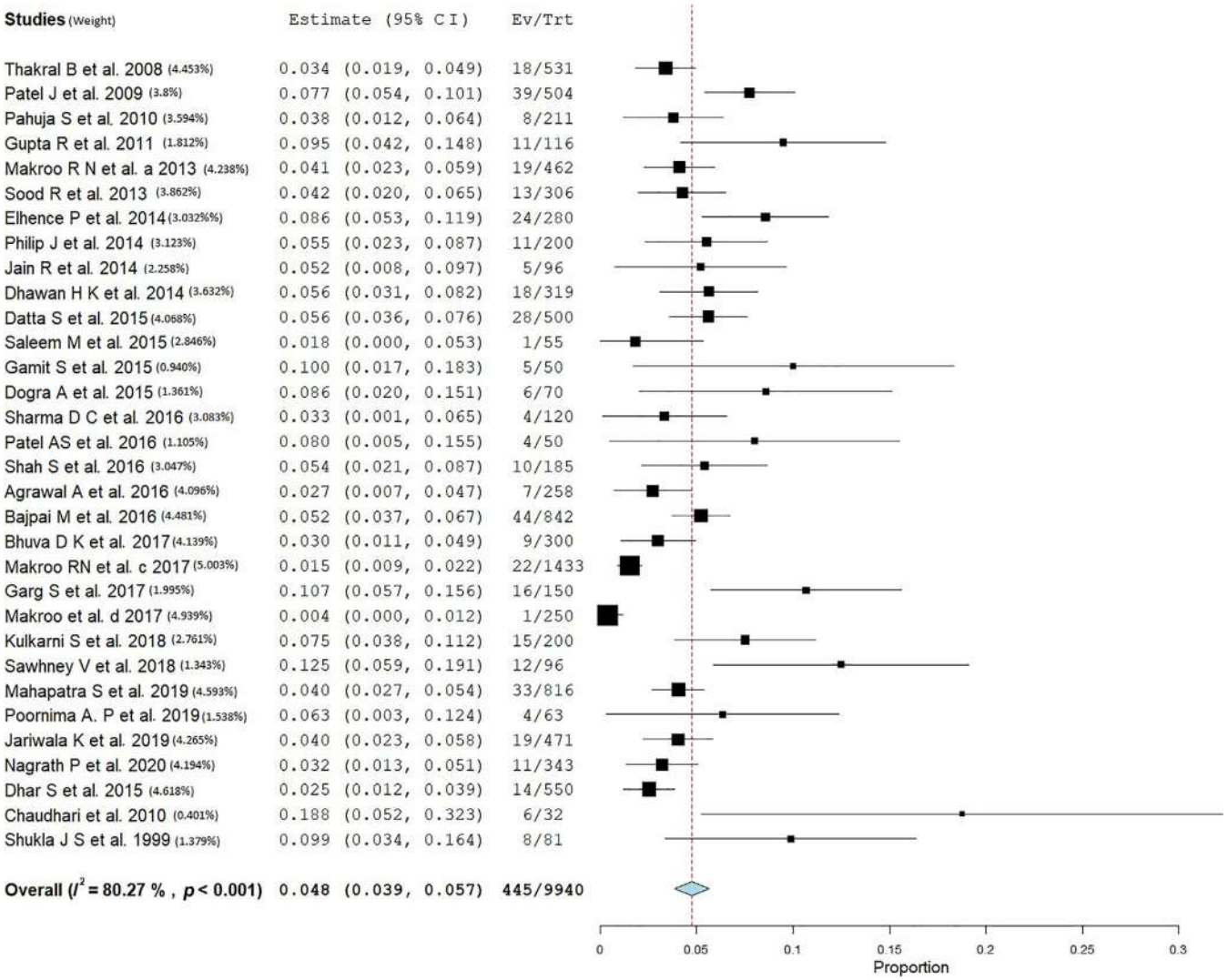

FIGURE 2 Forest plot of proportion estimate of alloimmunization among studies with only multiply-transfused patients

mentioning column agglutination technique (24 studies; 54.55%) followed by conventional tube technique (Six; 13.63%), solid-phase red cell adherence assay (four studies; 9.1%), electromagnetic bead assay (one study; 2.27%) and a combination of these methods (four studies; 9.1%). The technique to perform these tests was not mentioned in five studies (Table 1). [Correction added on 20 Jun 2022 after first online publication: The preceding paragraph was corrected in this version.].

A total of 44 studies, with a cumulative sample size of 309,986 patients, were included. The overall rate of alloimmunization among the studies which included multiply-transfused patients was 4.8 (95% confidence interval; 3.9–5.7) per 100 patients receiving transfusion with a zone-wise prevalence of 6.1%, 4.7%, 3.7%, 3.3% and 3.4% West, North, East, Central and South India, respectively (Figure 2). The overall prevalence in studies that included all patients requiring transfusion (hospital-based studies) was 0.5 (95% confidence interval; 0.3–0.8) per 100 patients tested for antibodies with a zone-wise prevalence of 0.6%, 0.6%, 0.5% and 0.1% in North, West, South and East India, respectively (Figure 3). A high rate of heterogeneity was observed between the studies in multiply-transfused and hospital-based studies with I^2 = 80.27% and 99.49%, respectively. Meta-regression revealed no significant difference of prevalence of alloimmunization between the different zones of the country in both the groups, with a p-value of 0.067 and 0.411 for hospital-based studies and multiply-transfused patients, respectively.

RBC alloantibody specificities

The details on the antibody specificities were mentioned in 93% of the studies (41/44). The details of RBC alloantibody specificity are as follows.

Overall study population

In the present review, as many as 1992 alloantibodies were identified among the 1846 alloimmunized patients. The antibody specificities were not mentioned in three studies, the details of

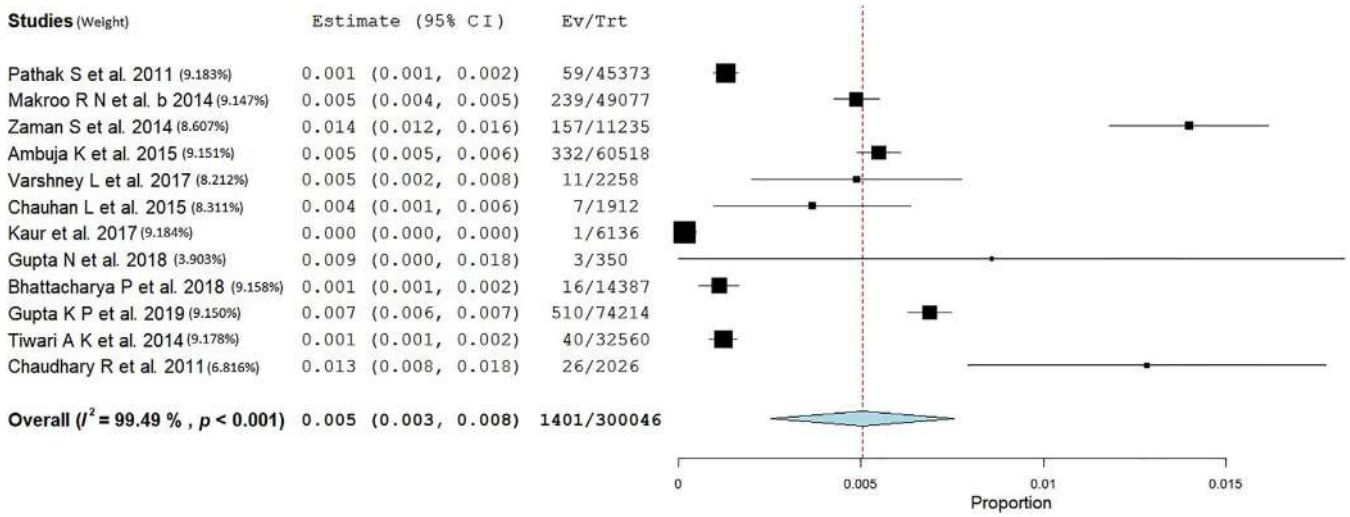

FIGURE 3 Forest plot of proportion estimate of alloimmunization among hospital based studies

TABLE 2 Specificity of red cell antibodies

Blood group system	Specificity	Antibody specificity among all studies, *n* (%)	Number of alloimmunized patients (Total: 1798), *n* (%)	Antibody specificity among multiply-transfused patients, *n* (%)	Number of alloimmunized patients in multiply-transfused group (Total: 397), *n* (%)	Antibody specificity in hospital-based studies (%)	Number of alloimmunized patients in hospital-based studies (Total: 1401), *n* (%)
Rh	D	481 (24.74)	481 (26.75)	30 (6.77)	30 (7.56)	451 (30.05)	451 (32.19)
	D + C	55 (2.83)	55 (3.06)	5 (1.13)	5 (1.26)	50 (3.33)	50 (3.57)
	C	81 (4.17)	81 (4.51)	26 (5.87)	26 (6.55)	55 (3.66)	55 (3.93)
	c	204 (10.49)	204 (11.35)	75 (16.93)	75 (18.89)	129 (8.59)	129 (9.21)
	E	451 (23.20)	451 (25.08)	127 (28.67)	127 (31.99)	324 (21.59)	324 (23.13)
	e	19 (0.98)	19 (1.06)	4 (0.90)	4 (1.01)	15 (1.0)	15 (1.07)
	Cw	18 (0.93)	18 (1.00)	7 (1.58)	7 (1.76)	11 (0.73)	11 (0.79)
Kell	K	167 (8.59)	167 (9.29)	60 (13.54)	60 (15.11)	107 (7.13)	107 (7.64)
	k	3 (0.15)	3 (0.17)	1 (0.23)	1 (0.25)	2 (0.13)	2 (0.14)
	Kpa	9 (0.46)	9 (0.50)	5 (1.13)	5 (1.26)	4 (0.27)	4 (0.29)
	Kpb	0	0	0	0	0	0
MNSs	M	114 (5.86)	114 (6.34)	10 (2.26)	10 (2.52)	104 (6.93)	104 (7.42)
	N	26 (1.34)	26 (1.45)	4 (0.90)	4 (1.01)	22 (1.47)	22 (1.57)
	S	45 (2.31)	45 (2.50)	9 (2.03)	9 (2.27)	36 (2.4)	36 (2.57)
	s	13 (0.67)	13 (0.72)	4 (0.90)	4 (1.01)	9 (0.6)	9 (0.64)
Duffy	Fya	24 (1.23)	24 (1.33)	5 (1.13)	5 (1.26)	19 (1.27)	19 (1.36)
	Fyb	13 (0.67)	13 (0.72)	7 (1.58)	7 (1.76)	6 (0.4)	6 (0.43)
Kidd	Jka	44 (2.26)	44 (2.45)	20 (4.51)	20 (5.04)	24 (1.6)	24 (1.71)
	Jkb	24 (1.23)	24 (1.33)	8 (1.81)	8 (2.02)	16 (1.07)	16 (1.14)
Lewis	Lea	52 (2.67)	52 (2.89)	12 (2.71)	12 (3.02)	40 (2.66)	40 (2.86)
	Leb	19 (0.98)	19 (1.06)	6 (1.35)	6 (1.51)	13 (0.87)	13 (0.93)
Lutheran	Lua	4 (0.21)	4 (0.22)	1 (0.23)	1 (0.25)	3 (0.2)	3 (0.21)
	Lub	0	0	0	0	0	0
	Xga	1 (0.05)	1 (0.06)	1 (0.23)	1 (0.25)	0	0
	V	1 (0.05)	1 (0.06)	0	0	1 (0.07)	1 (0.07)
	Mia	1 (0.1)	1 (0.06)	0	0	1 (0.07)	1 (0.07)
	Unknown	75 (3.86)	75 (4.17)	16 (3.61)	16 (4.03)	59 (3.93)	59 (4.21)
	Total	1944 (100)	–	443 (100)	–	1501 (100)	–

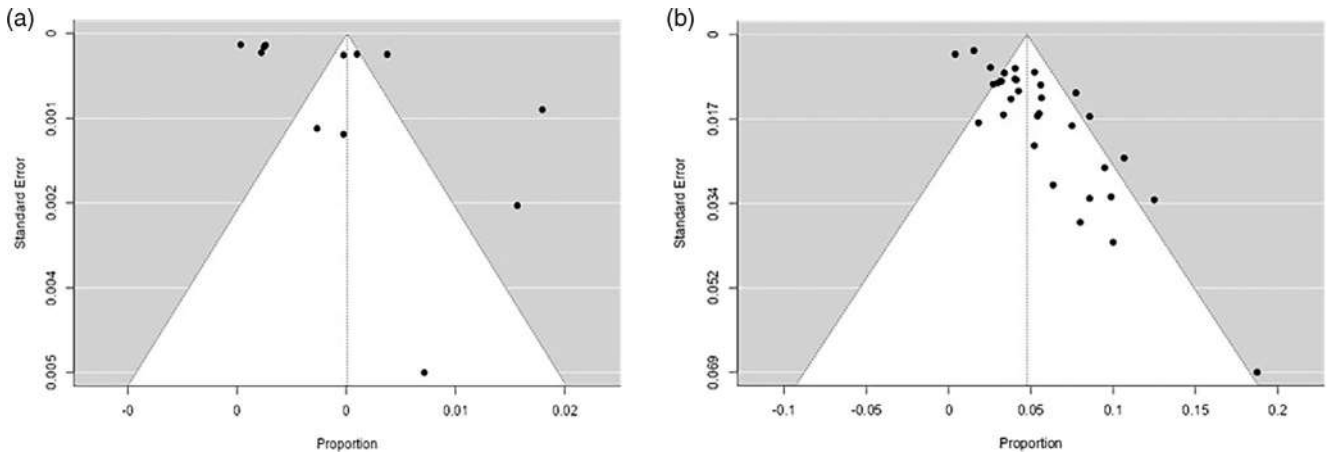

FIGURE 4 Funnel plot to examine the bias in the studies. (a) Funnel plot for hospital based studies. (b) Funnel plot for studies on multiply transfused patients

alloimmunization against individual antibody specificities were reported in 1798 patients with 1944 antibodies. The most common antibody identified was anti-D (481; 26.75%) followed by anti-E (451; 25.08%), anti-c (204; 11.35%), anti-K (167; 9.29%) and anti-M (114; 6.34%).

Multiply-transfused patients

A total of 491 alloantibodies were identified in 445 multiply-transfused patients. As the antibody specificities were not mentioned in 3 studies, alloimmunization details against individual antibody specificities could be extracted only in 397 cases among the multiply-transfused cohort. In this group of patients, the most common antibody identified was anti-E (127; 31.99%) followed by anti-c (75; 18.89%), anti-K (60; 15.11%), anti-D (30; 7.56%), anti-C (26; 6.55%) and anti-Jka (20; 5.04%).

Hospital-based studies

As many as 1501 alloantibodies were identified in 1401 alloimmunized patients among the hospital-based studies. In these studies, the most common antibody identified was anti-D (451; 32.19%) followed by anti-E (324; 23.13%), anti-c (129; 9.21%), anti-K (107; 7.64%) and anti-M (104; 7.42%).

Table 2 summarizes the frequency of antibody specificities among the total study population and the groups with multiply-transfused and hospital-based studies.

Multiple antibodies

Two studies did not mention the number of patients with multiple antibodies [31, 44]. Accordingly, approximately 10.82% (137/1266) of the total patients, 14.13% (53/375) of the multiply-transfused patients and 9.43% (84/891) of the patients in hospital-based studies had multiple alloantibodies.

Publication bias

Asymmetry was noted in the funnel plot drawn to examine the bias in the results. The observation was significant, as shown by the regression test (Fail-safe N Calculation Using the Rosenthal Approach) for funnel plot ($p < 0.001$), indicating the presence of publication bias with respect to the sample size (Figure 4).

The details of risk of bias were assessed using the parameters adopted from JBI critical appraisal (Table S1). In 12 studies, the study population was not appropriately described, that is, whether the patients had any triggering event for alloimmunization [12, 16, 18, 24, 32, 33, 36, 40, 41, 44, 48, 49]. In three studies, a valid method was used for the identification of alloimmunization; however, the specificity of the same was not confirmed by antibody identification. Hence these studies were described as not using valid methods for identification of the condition [9, 23, 27].

DISCUSSION

Systematic review and meta-analysis provides a more objective summary to guide policy-making than single-centre studies. The present systematic review included 44 studies on red cell alloimmunization among the recipients of blood transfusion from India. To the best of our knowledge, this is the first study of its kind from India. The frequency of the red blood cell antigens and thus the specificity of the antibodies differ by ethnicity. Hence it is important to review regional data on this subject and formulate guidelines on matching for the red blood cell antigens accordingly.

TABLE 3 Literature review on rate of alloimmunization in transfusion recipients

Region	Patient group	Prevalence	Common antibodies
Sub-Saharan Africa, Ngoma et al. (2016) [52]	Recipients of RBC transfusion	6.70%	Anti-E,[a] anti-K, anti-C and anti-D
Sub-Saharan Africa, Boateng et al. (2019) [53]	Sickle cell disease	7.40%	Anti-E,[a] anti-D, anti-C and anti-K
Iran, Darvishi et al. (2016) [54]	Thalassemia	10%	Anti-K,[a] anti-D and anti-E
Iran, Hosseini et al. (2020) [55]	Patients on regular transfusion	0–55%	Anti-K,[a] anti-E, anti-D, anti-C and anti-c
China, Chen et al. (2016) [56]	Prevalence of unexpected antibodies among Chinese population	0.23%	Anti-E,[a] anti- D, anti-C and anti-c
World, Franchini et al. (2019) [57]	Patients with transfusion dependent thalassemia	11.40%	Anti-K,[a] anti-E, anti-D, anti-c and anti-C
Brazil, Gomes et al. (2019) [58]	Patients with sickle cell disease	28.39% (mean incidence)	Rh followed by Kell system
Present study (2021)	Recipients of RBC transfusion (hospital-based studies)	0.5%	Anti-D,[a] anti-E, anti-c and anti-K
	Multiply-transfused patients	4.8%	Anti-E,[a] anti-c and anti-K

[a]Most common.

The specificity of the antibodies reported in the literature mostly is of the Rh and Kell blood group systems. In the present review, we considered a broad category of 'recipients of blood transfusion' as study subjects to review the rate of alloimmunization, and the commonest antibody noted was anti-D; it was anti-E among multiply-transfused patients. Ngoma et al. in a systematic review on the recipients of RBC transfusion of sub-Saharan Africans noted an alloimmunization rate of 6.7%, and anti-E was the commonest antibody in the study population [52]. A systematic review and meta-analysis of alloimmunization of patients with sickle cell disease of sub-Saharan Africa showed an overall proportion of alloimmunization of 7.4 (95% confidence interval: 5.1–10.0) per 100 transfused patients [53]. Antibodies against E, D, C and K antigens accounted for almost half of antibody specificities in their population. Darvishi et al. performed a systematic review and observed that the prevalence of alloimmunization in Iranian thalassaemia patients was 10%, the most prevalent alloantibody being anti-K (37%), followed by anti-D (29%) and anti-E (20%) [54]. Hosseini et al. reported a prevalence of 0%–55% of alloimmunization rate in patients on regular transfusion, with anti-K followed by anti-E, anti-D and anti-C being the most common antibodies identified [55]. A systematic review on the prevalence of unexpected antibodies in the Chinese population by Chen et al. reported an overall prevalence of 0.23%, with anti-E being the most common antibody followed by anti-D and anti-C [56]. Franchini et al. reported an overall alloimmunization rate of 11.4% among transfusion-dependent thalassemics from across the world, with anti-K being the most common antibody identified, followed by anti-E, anti-D, anti-C and anti-c [57]. Similarly, Gomes et al. reported an overall prevalence of 28.39% of alloimmunization among patients with sickle cell disease in Brazil, with most prevalent antibodies being against Rh followed by the Kell system [58]. Table 3 gives international data on the similar systematic reviews performed on different patient populations (patients with thalassemia, sickle cell disease, recipients of blood transfusion and patients on regular transfusions). All the studies reported that antibodies against Rh and Kell system were the most common alloantibodies detected, re-emphasizing the use of Rh- and Kell-matched blood in transfusion-dependant patients to prevent alloimmunization.

The alloimmunization rate in multiply-transfused patients was higher (4.8%) in comparison to the hospital-based patients, which included all patients requiring a transfusion (0.5%). This is most likely because the majority of patients in hospital studies had no prior exposure to RBC antigens either through transfusion or pregnancy.

In this study, we identified anti-D as the most common antibody, followed by anti-E, anti-c, anti-K and anti-M in hospital-based studies and in the total study population. About half (50%) of the antibodies were anti-D and anti-E. In India, the policy is to issue RhD antigen-matched red cell units to recipients; however, anti-D (26.5%) was the most common antibody identified in our study. Most of these studies did not report on the triggering event for anti-D production, that is, pregnancy or transfusion. Although we completely excluded studies on antenatal women, the articles included for the analysis had pregnancy-induced alloimmunized women receiving transfusion for various clinical conditions. Hence, this can be reason for higher rate of anti-D that we found in the present analysis among hospital-based studies. However, among the studies that included multiply-transfused patients, about half of the antibodies were anti-E and anti-c. There was a major difference in the prevalence of anti-D in hospital-based studies (32.19%) and studies that included only multiply-transfused patients (7.56%), probably because of a high frequency of pregnancy-induced antibodies, as stated earlier.

Prophylactic antigen-matched blood (PAM) transfusion is an effective strategy to prevent alloantibody formation and the subsequent negative consequences (Figure 5). PAM is important especially in transfusion-dependent patients such as those with warm autoantibodies, sickle cell disease, thalassemia, cancer, liver disease and so

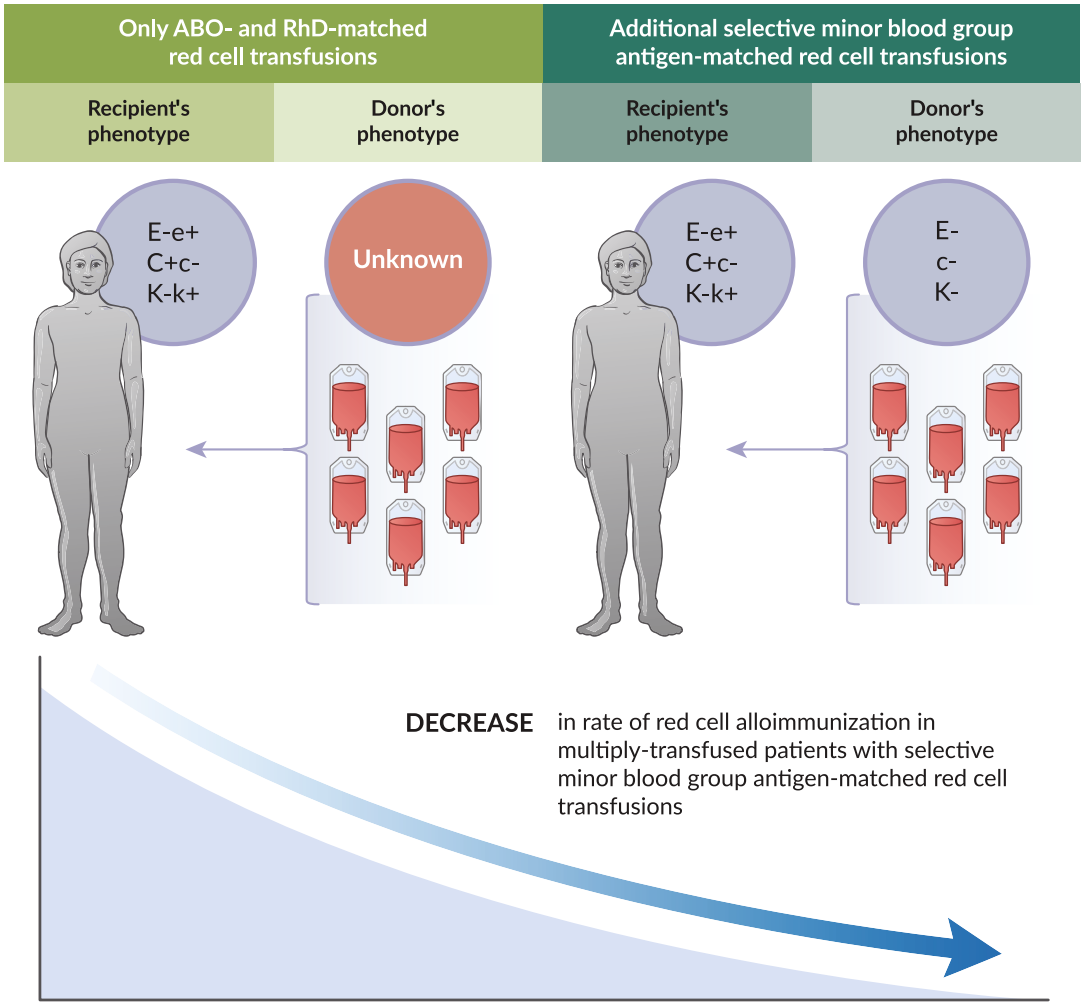

FIGURE 5 Red cell alloimmunization in multiply-transfused patients

on. They are at greater risk of developing alloantibodies especially when there is a high disparity in RBC antigens between the donor and the patient. As per British Committee for Standards in Haematology guidelines on pre-transfusion compatibility procedures in blood transfusion laboratories, it should be a local decision whether to provide red cells that have been additionally matched for Rh (CcEe) and K to minimize the risk of red cell alloimmunization [59].

Considering the prevalence of RBC alloimmunization among patients in Indian population, antibody screening should be made mandatory in pre-transfusion testing. Though it is mentioned in the guidelines and the NABH standards, it is not being followed in majority of the blood centres. Implementation of a routine antibody screening necessitates the use of reagent red cells and additional technical expertise in interpretation of the results. The imported reagent red cells, adds to the cost for transfusion, and this may be considered as a hindrance in the implementation of routine antibody screening all over India. Prophylactic antigen matching or genotype-matched blood transfusion is an ideal strategy to prevent alloimmunization. However, it may not be possible in a resource-poor setting, and hence we propose that as an economical strategy to reduce the risk of alloimmunization, we can consider providing matching for E and c antigens in addition to the RhD matching for patients requiring multiple transfusions. This requires only the use of additional antisera of anti-E and anti-c for screening the patient and the donor cells. Based on the present systematic review of the existing data on red blood cell alloimmunization in multiply-transfused patients among Indian population, this strategey would help in reducing the rate of alloimmunization. The probability of finding antigen-negative blood for a transfusion recipient is based on the prevalence of the corresponding antigen-negative individuals in the population. The prevalence of E and c antigens as per studies conducted in India is around 20% and 58%, respectively [60, 61]. Hence, the probability of finding E- and c-matched units is relatively high (1 out of 3 units cross-matched) and could be a cost-effective procedure in developing countries.

The comprehensive literature search was performed to include most of the studies conducted across India without the date restrictions and included studies published in most of the databases. It is more important to study the prevalence of alloimmunization in patients receiving blood transfusions than in multiply-transfused patients, because we may miss the other triggering responses for alloimmunization such as pregnancy or transplantation. In this

review, we included the studies on patients receiving blood transfusion to understand the overall prevalence of alloimmunization in the country.

Most of the studies were cross-sectional, which might have led to underestimation of the true prevalence of the alloimmunization. The risk factors for alloimmunization could not be analysed, as most of the studies did not report on pregnancy, history of transfusion and previous antibody testing.

Further studies are needed that provide information on parameters that have been shown to influence alloimmunization, such as gender, age of patients, number of transfusion events before alloimmunization occurred, history of pregnancy, age at first transfusion and age of RBCs transfused. This can be achieved when future studies use the standard guidelines of Strengthening the Reporting of Observational studies in Epidemiology (STROBE) for reporting the prevalence of alloimmunization.

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S.S. and D.C. designed the research study, performed the analysis and wrote the initial draft of the manuscript. S.S., S.D., D.C. and A.B. and their team performed data extraction. R.K.C. supervised the research and reviewed and edited the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Risk of transfusion-related acute lung injury and human immunodeficiency virus associated with donations from trans donors in Quebec, Canada

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Abstract

Background and Objectives: Blood operator must establish selection criteria according to the populations at risk of blood-related infections and complications. Therefore, this study aimed to assess the risks of transfusion-related acute lung injury (TRALI) and human immunodeficiency virus (HIV) associated with donations from trans persons.

Materials and Methods: Donor screening data from Héma-Québec were used. The risks of TRALI and HIV were estimated based on internal data and assumptions derived from the literature. The risk was assessed under four scenarios: a most likely scenario, an optimistic scenario and two pessimistic scenarios. All scenarios assumed no prior screening for trans donors.

Results: The trans population comprised 134 donors, including 94 (70.1%) trans men. Of the 134 donors, 58 (43.3%) were deferred from donating a blood-derived product because of an ongoing gender-affirming genital surgery, and the remaining 76 (56.7%) were eligible donors. The risk of having a TRALI-causing donation, given that it comes from a trans man, was estimated at one every 115–999 years for all scenarios. The risk of having an HIV-contaminated donation, given that it comes from a trans woman, was estimated at one every 1881–37,600 years for all scenarios.

Conclusion: This study suggests that donations from trans persons are associated with a negligible risk of TRALI and HIV.

KEYWORDS
blood safety, risk analysis, trans donors, transfusion medicine, transfusion-transmissible infection

HIGHLIGHTS

- Using a risk model simulation, this study helps understand the risks of transfusion-related acute lung injury (TRALI) and human immunodeficiency virus (HIV), potentially associated with donations from trans donors.
- Our analysis suggests that the risk of having a TRALI-causing donation from a trans man and that of having an HIV-contaminated donation from a trans woman is negligible.

- This study highlights the fact that it may be possible to adopt more inclusive practices for trans donors while negligibly affecting blood safety.

INTRODUCTION

Blood operators must establish selection criteria according to the populations at risk of blood-related infections. In particular, gender is associated with various risks related to blood transfusion. For example, the prevalence of human immunodeficiency virus (HIV) is higher among men who have sex with men [1]. The incidence of transfusion-related acute lung injury (TRALI) is highest in recipients of plasma or platelets donated by women with a pregnancy history because pregnancy increases the likelihood of developing anti-human leukocyte antigen (anti-HLA) antibodies [2].

These gender-specific risks are more difficult to assess with trans persons, since the gender identity of these individuals differs from the sex assigned at birth. Moreover, gender transition is unique: it can be social, medical (hormone therapy, gender-affirming genital surgery, etc.) and/or legal [3, 4]. Concerning the legal transition, in Canada, gender is categorized as 'male', 'female' or 'other' on federal-government-issued identification documents (IDs) and can be modified without any medical transition [5]. The same process applies in Quebec, but 'male' and 'female' are the only available categories in provincial-government-issued IDs.

Héma-Québec (a blood operator in Canada) also uses a gender-based classification scheme to screen trans donors prior to donation, which can lead to challenges. For example, trans men (for whom the sex assigned at birth was 'female') may not be asked about their pregnancy history, which may increase the risk of having a TRALI-causing donation. Similarly, trans women (for whom the sex assigned at birth was 'male') may not be asked about sexual contacts with other male partners, which may increase the risk of having a donation contaminated by HIV and other sexually transmitted infections given a higher prevalence among trans women due to unprotected receptive anal intercourse (URAI) [6, 7].

As the number of current and potential trans donors has increased in Canada, this study aimed to assess the risks of TRALI or HIV associated with donations from trans donors.

MATERIALS AND METHODS

Donor screening

At Héma-Québec, trans identity can be self-reported by the donor or captured through questions, such as (1) In the last 6 months, have you consulted a physician for health problems or for a surgery?; and (2) In the last three days, have you taken any medication?

Once trans status is identified, the Assistant Director of Medical Affairs interviews the donor (questions in Table S1) to determine the most suitable questionnaire (i.e., male- or female-specific) and donor eligibility. When gender on government-issued IDs differs from that deemed more suitable for the gender-specific questionnaire, an admissibility card is issued to the donor, which must be presented at each following donation.

Study population

The study population included all self-identified trans individuals who went through the donor screening process at Héma-Québec between 11 August 2015 and 25 August 2021. Eligible and deferred trans donors were included in the analysis, but Héma-Québec donors who self-identified as non-binary were excluded.

Study measures

The risks of TRALI and HIV were assessed based on internal data from Héma-Québec and assumptions derived from the literature.

Risk of TRALI

The risk of having a TRALI-causing donation was calculated using a deterministic approach, assuming the following: (1) Trans male donors who were asked about their pregnancy history are representative of the overall population; (2) There is no prior donor screening for trans donors, that is, the risk is calculated as if no trans men were identified; (3) Only trans men contribute to the risk of TRALI; and (4) Only platelet and plasma (excluding source plasma) donations contribute to the risk of TRALI.

Parameters

The risk of TRALI depends on the number of past pregnancies [8]. Consequently, the following parameters were used to estimate the risk of having a TRALI-causing donation from trans male donors: (1) the prevalence of pregnancy history among trans male donors who were asked about their pregnancy history; (2) the proportion of cis gender women with anti-HLA among those with a pregnancy history, as reported by Triulzi et al. [8]; and (3) the risk of TRALI among recipients of anti-HLA-containing donations, as reported by Kleinman et al. [2].

Therefore, the risk of having a TRALI-causing donation, given that the donation is from a trans man, was calculated as follows:

$$\begin{aligned} &P(\text{TRALI}|\text{Transgender man donation}) \\ &= P(\text{TRALI}|\text{anti}-\text{HLA}) \times P(\text{anti}-\text{HLA}|\text{Pregnancy}) \\ &\quad \times P(\text{Pregnancy}|\text{Transgender man donation}) \end{aligned}$$

The risk of having a TRALI-causing donation from a trans man, among all donations, was calculated as follows:

$$P(\text{TRALI}) = P(\text{TRALI}|\text{Transgender man donation}) \times P(\text{Transgender man donation})$$

where the probability of a donation from a trans man was defined as the mean annual number of donations from trans men at Héma-Québec from 2015 to 2021 divided by the number of platelet and plasma donations in 2019 (pre-pandemic).

Scenarios

The risk of TRALI was assessed under four main scenarios, which differed in the way parameters related to the prevalence of pregnancy history and the probability that a donation contains anti-HLA given a pregnancy history were elicited. In the *most likely scenario*, the prevalence of pregnancy history among trans men was set according to the number of donations from trans men with a pregnancy history between 2015 and 2021, and the probability of having an anti-HLA-containing donation (given a pregnancy history) was set as the mean obtained among all females with a pregnancy history, regardless of the number of pregnancies. In the *optimistic scenario*, the prevalence of pregnancy history was the same as that in the most likely scenario, and the probability of having an anti-HLA-containing donation, given a pregnancy history, assumed all individuals with a pregnancy history only had one previous pregnancy. In the *pessimistic scenario A*, the prevalence of pregnancy history was the same as that in the most likely scenario, and the probability of having an anti-HLA-containing donation, given a pregnancy history, assumed all individuals with a pregnancy history had four or more previous pregnancies. In the *pessimistic scenario B*, the prevalence of pregnancy was four times as high as that in the most likely scenario, and the probability of having an anti-HLA-containing donation was the same as that in the most likely scenario. An additional scenario ('pessimistic scenario C') was also evaluated (detailed in Data S2), with the same parameters as those in the most likely scenario except for the average number of annual donations from trans men. This scenario assumes a higher proportion of donations from trans men among all donations and therefore reflects the worst case for the overall risk of TRALI.

Risk of HIV infection

The risk of having an HIV-contaminated donation was also calculated using a deterministic approach assuming the following: (1) There is no prior donor screening for trans donors, that is, the risk is calculated as if no trans women were identified; (2) Trans female donors engaged in URAI at a rate similar to that of MSM in the last 3 months (to address the lack of published information on trans female donors); (3) Only trans women contribute to the risk of having an HIV-contaminated donation; and (4) Platelets, plasma (excluding source plasma) and red blood cell donations contribute to the risk of HIV infection.

TABLE 1 Trans donor characteristics

	Trans men		Trans women	
	N	(%)	*N*	(%)
Age group (years)[a]				
18–29	65	(48.5)	15	(11.2)
30–39	17	(12.7)	11	(8.2)
40–49	7	(5.2)	8	(6.0)
50–70	5	(3.7)	6	(4.5)
Ethnicity				
White	75	(56.0)	35	(26.1)
Other	9	(6.7)	1	(0.8)
Unknown	10	(7.4)	4	(3.0)
Donor status[b]				
Active	37	(27.6)	13	(9.7)
Inactive	30	(22.4)	16	(11.9)
Never donated	27	(20.1)	11	(8.2)
Deferral due to ongoing gender-affirming genital surgery				
Active	41	(30.6)	17	(12.7)
Ended	53	(39.5)	23	(17.2)
History of pregnancy				
Yes	3	(2.2)	0	(0.0)
No	43	(32.1)	6	(4.5)
Unknown[c]	48	(35.8)	34	(25.4)
Total	94	(70.1)	40	(29.9)

Abbreviation: HQ, Héma-Québec.
[a]Age at the time of the first donation.
[b]A donor was considered active if a donation was made in the last 24 months.
[c]Includes persons who never donated and donors who were not asked about pregnancy history.

Parameters

Risk assessment was stratified based on whether the donation was made during or outside the window period. Missed HIV-contaminated donations made during the window period are caused by undetectable viral loads. Missed HIV-contaminated donations made outside the window period might (theoretically) be caused by failures of nucleic acid tests (NATs), test transcription errors and clinical test errors, but are virtually impossible because of the performance of current tests [9]. This risk, which is essentially zero, has nevertheless been included. The probability of having an HIV-contaminated donation, among donations from trans women, was therefore:

$$P(\text{infectious donation}|\text{Transgender woman donation}) = P(\text{window period donation}|\text{Transgender woman donation}) + P(\text{Test and clinical error}|\text{Transgender woman donation})$$

TABLE 2 Parameters and estimates for the risk of TRALI

Parameter	Estimate				Step	Calculation/ reference
	Most likely scenario[a]	Optimistic scenario[b]	Pessimistic scenario A[c]	Pessimistic scenario B[d]		
Average number of donations from trans men within a year[e]	24	24	24	24	A	HQ database
Average number of donations from trans men with pregnancy history in a year[e,f]	0.67	0.67	0.67	2.67	B	HQ database
Prevalence of pregnancy history among donations from trans men	**0.0278**	**0.0278**	**0.0278**	**0.1111**	**C**	**=B/A**
Probability of anti-HLA among women with pregnancy history	0.244	0.112	0.322	0.244	D	Triulzi et al. [8]
Risk of TRALI among recipients of anti-HLA-containing donations	0.0134	0.0134	0.0134	0.0134	E	Kleiman et al. [2]
Total number of donations per year[g]	67,456	67,456	67,456	67,456	F	HQ database
Risk of TRALI among donations from trans men	**9.08×10^{-5}**	**4.17×10^{-5}**	**1.20×10^{-4}**	**3.63×10^{-4}**	**G**	**=E × D × C**
Number of donations from trans men per TRALI	11,011	23,987	8343	2753	H	=1/G
Number of years for one TRALI-causing donation[h]	**459**	**999**	**348**	**115**	**I**	**=H/A**
Risk of TRALI[h] among all donations	**3.23×10^{-8}**	**1.48×10^{-8}**	**4.26×10^{-8}**	**1.29×10^{-7}**	**J**	**=G·(A/F)**

Note: Bold indicates the main parameters or final risk estimates for TRALI.
Abbreviations: HQ, Héma-Québec; TRALI, transfusion-related acute lung injury.
[a]Most-likely scenario: The prevalence of pregnancy history among donations from trans men was set according to the number of donations from trans men with a pregnancy history between 2015 and 2021; the probability of having an anti-HLA-containing donation among women with a pregnancy history was set as the mean among all females with a pregnancy history, regardless of the number of pregnancies.
[b]Optimistic scenario: The probability of having an anti-HLA-donation among women with a pregnancy history was set as the mean among all females with one pregnancy.
[c]Pessimistic A scenario: The probability of having an anti-HLA-containing donation among women with a pregnancy history was set as the mean among all females with four or more pregnancies.
[d]Pessimistic B scenario: The prevalence of pregnancy history among donations from trans men was set as four times that in the most-likely scenario.
[e]Based on donations made from 2015 to 2021.
[f]Among trans men who were asked about pregnancy history.
[g]Based on the number of plasma and platelet donations in 2019, excluding source plasma.
[h]Only considering the risk of TRALI due to donations from trans men.

The HIV window period is only about 9 days with NAT [10]. So the probability of having an HIV-contaminated donation made during the window period depends on the number of incident infections over a year among trans female donors (i.e., seroconverters) and on the mean interval between donations (MID). The probability of having an HIV-contaminated donation made during the window period, given that the donation is from a trans woman, is therefore:

$$\begin{aligned} &P(\text{window period donation}|\text{Transgender woman donation}) \\ &= P(\text{window period donation}|\text{seroconverter}) \\ &\quad \times P(\text{seroconverter}|\text{Transgender woman donation}) \\ &= \frac{\text{WP}_{\text{length}}}{\text{MID}}(\text{Incidence}_{\text{repeat}} \times \%_{\text{repeat}} + \text{Incidence}_{\text{repeat}} \times \text{FT}_{\text{correction}} \times \%\text{FT}) \end{aligned}$$

To determine the incidence of HIV for first-time donors, an adjustment factor of 1.65 is applied for repeat donors, as proposed by Davison et al. [9]. The proportion of repeat and first-time donors was derived from Héma-Québec data on all trans female donors since 1986.

Trans female donors were assumed to have sexual behaviours similar to those of MSM in the last 3 months. Therefore, HIV incidence for trans female donors was assumed to be the same as that for MSM plasma donors who did not comply with the current 3-month deferral, as reported by Aubé et al. [11]. Uncertainty about the difference in HIV incidence for trans female donors versus MSM donors was addressed in the scenarios (see further below).

For donations made outside the window period, the risk of HIV infection was calculated as follows, based on the prevalence of HIV among trans female donors:

$$\begin{aligned} &P(\text{Test and clinical error}|\text{Transgender woman donation}) \\ &= P(\text{Test and clinical error}|\text{HIV positive donation}) \\ &\quad \times (\text{Prevalence}_{\text{repeat}} \times \%_{\text{repeat}} + \text{Prevalence}_{\text{FT}} \times \%\text{FT}) \end{aligned}$$

The prevalence of HIV among trans female donors was derived from the results of Aubé et al. for non-compliant MSM plasma donors [11].

The risk of an HIV-contaminated donation from a trans woman (among all donations) was also assessed.

$$\begin{aligned} P(\text{infectious donation}) = &P(\text{infectious donation}|\text{Transgender woman donation}) \\ &\times P(\text{Transgender woman donation}) \end{aligned}$$

The probability of having a donation from a trans woman was the annual mean number of donations from trans women between 2015 and 2021 divided by the total number of donations in the year 2019.

TABLE 3 Parameters and estimates for the risk of HIV infection

Parameter	Estimate				Step	Calculation/reference
	Most likely scenario[a]	Optimistic scenario[b]	Pessimistic scenario A[c]	Pessimistic scenario B[d]		
HIV prevalence among repeat, non-compliant MSM donors	1.55×10^{-4}	1.55×10^{-4}	1.55×10^{-4}	1.55×10^{-4}	A	Aubé et al. [11]
HIV prevalence among first-time, non-compliant MSM donors	0.001554	0.001554	0.001554	0.001554	B	Aubé et al. [11]
HIV incidence among repeat, non-compliant MSM donors	1.55×10^{-4}	1.55×10^{-4}	1.55×10^{-4}	1.55×10^{-4}	C	Aubé et al. [11]
Incidence adjustment factor	1	0.5	2	1	D	Scenario's adjustment
HIV incidence for trans women donors	$\mathbf{1.55 \times 10^{-4}}$	$\mathbf{7.77 \times 10^{-5}}$	$\mathbf{3.11 \times 10^{-4}}$	$\mathbf{1.55 \times 10^{-4}}$	**E**	**=C × D**
First-time donor adjustment factor	1.65	1.65	1.65	1.65	F	Davison et al. [9]
HIV window period (days)	9	9	9	9	G	O'Brien et al. [10]
Mean interval between donations (days)	228.125	228.125	228.125	228.125	H	HQ database
Proportion of repeat donors	87%	87%	87%	87%	I	HQ database
NAT, test transcription and clinical test error probability	1.01×10^{-5}	1.01×10^{-5}	1.01×10^{-5}	1.01×10^{-5}	J	Aubé et al. [11]
Number of donations from trans women per year	8	8	8	80	K	HQ database
Total number of donations per year[e]	272,248	272,248	272,248	272,248	L	HQ database
Residual risk of donations made during the window period among donations from trans women	6.64×10^{-6}	3.32×10^{-6}	1.33×10^{-5}	6.64×10^{-6}	M	=[E × I + E × F × (1 − I)](G/H)
Residual risk of donations made outside the window period among donations from trans women	3.38×10^{-9}	3.38×10^{-9}	3.38×10^{-9}	3.38×10^{-9}	N	=[A × I + B × (1 × I)] × J
Residual risk of HIV infection among donations from trans women	$\mathbf{6.65 \times 10^{-6}}$	$\mathbf{3.32 \times 10^{-6}}$	$\mathbf{1.33 \times 10^{-5}}$	$\mathbf{6.65 \times 10^{-6}}$	**O**	**=M + N**
Number of donations from trans women per HIV-contaminated donation	150,476	300,798	75,257	150,476	P	=1/O
Number of years per HIV-contaminated donation[f]	**18,809**	**37,600**	**9407**	**1881**	**Q**	**=P/K**
Residual risk of HIV infection[f] among all donations	$\mathbf{1.95 \times 10^{-10}}$	$\mathbf{9.77 \times 10^{-11}}$	$\mathbf{3.90 \times 10^{-10}}$	$\mathbf{1.95 \times 10^{-9}}$	**R**	**=O × (K/L)**

Note: Bold indicates the main parameters or final risk estimates for HIV.
Abbreviations: HIV, human immunodeficiency syndrome; HQ, Héma-Québec; MSM, men who have sex with men; NAT, nucleic acid testing.
[a]Most likely scenario: HIV incidence among trans female donors was set as the same as that among repeat, non-compliant MSM donors. The number of donations from trans women per year was set as the mean, annual number of deferral-free donations per trans female donor from January 1, 2018 to August 21, 2021.
[b]Optimistic scenario: HIV incidence among trans female donors was set as half that among repeat, non-compliant MSM donors.
[c]Pessimistic scenario A: HIV incidence among trans female donors was set as twice that among repeat, non-compliant MSM donors.
[d]Pessimistic scenario B: The annual number of donations from trans female donors was set as 10 times that in the most likely scenario.
[e]Based on the number of donations of plasma, platelets and red blood cells in 2019, excluding source plasma.
[f]Only considering the risk of HIV transmission due to donations from trans women.

Scenarios

The risk of HIV infection was assessed under four scenarios, which differed on the basis of the incidence of HIV among trans female donors and the annual number of donations from trans women. In the *most likely scenario*, the incidence of HIV among trans female donors was assumed to be the same as that among non-compliant MSM donors, and the number of donations from trans female donors was the mean annual number of deferral-free donations per trans female donor from 1 January 2018 to 21 August 2021. In the *optimistic scenario*, the incidence of HIV among trans female donors was half that among non-compliant MSM donors, and the annual number of donations from trans women was the same as that in the most likely scenario. In the *pessimistic scenario A*, the incidence of HIV among trans female donors was twice as high as that among non-compliant MSM donors, and the annual number of donations from trans women was the same as that in the most likely scenario. In the *pessimistic scenario B*, the incidence of HIV among donations from trans women was the same as that in the

most likely scenario, and the number of donations from trans women was 10 times that in the most likely scenario.

RESULTS

Trans donor characteristics

The Héma-Québec trans population comprised 134 donors, including 94 (70.1%) trans men. Fifty-eight (43.3%) were deferred from donating a blood-derived product because of an ongoing gender transition, more specifically gender-affirming genital surgery, and 76 (56.7%) were eligible donors (Table 1).

Risk of TRALI

In the most likely scenario, the risk of having a TRALI-causing donation, given that it comes from a trans man, was estimated at 9.08×10^{-5} (or 1 per 11,011 donations; Table 2). In the province of Quebec, the risk of having a TRALI-causing donation, among all donations, was estimated at 3.23×10^{-8} (or 1 per 30,946,905 donations), which is significantly lower than the above risk given the small proportion of trans men among all donors. The time required to observe one TRALI-causing donation from a trans man was estimated at 459 years.

In the optimistic scenario, the number of years required to observe one TRALI-causing donation was estimated at 999 years (Table 2). The risk of having a TRALI-causing donation from a trans man was estimated at one every 348 years in the pessimistic scenario A, and one every 115 years in the pessimistic scenario B. In the pessimistic scenario C, given the particularly large number of yearly donations from trans men, it was estimated at one every 46 years (Table S2).

Risk of HIV infection

In the most likely scenario, the risk of having an HIV-contaminated donation from a trans woman was estimated at 6.65×10^{-6} (or 1 per 150,476 donations), and the corresponding annual risk (among all donations) was 1.95×10^{-10} (or 1 per 5,120,841,305 donations; Table 3). The time required to observe one HIV-contaminated donation from a trans woman was estimated at 18,809 years in the province of Quebec.

In the optimistic scenario, the number of years required to observe one HIV-contaminated donation was estimated at 37,600 years. This number was estimated at 9407 years in the pessimistic scenario A and 1881 years in the pessimistic scenario B.

DISCUSSION

This study helps us to understand the risks of TRALI and HIV potentially associated with donations from trans donors. For TRALI, few of the trans male donors who were asked about pregnancy history had one (6.5%). Consequently, the probability of having a TRALI-causing donation from a trans man was particularly low, with one event every 459 years in the most likely scenario. The risk of having an HIV-contaminated donation from a trans woman was even lower, with one event every 18,809 years in the most likely scenario.

Overall, the risk of having a TRALI-causing donation ranged from one event every 999 years in the optimistic scenario to one event every 115 years in the most pessimistic scenario (i.e., when multiplying the incidence of pregnancy history by 4), excluding additional pessimistic scenario C in Data S2. Results were therefore robust to increases in the incidence of pregnancy history, for which there was significant uncertainty since this information was missing for 31.3% of trans male donors (excluding those who have never donated). As for the pessimistic scenario C, in which the annual number of donations from trans men was increased, the number of years to observe a TRALI-causing donation was, as expected, lower than in the other scenarios. However, the risk of TRALI among all donation remained very low at 3.23×10^{-7} (Table S2). Of note, these low risk estimates were obtained assuming none of the trans men disclosed a pregnancy history at a prior screening, which likely overestimates risk. Also, only platelet and plasma donations (excluding source plasma) were assumed to contribute to the overall risk, since those are the only deferred donations for women with a history of pregnancy at Héma-Québec.

Pregnancy is a significant risk factor for the development of anti-HLA, but the extent of this contribution differs across studies. Although some studies reported a higher probability of anti-HLA than reported by Triulzi et al. for women with a history of pregnancy [8, 12], the pessimistic scenario A assumed all women had four or more pregnancies and thus the parameters remain representative of the literature. As for the probability of TRALI given an anti-HLA-containing donation, the most recent estimate was 0.59% [2], which may be an overestimation since it includes all "possible TRALI" events, notwithstanding whether they were caused by transfusion.

The risk of having an HIV-contaminated donation from a trans woman ranged from one event every 37,600 years in the optimistic scenario to one event every 1881 years in the most pessimistic scenario B. This risk therefore appears to be largely theoretical and remained negligible when assuming large increases in the risk of HIV infection among trans women or increases in the number of donations from trans women. Assumptions on HIV incidence and prevalence for trans female donors were based on those of MSM donors who did not comply with Héma-Québec's 3-month deferral. However, not all trans female donors have sex with men and thus exhibited sexual behaviours that are at risk for HIV in the last 3 months. Furthermore, trans female donors who are aware of the current deferral of 3 months after male-to-male sexual contact may assume that they are also excluded, in which case the real incidence of HIV in the overall trans female population would be lower than that assumed in the current study. The risk of HIV infection remained low when assuming a substantial increase in the number of trans female donors; further, even this estimate was likely an overestimation

considering that the other assumptions on incidence were conservative. The risk of an HIV-contaminated donation missed because of NAT system failure, test transcription and clinical test errors could have been ignored considering their negligible contribution. It should be noted that people who donate generally consider their blood safe.

Despite these reassuring results, little is known about the incidence of HIV in the overall trans female population [13], and even less among trans donors. Among other things, although the risk was assessed regardless of gender-affirming genital surgery (Table S1), the incidence of HIV among trans women who have undergone gender-affirming genital surgery is unclear [7]. Among trans women, the incidence of HIV infection has also been shown to be higher for people of colour [6, 14], but this group accounted for only 2.25% of trans female donors.

Although deterministic and conservative, the TRALI and HIV risk assessments emphasize that trans donors pose a negligible risk even when assuming no prior screening. Efforts should be focused on screening individuals for high-risk behaviours, which could be done through a gender-neutral questionnaire. Such individualised risk assessment is already implemented in other countries, including in the United Kingdom following the FAIR (For the Assessment of Individualised Risk) steering group's recommendations [15–17]. It also did not significantly impact blood safety when introduced in Argentina [18], and no significant increase in the proportion of HIV-positive MSM donors was observed after the implementation in Italy [19]. Moreover, a recent study indicated low non-compliance with a 3-month MSM deferral and with the disclosure of other HIV behavioural risk factors in Quebec [20], which justifies transitioning to an individualised behavioural donor risk assessment. A gender-neutral approach may also be welcomed by the trans community, since past behaviours would not be associated with the person's gender, and there would be no need for the person to disclose details on gender transition that are not needed for the screening of high-risk behaviours [21].

This study is subject to some limitations. In both risk analyses, parameters were fixed, which limits the interpretability of the estimates and their accuracy. However, parameters were varied in the scenarios, and so additional variations were arguably not necessary. The use of conservative assumptions and the inclusion of pessimistic scenarios ensured that the risks were likely overestimated; since the risks of TRALI and HIV were both low, conclusions could be drawn despite this deterministic approach.

Also, most trans donors who agreed to go through the screening process were likely motivated and confident in the safety of their blood. Their sexual behaviour could therefore be less risky than that of potential trans donors who were not screened. Nonetheless, this would not significantly impact the HIV risk analysis since trans donors were assumed to have the same HIV prevalence and incidence as non-compliant MSM donors.

The risks of TRALI and HIV were estimated based on the past number of trans donors, which may not adequately reflect the actual and future size of the trans donor population.

For the TRALI risk analysis, the Héma-Québec database did not include information on the number of previous pregnancies, and this parameter influences the presence of anti-HLA. This lack of information was addressed using scenarios in which the number of pregnancies was varied.

Finally, the (few) published estimates on the prevalence and incidence of HIV infection among trans women may be outdated, hence the use of data among MSM donors. This knowledge gap highlights the need to consider trans women as a distinct population in future studies on HIV infection prevalence and incidence.

In conclusion, to the best of our knowledge, this is the first study to assess the risks of TRALI and HIV associated with donations from trans persons. Our analysis suggests that the risk of having a TRALI-causing donation from a trans man and that of having an HIV-contaminated donation from a trans woman are negligible, even when assuming no prior screening for trans donors. Therefore, it may be possible to adopt more inclusive practices for trans donors while negligibly affecting blood safety.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Understanding the experiences of plasma donors in Canada's new source plasma collection centres during COVID-19: A qualitative study

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Abstract

Background and Objectives: To address a national concern over the sufficiency of plasma, Canadian Blood Services (CBS) initiated a proof-of-concept programme with three new source plasma collection centres, aiming to demonstrate a cost-effective template for future source plasma collection and to alleviate the concerns and risks associated with the dependence on the United States. This study uses social capital as a framework to assess the success of the proof-of-concept collection centres.

Materials and Methods: One-hundred and one qualitative interviews with source plasma donors in three new source plasma centres in Canada were carried out.

Results: CBS played a critical role in motivating whole-blood donors to switch to plasma donation by building on their identity as a donor and facilitating access. Community was central to ensuring that donors returned. The importance of the social network was apparent through relationships participants developed with staff and through the relationships that staff had with each other. Donors wanted to understand more about the uses of plasma so that they could promote donation through their social networks outside the centre.

Conclusion: Campaigns to convert existing blood donors to plasma donors should build on their identity as a donor and structure the centre as a safe and welcoming place. To retain donors, blood collection agencies should emphasize community by facilitating staff ability to work well together and connect with the donor. Blood operators have the potential to expand existing social networks and foster trust through the dissemination of knowledge about plasma more broadly in more diverse communities.

KEYWORDS
COVID-19, plasma sufficiency, qualitative research, social networks, source plasma donors

Highlights

- Canadian Blood Services successfully converted whole-blood donors to plasma donors by building on existing social networks and trusted communities.
- Campaigns to convert existing blood donors to plasma donors should build on the donors' identity as a person who wants to help others through donation and structure the centre as an accessible, safe and welcoming place.
- Plasma donor retention should emphasize social networks by facilitating staff to work together well and connect with the donor.

INTRODUCTION

Like many jurisdictions globally, Canada relies on paid donation in the United States to supply more than 80% of the plasma used to create plasma-derived products to treat many illnesses. To address a national concern over the sufficiency of plasma, Canadian Blood Services (CBS) has initiated a proof-of-concept programme with three new source plasma collection centres, aiming to demonstrate a cost-effective template for future source plasma collection and alleviate the concerns and risks associated with the dependence on the United States. This study addresses the experiences of donors in the first three proof-of-concept centres as CBS opens eight more new plasma centres across the country.

Literature on plasma donation in a voluntary, non-remunerated setting has assessed motivations and deterrents for plasma donation, indicating that plasma donors want to help others or save lives [1–6] and are motivated by the blood collection agencies' (BCAs') need for plasma [2, 7, 8]. Plasma can be donated more frequently than whole blood, which facilitates a routine and relationships with staff [2, 9]. Regular plasma donors work to fit donation into their lives [6]. Given the need for plasma, there has been some scholarship measuring BCAs' efforts to convert donors from whole blood to plasma donors and retain them as ongoing donors [2, 3, 9–11]. BCAs are encouraged to build retention through providing social and informational support and structuring the donation process to encourage interpersonal relationships [12].

For the proof-of-concept programme, CBS closed long-standing whole-blood collection centres in the smaller Canadian towns of Sudbury (Ontario), Lethbridge (Alberta) and Kelowna (British Columbia), and opened new source plasma collection centres, asking donors to switch to plasma donation. The centres were in a new space with 12–16 beds (from 4 to 6 beds in whole-blood centres), designed with the organization's 'Donor Centre of the Future' standards meant to increase and retain the donor base while providing safe and efficient operations. Each centre has dedicated staff responsible for engaging the community through schools, workplaces, faith-based organizations, charities and local businesses, and the centres are designed to foster relationships through an open seating area, a conference room for book clubs and community groups and food from local businesses. However, just as the first centre was to open, the COVID-19 pandemic began. CBS adopted measures to ensure that the donation experience was safe, by including masks, physical distancing and restrictions on eating and drinking. Community outreach had to take place almost entirely online. Despite these challenges, whole-blood donors in these sites have converted to plasma donation. As of March 2022, of the donors who had contributed whole blood in the year prior to the opening of the plasma centre, 74% converted to plasma donation in Sudbury, 49% in Lethbridge and 64% in Kelowna. The proof-of-concept centres have collected approximately 32,300 L of source plasma, representing 88% of the target established before the pandemic.

This qualitative study of source plasma donors explores their interest in donating and experiences in the centre; it also examines social and informational support for plasma donation through interpersonal relationships, using a sociological approach. This approach is informed by Charbonneau's [13] position that blood donation is fundamentally social, linked to personal, political, and cultural meanings, as well as to trust in the state. Analysis draws on the theory of social capital to understand blood donation as a social phenomenon that is embedded in the context of community [14] and considers the essential role of trust in healthcare institutions in the context of the COVID-19 pandemic [15, 16]. The concept of social capital has been used to argue that networks of social interaction can lead to trust in the community and a generalized reciprocity [17]. This conceptualization of social capital is central for a national blood service that relies on voluntary non-remuneration; a level of trust and community integration is essential for maintaining the blood system, particularly during a transition, as the one CBS is currently undertaking with plasma.

MATERIALS AND METHODS

This investigation is part of a broader ethnographic process evaluation [18] of the CBS plasma proof-of-concept programme, involving interviews with key informants associated with the project through CBS, document analysis and interviews with staff in the first proof-of-concept centre in Sudbury. This paper presents findings from qualitative semi-structured interviews with source plasma donors in three new source plasma centres in Canada. This study was approved by the CBS Research Ethics Board.

Recruitment of participants

Purposive sampling was used to identify and select individuals who experienced the three new plasma centres. Donors who had donated plasma in the first 6 months of the new plasma collection centres in Sudbury, Lethbridge and Kelowna were contacted by email or received a recruitment flyer in the centre. Inclusion criteria were 18 years or older, able to speak and understand English, donated source plasma in Sudbury, Lethbridge or Kelowna and had not been recruited in the previous 6 months to participate in a study. A total of 1682 eligible donors received an email about the study and were asked to contact the investigator by email if they were interested in participating. Participants were recruited one centre at a time. In total, 249 donors reached out about the study, and KH responded to 148 of them in the order that emails were received with the letter of information and informed consent form and an invitation to participate in an interview, with the aim of obtaining a sample of $n = 30$ source plasma donors per centre, since 20–30 interviews are generally sufficient to achieve saturation of themes [19].

Data collection and analysis

Sudbury's centre opened in August 2020, and interviews were conducted between December 2020 and February 2021; Lethbridge's centre opened in December 2020, and interviews were conducted

between February 2021 and April 2021. Kelowna's centre opened in June 2021, and interviews were conducted between September 2021 and October 2021. Semi-structured one-on-one interviews were conducted by KH by video conference or telephone between December 2020 and October 2021. The interviews lasted between 30 and 70 min. Participants were asked (1) how they experienced the switch to plasma donation in the midst of a pandemic, (2) their reasons for donating plasma, (3) their experiences in the centre, (4) their understanding of community in relation to donation, (5) their knowledge about plasma and (6) their thoughts on how CBS can promote plasma donation. Each participant filled out a consent form and emailed it to KH. Interviews were recorded and transcribed verbatim and checked against the audio recording for inconsistencies. Participants were asked about why they donate, what donation means to them, their experience in the centre, whether they encourage others to donate, how they think CBS should encourage others and their knowledge about plasma. All interview transcriptions were entered into Nvivo, a qualitative software analysis tool, and coded by KH. Interview data were analysed using thematic analysis informed by grounded theory and abductive analysis, which move between gathering and analysing data [20]. With this approach, the researcher is situated in a knowledge and understanding of the literature and theory in the area of study, and checks these contributions against what is being observed in the field throughout the research process [20].

RESULTS

Sample description

A description of the participants is summarized in Table 1. Semi-structured interviews were conducted with 33 participants in Sudbury, 32 participants in Lethbridge and 36 participants in Kelowna, for a total of 101 participants. Most participants were between 50 and 70 years old. Of the participants, 52% were male, 47% were female and 1% were other. Also, 82% of the participants were white, and the second most prevalent ethnicity was Metis (4%). There was a range of educational background, with most donors having obtained post-secondary education. In the following, the quotations from participants are numbered and assigned a letter, PS = Sudbury, PL = Lethbridge, and PK = Kelowna.

Analytical results are presented below according to key areas of discussion with the participants, and the primary themes emerging from the study are as follows—Conversion: responding to an ask and establishing trust; Reasons for donating: continuing the donor identity by making donation easy; Retention: connecting with staff, safety, and the need and Promotion: from the positive experience to an interest in plasma.

Conversion: Responding to an ask and establishing trust

CBS was centrally involved in converting whole-blood donors to plasma donors in the three centres. Most participants became aware of plasma when they were donating whole blood, before the whole-blood centre closed. Staff in the centre talked to them about the closure, the opening of a new plasma centre and the reasons for the change. As one participant from Lethbridge explained,

> the ladies at the clinic were talking about it, when it was still a blood donor clinic, […] I just thought, oh that's good, that's, nothing wrong with that, I can do that (PL20).

Ninety-five percent of the plasma donors in the new centres had donated whole blood with CBS. When asked if it was difficult to switch, most said it was not. They noted that it took more time, but also felt they did not feel as tired after the donation. In plasma donation, red blood cells are returned to the donor, and therefore, some reported that they did not feel as depleted.

When participants talked about switching from whole blood, they highlighted the importance of the staff in the centre explaining everything to them. Plasma donation differs from whole-blood donation in that blood goes into a centrifuge that separates whole blood and plasma, and then red blood cells and saline are returned to the donor during the donation process. When they came to donate plasma for the first time, the donors felt welcome, and the staff answered all of their questions about the collection process. This relationship with staff often existed prior to the first plasma donation because donors recognized staff who carried over from the whole-blood centre. Their interactions with the staff in the plasma centre were based on a trust, which was central to the conversion process. Particularly for first-time donors, members of the staff at the three new centres would stay with them throughout the duration of their visit. For new donors, this was reassuring:

> Because I'd never done it before and because there was all these, tubes and whatever, you know, blood going in and then coming out, and so it was just, nice to have that support (PK31).

Reasons for donating: Continuing the donor identity by making donation easy

Participants' reasons for donating plasma were rooted in their identify as whole-blood donors. When participants were asked why they are a plasma donor, the most common response was that they wanted to help someone who needs their donation. This reason was combined with other reasons, as demonstrated by this participant from Kelowna, who identifies feeling good, helping people and benefit weighted against the duration of the commitment:

> it makes me feel good, and it helps save lives, and really for what takes maybe about an hour, an hour and a half process, it's, it's worth doing (PK16).

Other reasons participants discussed included giving back to the community, the ability to donate more frequently than whole blood, the

TABLE 1 Sample description and total donors in the first 6 months

	Participant characteristics				Total donors in the first 6 months[a]			
Category	**Sudbury, *n***	**Lethbr, *n***	**Kelown, *n***	**%**	**Sudbury, *n***	**Lethbr, *n***	**Kelown, *n***	**%**
Total	33	32	36	101	1714	2000	2502	6216
Donated before	28	32	35	94%	1436	1798	2091	86%
New donor	5	0	1	6%	278	202	411	14%
Age								
18–30	4	1	4	8%	424	559	637	26%
31–40	8	4	4	16%	283	370	437	18%
41–50	4	4	4	12%	255	282	367	17%
51–60	7	8	11	25%	344	331	470	18%
61–69	7	11	8	26%	285	321	422	17%
70 and older	3	4	5	12%	123	137	169	7%
Gender[a]								
Male	15	19	19	52%	795	961	1150	47%
Female	18	13	16	47%	919	1039	1352	53%
Other	0	0	1	1%				
Ethnicity								
White	23	29	31	82%				
Jewish	0	1	0	1%				
Hispanic	0	0	1	1%				
White/Chinese	0	1	0	1%				
White/Turkish	0	0	1	1%				
Metis	1	0	3	4%				
Metis/White	1	0	0	1%				
Italian/Filipino	1	0	0	1%				
South Asian	1	0	0	1%				
Mullato/Black	1	0	0	1%				
Chinese	1	0	0	1%				
White/French Canad	1	0	0	1%				
French Canad	1	0	0	1%				
English	1	0	0	1%				
Missing	0	1	0	1%				
Education								
High school	0	5	5	10%				
College (at least some)	8	8	10	26%				
University (at least some)	19	13	13	45%				
Graduate degree	6	6	8	20%				

[a]CBS only collects gender identities male and female. This policy is being re-examined in order to ensure diversity and inclusion.

fact that they or a member of their family might need it 1 day and the ease of the process. Participants gave similar answers to the question of what donation *means to them*. The most common answer was that donation helps saves lives.

The desire to help was often coupled with the claim that donation is easy. Participants talked about the donation centre being at a short distance from their home, located in a part of the city that was accessible, with parking, or they said that they were retired, so had time. A few said switching to plasma donation was 'not difficult, *but*…' and indicated that the process was a little more invasive than whole-blood donation because the needle is longer, it takes longer, or they miss the social time in the whole-blood centre (COVID-related). Those who noted the length of time said it was difficult given work and caregiving responsibilities.

When talking about why they donate plasma and what donation means to them, very few participants talked about the specific uses of plasma. They donated plasma as a continuation of a history of donation for the purpose of helping someone in need. Many donated

because they were long-time whole-blood donors, and they wanted to continue to give:

> I don't know exactly what all the different things that are used, you know, use plasma to manufacture or whatever, but, I would think, but I, I would think that [...] whatever they can use it for to help someone out, I'm just glad to help (PL1).

Thus, participants' motivations for donating whole blood carried over to plasma.

Retention: Connecting with staff, safety, and the need for plasma

Plasma donors can donate every week, and to meet sufficiency, CBS is asking them give monthly, with a target of eight donations per year. This means that aside from getting them into the door, CBS needs to retain them. Ninety-seven participants had donated more than once; 21 had donated more than five times.

When asked what keeps them coming back to the centre, participants talked about the atmosphere in the centre, the need for plasma and the ease of the process as interconnected—it was easy because it was close to them and did not take long, and it was important. The combination inspired them to return.

In all centres, participants emphasized their relationships with members of the staff, and a large majority said they had positive interactions with staff. Plasma donors can donate more frequently, and they are in the centre for a longer period, so they have more time to get to know the staff. This was an essential part of the experience for them, particularly as they transitioned from whole blood to plasma—they found the staff knowledgeable and helpful about the process and the growing need for plasma. In each centre, participants primarily emphasized that the staff were friendly, meaning that they were empathetic, kind, remembered the donor's name and thanked them for their donation. Participants also emphasized that the staff were professional and knowledgeable, and this helped put them at ease.

While this came across in every centre, participants from Lethbridge noted that they sensed some tensions within the staff because the transition from a whole-blood centre to a plasma centre meant some disputes over staffing with the union representing nurses and plasma associates. Participants still reported that their interactions with the staff were positive, and those close relationships meant that they were keenly aware of the staffing issues.

Despite the COVID precautions, a large majority of participants felt a sense of community in the centre, and this was very much linked to relationships that they developed with the staff, and between staff. The primary reason for the centre feeling like a community was that members of the staff got along with each other. A participant from Kelowna also said the community feeling came from staff working together, and then connected this to feeling safe in the centre:

> I think that just also adds to the safety factor where, if they're all working together the way they should, then things should run smoothly, and less chance of, you know, any kind of upset, or mistakes (PK23).

Further, participants talked about a sense of community from their own relationships with the staff. For some, it was because they recognized the staff from the whole-blood centre and had already developed a relationship with them.

> so when I walk in, it's like a family, like I, because I go every two weeks, I'm on the same rotation as the staff. And even though we have our masks on, we all know who we are (PS27).

Participants also indicated that their relationships with each other give them a sense of community. For some, the efforts to create a centre that was a community hub was successful. The frequency of donation means that donors with a similar schedule start to see each other and recognize each other. At the same time, some said that COVID had an impact on their ability to communicate with other donors.

> We're too spread out, you can't even talk to anybody who's in there. [...] before COVID, on a busy day, there could be four or five people sitting in a row of chairs, waiting to start the process. And sometimes you talk to people, [...] I think post-COVID, things will go back to be a lot more relaxed, and there'll be a lot more sharing and communication (PL7).

Participants also appreciated that the new plasma centres were open, bright, spacious, modern and clean, and these features encouraged them to return. While some participants missed the ability to socialize with one another because of the COVID precautions, they understood why the measures were in place, and all said they felt safe in the centre amid the pandemic. For some, it was one of the only places they could go, and they appreciated the social time it afforded when they had largely been alone. They were also impressed by the food. Plasma centres offer a menu to donors, where they can select a drink and several food options including local baked goods and healthy snacks, to take with them. Participants liked that the centre was promoting local businesses by offering their food, particularly during the pandemic when local businesses in their town were struggling.

Finally, the need for plasma encouraged retention. Participants referred to the ongoing need for plasma, as emphasized in the marketing materials distributed in the community with the message 'the need for plasma is more than four times what is currently donated in Canada'. That message is reinforced in the centre with TV monitors highlighting why plasma is needed and how it is helping recipients. Further, participants had conversations with members of the staff where they learned about plasma.

Promotion: From the positive experience to an interest in plasma

A large majority of participants encourage others to donate by talking about their positive experience, emphasizing the relaxing environment and the snacks. They say donating plasma is easy and try to demystify the experience. They also talk about helping people. A few make a connection to how plasma has had an impact on their family and friends. A large majority of participants understand that there is a need for plasma, and this is important to them. However, very few know how plasma is used, what happens to their donation and how plasma is turned into treatments. At the same time, they desire to know more about these things, and they said this would help them promote plasma donation in the community:

> I think [more knowledge] also would give ammunition to encourage others to donate. If you know what it's for, then you can explain what it's for to other people, thereby creating more interest (PK4).

Many participants wanted to learn more through conversations with staff in the centre. They liked that the staff could answer their questions about what plasma is and why there is this new centre. When asked about how CBS can better promote plasma, participants across centres emphasized demystifying plasma donation (showing the centre, addressing the fear of needles, communicating the time commitment and clarifying COVID safety measures), doing more community outreach (events in schools, workplaces and places of worship) and emphasizing the need for plasma. As a participant from Kelowna put it:

> right now, you just, you put your blood or your plasma into a bag or bottle, and you never see it again and you, you know, you lose this connection of where it goes and what it's being used for. I think if they knew that, the donor knew that hey, or the public knew for that matter, like hey, we will tell you when we can that it went to these people, I think it means more, it means that you helped someone directly (PK2).

Participants wanted to be able to connect their donation to the story of a recipient.

DISCUSSION

This study explored the experiences of donors who converted from whole-blood donation to plasma donation in three proof-of-concept centres in Canada. Smith et al. [15] use the theory of social capital to explain why people are more likely to donate blood when they are embedded in trusted social networks that value blood donation. CBS relied on a community of people who were already familiar with donation, and where they had already developed trust, and then reinforced that trust through a model that supported their transition, and that ensured their safety during a pandemic. This was demonstrated through the way that participants expressed a willingness to switch to plasma because they were asked. It was fostered by the role of the staff in the centre: being friendly, knowledgeable and walking the donor through the process so that it was not intimidating—putting the donor at ease. The centre itself was accessible, comfortable and safe amidst the pandemic. These findings resonate with Healy's [21] scholarship from a social-organizational approach that the BCA structure promotes and makes altruism logistically possible. Participants in this study were motivated by a desire to help people. However, this motivation did not stand alone. It was often coupled with factors identified by scholarship in this area: ease of access and the ability to make it a part of their lives [6], the need for plasma [2, 7, 8] and the ability to retain their identity as a donor [4].

Charbonneau and Queniart [14] have demonstrated that blood donors are more likely to commit to a career as a regular donor if they are supported and encouraged by the BCA and are convinced of the practical value of donation. This study demonstrates that participants felt encouraged and supported in the transition to plasma. Trust in and familiarity with the institution helped bring donors into the plasma centre, and community was central to ensuring that donors returned. The importance of the social network was apparent through relationships participants developed with staff, and through their appreciation of the relationships that staff had with each other.

At the same time, donors were not aware of the practical value of donating plasma. They expressed an interest in promoting plasma donation through their social networks, but needed to better understand what plasma is and does to fully engage in those conversations. That education should take place through the relationships with staff. As Smith et al. found, staff play a central role in developing the community [15, 22]. This study demonstrates that the trust relationship developed with staff who are seen as knowledgeable can be used to facilitate better knowledge about plasma, to further promote donation through the donors' social networks.

While social capital allows us to understand why this population of donors were willing to donate plasma, it does not explain why most of the population does not donate. The limitations of this study are that there were not enough new donors in the sample to understand how to promote donation beyond existing whole-blood donors, and since the sample was not ethnically diverse, it does not offer insight into diverse social networks. A more critical approach to the concept of social capital points to how social networks are accumulated through historical relations of power [23]. It is possible that people who do not donate want to help others but lack the institutional trust and social networks that support donation.

In conclusion, BCA campaigns to convert existing blood donors to plasma donors should build on the donors' identity as a person who wants to help people through donation, and structure the centre as an accessible, safe, and welcoming place. To retain donors, BCAs should emphasize community by facilitating staff's ability to work well together, and connect with the donor. They should promote plasma

through educational settings, workplaces, and community events, emphasizing the need by making a connection to the recipient. Donors want to help with this work by encouraging friends and family, but they need more education about what plasma is and does. This education could also happen at the centre, supported by the close relationships developed with staff. CBS's demonstrated success in building on existing social networks and trusted communities to convert donors to plasma means that the organization can also expand these networks and foster trust through the dissemination of knowledge about plasma more broadly through outreach in diverse communities. As the demand for plasma protein products increases, there is mounting pressure to recruit new plasma donors. Future work should investigate who is in and out of the networks and communities that have accrued social capital in relation to donation and understand how to expand those networks.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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ORIGINAL ARTICLE

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Whole blood donor return rates after deferral for tattooing or body piercing—Survey across blood donation services: The BEST collaborative study

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Abstract

Background and Objectives: To protect transfusion recipients from transfusion-transmissible infections, blood donors are deferred from donating after recent tattooing or piercing. To explore to what extent and how this deferral impacts donor availability, we performed an international study to investigate how many donors were deferred for a recent tattoo or piercing and how many of these donors returned to donate.

Materials and Methods: We surveyed blood centre members of the Biomedical Excellence for Safer Transfusion (BEST) Collaborative and the European Blood Alliance Donor Studies Working Group on their numbers of donations, tattoo and piercing deferrals, and return rates in the year 2017.

Results: Eight blood centres participated. Overall, deferral rates were lower for repeat donors compared to new donors. Repeat donors were more likely to return than new donors. Women and young donors were more often deferred than male and older donors. Men were more demotivated by tattoo or piercing deferral, resulting in lower return rates compared to women. Return rates differed greatly between blood centres.

Conclusion: Tattoo and piercing deferrals lead to missed donations and result in lower return rates. However, the numbers vary largely internationally, probably due to cultural and policy differences. Shortening deferral periods after tattooing or piercing may reduce the impact on donor availability, which should be investigated in single-centre studies.

KEYWORDS
blood donors, donor deferral, donor return

Highlights

- Donor return related to tattoo and piercing deferral differs internationally.
- New donors return less often after a tattoo or piercing deferral than repeat donors.
- Shortening deferral duration after tattoo or piercing may improve donor return, but this should be investigated in single-centre studies.

INTRODUCTION

Transfusion-transmissible infections (TTIs), such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV), can put recipients of blood products at risk [1]. Prior to their donation, donors are required to fill in a donor health questionnaire to assess their TTI risk to prevent TTI transfer to vulnerable patients. When this risk is considered high, donors are deferred from donating blood products. Tattooing and piercing are such potential risk factors for TTI [2, 3]. Tattooing and piercing have become popular decorative procedures, but the percentage of individuals undergoing such procedures varies between countries [4–6]. A large proportion of individuals do not associate tattooing with health risks [7].

As a result of improved hygiene, sterilization methods and single-use material and equipment for tattooing and piercing, these procedures only account for a very small proportion of HIV, HBV and HCV infections in blood donors [8, 9]. Nowadays, most infections due to tattooing or piercing occur in non-sterile environments, such as prisons or non-professional settings [2]. Therefore, several blood centres do not apply a deferral period when the tattoo or piercing was set by a tattoo or piercing shop meeting the country or state's hygiene regulations since the risk of infection is negligible in a controlled setting. A recent systematic review and meta-analysis found low-quality evidence for an increased risk for HCV in donors receiving a tattoo or piercing. High-quality evidence is, however, lacking [3]. Moreover, a recent study shows that TTIs are not associated with recent tattoos or piercings but can often be attributed to other risk behaviours, such as injected drug use or needle-stick incidents [10, 11].

Additionally, the introduction of nucleic acid testing (NAT) significantly shortened window periods for TTIs, which can consequently lead to the shortening of deferral periods after a TTI risk. However, donor deferral practices are not easily adapted to reflect the increased sensitivity of NAT that can detect TTIs, resulting in deferral periods that might be too long and may cause donors to stop donating [12]. This might be an even greater reason for concern with respect to new donors, who have a higher chance of being deferred and are more likely to stop donating after deferral [13]. In the Netherlands, return rates after a deferral for recent tattooing, body piercing or acupuncture are lower compared to after a deferral for other reasons [10]. To explore whether this only applies locally or whether deferrals after tattooing and piercing are universally discouraging donors, we performed an international study to investigate how many whole blood (WB) donors were deferred for a recent tattoo or piercing and how many of these donors returned to donate.

METHODS

Study design

This study is a collaboration among members of the Biomedical Excellence for Safer Transfusion (BEST) Collaborative Donor Studies team and the European Blood Alliance (EBA) Donor Studies Working Group. The BEST Collaborative brings together an international group of scientists, physicians, and industry members to perform collaborative studies in transfusion medicine. The EBA is an association of not-for-profit blood centres. We surveyed blood centre members of both groups on their numbers of WB donations, tattoo and piercing deferrals, and return rates.

Study population and data collection

Participating blood centres were asked to report their total numbers of WB donation attempts, tattoo and piercing deferrals, and other deferrals (including haemoglobin [Hb] deferral) between 1 January and 31 December 2017. A donation attempt was defined as a donor presenting at the blood bank with the purpose of making a blood donation or undergoing a new donor screening. For each of those donation attempts, numbers of donor returns were reported. Donor return was defined as a donor visiting the blood centre to donate WB or blood-derived products within 12 months after (a) the end of the longest applicable deferral period or (b) the end of the minimum donation interval following their on-site donation attempt in 2017. Return rates were collected for donor return after a tattoo or piercing deferral, any other deferral and no deferral for comparison.

Numbers were split by sex, new or repeat donor, and age group (aged ≤24 years, 25–39 years, 40–54 years, and ≥55 years). A new donor was defined as a donor visiting the blood centre for the first time, either for a pre-donation screening or for a WB donation. A repeat donor was defined as a donor visiting the blood centre for a consecutive WB donation attempt. Chi-square tests were used to test the differences between groups.

RESULTS

Of 21 blood centres invited, eight blood centres participated (38%). Three blood centres were able to distinguish between tattoo and piercing deferrals (Table 1). Five blood centres had one deferral code for both tattoo and piercing deferrals (Table 2).

New donors had deferral rates ranging from 0.14% to 2.86% for piercings and 0.84% to 2.46% for tattoos. Repeat donors were as often deferred for tattoos as for piercings (0.07%–0.24% for tattoos vs. 0.04%–0.12% for piercings). Among donors deferred because of tattooing or piercing, the return rates 47.8%–77.1% and 50%–78.6% were higher for repeat donors compared to 4.8%–60.5% and 0%–62.0% for new donors, respectively (for tattooing: $p < 0.001$, df $= 1$, $\chi^2 = 147.1$, for piercing: $p < 0.001$, df $= 1$, $\chi^2 = 207.6$). Of note, the return rates of new donors donating at Siro-Libanês Blood Bank were very low overall.

Results were generally similar for blood centres using the same deferral code for both tattoo and piercing deferral. The deferral rates for tattooing or piercing were higher in new donors (0.22%–1.94%) compared to repeat donors (0.05%–0.51%; $p < 0.001$, df $= 1$, $\chi^2 = 15{,}510$). Return rates after tattoo or piercing deferral were lower

TABLE 1 Deferral and return rates of new and repeat donors for tattooing and piercing

Blood centre	Months deferral after tattoo/piercing[a]	WB donation attempts		Tattoo deferral rate (%)		Piercing deferral rate (%)		Tattoo return rate (%)		Piercing return rate (%)		Not deferred return rate (%)		Other deferral return rate (%)	
		ND	RD	ND	RD	ND	RD	ND	RD	ND	RD	ND	RD	ND	RD
Canadian Blood Service, Canada	6	180,431	848,436	0.84	0.07	0.98	0.06	24.5	47.8	24.8	50.6	44.2	75.9	18.2	45.5
Sanquin Blood Bank, the Netherlands	6	43,212	464,112	2.46	0.10	2.86	0.12	60.5	77.1	62.0	78.6	78.4	90.3	58.6	82.5
Sírio-Libanês Blood Bank, Brazil	12 (6 for piercing)	3663	4603	1.15	0.24	0.14	0.04	4.8	54.6	0	50.0	3.9	68.7	11.0	68.0

Abbreviations: ND, new donor; RD, repeat donor; WB, whole blood.
[a]In 2017.

TABLE 2 Deferral and return rates of new and repeat donors for tattooing and piercing combined

Blood service	Months deferral after tattoo/piercing[a]	WB donation attempts		Tattoo/piercing deferral rate (%)		Tattoo/piercing return rate (%)		Not deferred return rate (%)		Other deferral return rate (%)	
		ND	RD	ND	RD	ND	RD	ND	RD	ND	RD
Héma Québec, Canada	3	35,793	219,387	0.83	0.35	31.5	59.1	41.2	74.5	16.9	59.5
National Blood Transfusion Service, Malta	6	21,171[b]		n.a.	n.a.	11.1	30.7	n.a.	n.a.	n.a.	n.a.
OneBlood Inc., USA	12[c]	247,978	675,173	0.22	0.05	25.7	38.3	n.a.	n.a.	n.a.	n.a.
Red Cross Flanders, Belgium	4	21,062[d]	156,522[d]	n.a.	n.a.	43.6	62.2	n.a.	n.a.	30.0	67.6
American Red Cross, USA	12[a]	991,062	1,522,281	1.94	0.51	22.2	39.3	58.3	80.5	25.9	58.0

Abbreviations: ND, new donor; RD, repeat donor; WB, whole blood.
[a]In 2017.
[b]No data available for new or repeat.
[c]Donors are not deferred if they received a tattoo in the past 12 months if the tattoo is applied by a state-regulated entity with single needles and non-reusable ink. Donors are not deferred if they had a piercing in the past 12 months if it was done using a single-use equipment.
[d]Total number of blood donors who donated in the given time frame.

in new donors (11.1%–43.6%) than in repeat donors (38.3%–62.2%; $p < 0.001$, df = 1, $\chi^2 = 225.4$). Overall, the return rates for repeat donors deferred for tattooing or piercing were lower compared to donors deferred for other reasons, with the exception of the Canadian Blood Service. This contrasts with the pattern seen in new donors, where, in four out of six cases, the return rates after a deferral for tattoo or piercing were higher than after a deferral for other reasons. Donors who were not deferred returned more often than donors deferred for a tattoo or piercing event, with the exception of new donors at Sanquin Blood Bank and Síro-Libanês Blood Bank.

Deferral rates for both tattoo and piercing were highest in young donors (aged ≤ 24 years) for both new (0.4%–3.7%) and repeat (0.2%–2.1%) donors. For every age group, return rates were higher in repeat donors compared to new donors. Deferral rates were higher for women in all blood centres. Return rates were also higher for women compared to men, with the exception of repeat male donors at Red Cross Flanders and new male donors at the Canadian Blood Service.

DISCUSSION

In this study, among eight blood collection services, we studied to what extent blood donors were deferred for tattooing or piercing

procedures and whether these donors returned for a donation after their deferral period ended. In this study, we give an overview of the deferral rates for tattooing or piercing of several blood collection services in the year 2017. Overall, deferral rates were lower for repeat donors compared to new donors. Repeat donors were more likely to return than new donors. Women and young donors were more often deferred than male and older donors. Men were more demotivated by tattoo orpiercing deferral, resulting in lower return rates compared to women.

The highest deferral rates found were 2.5% for tattoos and 2.9% for piercings, both in new donors in the Netherlands. In other participating blood centres, deferral rates were lower, even when tattoo and piercing deferrals were combined. These deferrals lead to missed donations and eventually could cause donors to stop donating. We found that new donors are more often deferred for tattooing or piercing than repeat donors. This could be due to unfamiliarity with the blood donation process and donor eligibility criteria. Providing new donors with clear information about donor eligibility and an online donor screening, which can be done at home, might prevent donor deferral on site. We observed lower return rates after tattoo or piercing deferral in new donors compared to repeat donors. Other studies investigating blood donor return found similar results [12, 13]. However, in new donors, the return rates after tattoo or piercing were higher compared to return rates after other deferrals. This might be explained by the fact that new donors deferred for other reasons are more often permanently deferred compared to deferred repeat donors, for example, because they have a medical condition that excludes them from donating. To diminish the loss of donors it is important to know how donors feel after a deferral and in which way this effects their willingness to donate again. Gemelli et al. showed that deferred donors show strong emotions, such as feelings of rejection and disappointment [14]. Additionally, donors showing negative emotions after a deferral were less inclined to return for the next donation.

Since the return rates are so different between the blood services for both non-deferred donors and deferred donors, it is difficult to determine the impact of the length of the deferral period on the likelihood of donor return. It would be easier to examine this by evaluating data from one blood service over time when deferral periods are changed. Differences in deferral rates between blood centres could be caused by different ways of communicating with donors. Efforts should be made to retain deferred donors, especially for new donors with the highest risk to stop donating. Also, shortening deferral periods might increase donor returns. A Canadian study showed no increase in TTIs after shortening the deferral period from 12 to 6 months [15]. This remained true after further shortening the deferral period to 3 months [16]. Depending on the quality of tests used for TTIs, deferral periods for tattoos or piercings could be shortened. The United States completely removed the deferral period for receiving a tattoo or piercings if the tattoo or piercing procedure was done at an entity compliant with state regulations; otherwise, the deferral period is 12 months. Also, in the Netherlands and Canada, the deferral period for tattooing and piercing has been shortened to 4 and 3 months in 2019 and 2018, respectively. The effect of this policy change on deferral and return rates should be investigated in the near future.

A limitation of this study is the amount of missing data and the limited number of participating blood centres. This prevents us from looking at the effects of deferral time on the return rate. We did not perform more complex statistical analyses on these data because of the limited sample size and heterogeneity among datasets, which hamper possibilities to adjust for important factors, such as deferral time, and thereby impede a reliable interpretation. Also, with four participating blood centres (50%) from North America, our participating set of blood centres is not diverse. Also important to mention is that individuals with tattoos and piercings often have more than one. Donors who are aware of the deferral periods after tattooing and piercing could self-defer, and therefore, not make a donation attempt. Therefore, the deferral of these donors is not registered, and consequently, we might underestimate how many donors are not available due to recent tattooing or piercing procedures. In addition, on-site versus off-site deferrals were not captured, so we could not distinguish between donors who were deferred while trying to make a donation at the donation site (on-site) and donors who were deferred via telephone or e-mail (off-site). Off-site deferral might have a less negative impact on donor return because the donor need not make an effort to come to the collection site, only to be turned away.

Prinsze et al. investigated the risk behaviours of donors who have tested positive for the presence of a TTI and concluded that donors who have had a tattoo or piercing often also showed other risk behaviours [10]. These risk behaviours were, among others, intravenous drug use, being born in a TTI high-endemic area or a previous TTI diagnosis. It would be valuable do to such a risk assessment in multiple blood centres, especially in those applying strict regulations for tattoo or piercing shops for TTI prevention. The outcome of this assessment could eventually loosen the strict deferral criteria for tattoos or piercings and prevent donor deferral and loss of donors.

In conclusion, repeat donors have lower deferral rates and return more often than new donors. Additionally, women and young donors were more often deferred than male and older donors, but men were more demotivated by tattoo or piercing deferrals, resulting in lower return rates compared to women. These findings were quite consistent across countries, and therefore, show border-crossing trends. However, between-country differences are large, indicating potentially large effects of cultural and policy differences that should be taken into account in subsequent studies.

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All of the authors critically revised and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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ORIGINAL ARTICLE

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Seronegative human T-cell lymphotropic virus 1 carriers in blood banks: A potential viral source for silent transmission?

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Abstract

Background and Objectives: Transfusion-transmitted viruses count among the greatest threats to blood safety. In Argentina, current laws oblige testing all donated blood for the presence of antibodies against human T-cell lymphotropic viruses 1 and 2 (HTLV-1/2). In endemic zones of the country, a high rate of seronegative HTLV-1 individuals with clear evidence of infection because of symptoms and/or presence of *tax* sequences of HTLV-1 and/or IgG anti-Tax antibodies has been recently described. Migration from endemic to nonendemic zones of Argentina is very frequent.

Materials and Methods: During a 1-year period, in the blood bank of Córdoba city, we performed molecular screening of all donors who were born in or arose from endemic zones for HTLV-1/2 in Argentina and neighbouring countries.

Results: By screening 219 bp of HTLV-1/2 *tax* gene, 0.6% (2/317) of the blood donors proved to be positive for HTLV-1 *tax* sequence. One of the donors presented anti-Tax antibodies, demonstrating the transcriptional activity of the *tax* gene, and the other donor was also positive for LTR and *pol* gene sequences. The HTLV-1 genetic analysis of the LTR sequence determined that it belonged to the Cosmopolitan subtype HTLV-1aA.

Conclusion: These findings suggest potential limitations of some currently approved screening assays for HTLV-1 detection applied in some donor populations and the possibility of an HTLV-1 seronegative carrier state with the potential for silent transmission by blood.

KEYWORDS
Argentina, blood safety, nonendemic HTLV-1 zone, seronegative HTLV-1 carriers

Highlights

- Seronegative human T-cell lymphotropic virus 1 (HTLV-1) carriers in the blood banks of Argentina were detected.
- Possible limitations of some current blood screening assays for HTLV-1 detection.
- It is uncertain if seronegative HTLV-1 carriers represent a threat to transfusion safety.

[Correction added on 04 Aug 2022 after first online publication: Highlights section has been updated in this version.]

María C. Frutos and Sandra Gallego are scientific members of CONICET.

INTRODUCTION

Human T-cell lymphotropic virus 1 (HTLV-1) is the etiological agent of adult T-cell leukaemia and tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM) [1]. However, most people infected with this virus never develop any sign or symptom of the disease, and if they do, it is usually late in life [2].

HTLV-1 infection has been reported in almost all South American countries, including Brazil, Colombia, Argentina, Peru, French Guyana and Chile [3]. Moreover, some areas of South America, such as northeast of Brazil and northwest of Argentina, are considered endemic to HTLV-1 [4]. Concerning specifically to HTLV-1 infection in Argentina, there are two different areas: one endemic zone in the Northern part of the country, where blood banks report the highest prevalence of HTLV-1/2 infection (0.6%–1.0%), and a nonendemic area in central and southern regions of the country where the prevalence of HTLV-1/2 infection in blood banks is lower than 0.1% [5, 6].

In a recent publication, we describe for the first time the existence of seronegative HTLV-1 carriers in highly endemic areas of our country [7]. In this study, 64.5% of the subjects were seronegative for HTLV-1 infection but carried proviral sequences of HTLV-1. Besides, 35.7% of these subjects presented antibodies to Tax protein of HTLV-1 [7], and the Tax antigen is not included in commercially available HTLV kits for serological testing. This issue opened the question of whether the prevalence of HTLV-1 infection in Argentina may be greater than detected by currently used serologic tests.

Earlier studies have reported the condition of seronegative HTLV-1 carriers in intravenous drug users [8], seronegative TSP/HAM patients [9, 10], subjects with mycosis fungoides [11], patients with infective dermatitis [12], and healthy blood donors as well [13, 14].

Although in Argentina, the current blood laws oblige testing every donated blood for antibodies against HTLV-1/2, the finding of seronegative HTLV-1 carriers in the general population of highly endemic areas in Argentina, which cannot be detected by conventional serological screening, warns us about a potential hazard for blood banks.

Migration from endemic to nonendemic zones of Argentina is very frequent. During a 1-year period, in the blood bank of Córdoba city (capital of a nonendemic province), we performed molecular screening to all donors born in or had grown up in the endemic zones for HTLV-1/2 in Argentina and neighbour countries.

Córdoba city, capital of Córdoba province (1.3 million inhabitants), usually receives a large influx of subjects from endemic areas.

MATERIALS AND METHODS

Samples

Blood samples were collected between August 2015 and August 2016 at Fundación Banco Central de Sangre, Córdoba, Argentina. This is a blood bank that centralizes different blood transfusion departments working all over the 165.321 km^2 of Córdoba province. Serological and molecular pre-transfusion screening of almost 50% of the blood units collected throughout the province is performed in this blood bank.

A total of 317 healthy adults without risk factors for transfusion-transmitted infections were studied. All donors donated along 1 year at Fundación Banco Central de Sangre; they were born in or had grown up in the endemic zones for HTLV-1/2 in Argentina or other countries; all were seronegative for HTLV-1/2 antibodies when screened with Architect rHTLV-I/II assay (Abbott Laboratories Wiesbaden, Germany). Thus, 75.7% (240/317) had come from endemic zones of Argentina (Jujuy, Salta, Formosa, Chaco and Misiones) and 8.2% (26/317) from Peru, 4.1% (13/317) from Paraguay, 2.5% (8/317) from Bolivia, 2.2% (7/317) from Venezuela, 1.9% (6/317) from Chile, 1.6% (5/317) from Brazil, 1.6% (5/317) from Colombia, 1.6% (5/317) from Mexico, 0.3% (1/317) from Ecuador and 0.3% (1/317) from Guatemala. The study population included 69% (219/317) males and 31% (98/317) females aged 18–64 years. These proportions reflect the characteristics of regular blood donors in Argentina, showing that males constitute more than 60% of the blood donor population in this country [15].

The samples were codified as H followed by a number and were an aliquot of blood obtained from the same tube used for triplex nucleic acid amplification testing studies in the routine pre-transfusion screening of all blood donors. Thereby, the quality of samples for molecular analysis was guaranteed.

This study complied with the principles outlined by the Declaration of Helsinki and was approved by the Ethics Committee of OULTON Institute (10/2015) of Córdoba, Argentina. Written informed consent was signed by all the participants prior to sample collection.

Polymerase chain reaction assays

DNA was extracted from whole-blood samples of the 317 selected blood donors. Nested polymerase chain reaction (PCR) was carried out to amplify the 219-bp sequence of the *tax* gene following protocols described by Vandamme et al. [16]. The HTLV-1/2 positive samples by generic nested PCR were subsequently typed by specific nested-PCR for HTLV-1 (100 bp) and HTLV-2 (151 bp), targeting the *tax* region [16]. Also, an additional PCR was carried out to amplify 100-bp of the HTLV-1 *tax* gene following protocols previously described using primers designed for the detection of HTLV-1 strains prevalent in Argentina [17].

The PCR products were separated on a 2% agarose gel with SYBR Safe DNA gel (Invitrogen) staining and visualised under UV light.

Amplification of 1119 bp of the *tax* region from the proviral genome was performed in all *tax*-positive samples [18]. The other two sequences, 561 bp and 672 bp from the *env* gene in addition to the LTR region, were also amplified using nested-PCR [19]. The reaction to detect the *pol* gene (107 bp) of HTLV-1 was performed using real-time PCR developed by Andrade et al. [20].

Sequencing

PCR products corresponding to *tax* sequences and LTR region were purified using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. *Tax* (100–219 bp) and LTR fragments (672 bp) were subjected to direct nucleotide sequencing reaction in both directions using the internal PCR primers by Macrogen, Inc. (Seoul, Korea). The alignment of the sequences from seronegative HTLV-1 donors was performed using the Clustal W program (Conway Institute UCD Dublin, Dublin, Ireland) and compared with Pairwise/Blast/NCBI. The sequence was deposited in GenBank (MZ687332).

The maximum likelihood tree was constructed with the PhyML 3.0 software (Université de Montpellier, Montpellier, France) [21]. The model of nucleotide substitution was selected according to the Akaike Information Criterion implemented in the ModelTest 3.7 software (Universidad de Vigo, Galicia, Spain) [22] for the data set analysed.

Molecular signatures on LTR seronegative HTLV-1 carrier sequence were analysed with VESPA software [23].

The identity matrix was calculated using the Distance Matrix tool (IVisTMSA) [24].

Serological assays

Samples that resulted positive for HTLV-1 by molecular assays were re-tested for HTLV-1/2 antibodies by PA assay (Serodia Fujirebio Inc., Tokyo, Japan) and also analysed by an "in-house" IFA on MT-2 cell line [25]. Besides, samples that were positive for HTLV-1 by molecular assays were further tested for IgG anti-Tax antibodies using anti-Tax-IgG enzyme-linked immunosorbent assay (ELISA) [26]. The sensitivity and specificity of this assay had been previously reported [7]; it was performed at the Laboratório de Virologia Básica e Aplicada, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

Statistical analysis

Distribution of frequencies for each variable was analysed with the Kruskal–Wallis test and Dunn's post-test. Statistical analyses for IgG anti-Tax antibodies were conducted using GraphPad Prism 8.0.1 software (GraphPad Software Inc., San Diego, CA). Analysis of unpaired *t*-test for anti-Tax IgG reactivity was performed with Mann–Whitney's post-test. Significance was assumed at $p < 0.05$.

RESULTS

Among the 317 donors analysed, 2 (0.63%) tested positive for HTLV-1 sequences in blood, detected by two PCRs targeting different sequences of the *tax* gene, both of 100 bp [16, 17]. These subjects were also HTLV-1/2 negative for antibodies by PA and IFA assays (Figure 1).

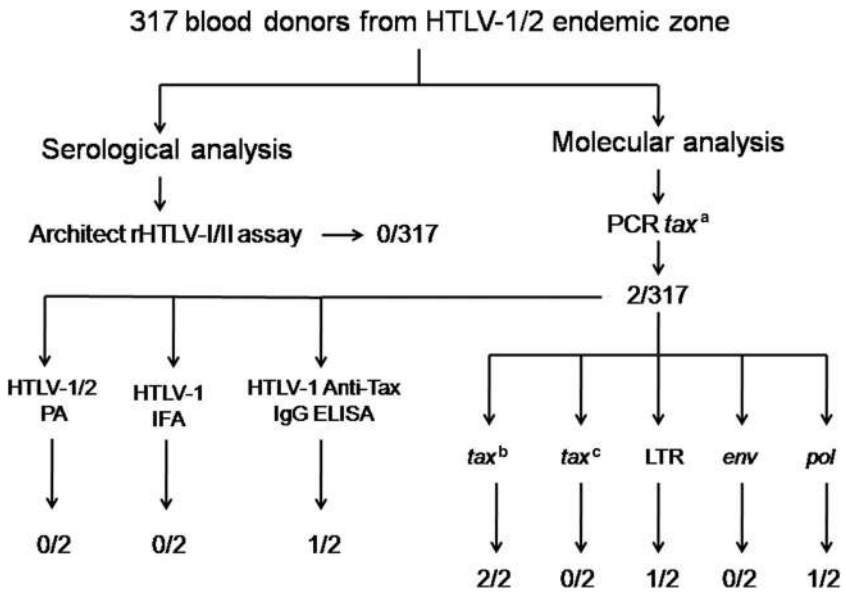

FIGURE 1 Human T-cell lymphotropic virus (HTLV) serology and polymerase chain reaction (PCR) analysis of samples from blood donors coming from HTLV-1/2 endemic areas. Blood samples from donors who were negative for HTLV-1/2 antibody screening by Architect rHTLV-I/II assay at the blood bank were further analysed at InViV with a nested-PCR for the HTLV *tax* gene. Blood samples that resulted positive for a sequence of *tax* were further tested for another *tax* region by PCR, *env* gene and LTR regions by nested-PCR, and *pol* gene by qPCR. Also, plasma samples of *tax* positive donors were subsequently retested serologically for antibodies against structural antigens by HTLV-1/2 PA (Serodia Fujirebio Inc., Tokyo, Japan), HTLV-1 IFA (in house) and tested by HTLV-1 non-structural antibodies anti-Tax IgG enzyme-linked immunosorbent assay (in house). [a]215 bp and 100 bp [16]. [b]100 bp [17]. [c]1100 bp [18]

In one of the blood samples, sequences from the LTR region (672 bp) and *pol* gene (107 bp) were also amplified. This donor was a 48-year-old male (H94) born in Jujuy province (Argentina). The other *tax*-positive donor was a 28-year-old female (H256) native from the Chaco province (Argentina); IgG anti-Tax antibody was also detected in her blood sample (Figure 2).

The HTLV-1 *tax* sequence detected in both donors (100 bp) was highly homologous to prototypic ATK-1 HTLV-1 *tax*, also showing high homology with other isolates from the endemic zone of Argentina (>97%) and strains from neighbour countries (Table 1).

The genetic analysis of the HTLV-1 LTR region showed that the sequence from the donor H94 belonged to the Cosmopolitan subtype HTLV-1a Transcontinental subgroup A within the Latin American and Jujuy subclusters (Figure 3).

The VESPA analysis of HTLV-1 LTR sequences showed that, as compared to the reference strain ATK-1, the seronegative HTLV-1 carrier sequence contained not only the polymorphisms typical for the Transcontinental HTLV-1aA subgroup (T246C, C306G, T479C, A529G and G675A) and deletion at position A209 but also a singular position T188C (data not shown). These polymorphisms were identical to those previously described in infected HTLV-1 seropositive subjects from Jujuy.

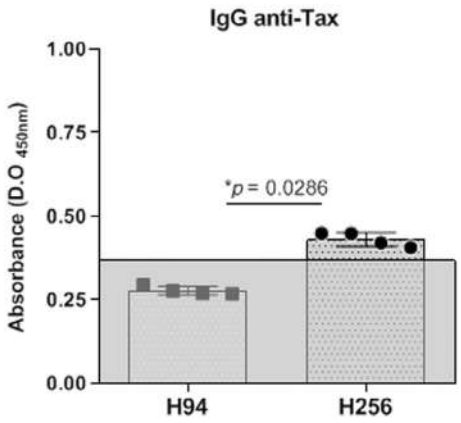

FIGURE 2 IgG anti-Tax antibodies against Tax protein of human T-cell lymphotropic virus 1 detected by enzyme-linked immunosorbent assay in seronegative blood donors. The grey area represents the cutoff point optical density (OD = 0.371) of the assay

DISCUSSION

Herein, we describe for the first time the existence of seronegative HTLV-1 carriers in the blood banks of Argentina. Among the 317 blood donors born in or arose from endemic zones for HTLV-1/2 of Argentina or other countries recognized as endemic for the virus, we detected 2 donors (0.63%) harbouring *tax* sequences with the absence of antibodies evidenced by commercially available CE-marked or FDA-approved HTLV-1/2 assays.

To our knowledge, there is only one published article regarding HTLV-1 carriers in blood banks, which reports cases from a blood bank in the United States [14]. The researchers found a higher prevalence of seronegative HTLV-1/2 carriers (8.6%) detected by molecular screening of 250 plasma samples from healthy blood donors. In contrast with this study, in which only sequences of *tax* genes were found, sequences of other genes were detected (LTR and *pol*) in one donor of our study as well. In addition, other investigators sought donors who were seronegative HTLV-1/2 carriers in a blood bank, but they did not find any [27].

TABLE 1 Matrix identity of human T-cell lymphotropic virus 1 (HTLV-1) *tax* gene sequences in blood samples and comparison with isolates from different sources

	ATK-1	H94	H256	ArJ03-06	ArJ13-01	HN1	B1033	HAM16	BRSP65679	LC210018	ArJ54-2	ArJ17-2
ATK-1	100											
H94	97.2	100										
H256	98.3	96.6	100									
ArJ03-06	100	97.2	98.3	100								
ArJ13-01	100	97.2	98.3	100	100							
HN1	100	97.2	98.3	100	100	100						
B1033	99.4	96.6	97.7	97.7	99.4	99.4	100					
HAM16	100	97.2	98.3	100	100	100	99.4	100				
BRSP65679	99.4	96.6	97.7	99.4	99.4	99.4	99.4	99.4	100			
TT0021	100	97.2	98.3	100	100	100	99.4	100	99.4	100		
ArJ54-2	100	97.2	98.3	100	100	100	99.4	100	99.4	100	100	
ArJ17-2	100	97.2	98.3	100	100	100	99.4	100	99.4	100	100	100

Note: ATK-1 (J02029): pattern sequence of HTLV-1; ArJ03-06 (MK63897) and ArJ13-01 (MK638974): seronegative HTLV-1 carriers from Jujuy (endemic zone of Argentina); ArJ54-2 (DQ227188) and ArJ17-2 (DQ227165): seropositive HTLV-1 subjects from Jujuy. HN1 (KC807984): HTLV-1 infected patients from China. TT0021 (LC210018): HTLV-1 infected blood donors from Japan. HAM16 (KY007274): HAM patient from Brazil. B1033 (AB513134): HTLV-1 patient with adult T-cell leukaemia from Japan. BRSP65679 (KY928595): sequence from patient coinfected with HTLV-1 and HIV-1 from Brazil.

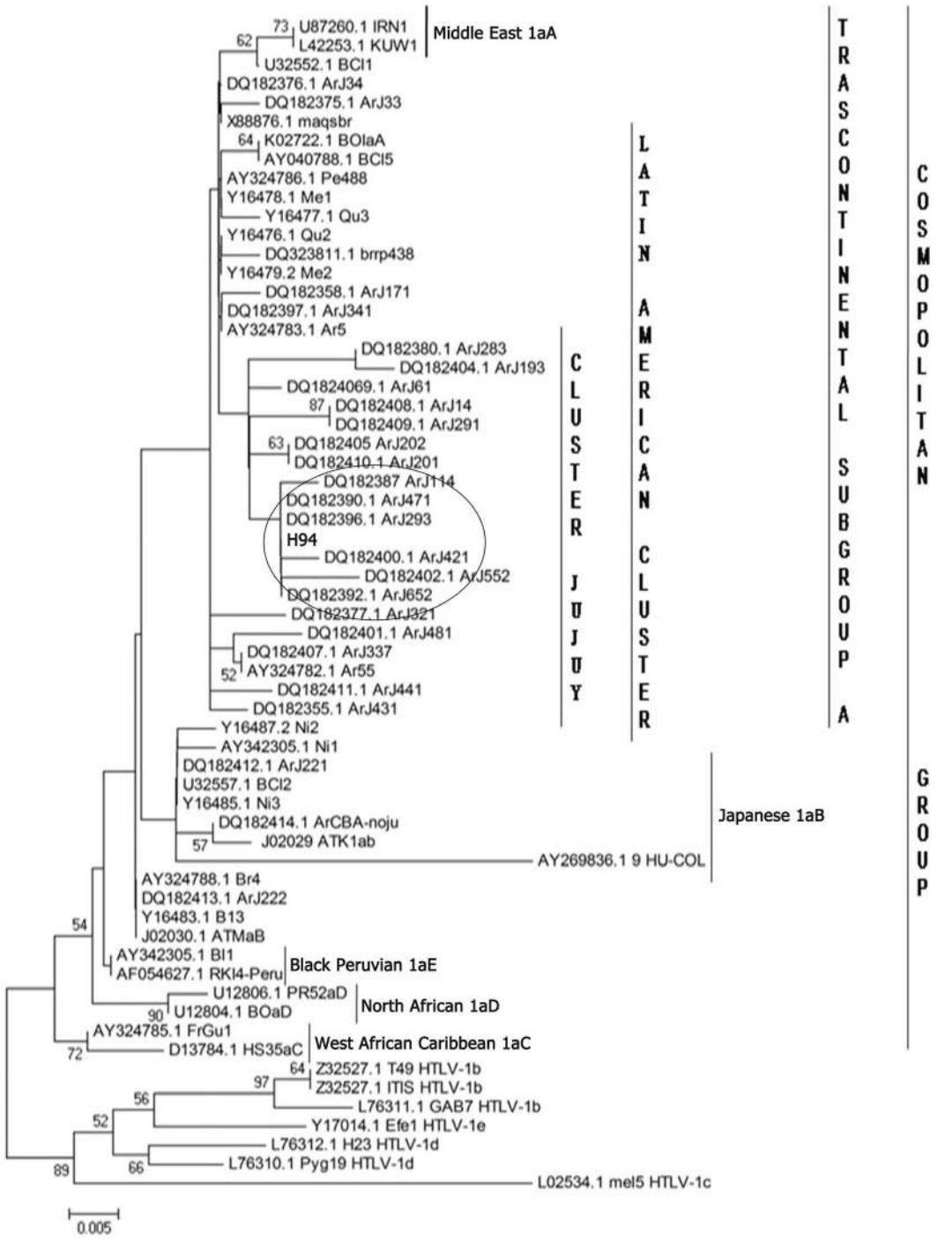

FIGURE 3 Maximum likelihood dendrogram for the human T-cell lymphotropic virus 1 (HTLV-1) LTR sequences. HTLV-1 LTR (630 bp) genetic tree comparing sequences from seronegative HTLV-1 blood donors and worldwide sequences, including HTLV-1 reference. It was constructed using TIM2 + G as a model of nucleotide substitution with parameters suggested by ModelTest 3.7 (PhyML software). The strain that belongs to this study begins with H and is written in bold. Numbers above branches: bootstrap values over 1000 bootstrap pseudoreplicates. Only bootstrap values >50% are shown at nodes

It has been suggested that the lack of LTR sequences may explain the replication incompetence and inexpression of HTLV-1 antigens and the consequence of the absence of immune response. Thus, the authors propose that TSP/HAM patients carry a defective HTLV-1 provirus, probably as a consequence of a vigorous immune response early in the infection, which successfully eradicates the infected cells, leaving only those with defective sequences [28]. Despite the case of our donor, in whom sequences from three different viral genes were detected (LTR, *pol* and *tax*), the possibility of a defective HTLV-1 provirus cannot be discarded in the face of the absence of immune response.

Furthermore, it is not surprising that the *tax* gene is always found in these cases, and in many cases, this is the only one. The genetic stability of the HTLV-1 *tax* gene has been determined through different studies [29]; this is why several PCRs targeting *tax* sequences have been developed and largely used for molecular diagnosis and investigation of HTLV infection [17, 20, 30]. However, the genetic versatility of HTLV-1 also reaches *tax* sequences, as we have recently demonstrated in the case of infected people with missed *tax* genes [31].

The specificity of the HTLV-1 *tax* gene sequences amplified from the two seronegative blood donors by different PCRs was confirmed by nucleotide sequencing (100 bp). HTLV-1 is genetically very stable; a low degree of genetic variation (0.5%–3%) has been described for HTLV-1 strains from Africa, Japan, the Caribbean basin, and the Americas [32, 33]. The *tax* sequences from the two donors in this study showed 97.2%–98.3% homology to the ATK-1 sequence, also demonstrating high homology with other isolates from endemic zones of Argentina. Thus, the high homology found between the strains corroborated that the amplified sequences corresponded to HTLV-1.

HTLV-1aA is the prevalent subgroup in South American countries, such as Colombia, Peru, Chile and Brazil [32, 34]. In our study, the analysis of the amplified LTR sequence from one of the seronegative donors identified it as HTLV-1, Cosmopolitan Group (a), and Transcontinental subgroup (A). This sequence was very similar to that found in previous studies regarding HTLV-1 in Jujuy province and grouped in a particular cluster within the Latin American/Transcontinental subgroup, named Jujuy subcluster [35]. In accordance with this finding, the blood donor was a native of the Jujuy province.

Although we did not demonstrate the transmission of HTLV-1 from these carriers to blood recipients, this possibility cannot be excluded. In this sense, Zucker-Franklin showed transmission of *tax* to rabbits by transfusion of PBMC from *tax only* HTLV-1 seronegative blood donors [36]. Moreover, a seronegative status with stable HTLV-1 infection has been established in an animal model [37]. The major finding of this study was that the persistent presence of HTLV-1 without antibody response was successfully established, experimentally, in syngeneic rats inoculated with an HTLV-1-infected cell-line scarcely expressing major HTLV-1 structural proteins but preferentially expressing Tax.

Undoubtedly, seronegative carriers of HTLV-1 from which some proviral sequences are deleted exist, and this state may be associated with disease [9–12]. Moreover, we have recently described high rates of seronegative symptomatic and asymptomatic HTLV-1 carriers in Argentina, harbouring only tax sequences [7].

Since most carriers of deleted HTLV-1 sequences seem to retain the tax sequence and/or its gene product, p40tax [7, 9–11, 14], and considering that tax is the transcriptional transactivator of HTLV-1 and has a role in the upregulation of innumerable cellular growth factors, cytokines, and oncogenes [38–40], the transmission of tax is an important question that requires attention.

In our study, a second donor resulted positive for *tax* sequences and also positive for IgG anti-Tax antibodies. The presence of antibodies anti-Tax in the same individual may help to alleviate concern about the possibility of PCR contamination. This last possibility was dismissed by the repeated collection of samples in the donor without anti-Tax antibody and analysis of specimens obtained from the same donors at different times and handled by different personnel in a blind manner.

The evidence of IgG anti-Tax in this donor also confirmed that despite a short sequence of *tax* genes detected in the absence of other gene sequences, this individual probably had an active infection at some point in life. It has been suggested that anti-Tax antibodies are involved in TSP/HAM pathogenesis [41], and researchers suggest that the presence of anti-Tax antibodies contributes to the aggravation of HTLV-1 infection and is a marker of disease evolution [41, 42].

In Argentina, many efforts are being made for the implementation of nucleic acid techniques for viral screening in blood banks and highly sensitive tests for the detection of antigens and antibodies, which are efficient tools that reduce residual risks of infection-transmission through blood transfusions. In this sense, screening of antibodies against HTLV-1/2 is nowadays mandatory all over the country (Law 22990) since Argentina has some areas well known as endemic for HTLV-1 and HTLV-2 infection, and also because these viruses have been detected in different blood banks across the country [6]. The decision to screen blood donations for a particular pathogen should be based on the risk assessment of transfusion-transmitted infections determined by the prevalence of such pathogens in the donor population, susceptibility of the recipients, and the reported number of transfusion-transmitted cases in each region. Thus, the question raised in our study is if seronegative HTLV-1 carriers are capable of transmitting the infection by blood transfusion and, as a consequence, if it is necessary to implement further techniques for serological screening or incorporate molecular screening specific for this virus. In anti-HTLV routine screenings, positive tests can occur, as well as non-reactive results, taking into account the use of reagents containing only viral envelope proteins. This sensitivity can be augmented using chimeric antigen from *env*, *gag* and *pX* HTLV-1/2 regions [43].

In Argentina, leukoreduction of blood products is not mandatory, and in consequence, it is not performed routinely in local blood banks. It is reasonable to think that leukoreduction substantially reduces the risk of HTLV-1 transmission, and since the provirus is integrated into the CD4+ lymphocytes, there are few cell-free viruses. In this sense, a look-back study of blood transfusions in the United Kingdom found that filter-leukoreduced or buffy coat reduced blood product transmission of HTLV-1 by 93% [44]. Other studies demonstrated that leukoreduced blood products, although safer, still carry a theoretical risk of HTLV-1 transmission from donors with high proviral load [45]. Thus, in countries with a high prevalence of HTLV, like Argentina and Brazil [46], where universal leukoreduction is not recommended, it is important to be aware of any residual risk for HTLV transmission.

The findings suggest potential limitations of some currently approved screening assays for HTLV-1 detection applied in some donor populations and the possibility of an HTLV-1 seronegative carrier state with the potential for silent transmission by blood. Therefore continuous epidemiological surveillance in blood banks, including follow-up of positive blood donors and recipients of positive blood, should be

performed. This is mainly worrying in Argentina and neighbouring countries with endemic areas for this virus. Assessment of the epidemiological risk through investigation and surveillance of agents with potential for blood transmission is critical to determine the infectious risk and implement newer interventions to ensure safe blood supplies [47].

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CONFLICT OF INTEREST

There are no conflicts identified.

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ORIGINAL ARTICLE

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Is intravenous immunoglobulin a risk factor for necrotizing enterocolitis in neonates with haemolytic disease of the newborn? A retrospective cohort study

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Abstract

Background and Objectives: To assess whether the use of intravenous immunoglobulin (IVIG) in late-preterm and term newborns with haemolytic disease of the newborn (HDN) is associated with an increased risk of necrotizing enterocolitis (NEC).

Materials and Methods: A retrospective cohort study was conducted in a tertiary centre. Infants with HDN during early neonatal period (<7 days) who were of ≥34 weeks' gestation and born between January 2019 and October 2021 were included. Propensity score, interaction as well as univariate and multiple logistic regression analyses were employed.

Results: One-thousand two-hundred and fifty-nine infants with HDN were enrolled, of whom 192 (15.3%) received IVIG. NEC was diagnosed in 29 (2.3%) patients with 5 (2.6%) in the IVIG group and 24 (2.2%) in the non-IVIG group. No significant association between IVIG administration and confirmed NEC was observed using univariate analysis ($p > 0.05$). The possible predictors of NEC, as assessed by multivariate analysis, were caesarean delivery, haemoglobin on admission <130 g/L and patent ductus arteriosus (PDA). There was no interactive effect of IVIG against NEC for prematurity, low birth weight, caesarean delivery, haemoglobin on admission <130 g/L and PDA.

Conclusions: In late-preterm and term infants with HDN, there was no evidence that the early use of IVIG led to the development of NEC.

KEYWORDS
haemolytic disease of the newborn, intravenous immunoglobulin, necrotizing enterocolitis, risk factor

Highlights

- A retrospective cohort study was conducted to examine the possible association between intravenous immunoglobulin therapy and the development of necrotizing enterocolitis (NEC) in late-preterm and term infants with haemolytic disease of the newborn (HDN).
- Different statistical tools were used to control for confounding.
- In late-preterm and term infants with HDN, there was no evidence that the early use of IVIG led to the development of NEC.

INTRODUCTION

Haemolytic disease of the newborn (HDN) is the main cause of early neonatal hyperbilirubinaemia [1]. Phototherapy and, in severe cases, exchange transfusion (ET) are used to prevent kernicterus and to reduce perinatal mortality [2]. However, it is recognized that ET is potentially harmful because it is associated with a mortality and morbidity of 0.5%–4.7% and 2.8%–23.5%, respectively [3]. Intravenous immunoglobulin (IVIG) administration has emerged as a therapeutic modality in neonates with HDN after it was recommended by the American Academy of Pediatrics (AAP) in 2004 [1].

IVIG acts by blocking Fc receptors on macrophages, thereby reducing the breakdown of antibody-coated red blood cells (RBCs) and also enhancing the clearance of maternal antibodies, which have been shown to decrease the need for ET [1]. IVIG is usually given at a dose of 500–1000 mg/kg infused over 2–6 h [2], but the timing of administration and dose used are still controversial [4]. IVIG is purified and concentrated immunoglobulin derived from pooled plasma of the donor population [5, 6]. However, in clinical work, more than one study has pointed out that IVIG treatment may increase the risk of necrotizing enterocolitis (NEC) in haemolytic patients [7]. NEC is one of the most devastating diseases encountered in neonates with high morbidity and mortality rates, and it has generated a significant debate about IVIG safety in HDN. There is wide agreement that NEC is a complicated syndrome characterized by intestinal injury, inflammation and necrosis. It can also be characterized by a diversity of alterations in mucosal defences, gastrointestinal microbiota and imbalances of inflammatory responses [7]. Ree et al. reported that the HDN population should be concerned as a distinct entity, with potentially distinct risk factors contributing to the development of NEC [8]. One of the main possible mechanisms related to NEC associated with IVIG is the thrombotic effect, which can result from hyper-viscosity of the IVIG solution used. However, other studies have shown no changes in intestinal blood flow as assessed by ultrasound after IVIG administration [9, 10].

We searched in 'PubMed' with the keywords 'necrotizing enterocolitis', 'immunoglobulin', 'IVIG', 'NEC' and 'hemolysis'. Only one retrospective study was reported by Figueras-Aloy et al. in 2010. The authors concluded that IVIG was a risk factor for NEC in severe HDN. However, this study did not look at certain potentially useful confounding factors such as anaemia as a predictor of NEC. In addition, some factors such as sample size, study population, comorbidities and severity of HDN may also have an impact on the variability of the results. To date, these doubts still remain. Our hypothesis was that the use of IVIG in HDN might increase the risk of developing NEC.

We conducted a retrospective, single-centre observational study and aimed to explore more comprehensively whether there was an association between early administration of IVIG and NEC in late-preterm and term newborns with HDN. Our second aim was to explore the relative importance of several neonatal factors in predicting the development of NEC in infants with HDN.

MATERIALS AND METHODS

Study subjects

This was a single-centre, retrospective cohort study of HDN with and without IVIG conducted in a tertiary-care hospital in Chongqing, China. The Institutional Review Board of the hospital approved this retrospective study and waived written informed consent. Our neonatal diagnostic centre serves >4000 neonates every year.

Data collection was conducted through a review of the patients' medical records. The medical records of all HDN cases with jaundice as the main complaint from January 2019 until October 2021 were reviewed. Infants were placed into an IVIG or a non-IVIG group. Exclusion criteria were gestational age less than 34 weeks, admission after the age of 7 days, as well as infants with major congenital anomalies including gastrointestinal malformations and congenital heart disease, but not including patients with patent ductus arteriosus (PDA). Patients with insufficient relevant information were also excluded.

Treatment

Infants with incompatibilities other than ABO began phototherapy immediately upon admission to our unit based either on their Bhutani nomograms (gestational age ≥ 35 weeks) or their total serum bilirubin (TSB) levels for age and birth weight (gestational age <35 weeks) [11]. However, all ABO-incompatible infants began phototherapy when their TSB (mg/dl) plus 2 mg/dl reached the cutoff value.

Phototherapy was carried out using an LED phototherapy system (Zhengzhou Dison Instrument and Meter Co., Ltd, Henan, China, intensity ≈ 10 μW/cm^2/nm, spectral range 450–480 nm). Infants had regular TSB (every 24–48 h) and transcutaneous bilirubin (TCB) (every 8–12 h) tests, which were performed with a twin-beam microbilimeter (Ningbo David Medical Device Co., Ltd, Zhejiang, China). Phototherapy was terminated when either the TSB or TCB decreased 2 mg/dl below the cutoff for phototherapy.

ET was indicated when either the TSB reached 5 mg/dl more than the ET curve line recommended by the AAP in 2004 or the infants showed signs of kernicterus [11]. When the infants had anaemia and the TSB reached the phototherapy cutoff, use of IVIG was considered. However, the final decision was made by the clinician and based on the wishes of the infant's parents. Infants received one infusion of IVIG (either 0.5 or 1 g/kg; Yuanda Shuyang Pharmaceutical Co., Ltd, Sichuan, China) within 2–4 h after admission, followed by a second infusion 24 h later, if required. According to the total dose of IVIG, the patients' data were analysed based on the use of 0.5 and 1 g/kg of IVIG as the low- and high-dose groups, respectively.

Observation indicators

Information regarding observational factors was obtained from the electronic medical record system of the Women and Children's

Hospital of Chongqing Medical University, and was anonymized before analysis. Data were collected for each infant from the time of admission to the day before the discharge date, and analysed for cases from admission to the day before the onset of NEC and for controls from admission to discharge. Demographic characteristics, perinatal features, treatment and neonatal complications before NEC were reviewed. Information on abdominal radiographic and sonographic results as well as surgical factors was also collected.

Definitions

HDN was confirmed by a positive direct antiglobulin test (DAT) and/or positive antibody release test indicating the presence of antibodies on RBCs. The DAT and antibody release tests were performed by using the gel microcolumn assay, which is a sensitive method to detect RBC alloantibodies. NEC was diagnosed with the accepted combination of clinical signs and radiographic findings via the Bell's modified staging criteria [12]. The cutoff value of anaemia on admission in our study was defined as a haemoglobin (Hb) level <130 g/L or haematocrit (Hct) <39% of the venous peripheral blood [13, 14]. The rate of Hb decline in the NEC group meant disparity from admission to the onset of NEC, whereas in the group without NEC this referred to disparity from admission to discharge. A rise in carboxyhaemoglobin was defined as carboxyhaemoglobin >1.2% [15].

Hypoalbuminaemia was defined as serum albumin <25 g/L [14]. Blood eosinophilia was defined when the eosinophil count was more than 0.5×10^9 cells/L of the total white blood cells (WBCs) counted for ≥2 days before the onset of NEC [16]. Tense phototherapy was defined as an illumination intensity >30 μW/cm^2/nm and spectral range 450–480 nm [11]. Erythrocyte suspension transfusion was considered based on Hb, Hct, age and clinical condition [17]. G6PD deficiency was confirmed with an enzyme activity of below 12.5 U/g haemoglobin [13]. PDA was diagnosed on the basis of echocardiographic findings. The presence of sepsis was confirmed by cultures and a clinical diagnosis [14]. Small for gestational age (SGA) was defined as a birth weight of less than 10th percentile by gestational age and gender [18].

Statistical analysis

Univariate analyses included the comparison between the groups with and without IVIG and the groups with and without NEC. Categorical variables were expressed as numbers and percentages. The chi-square test was used for groups. Continuous variables were expressed as the means ± standard deviations (mean ± SD) or as the medians and interquartile ranges (IQRs), and were analysed using Student's *t*-test or the Mann–Whitney *U*-test depending on the underlying distribution of the data. We established a propensity score for IVIG to avoid attributing factors to IVIG that were attributable to other variables [19]. This score was obtained using a logistic regression model that included the demographic variables, which in the bivariate analysis were associated with IVIG ($p < 0.3$) and not considered to be covariables or confounders. Covariables were the variables that were probably related to NEC at $p < 0.1$ in univariate analysis, whereas confounders were the variables that at the same time had a $p < 0.3$ in infants who were classified by exposure (IVIG) and by outcome (NEC). Variables that were entered in the logistic regression model included the covariables, the potential confounding variables, the propensity score and the use of IVIG [2].

Stepwise forward logistic regression was conducted to identify a parsimonious model. As a confirmatory analysis, a stepwise backward

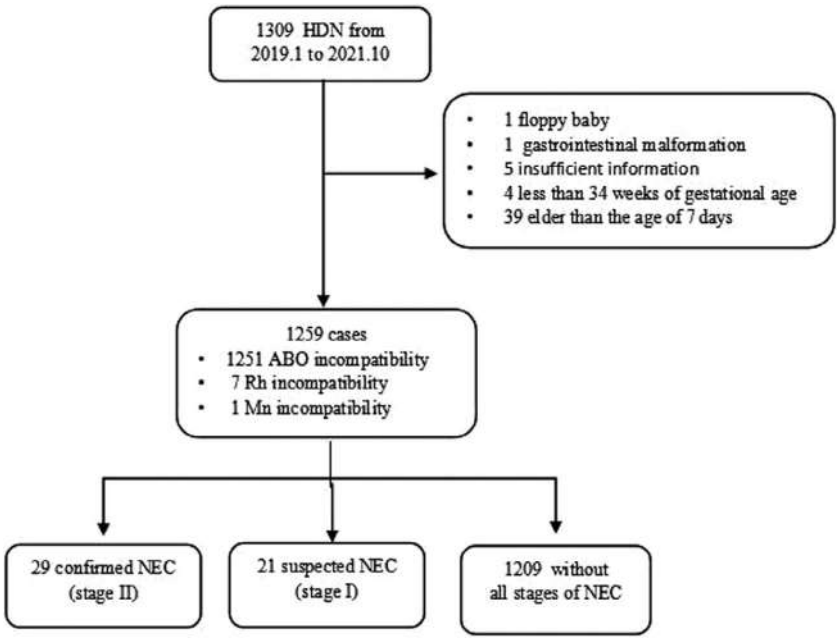

FIGURE 1 Flow diagram of study population. HDN, haemolytic disease of the newborn; NEC, necrotizing enterocolitis

logistic regression model was also conducted. The final parsimonious model was used to identify independent predictors for NEC. As a retrospective study, subgroup interaction analyses with the Mantel–Haenszel test were used in order to control the bias and to validate the multivariate regression analysis. The risks for adverse outcomes were expressed by odds ratios (ORs) and 95% confidence intervals (CIs). Statistical analysis was conducted using a SPSS V26.0 (IBM, Armonk, NY) and $p < 0.05$ by two-sided was considered to be statistically significant.

RESULTS

A total of 1309 late-preterm and term newborns were admitted for HDN from January 2019 to October 2021 at our hospital, and 1259 of them were finally enrolled into the study according to inclusion and exclusion criteria (Figure 1). These included 1251 cases of ABO incompatibility, 7 cases of Rh incompatibility and 1 case of Mn incompatibility.

One-hundred and ninety-two infants (15.3%) received IVIG, of whom 17 and 175 were given a low and a high dose of IVIG, respectively. Confirmed NEC (stage II) was diagnosed in 29 (2.3%) patients, of whom 4 required an operation because of the failure of medical treatment and the rest gradually recovered after conservative therapy. Suspected NEC (stage I) was recorded in 21 (1.7%) patients, all of whom recovered after conservative therapy; 6 infants received erythrocyte suspension transfusion and 1 contracted NEC and received a transfusion after the onset. Sixty-four (5.1%) patients reached the ET curve line with a mean maximum TSB of 338 μmol/L (IQR: 281.3–390.7 μmol/L), and were given tense phototherapy. No patient received ET.

The clinical data are summarized in Table 1. In univariate analysis, NEC was diagnosed in 2.6% and 2.2% of the IVIG and non-IVIG groups, respectively ($p = 0.793$). With respect to all stages of NEC, the incidence was 4.7% and 3.8% in the IVIG and non-IVIG groups,

TABLE 2 Multivariate regression analysis for confirmed NEC

Variable	*B*	*p*-Value	OR	95% CI
Caesarean delivery	1.195	0.007	3.30	1.38–7.89
Hb < 130 g/L[a]	1.631	<0.001	5.11	2.12–12.33
PDA	1.286	0.030	3.62	1.14–11.54

[a]At admission.

TABLE 1 Variables associated with use of IVIG and confirmed NEC by univariate analysis

	Use of IVIG			Confirmed NEC		
Variable	With ($n = 192$)	Without ($n = 1067$)	*p*-Value	With ($n = 29$)	Without ($n = 1230$)	*p*-Value
Clinical information						
Male	84 (43.8)	518 (48.5)	0.221	13 (44.8)	589 (47.9)	0.851
Gestational age, median (IQR), weeks	39.1 (38.3–40.1)	39.0 (38.4–40.0)	0.459	38.6 (37.5–39.7)	39.1 (38.4–40.0)	0.056
Caesarean delivery	78 (40.6)	505 (47.3)	0.086	22 (75.9)	561 (45.6)	0.02
Laboratory data						
TSB ≥ 342 μmol/L	20 (10.4)	32 (3)	<0.001	2 (6.9)	50 (4.1)	0.339
Hb < 130 g/L[a]	33 (17.2)	38 (3.6)	<0.001	8 (27.6)	63 (5.1)	<0.001
Hct < 39%[a]	32 (16.7)	36 (3.4)	<0.001	7 (24.1)	61 (5.0)	0.001
Low albumin	1 (0.5)	3 (0.3)	0.485	1 (3.4)	3 (0.2)	0.089
Decreased rate of Hb, median (IQR), g/L days	3.8 (2.3–5.0)	3.1 (1.6–4.3)	<0.001	3.2 (2.1–4.1)	3.2 (1.8–4.4)	0.928
Treatment						
Phototherapy ≥24 h	169 (88.0)	775 (72.6)	<0.001	24 (82.8)	920 (74.8)	0.328
Tense phototherapy	29 (15.1)	35 (3.3)	<0.001	2 (4.5)	62 (5.1)	1
IVIG				5 (17.2)	187 (15.2)	0.793
Comorbidity						
HDN except ABO compatibility	5 (2.6)	3 (0.3)	0.003	0 (0)	8 (0.7)	1
Prematurity	4 (2.1)	45 (4.9)	0.091	0 (0)	49 (4.5)	0.62
PDA	8 (4.2)	36 (3.4)	0.582	7 (15.9)	37 (3.0)	0.001
Confirmed NEC	5 (2.6)	24 (2.2)	0.793			
All-stage NEC (I and II)	9 (4.7)	41 (3.8)	0.581			

Abbreviations: ET, exchange transfusion; Hb, haemoglobin; Hct, haematocrit; IQR, inter quartile range; PDA, patent ductus arteriosus; SGA, small for gestational age; TSB, total serum bilirubin.
[a]At admission.

respectively ($p = 0.581$). Patients who received IVIG had more severe HDN with higher TSB as well as more anaemia, carboxyhaemoglobin > 1.2%, and Hct < 39% on admission than infants who were treated only with phototherapy ($p < 0.05$). Caesarean section, Hb < 130 g/L and Hct < 39% were independent variables associated with both IVIG therapy and NEC ($p < 0.3$, confounders). Gestational age, hypoalbuminaemia and PDA were independent variables associated with NEC ($p < 0.1$, covariates). Being male, admission age < 3 days, TSB ≥ 342 μmol/L, eosinophilia before NEC, phototherapy >24 h, tense phototherapy, carboxyhaemoglobin>1.2%, decline rate of Hb and non-ABO compatibility were used to obtain the propensity score ($p < 0.3$ only for IVIG therapy).

In the multivariate analysis, caesarean delivery (OR: 3.30 [95% CI: 1.38–7.89]), Hb < 130 g/L (OR: 5.11 [95% CI: 2.12–12.33]) and PDA (OR: 3.62 [95% CI: 1.14–11.54]) were independent factors that were significantly associated with confirmed NEC ($p < 0.05$; Table 2). There was no significant effect on the use of IVIG leading to the development of NEC.

Additional analyses including those at all stages of NEC were conducted. Caesarean delivery (OR: 2.03 [95% CI: 1.10–3.74]), Hb < 130 g/L (OR: 4.31[95% CI: 2.04–9.10]), SGA (OR: 8.54 [95% CI: 1.75–41.64]) and PDA (OR: 4.31 [95% CI: 1.74–10.68]) were independent factors significantly associated with all stages of NEC ($p < 0.05$).

Seventeen infants received low dose of IVIG and one developed NEC stage II. One-hundred and seventy-five received high dose of IVIG, four developed NEC stage II and four developed NEC stage I. The demographic and clinical data including gestational age, birth weight, gender, delivery mode, the severity of HDN between the low- and high-dose group were not different in bivariate analysis ($p > 0.05$). A two-dose regimen of IVIG did not have a significantly different effect on NEC stages I and II or on all stages of NEC ($p > 0.05$; Table 3).

There was no interactive effect of IVIG against confirmed NEC according to gestation age, birth weight, delivery mode, TSB reaching ET line, Hb and PDA (interaction $p > 0.05$) (Table 4). Additional analyses including all stages of NEC were conducted, and there was also no interactive effect of IVIG against all stages of NEC with respect to gestation age, birth weight, delivery mode, gender, TSB reaching ET line, Hb, PDA, SGA and phototherapy time ($p > 0.05$).

TABLE 3 Association between two regimens of IVIG and all stages of NEC in HDN

NEC	Low dose of IVIG ($n = 17$)	High dose of IVIG ($n = 175$)	p-Value	Total ($N = 192$)
Stage I	0 (0)	4 (0.6)	1.00	4 (2.1)
Stage II	1 (5.9)	4 (2.3)	0.374	5 (2.6)
Stage I and II	1 (5.9)	8 (4.6)	0.574	9 (4.7)

TABLE 4 Subgroup analysis of IVIG on confirmed NEC in HDN

	IVIG n/total (%)	PT n/total (%)	p-Value	Relative risk [95% CI]	Interaction p-Value	Total n/total (%)
Gestational age						
<37 weeks	0/7 (0)	3/84 (3.6)	0.784	0.92 [0.87–0.98]		3/91 (3.3)
≥37 weeks	5/185 (2.7)	21/983 (2.1)	0.589	1.27 [0.47–3.42]	0.731	26/1168 (2.2)
Birth weight						
<2500 g	0/1 (0)	3/22 (13.6)	1	0.95 [0.86–1.05]		3/23 (13.0)
≥2500 g	5/191 (2.6)	21/1045 (2.0)	0.582	1.31 [0.49–3.52]	0.646	26/1236 (2.1)
Delivery mode						
Vaginal delivery	1/114 (0.9)	6/562 (1.1)	1	0.82 [0.10–6.88]		7/676 (1.0)
Caesarean	4/78 (5.1)	18/505 (3.6)	0.519	1.46 [0.48–4.44]	0.636	22/583 (3.8)
TSB reached ET line						
Yes	0/29 (0)	2/35 (5.7)	0.497	0.53 [0.42–0.67]		2/64 (3.1)
No	5/163 (3.1)	22/1032 (2.1)	0.401	1.45 [0.54–3.89]	0.828	27/1195 (2.2)
Hb[a]						
<130 g/L	4/33 (12.1)	4/38 (10.5)	1	1.17 [0.27–5.11]		8/71 (11.3)
≥130 g/L	1/159 (0.6)	20/1029 (1.9)	0.343	0.32 [0.04–2.40]	0.453	21/1188 (1.8)
PDA						
With	2/8 (25)	2/36 (5.6)	0.145	5.67 [0.66–48.33]		4/44 (9.1)
Without	3/184 (1.6)	22/1031 (2.1)	1	0.76 [0.23–2.57]	0.800	25/1215 (2.1)

[a]At admission.

DISCUSSION

Anaemia and associated hyperbilirubinaemia in neonates are the most common clinical manifestations of HDN [5, 20]. IVIG has been proposed as a potential intervention that can decrease the severity of haemolysis and the associated hyperbilirubinaemia. In their report in 2004, the AAP recommended the use of IVIG for HDN. The dose suggested was 500 mg for each kg of body weight given via the intravenous route to be infused over 2 h [5]. Two Cochrane systematic reviews in 2014 and 2018 did not support the AAP's recommendations and concluded that the efficacy of IVIG was not conclusive in Rh HDN and that its role in ABO disease was not clear [1]. In this study, IVIG was given to 15.3% of infants with HDN, and haemolysis in the IVIG group seemed more severe, with 15.1% of infants at admission reaching the ET line corresponding to 3.3% in the group without IVIG ($p < 0.001$). Excluding one infant who received transfusion after the onset of NEC, five infants received erythrocyte suspension transfusion (1 [0.5%] and 4 [0.4%] of the IVIG and non-IVIG groups, respectively) ($p = 0.563$) before being discharged from our hospital. Our study partially supports the finding that the use of IVIG decreases the erythrocyte suspension transfusion in children with HDN.

IVIG administration in neonates is considered generally safe. But some studies have reported that IVIG treatment may increase the risk of NEC in HDN. Navarro et al. first reported that NEC occurred 6–18 h after IVIG administration in three patients with ABO incompatibility [21]. A retrospective study by Figueras-Aloy et al. found that a high dose of IVIG was an independent risk factor for NEC in children with HDN [2]. However, IVIG can also be a sign of anaemia, and Figueras-Aloy et al. did not take into account the Hb levels or anaemia in their studies. A meta-analysis by Yang et al. in 2016 of IVIG in HDN indicated that IVIG treatment for haemolysis may increase the risk of NEC in infants [7]. Among the five studies used in their analysis, only one included TSB and Hb levels and showed no association between IVIG and NEC. The other four studies, without TSB and Hb data, all reported a significant association between IVIG and NEC. No potential mechanisms for this association were suggested.

Some previous investigations hypothesized that intestinal blood flow changes could be caused by IVIG and this would precede the development of clinical manifestation of NEC. However, no blood flow changes were observed immediately after the infusion [7]. Mesenteric vessel thrombosis and high viscosity due to IVIG administration have also been suggested as potential risk factors that can increase the risk of NEC. However, the high viscosity of IVIG is unlikely to cause thrombosis simply, and it has only been reported in vascular inflammatory diseases such as Kawasaki disease and immune platelet purpura [9]. Another possible explanation is that the high levels of bilirubin indicate a more severe degree of haemolysis, which can lead to an increased haemodynamic disruption to the intestinal blood flow and, therefore, a higher risk for NEC [5]. Our results rejected this possibility, as we observed no association between NEC and the severity of HDN because TSB, carboxyhaemoglobin > 1.2% and the decreased rate of Hb, and phototherapy for ≥24 h were not different between the NEC and non-NEC groups. In agreement with our study, Figueras-Aloy et al. showed no statistically significant difference in TSB levels in the NEC and non-NEC groups. This finding suggests that NEC was unrelated to the severity of haemolysis [2, 7]. In addition, a review of IVIG for preventing infection in preterm infants in 2020 showed there was no significant difference in the incidence of NEC [22]. A systematic review of IVIG in HDN by Zwiers et al. in 2018 including nine studies reported that none of the adverse reactions of NEC was associated with IVIG [4]. In our experience, IVIG in two-dose regimens does not increase the incidence of NEC and has no effect on the severity of NEC. PDA and anaemia were reported as risk factors for NEC; it is possible that these may affect the gastrointestinal blood flow [9, 14, 23, 24], consistent with our outcomes. To explore the potential blood flow changes between IVIG and NEC, we further analysed a subgroup of babies with either PDA or Hb < 130 g/L. IVIG did not have a significant effect on NEC in these infants.

The incidence of NEC in neonates with HDN reported by Figueras-Aloy et al. with gestation ≥34 weeks and by Ree et al. with gestation ≥30 weeks was 2.2% and 1.3%, respectively, [2, 8] which are slightly lower than our results. The pathophysiology of NEC is complex, and it is a multifaceted disorder. Risk factors such as prematurity, low birth weight, ischaemia, infection, PDA, caesarean section, hypotension and feeding practices can be involved in the development of NEC [6, 12, 14, 23, 25, 26]. In our study, caesarean delivery, Hb < 130 g/L on admission and PDA were independent risk factors for the development of diagnosed NEC in infants with HDN. Caesarean delivery, Hb < 130 g/L on admission, PDA and SGA were independent risk factors for the development of all-stage NEC in infants with HDN. An explanation for the association between NEC and caesarean section may be due to the less stress during delivery [23]. Emerging literature suggests the potential for the gut microbiome to develop before delivery and that the effect of the mode of delivery on NEC is likely complex and multifactorial [27]. Anaemia and RBC transfusions were reported to be associated with an increased risk of NEC due to diminishing mesenteric blood flow, which could lead to intestinal hypoxia [14, 27]. The mechanism whereby PDA increases NEC may be that it could induce an increased left-to-right shunting through the ductus [24].

There are a few limitations to our study. Firstly, the retrospective design of the study is inferior to any kind of prospective study. Secondly, while we reported on a large sample of HDN, we acknowledge the small sample of 29 infants with NEC, and caution must be applied to any conclusions drawn. This low sample of NEC in our study possibly prevents reliable risk factor analysis and perhaps the section on predictors should be considered as an extended outcome. Thirdly, there is a lack of data on the long-term outcomes of the infants with HDN. Thus, prospective studies with a larger sample of NEC focusing on the long-term outcomes of HDN are needed.

In conclusion, our study suggests that in early preterm and term neonates with HDN, the early use of IVIG was not significantly associated with a higher risk of NEC. Caesarean delivery, haemoglobin at admission <130 g/L, and PDA are possible risk factors for the development of NEC. Therefore, when assessing the use of IVIG in infants with HDN, the risk of NEC should be discounted.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

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Practice patterns of ABO-matching for cryoprecipitate and patient outcomes after ABO-compatible versus incompatible cryoprecipitate

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Abstract

Background and Objectives: This sub-study of the FIBRES trial sought to examine the patterns of ABO-compatible cryoprecipitate administration and to identify adverse consequences of ABO-incompatible cryoprecipitate.

Materials and Methods: This was a post hoc analysis of data collected from the FIBRES randomized clinical trial comparing fibrinogen concentrate with cryoprecipitate in the treatment of bleeding related to hypofibrinogenemia after cardiac surgery. The primary outcome was the percentage of administered cryoprecipitate that was ABO-compatible. Secondary outcomes were adverse events at 28 days. A follow-up survey was distributed to the FIBRES participating sites to examine the rationale behind the identified cryoprecipitate ABO-matching practice patterns.

Results: A total of 363 patients were included: 53 (15%) received ABO-incompatible cryoprecipitate and 310 (85%) received ABO-compatible cryoprecipitate. There was an increased incidence of post-operative anaemia in the ABO-incompatible group (15; 28.3%) in comparison to the ABO-compatible (44; 14.2%) group ($p = 0.01$) at 28 days, which was unrelated to haemolysis, without a significant difference in transfusion requirement. In the multivariable logistic regression models accounting for clustering by site, there was no observed statistically significant association between the administration of ABO-incompatible cryoprecipitate and any other adverse outcomes. Nine out of 11 sites did not have a policy requiring ABO-matched cryoprecipitate.

Conclusion: This sub-study demonstrated that most cryoprecipitate administered in practice is ABO-compatible, despite the absence of guidelines or blood bank policies to support this practice. A signal towards increased risk of post-operative anaemia may be explained by higher rates of urgent surgery (vs. elective) in the ABO-incompatible group. Future studies should prospectively examine the impact of ABO-compatible versus incompatible cryoprecipitate to conclusively establish if there is a meaningful clinical impact associated with the administration of ABO-incompatible cryoprecipitate.

KEYWORDS
ABO-matching, cryoprecipitate, safety, transfusion

Highlights

- Most cryoprecipitate administered in practice is ABO-compatible, despite the absence of guidelines or blood bank policies to support this practice.
- There was no statistically significant association between administration of ABO-incompatible cryoprecipitate and adverse outcomes such as death, stroke, liver injury, kidney injury, or thromboembolic events.
- A signal towards increased risk of post-operative anemia, unrelated to hemolysis, may be explained by higher rates of urgent surgery (vs. elective) in the group receiving ABO-incompatible cryoprecipitate.

INTRODUCTION

Cryoprecipitate refers to the insoluble proteins prepared by thawing frozen plasma at 1–6°C. After the product is thawed and centrifuged, the supernatant is removed, leaving the cold insoluble precipitate plus 5–15 ml of plasma in the original bag. The cryoprecipitate formed contains fibrinogen, factor VIII, fibronectin, factor XIII and von Willebrand factor (VWF) [1, 2]. Clinically, cryoprecipitate is administered to patients with acquired hypofibrinogenemia of various aetiologies, including disseminated intravascular coagulation (DIC), post-cardiac surgery, liver transplant and post-partum haemorrhage [3].

The incidence of adverse effects with cryoprecipitate administration has been reported at 6.57 events per 10,000 units [3]. Adverse effects include haemolysis, sepsis, thrombosis, renal dysfunction, cholestasis in liver transplant recipients and transfusion-related acute lung injury (TRALI) [3, 4]. Haemolysis and a positive direct antiglobulin test have only rarely been reported in patients receiving ABO-incompatible cryoprecipitate due to anti-A and anti-B antibodies reacting with A and B antigens on recipient red cells. This is of particular concern when group O cryoprecipitate is transfused to non-group O transfusion recipients, especially if a large volume of ABO-incompatible cryoprecipitate is administered [5].

There is currently limited evidence to suggest ABO-incompatible cryoprecipitate causes adverse reactions. Hadjesfandiari et al. [6] examined anti-A and anti-B antibody titres in cryoprecipitate, identifying a likelihood of 1 in 3 million that a transfused pool of 10 cryoprecipitate units would contain a titre higher than 1:100 (the titre below which many hospitals will label the product as low titre) for either antibody [7].

Despite various organizations stating ABO matching is not required for cryoprecipitate, this is still a common practice, and some hospital policies require ABO matching. Recent data from Canadian Blood Services indicated that 57% of surveyed Canadian hospitals were willing to issue unmatched cryoprecipitate to patients with unknown blood groups [8]. Most guidelines suggest that universal ABO matching for cryoprecipitate is not required, citing the lack of evidence demonstrating significant adverse complications (Table 1). However, as yet, data surrounding the real-world application of such guidelines are lacking. The fibrinogen replacement in surgery (FIBRES) trial was conducted at 11 Canadian hospitals and randomized

TABLE 1 Recommendations on cryoprecipitate ABO compatibility from transfusion medicine organizations and societies

Organization	Recommend ABO-matched cryoprecipitate	Comments
AABB [9]	No	
Australian and New Zealand Society of Blood Transfusion [10]	Yes	
American Red Cross [11]	No recommendation	
British Society of Haematology [12]	No recommendation	For plasma, donors with identical ABO blood groups to the recipient should be used as the first choice. If this is not possible, ABO non-identical plasma is acceptable if it has 'low-titre' anti-A or anti-B activity.
Canadian Blood Service [13]	No recommendation	Plasma products such as cryoprecipitate must be ABO compatible with the recipient's blood type but not necessarily be group specific. The plasma product, to be compatible, should not contain ABO antibodies that may be incompatible with the ABO antigens on the patient's red blood cells.
Canadian Society of Transfusion Medicine [14]	No	Adult recipients can be transfused with any ABO group of cryoprecipitate. ABO compatible cryoprecipitate should be used for neonates.
World Health Organization [15]	Yes	

735 adult patients who underwent cardiac surgery and developed clinically significant bleeding with hypofibrinogenemia post-cardiopulmonary bypass to cryoprecipitate versus fibrinogen concentrate [16]. This sub-study of the FIBRES trial seeks to examine the patterns of ABO-matched cryoprecipitate administration and to explore if there are any adverse consequences of ABO-incompatible cryoprecipitate.

MATERIALS AND METHODS

This was a post hoc analysis of data prospectively collected from the FIBRES randomized clinical trial comparing fibrinogen concentrate with cryoprecipitate (as control) in the treatment of bleeding related to hypofibrinogenemia after cardiac surgery [16]. Ethics approval for the post hoc data analysis was obtained from the University Health Network research ethics board.

In this sub-study, ABO-incompatible cryoprecipitate was defined as group O cryoprecipitate to A, B and AB patients; group A cryoprecipitate to B and AB patients; and group B cryoprecipitate to A and AB patients. Patients who received only ABO-compatible cryoprecipitate were included in the 'ABO-compatible' group, whereas patients who received incompatible or a combination of compatible and incompatible were included in the 'ABO-incompatible' group. Data collected during the FIBRES study were analysed to determine the current practice patterns in cryoprecipitate ABO-matching at institutions across Canada. During the study, institutional transfusion policies were not modified, aside from dose standardization (10 units per dose) [17].

The primary outcome of interest in this sub-study was the percentage of administered cryoprecipitate at participating sites that was ABO compatible. Secondary outcomes were adverse events at 28 days as defined by the *Medical Dictionary for Regulatory Activities* (Version 21.1) system of nomenclature, including death, myocardial infarction (MI), stroke, liver injury, kidney injury, thromboembolic events (defined as deep vein thromboses, pulmonary emboli, MI or strokes), coagulopathy/DIC, anaemia (haemoglobin <7 g/dl)/severe haemolysis and hyperbilirubinemia. A composite outcome of kidney injury, liver injury, MI, stroke, thromboembolic events or death was also examined. Post hoc, post-operative anaemia (haemoglobin ≤7.5 g/dl) on post-operative day 1 was included as an adverse outcome; a cut-off of haemoglobin ≤7.5 g/dl was selected to align with available cardiac surgery data, including from the TRICS-III trial [18]. As patients may have had more than one haemoglobin measurement on post-operative day 1 (POD1), the last POD1 measurement in each individual patient was designated the POD1 haemoglobin.

Continuous variables were handled as means and standard deviations, and where non-normality of data distributions was found, medians and interquartile ranges were determined. Categorical variables were handled as frequencies or percentages. Adjusted multivariable logistic regression models incorporating a priori specified confounders were developed. For each logistic regression model, we used global measures of model fit based on information criterion statistics. Model discrimination was assessed using the C-statistic. Model calibration was assessed through the use of the Hosmer–Lemeshow test by comparing the observed events within prespecified risk groupings (deciles of risk), with higher *p*-values indicating better calibration. Clustering by the centre was accounted for by the use of mixed models. For multilevel models accounting for clustering by site, quasi-likelihood under the independence criterion statistics were examined [19]. Models were adjusted for age, sex, critical preoperative status, preoperative haemoglobin level, preoperative creatinine clearance and bleeding severity as defined by the Universal Definition of Perioperative Bleeding in Cardiac Surgery (UDPB), which was modified as per the main FIBRES analysis [17]. In our models, we defined severe bleeding as UDPB category 3 or higher. Special data handling methods were not employed for dealing with missing data for the predictor or outcome variables, as generally, there was minimal missing data in the FIBRES dataset. Parameter estimates are unlikely to be biased when data are at least missing at random. SAS University Edition (SAS Institute, Inc., Cary, NC) was used for all statistical analyses. All reported *p*-values were two-sided, and values of $p < 0.05$ were considered to be statistically significant.

Two separate analyses were pursued. The first included all FIBRES patients randomized to receive cryoprecipitate, regardless of the dose received. The second analysis was restricted to patients receiving 20 or fewer units of cryoprecipitate to eliminate the bias of higher proportions of ABO-incompatible cryoprecipitate in higher dose recipients.

A follow-up survey was distributed to the 11 FIBRES participating sites in September 2020 to examine the rationale behind the identified cryoprecipitate ABO-matching practice patterns. The survey questions are included in Appendix S1.

RESULTS

Post-FIBRES survey data

The survey was distributed to the 11 participating sites with a 100% response rate. Nine hospitals had policies that did not require ABO compatible cryoprecipitate; one site required ABO-compatibility, and one additional site required ABO compatibility only for transplant patients. ABO-compatible cryoprecipitate coincidentally being available in inventory was the main (5/9, 55%) reason for preferential administration of ABO-compatible cryoprecipitate, despite the absence of such a policy. Medical laboratory technologists (MLTs) independently selecting ABO-compatible cryoprecipitate was also a frequently cited reason (4/9; 45%). No respondents attributed this to electronic alerts from the blood bank system. If no ABO-compatible cryoprecipitate was available in the inventory, 10 (90.1%) respondents stated their blood bank would administer ABO-incompatible cryoprecipitate, and 1 (9.9%) stated their blood bank would administer fibrinogen concentrate instead. If the blood group was unknown, seven (70%) stated they would administer any ABO-group of cryoprecipitate, based on inventory availability, two (20%) stated they would

TABLE 2 Baseline patient characteristics associated with ABO-compatible cryoprecipitate use

	ABO-incompatible cryoprecipitate (%) ($n = 53$)	ABO-compatible cryoprecipitate (%) ($n = 310$)	*p*-value
Age, median (IQR), years	65 (58, 75)	64 (52, 71)	0.10
Sex, *n* (%)			
Male	48 (90.6%)	210 (67.7%)	0.001
Female	5 (9.4%)	100 (32.3%)	
NYHA heart failure class			
I	12 (22.6%)	100 (32.3%)	0.56
II	18 (34.0%)	90 (29.0%)	
III	16 (30.2%)	87 (28.1%)	
IV	7 (13.2%)	33 (10.7%)	
Critical state	3 (5.7%)	34 (11.0%)	0.33
Ejection fraction, %			
>50	41 (78.9%)	222 (75.3%)	0.37
31–50	10 (19.2)	47 (15.9%)	
21–30	1 (1.9%)	11 (3.7%)	
<21	0 (0.0%)	15 (5.1%)	
Pulmonary hypertension (mmHg), *n* (%)			
<30	40 (75.5%)	235 (75.8%)	0.82
31–55 (moderate)	11 (20.8%)	57 (18.4%)	
>55 (severe)	2 (3.8%)	18 (5.8%)	
Renal impairment			
eGFR (ml/min per 1.73 m^2), *n* (%)			
>90	12 (22.6%)	99 (32.0%)	0.05
60–89	23 (43.4%)	100 (32.4%)	
45–59	6 (11.3%)	44 (14.2%)	
30–44	1 (1.9%)	30 (9.7%)	
15–29	5 (9.4%)	22 (7.1%)	
<15 or dialysis	6 (11.3%)	14 (4.5%)	
Preoperative laboratory values			
INR, median (IQR)	1.0 (1.0, 1.1)	1.0 (1.0, 1.2)	0.33
Haemoglobin (g/dl), median (IQR)	12.6 (10.9, 14.7)	13.6 (12.0, 14.8)	0.13
Platelets ($\times 10^9$), median (IQR)	180 (146, 250)	185 (155, 227)	0.94
Post-operative laboratory values (post-operative day 1)			
INR, median (IQR)	1.2 (1.1, 1.4)	1.2 (1.1, 1.3)	1.00
Haemoglobin (g/dl), median (IQR)	8.8 (8.1, 9.6)	9.1 (8.3, 10.1)	0.19
Platelets ($\times 10^9$), median (IQR)	117 (94, 155)	114 (92, 142)	0.26
Fibrinogen replacement[a]			
Pre-treatment fibrinogen level (g/L), median (IQR)	1.9 (1.5, 2.4)	1.7 (1.4, 1.9)	0.005
Post-treatment fibrinogen level (g/L), median (IQR)	2.5 (2.0, 3.1)	2.3 (2.1, 2.6)	0.06
Surgical factors			
Elective	28 (52.8%)	207 (66.8%)	<0.001
Urgent	22 (41.5%)	54 (17.4%)	
Emergent	3 (5.7%)	49 (15.8%)	
Complex	34 (64.2%)	226 (72.9%)	0.19
Redo sternotomy	11 (20.8%)	81 (26.1%)	0.41
Pre-operative IABP	0 (0.0%)	3 (1.0%)	1.00
Pre-operative VAD	1 (1.9%)	8 (2.6%)	1.00
UDPB bleeding severity categories			
Universal definition of perioperative bleeding[b] (UDPB) severity category (≥3 or higher)	31 (58%)	138 (44%)	0.06
Allogeneic product transfusion up to 7 days post-operatively			
Packed red cells, median (IQR)	4 (2, 8)	4 (2, 7)	0.51
Frozen plasma units, median (IQR)	3 (2, 6)	4 (0, 6)	0.72
Platelet units, median (IQR)	3 (1, 4)	2 (1, 4)	0.71

Abbreviations: eGFR, estimated glomerular filtration rate; IABP, intra-aortic balloon pump; INR, international normalized ratio; NYHA, New York Heart Association; UDPB, Universal Definition of Perioperative Bleeding; VAD, ventricular assist device.

[a]Pre-treatment and post-treatment fibrinogen for repeat administrations (≥2) where multiple measurements were available was recorded as the average per patient.

[b]UDPB categories are defined as: 0 = insignificant; 1 = mild; 2 = moderate; 3 = severe; 4 = massive.

administer fibrinogen concentrate, and one (10%) stated they would administer group A cryoprecipitate, if available; one site declined to answer this question. Since completion of the FIBRES trial, no respondents had changed their cryoprecipitate ABO-matching policy, but

eight (80%; one respondent declined to answer) had changed their fibrinogen replacement policy to utilize fibrinogen concentrate as the preferred product over cryoprecipitate in all patients.

Patient outcomes after ABO-compatible versus incompatible cryoprecipitate

In FIBRES, 412 patients were randomized to receive cryoprecipitate, of whom 383 received the intervention as randomized, and 29 did not (the patients had ceased bleeding by the time the product arrived). An additional 20 patients were lost to follow-up. Thus, 363 patients were included in this sub-study, which were part of the primary FIBRES analysis dataset. Of these, 53 (15%) received ABO-incompatible cryoprecipitate, and 310 (85%) received ABO-compatible cryoprecipitate. Ten of 11 (90.9%) sites transfused more ABO-compatible than ABO-incompatible cryoprecipitate.

Among the included patients, 258 (71.1%) were male, and 105 (28.9%) were female. The median age was 64 years. Aside from a baseline imbalance in sex and renal function (a greater proportion of males and renal dysfunction in the ABO-incompatible group), there were no statistically significant differences in baseline preoperative characteristics between those patients that received ABO-compatible versus ABO-incompatible cryoprecipitate (Table 2).

Regarding surgical factors (Table 2), patients in the ABO-compatible group (207; 66.8%) were more likely ($p < 0.001$) to undergo elective surgery than the ABO-incompatible group (28; 52.8%). However, there were no significant differences in rates of complex (procedures other than coronary artery bypass graft surgery alone, single valve surgery alone or atrial septal defect repair alone) surgeries, redo sternotomies or any specific cardiac surgeries.

There were no statistically significant differences in bleeding severity as defined by UDPB (Table 2) between ABO-compatible and incompatible groups ($p = 0.06$). Similarly, there were no significant differences in individual allogeneic blood product transfusion, including red blood cells, platelets, or frozen plasma (FP), between the two groups. This held true for the sum of these allogeneic transfusions within 24 h or within 7 days of the index surgery.

In total, there were 466 cryoprecipitate administrations; 296 patients (81.5%) received 1 dose, 49 (13.5%) received 2 doses, 10 (2.8%) received 3 doses, 6 (1.7%) received 4 doses and 1 patient each (0.3%) received 6 and 12 doses, respectively. The distribution of cryoprecipitate administrations by patient blood group and cryoprecipitate blood group are summarized in Table S2.

The unadjusted analysis demonstrated an increased incidence of post-operative anaemia (at 28 days) in the ABO-incompatible group (15; 28.3%) in comparison to the ABO-compatible (44; 14.2%) group ($p = 0.01$; Table S3), without different rates of post-operative day 1 anaemia (defined as a haemoglobin level ≤7.5 g/dl). All post-operative anaemia events at 1 and 28 days were non-haemolytic anaemias. In the final multivariable logistic regression models accounting for clustering by study site, there was no observed statistically significant association between the administration of ABO-incompatible cryoprecipitate and any adverse outcomes (28-day mortality, MI, stroke, liver injury, kidney injury, thromboembolic events, nor the composite outcome; Table 3) except for post-operative anaemia at 28 days (aOR 0.51, 95% CI 0.34–0.77; $p = 0.001$).

TABLE 3 Multivariable analysis of outcome data adjusted for differences in baseline characteristics

Outcome	aOR (95% CI)	*p*-value
28-day mortality	0.62 (0.27, 1.42)	0.26
MI	*Too few outcome events, model did not converge*	
Stroke	0.52 (0.18, 1.49)	0.22
Liver injury	0.65 (0.34, 1.23)	0.18
Kidney injury	0.76 (0.28, 2.09)	0.56
Thromboembolic events	1.08 (0.45, 2.68)	0.85
Original composite (AKI, liver injury, MI, stroke, TE, or death)	0.88 (0.62, 1.24)	0.46
Post-operative day 1 haemoglobin ≤7.5 g/dl	1.17 (0.57, 2.43)	0.67
Post-operative haemoglobin <7 g/dl up to 28 days	0.49 (0.32, 0.72)	<0.001
Composite 1 (TE, stroke, MI)	1.13 (0.56, 2.31)	0.73
Composite 2 (Stroke, TE, MI, or death)	0.73 (0.45, 1.30)	0.32

Note: Odds ratios presented for ABO-compatible cryoprecipitate group, with the ABO-incompatible group serving as the reference. Models were adjusted for age, sex, preoperative haemoglobin level, preoperative creatinine clearance, Universal Definition of Perioperative Bleeding (UDPB) severity category (≥3 or higher), and critical preoperative status [critical preoperative status was determined by blinded adjudication on patients who underwent emergency surgery and had any of the following conditions: (1) ventricular tachycardia or fibrillation or cardiac arrest; (2) preoperative cardiac massage; (3) preoperative ventilation before anaesthetic room; (4) haemodynamic support requiring preoperative inotropes of ventricular assist devices; (5) preoperative acute renal failure (anuria or oliguria).] The final model for thromboembolic events was adjusted for age, sex, preoperative creatinine clearance, preoperative haemoglobin level, and UDPB category (≥3 or higher).
Abbreviations: AKI, acute kidney injury; aOR, adjusted odds ratio; MI, myocardial infarction; TE, thromboembolism.

The unadjusted analysis of the sub-group of patients receiving ≤2 doses of cryoprecipitate demonstrated an increased risk of anaemia in the ABO-incompatible group, with no haemolytic events reported. The multivariable analysis adjusted for age, sex, UDPB and critical preoperative status did not demonstrate an increased risk of any adverse outcome.

DISCUSSION

This study demonstrated that during the FIBRES trial, 85% of patients received ABO-compatible cryoprecipitate despite participating Canadian blood banks not having a policy in place to require

ABO-compatible cryoprecipitate. The main reason for this appears to be that ABO-compatible cryoprecipitate was coincidentally available in inventory, and MLTs preferentially selected it. In addition, blood groups O and A represent 85%–90% of the cryoprecipitate donor pool, likely also contributing to this observation. However, if matched cryoprecipitate was unavailable or the blood group was unknown, most blood banks would administer any ABO group of cryoprecipitate based on inventory availability. Since the completion of the FIBRES trial, 80% of blood banks changed their policy to prioritize the use of fibrinogen concentrate in all patients.

With regard to adverse events related to ABO-incompatible cryoprecipitate administration, there was no statistically significant increase in death, stroke, liver injury, kidney injury, thromboembolic events, or the composite outcome incorporating these endpoints after adjustment for potential confounders. There was a signal of association of ABO-incompatible cryoprecipitate with post-operative anaemia at 28 days, despite similar pre-operative and post-operative day 1 haemoglobin levels. One possible explanation is the higher rates of urgent as opposed to elective surgeries in the ABO-incompatible group, which were associated with larger volume bleeding (and anaemia) and subsequent transfusion ($p < 0.001$) [20]. Additionally, the ABO-incompatible group had a higher incidence of renal dysfunction at baseline, which may predispose to post-operative anaemia. All post-operative anaemia events in the cryoprecipitate group of the FIBRES modified intention-to-treat analysis cohort were non-haemolytic anaemias. Given the lack of haemolysis, which would be the presumed mechanism of cryoprecipitate-induced post-operative anaemia, and the small number in this cohort, this finding may be due to residual confounding or chance alone and should be interpreted with caution.

Reports have illustrated transfusing ABO-incompatible plasma can result in clinical and laboratory evidence of haemolysis [21]. Given the presence of residual plasma in cryoprecipitate units, concern for haemolysis has been attributed to the transfusion of ABO-incompatible cryoprecipitate. In in vitro studies, the formation of ABO immune complexes may also contribute to haemolysis [22]. A retrospective study in surgical patients receiving blood products suggested patients receiving ABO non-identical platelets, FFP, and cryoprecipitate required 50% more red cell transfusions [23], although this has not been reproduced in other reports [24]. In this study, there was no increase in reported adverse events, including haemolysis, DIC or hyperbilirubinemia related to ABO-incompatible cryoprecipitate administration, nor was there an increased rate of transfusion.

This study was limited by its retrospective nature, including analysis of post hoc data, with potential selection bias related to the non-randomized distribution of patients between the ABO-compatible and ABO-incompatible cryoprecipitate groups. Although baseline demographic and other characteristics were similar between groups, the possibility of residual confounding remains. A further limitation was the low number of patients receiving ABO-incompatible cryoprecipitate; however, there has not been a study to date that has described a similar cohort. The low event rates also limited the ability to accurately identify true differences between groups. While we attempted to compensate for this through this use of a priori specified composite endpoints combining clinically important adverse events, our sample size may still have been underpowered to detect a clinically important difference between groups. In addition, there was no active surveillance for haemolysis (such as measurements of biochemical measures of haemolysis), and therefore, an increased incidence of haemolysis cannot be excluded. Further, ABO types of the platelets and plasma were not available for analysis to account for the transfusion of additional ABO-incompatible plasma. Last, only FIBRES participating sites were surveyed, which could have resulted in a bias towards academic centres.

In summary, this sub-study demonstrated that most cryoprecipitate administered in practice is ABO-compatible, despite the absence of guidelines or widespread blood bank policies to support this practice. Future studies in countries that continue to rely on cryoprecipitate should prospectively examine the impact of ABO-compatible versus incompatible cryoprecipitate, and may consider similar analyses with a focus on adverse patient outcomes to conclusively establish if there is a meaningful clinical impact associated with the administration of ABO-unmatched cryoprecipitate.

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CONFLICT OF INTEREST

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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The spectrum of ABO haemolytic disease of the fetus and newborn in neonates born to group O mothers

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Abstract

Background and Objectives: ABO haemolytic disease of the fetus and newborn (HDFN) is a lesser recognized entity; however, the severity may vary in neonates. This prospective observational study was performed to determine the severity and risk of ABO-HDFN in neonates born to O group mothers.

Materials and Methods: A total of 260 neonates born to non-alloimmunized blood group O mothers were recruited. Blood group O neonates were excluded from the study. Neonatal direct antiglobulin test (DAT) was performed using the column agglutination technique. They were monitored for clinical and laboratory parameters and followed up at 6–8 weeks. The maternal anti-A and anti-B titres (IgM and IgG) were also done.

Results: A total of 176 neonates with blood group A (77/260; 29.6%) and B (99/260; 38.1%) were finally included in the study, and 15 (8.5%) of them were DAT positive. Overall, 26.7% (47/176) neonates received phototherapy, 172 (97.7%) survived and none required readmission. The median (inter-quartile range [IQR]) maternal IgG anti-B titre (32 [32–64]) was significantly higher ($p < 0.001$) than the IgG anti-A titre (16 [8–64]). The maximum total serum bilirubin in neonates had a significant positive association with neonatal birth weight ($p = 0.045$), positive DAT ($p = 0.006$) and requirement of phototherapy ($p < 0.001$). The relative risk (95% CI) of a DAT-positive neonate requiring phototherapy was 4.55 (3.12–6.33).

Conclusion: The frequency of ABO incompatibility in neonates born to group O mothers was 67.69% (176/260). The maternal IgG titre of ≥64 could be a good predictor for identifying the neonates at risk of developing hyperbilirubinaemia requiring phototherapy.

KEYWORDS
ABO-HDFN, antibody titre, direct antiglobulin test, haemolytic disease of the fetus and newborn, IgG antibody

Highlights

- The overall frequency of ABO incompatibility between mother and neonate was 67.7% (176/260).

- There was a significant difference between the direct antiglobulin test (DAT)-positive and DAT-negative groups of neonates in terms of the maximum total serum bilirubin (15.04 vs. 11.72 mg/dl; $p = 0.006$) and need for phototherapy (93.3% [14/15] vs. 20.5% [33/161]; $p < 0.001$).
- The relative risk of need for phototherapy when the maternal IgG titre was ≥64 was 2.98 (95% CI 1.86–4.5). Thus, maternal IgG titre of anti-A/anti-B of 64 or more could be a good predictor for identifying neonates at risk, and combining this with neonatal DAT has a significant role in determining the neonates at risk for developing hyperbilirubinaemia and requiring further management.

INTRODUCTION

Haemolytic disease of the fetus and newborn (HDFN) is caused by the binding of transplacentally transmitted maternal IgG antibodies to fetal red blood cells (RBCs), leading to haemolysis or suppression of erythropoiesis, which results in fetal and/or neonatal anaemia [1]. The severity in the newborn may be variable depending upon the expression of the corresponding antigens on neonatal RBCs, antigen maturity, immune antibody titre and avidity.

ABO-HDFN is a frequent event and usually a problem of the neonate rather than the fetus; however, it is difficult to predict the disease severity. About 15%–25% of pregnancies can have ABO incompatibility, whereas only 10% develop HDFN [2, 3]. ABO-HDFN is a relatively less recognized entity, as routine antenatal screening for high-titre anti-A and anti-B is not recommended because of poor reproducibility. In addition, it is rarely severe enough to warrant exchange transfusion (ET) in the neonate. In a study from southern India by Bhat et al. [4], ABO incompatibility was seen in 17.3% (151/878) of pregnancies, and 46 (30.4%) neonates required phototherapy; however, none required ET. In another study by Usha et al. [4] on 100 antenatal O group mothers, ABO-HDFN was encountered in 3 neonates with high titres of both IgG and IgM anti-A and anti-B antibodies. Thus, there is a need to increase awareness about ABO-HDFN for optimizing care in terms of early diagnosis and adequate monitoring. Therefore, this study was aimed at determining the

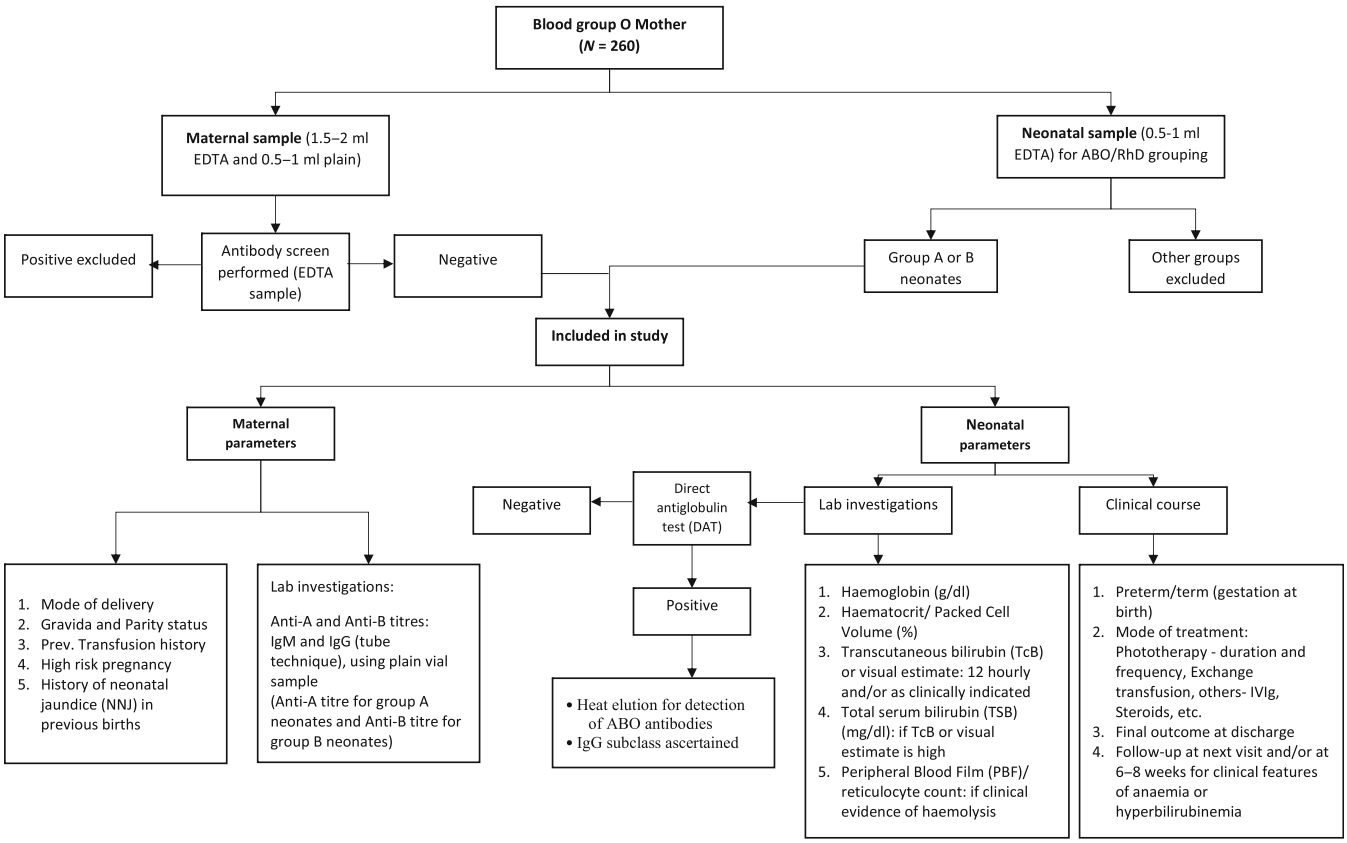

FIGURE 1 Study design and testing protocol. ABS, antibody screen; DAT, direct antiglobulin test; ET, exchange transfusion; Hb, haemoglobin; PCV, packed cell volume; PT, phototherapy.

severity and risk of ABO-HDFN in group A and group B neonates born to non-alloimmunized O group mothers.

MATERIALS AND METHODS

This prospective observational study was carried out from February 2020 to May 2021 and was approved by the Institutional Ethics Committee (vide letter no. INT/IEC/2020/SPL-490 dated 22 April 2020). A written informed consent was also taken from the mothers enrolled in the study. A total of 260 neonates born to blood group O mothers were recruited (Figure 1). All the mothers with blood group O who presented during the study period could not be enrolled because of the ongoing COVID-19 pandemic, as patient enrollment was quite challenging due to periodic lockdowns and travel restrictions. Thus, the sample size was reduced to 260 blood group O mothers who could finally be enrolled during the study period. The RBC antibody screen (ABS) was performed on maternal sample by a three-cell panel (Diacell, Bio-Rad, Morat, Switzerland) using the column agglutination technique (CAT) (LISS Coomb's AHG gel card, Bio-Rad). Only those with a negative ABS screen were further evaluated in the study, to avoid the influence of alloantibody(ies) directed towards antigens other than ABO. The neonates of group O mothers were either born in the 'clean labour room' of our institute ('in-borns') or born outside and later referred to our institute ('out-borns'). For in-born neonates, a cord sample (0.5–1 ml EDTA) was collected, and for out-borns, the first available sample (0.5–1 ml EDTA) was taken for testing. The neonates with blood group O were excluded from the study.

A neonatal direct antiglobulin test (DAT) was performed using CAT (Bio-Rad) [5, 6]. Further, for DAT-positive samples, the IgG

TABLE 1 Maternal IgM and IgG anti-A and anti-B titres

	Maternal IgM titre[a]			Maternal IgG titre[a]		
	Anti-A ($n = 77$)	Anti-B ($n = 99$)	*p*-Value	Anti-A ($n = 77$)	Anti-B ($n = 99$)	*p*-Value
Mean (±SD)	46.29 (54.32)	49.76 (57.18)	0.958	43.14 (69.67)	63.23 (67.29)	<0.001[b]
Median (IQR)	32 (16–64)	32 (16–64)		16 (8–64)	32 (32–64)	
Range	2–256	2–256		2–512	4–512	

Abbreviations: IQR, inter-quartile range; SD, standard deviation.
[a]Anti-A IgG titre in mothers who gave birth to neonates with blood group A, while anti-B IgG titre in mothers who gave birth to neonates with blood group B.
[b]Statistically significant.

TABLE 2 Distribution of subclass of IgG antibody at various dilutions for DAT-positive samples ($n = 15$)

Antibodies (dilution)	Negative	1+	2+
IgG1 (1:1)	12 (80%)	2 (13.3%)	1 (6.7%)
IgG1 (1:100)	15 (100%)	0	0
IgG3 (1:1)	14 (93.3%)	1 (6.7%)	0
IgG1 (1:100)	15 (100%)	0	0
Control	15 (100%)	0	0
IgG (1:10)	10 (66.7%)	3 (20%)	2 (13.3%)

TABLE 3 Neonatal investigations and outcomes

	N	Mean (±SD)	Median (IQR)	Range
Haemoglobin (g/dl)	176	18.09 (±3.51)	18 (16.38–20)	9.9–38
PCV (%)	176	54.14 (±10.17)	55 (49.53–60.25)	13–78
TSB (mg/dl) on day 2	86	9.15 (±3.97)	8.7 (5.9–12.17)	2.4–19.6
Maximal TSB (mg/dl)	71	12.38 (±3.98)	12.7 (9.4–14.9)	5.7–22.9
Age at maximum TSB (days)	71	3.56 (±1.65)	3 (2.5–4)	2–10
Lowest PCV (%)	71	49.79 (±10.11)	52 (46–55)	12.3–72
Age at lowest PCV (days)	71	49.79 (±10.11)	52 (46–55)	12.3–72
Duration of phototherapy (h) in neonates	47	34.17 (±25.67)	24 (12–48)	12–120
Age at discharge	172	7.70 (±12.32)	4 (3–7)	1–120

Abbreviations: IQR, inter-quartile range; PCV, packed cell volume; SD, standard deviation; TSB, total serum bilirubin.

subclass of anti-A or -B was determined using DAT IgG1/IgG3 screening cards (Bio-Rad) [5]; heat elution at 56°C [5] was also performed to determine the type of ABO antibody implicated. By monitoring the neonatal total serum bilirubin (TSB), the decision for phototherapy and/or ET was taken by the clinician based on American Association of Pediatrics (AAP) charts [7]. In neonates with a progressive rise in TSB, a reticulocyte count (in percentage), packed cell volume (PCV) (in percentage), and a peripheral smear were also performed for the evaluation of haemolysis. The haemoglobin (Hb) (in grams per decilitre) and PCV (in percentage) were determined using another EDTA (0.5 ml) sample using a cell counter (Orion 60 Haematology analyser). In view of the ongoing COVID-19, a telephonic follow-up of

TABLE 4 Neonatal profile and outcomes according to blood group A and B

	Neonatal ABO blood group ($n = 176$)		
Parameter	**A ($n = 77$)**	**B ($n = 99$)**	***p*-Value**
Birth weight (kg)	2.60 ± 0.64	2.38 ± 0.76	0.068[a]
<1	1 (1.3%)	6 (6.1%)	0.227[b]
1–1.5	4 (5.2%)	8 (8.1%)	
1.5–2.5	25 (32.5%)	37 (37.4%)	
≥2.5	47 (61.0%)	48 (48.5%)	
Baby gender			
Male	46 (59.7%)	68 (68.7%)	0.218[c]
Female	31 (40.3%)	31 (31.3%)	
Fetal maturity			
Term	52 (67.5%)	60 (60.6%)	0.343[c]
Pre-term	25 (32.5%)	39 (39.4%)	
DAT positive ($n = 15$)	8 (10.4%)	7 (7.1%)	0.434[c]
Strength of positive DAT			
1+	3 (37.5%)	3 (42.9%)	0.293[b]
2+	4 (50.0%)	1 (14.3%)	
3+	1 (12.5%)	3 (42.9%)	
Elution of antibody*** (in DAT-positive neonates)			
Negative	4 (50.0%)	2 (28.6%)	0.024[b,d]
Anti-A	4 (50.0%)	-	
Anti-B	-	5 (71.4%)	
Anti-IgG (1:10)			
Negative	5 (62.5%)	5 (71.4%)	0.135[b]
1+	3 (37.5%)	0 (0.0%)	
2+	0 (0.0%)	2 (28.6%)	
Lowest PCV (%)	48.54 ± 10.43	50.76 ± 9.88	0.312[a]
Maximum TSB (mg/dl)	12.65 ± 4.06	12.16 ± 3.96	0.612[e]
Received phototherapy ($n = 47$)	17 (22.1%)	30 (30.3%)	0.221[d]
Duration of phototherapy (h)	40.59 ± 31.57	30.53 ± 21.38	0.407[a]
Exchange transfusion ($n = 1$)	1 (1.3%)	0 (0.0%)	0.438[b]
Associated morbidity	14 (18.2%)	25 (25.3%)	0.263[c]
Outcome: survived	74 (96.1%)	98 (99.0%)	
Age at discharge (days)	5.64 ± 4.85	9.27 ± 15.63	0.255[a]

Note: Data presented as mean ± standard deviation (SD) or as number (in percentage).
Abbreviations: DAT, direct antiglobulin test; PCV, packed cell volume; TSB, total serum bilirubin.
[a]Wilcoxon–Mann–Whitney *U* test.
[b]Fisher's exact test.
[c]Chi-squared test.
[d]Adjusted *p*-value (anti-A vs. anti-B).
[e]*t*-test.
***Significant at $p < 0.05$.

the neonates was done at 6–8 weeks after discharge. The parents were asked about commonly encountered signs and symptoms of anaemia and hyperbilirubinaemia, including poor feeding, lethargy, general appearance and yellowish coloration of skin, noticed in the neonates.

The maternal sample was also tested for anti-A or anti-B titres (IgM and IgG) by the tube technique using serial doubling dilution method [5]. The IgG titre was determined by treating the maternal serum sample with 0.01 M dithiothreitol (Himedia Lab, Mumbai, India) to inactivate the IgM component. Appropriate controls were used to validate the results.

The data analysis was carried out using SPSS (Statistical Package for Social Studies) for Windows, version 20.0. Normally distributed data was presented as means and standard deviation (SD) or 95% confidence interval (CI). All tests were performed at a 5% level of significance; thus, an association was significant if the *p*-value was <0.05. Pearson association analysis was used to calculate the relation between quantitative variables. For qualitative variables, chi-square test was applied.

RESULTS

Out of the 260 group O mothers, none had positive ABS. The neonatal ABO blood groups were as follows: O 84 (32.3%), A 77 (29.6%) and B 99 (38.1%). Thus, the overall frequency of ABO incompatibility between mother and neonates was 67.69% (176/260). The 84 neonates with blood group O were excluded, and the remaining 176 neonates (blood group A and B) were finally included in the study. These neonates ($n = 176$) were the outcome of singleton pregnancies. Thus, out of 176 neonates, 77 (43.8%) were group A and 99 (56.2%) were group B. Of the 176 mothers, 100 (56.8%) delivered through the vaginal route and 76 (43.2%) underwent a lower-segment caesarean section. In terms of gestation, 3 (1.7%) delivered at <28 weeks, 11 (6.2%) between 28 and 31 weeks, 48 (27.3%) between 32 and 36 weeks and 114 (64.8%) at ≥37 weeks. Out of the 176 mothers, 76 (43.2%) were primigravida and 11 (6.2%) had a history of blood transfusion. The maternal anti-A/anti-B titres are shown in Table 1.

Among the 176 neonates, 15 (8.5%) had a positive DAT, out of which 6 (40.0%) had 1+, 5 (33.3%) had 2+ and 4 (26.7%) had 3+ agglutination strength. The results of IgG subclass distribution are given in Table 2 and the other neonatal parameters in Tables 3 and 4. Reticulocyte count (reference range: 3.3%–7.3%) was done for 14 (7.95%) neonates with clinical evidence of haemolysis. Among them, 3 (21.43%) had a positive DAT. Serial reticulocyte count was not done. As per the clinician's advice, 28 (15.9%) neonates were screened for G6PD deficiency, and all had normal values. Of them, 7 (25%) were DAT positive. Similarly, as per the clinical indication, the peripheral blood film was observed for 17 (9.65%) neonates, of whom 8 (47.06%) were DAT positive. Overall, 26.7% (47/176) neonates received phototherapy, only 1 (0.6%) required ET and 172 (97.7%, 95% CI: 93.9–99.3) survived. None of them required readmission, and there was no history suggestive of either anaemia or hyperbilirubinaemia.

TABLE 5 Maternal IgG titre distribution across relevant neonatal parameters

Parameters	Maternal IgG titres ($n = 176$)					
	≤16 ($n = 58$)	32 ($n = 48$)	64 ($n = 44$)	128 ($n = 20$)	256 ($n = 4$)	512 ($n = 2$)
Neonatal birth weight (kg)						
<1	1 (1.7%)	2 (4.2%)	2 (4.5%)	1 (5%)	0	1 (50%)
1–1.5	1 (1.7%)	5 (10.4%)	3 (6.8%)	3 (15%)	0	0
1.5–2.5	20 (34.5%)	15 (31.2%)	18 (40.9%)	8 (40%)	1 (25%)	0
≥2.5	36 (62.1%)	26 (54.2%)	21 (47.7%)	8 (40%)	3 (75%)	1 (50%)
Neonatal ABO						
A ($n = 77$)	41 (70.7%)	15 (31.2%)	12 (27.3%)	7 (35%)	1 (25%)	1 (50%)
B ($n = 99$)	17 (29.3%)	33 (68.8%)	32 (72.7%)	13 (65%)	3 (75%)	1 (50%)
DAT positive ($n = 15$)	2 (3.4%)	2 (4.2%)	3 (6.8%)	3 (15%)	3 (75%)	2 (100%)
Lowest PCV (%)	51.7 ± 5.1	52.4 ± 6.6	50.4 ± 9.2	46.9 ± 14.5	37.8 ± 15.4	50.0 ± 21.2
Maximum TSB (mg/dl)	11.1 ± 3.4	11.8 ± 4.0	11.9 ± 3.2	13.3 ± 3.9	17.5 ± 3.9	15.4 ± 9.4
Age at maximum TSB (days)	3.1 ± 1.1	3.5 ± 1.4	3.8 ± 1.9	3.8 ± 2.2	3.3 ± 1.3	4.5 ± 2.1
Phototherapy (PT)	5 (8.6%)	8 (16.7%)	18 (40.9%)	10 (50%)	4 (100%)	2 (100%)
Duration of PT (h)	19.2 ± 6.6	30.0 ± 17.9	31.6 ± 24.9	47.4 ± 34.2	48.0 ± 27.7	18.0 ± 8.5
Exchange transfusion	0	0	0	0	0	1 (50%)
Outcome: survived	57 (98.3%)	48 (100%)	44 (100%)	18 (90%)	4 (100%)	1 (50%)

Note: Data presented as mean ± standard deviation (SD) or as number (in percentage).
Abbreviations: PCV, packed cell volume; PT, phototherapy; TSB, total serum bilirubin.

TABLE 6 Association between maternal IgG titre and other parameters

Parameters	Maternal IgG titre	*p*-Value
Fetal maturity		0.179[a]
Term	50.07 ± 63.59	
Pre-term	62.09 ± 77.18	
Neonatal parameters		
ABO group***		<0.001[a]
A	43.14 ± 69.67	
	16 (8–64)[b]	
B	63.23 ± 67.29	
	32 (32–64)[b]	
DAT***		<0.001[a]
Positive	164.27 ± 164.78	
	128 (48–256)[b]	
Negative	44.21 ± 39.70	
	32 (16–64)[b]	
PCV (%)***	$r = -0.19$	0.013[c]
Maximum TSB (mg/dl)***	$r = 0.29$	0.015[c]
Phototherapy***		<0.001[a]
Yes	102.47 ± 108.35	
	64 (32–128)[b]	
No	36.95 ± 33.33	
	32 (16–64)[b]	
Duration of phototherapy (h)	$r = 0.25$	0.084[c]
Age at discharge (days)***	$r = 0.2$	0.010[c]

Note: Data presented as mean ± standard deviation (SD) or as number (in percentage).
Abbreviations: DAT, direct antiglobulin test; IQR, inter-quartile range; PCV, packed cell volume; *r*, correlation coefficient; TSB, total serum bilirubin.
[a]Wilcoxon–Mann–Whitney *U* test.
[b]Median (IQR).
[c]Spearman correlation.
***Statistically significant ($p < 0.05$).

The relevant neonatal parameters stratified over maternal IgG titres are given in Table 5. Association between maternal IgG titre (combined anti-A and anti-B) and other relevant parameters are given in Table 6 and Figure 2. The maximum TSB in neonates had a significant positive association with neonatal birth weight (in kilogram) ($p = 0.045$), maturity at birth ($p = 0.037$), positive DAT ($p = 0.006$), neonatal age at which lowest PCV values were recorded ($p = 0.046$), requirement of phototherapy ($p \leq 0.001$) and associated morbidity ($p = 0.046$). Neonatal DAT positivity was significantly associated with maternal IgG titres ($p \leq 0.001$), neonatal PCV ($p = 0.017$), lowest recorded PCV ($p = 0.043$), maximum TSB ($p = 0.006$), day of life at which the neonate had the maximum TSB ($p = 0.014$), and requirement ($p \leq 0.001$) and duration of phototherapy ($p = 0.024$). The relative risk (95% CI) of a DAT-positive neonate requiring phototherapy was calculated to be 4.55 (3.12–6.33).

DISCUSSION

ABO incompatibility is now the most common cause of HDFN in the developed world [8, 9]. It is a common cause of morbidity in the neonatal set-up because of the recent practice of early neonatal discharge, and ABO incompatibility leading to hyperbilirubinaemia is one of the most common causes for neonatal readmission [10]. It is typically observed in blood group O mothers who have IgG anti-A/anti-B, which could traverse the placenta and act against the fetal RBCs having A or B antigen [11, 12]. The overall frequency of ABO incompatibility between mother and neonates in our study was 67.69% (176/260), which is higher than those reported in earlier studies (Table 7). This could be attributed to the ethnic and racial differences among the population groups in various studies.

Das et al. [16] reported that early rise in TSB levels showed a positive association with maternal IgG antibody titres. We also observed that the day-2 TSB in neonates had a significant positive association with the maternal IgG titres ($r = 0.28$, $p = 0.010$); similar was the association of the maximum TSB ($r = 0.3$, $p = 0.044$). Bakkeheim et al. [14] pointed out that in ABO-incompatible HDFN, IgG antibody titre below 512 rarely requires aggressive treatment. ABO antibody titre of more than 512 had a sensitivity of 90% and a specificity of 72% for predicting immunoglobulin treatment. We found that the median maternal IgG titre for the neonates requiring phototherapy was 64 (range: 16–512), while it was 32 (range: 16–64) in those who did not require phototherapy ($p \leq 0.001$). The median (IQR) maternal IgG anti-B titre was 32 (32–64; range: 4–512), while the IgG anti-A titre was 16 (8–64; range: 2–512), $p \leq 0.001$. Implication of IgG anti-A in causing ABO-HDFN is more common than anti-B in most of the Western population studies [14]. However, in our study, IgG anti-B was more common in causing the ABO-HDFN as indicated by greater number of B group neonates (30/99; 30.3%) requiring phototherapy than A group neonates (17/77; 22.1%); however, the difference was not statistically significant ($p = 0.221$). This could possibly be due to a higher number of B group neonates (99/176) than A group (77/176) in our study, which is consistent with our population frequency, whereas in most of the studies done in Western population, the frequency of blood group A is far higher than that of blood group B. We also observed that the maximum TSB values were lower for pre-term neonates than term neonates ($p = 0.037$). This has been observed in earlier studies [17] and is probably due to fewer A or B antigenic sites on RBCs of pre-term neonates. Also, with increasing gestational age, transplacental antibody transfer becomes more significant, leading to DAT-positive results in a larger number of neonates and higher incidence of neonatal jaundice.

Madan et al. [18] observed that routine DAT of term non-jaundiced infants born to O RhD-positive mothers is not necessary. In a study by Das et al. [16], 11.2% of ABO-incompatible neonates had a positive DAT (O-A > O-B). These findings are consistent with our study, where 8.5% (15/176) neonates were DAT positive and higher in group A (8/15) neonates than in group B (7/15) neonates ($p = 0.434$). A significantly higher number of newborns required phototherapy in the DAT-positive group compared to the DAT-negative

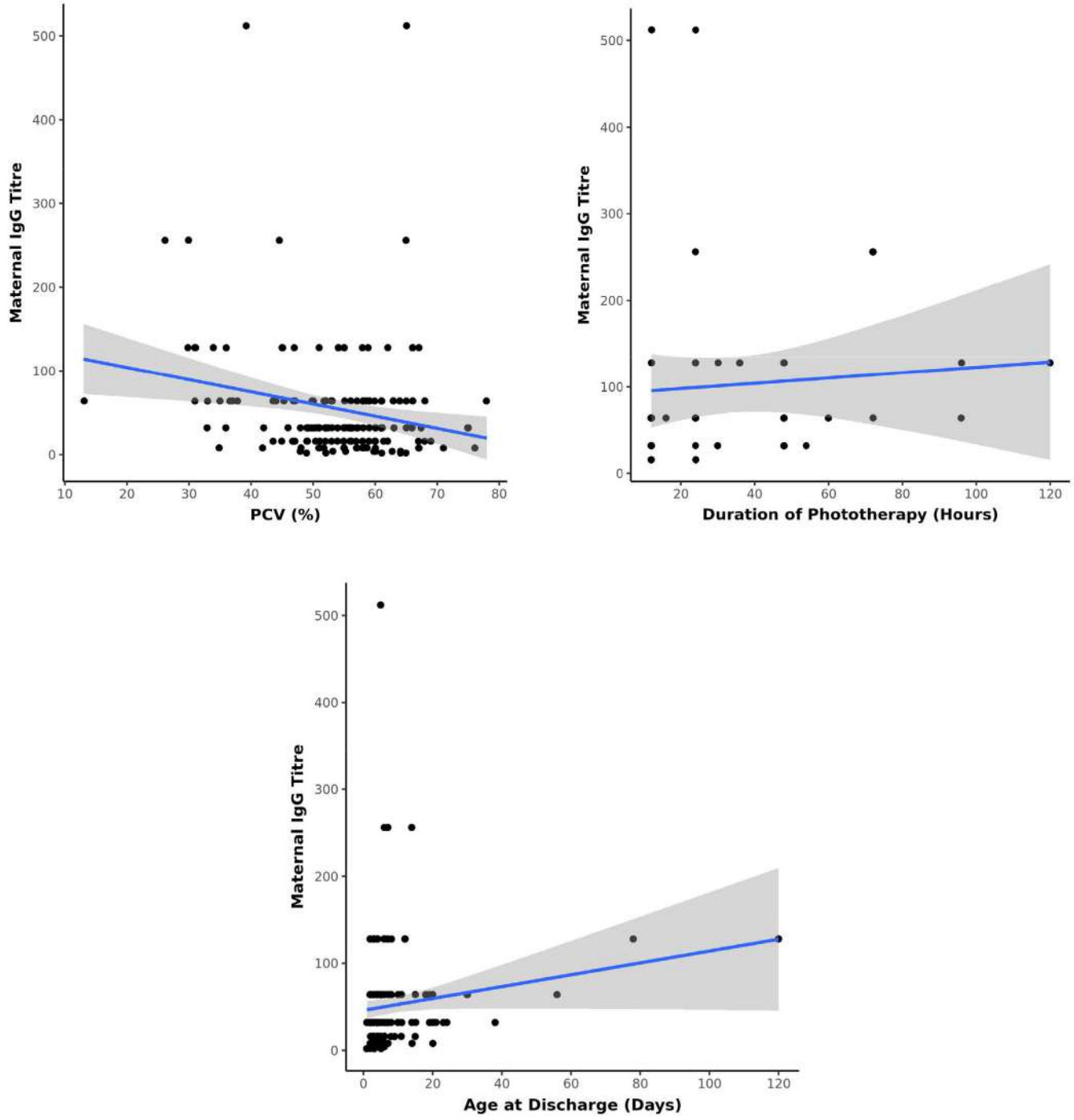

FIGURE 2 Scatterplot depicting the correlation between maternal IgG titres (combined anti-A and anti-B) and neonatal parameters—neonatal PCV (in percentage), duration of phototherapy (in hours), and age at discharge (in days)

TABLE 7 ABO incompatibility between group O mothers and their neonates in various studies

Study	Sample size	ABO incompatibility: number (%)		
		Total	O-A	O-B
Bhat et al. [4]	878	151 (17.3%)	76 (8.66%)	75 (8.54%)
Han et al. [13]	623	251 (40.28%)	141 (22.6%)	110 (17.65%)
Bakkeheim et al. [14]	253	98 (38.74%)	75 (29.64%)	23 (9.09%)
Oseni et al. [15]	130[a]	50 (38%)	Not determined separately	
Present study	260	176 (67.69%)	77 (29.6%)	99 (38.6%)

[a]All blood group mothers (O, A, B and AB) were included in the study.

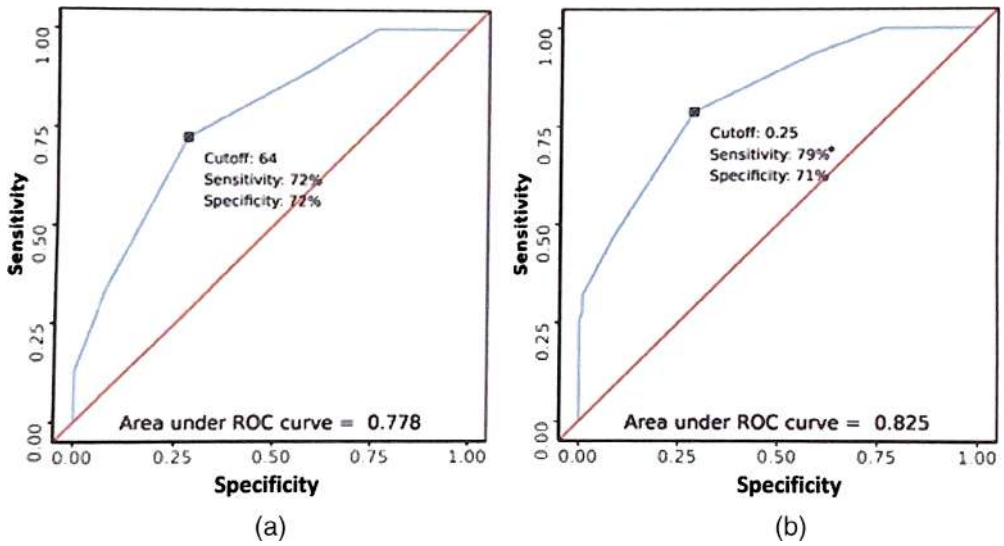

FIGURE 3 Receiver operating characteristic (ROC) curve analysis showing the (a) performance of maternal IgG titre (anti-A and anti-B) and (b) combined performance of maternal IgG titre and neonatal direct antiglobulin test (DAT), in predicting the requirement of phototherapy in neonates (asterisk denotes rounded off; exact value = 78.7%)

group (45.4% vs. 24.4%). We also observed that there was a significant difference between the DAT-positive and DAT-negative groups of neonates in terms of maximum TSB (15.04 vs. 11.72 mg/dl; $p = 0.006$) and requirement of phototherapy (93.3% [14/15] vs. 20.5% [33/161]; $p < 0.001$). We found that the neonates with a positive DAT had a significant positive association with the maximum TSB ($p = 0.006$) and it reached earlier (2.79 days) than in the neonates with negative DAT (3.75 days; $p = 0.014$). Therefore, a regular monitoring of TSB in neonates with a positive DAT may be effective in the timely detection of clinically significant hyperbilirubinaemia. The DAT-positive neonates had higher maternal IgG anti-A/anti-B titre as compared to DAT-negative neonates ($p < 0.001$); however, the strength of the positivity was not significantly associated ($p = 0.105$). The only neonate who required ET was born at 37 weeks of gestation by normal vaginal delivery (weight 3 kg), had a DAT of 1+, maternal IgG anti-A titre of 512, developed jaundice on day 4 of life, and was referred to our institute on day 6 of life with a TSB of 21 mg/dl and received phototherapy as well for 24 h.

To further analyse the role of maternal IgG titre and neonatal DAT in predicting the requirement of phototherapy, the receiver operating characteristic (ROC) curves and prediction analysis were performed (Figure 3). To determine a critical titre for establishing a cut-off maternal IgG titre for predicting the requirement of phototherapy in the neonates, the area under the ROC curve (AUROC) for maternal IgG titre was calculated and found to be 0.778 (95% CI: 0.706–0.85), demonstrating a fair predictive potential ($p \leq 0.001$). Thus, at a cut-off of maternal IgG titre ≥64, it predicted the requirement of phototherapy with a sensitivity of 72.3% and a specificity of 72%. The relative risk (95% CI) for the requirement of phototherapy when the maternal IgG titre was ≥64 was 2.98 (95% CI: 1.86–4.5). The combined sensitivity of maternal IgG titre and neonatal DAT was found to be 78.7% in predicting the need for phototherapy. Therefore, using neonatal DAT testing along with maternal IgG titre would be a better approach for identifying the neonates at risk of requiring phototherapy.

Ukita et al. [19] observed that IgG3 had a greater binding ability than IgG1 to the Fc receptor of phagocytic cells and cause haemolysis, while IgG2 and IgG4 cannot bind to these receptors and therefore cannot cause haemolysis. They also concluded that in group A or B infants born to O mothers, when only IgG2 anti-A or anti-B antibodies can be detected or all IgG subclasses are below the detectable level, ABO-HDFN will not develop, although a DAT of the cord RBCs may be positive. This helps in explaining the test for polyclonal IgG DAT to be positive in a larger number of neonates (5/15), even though they tested negative for IgG1 and IgG3, in our study.

Richa et al. [20] observed that the DAT strength was associated with a borderline statistical significance when the eluate testing was done for detecting new alloantibodies ($p = 0.052$). In our study, we also observed a significant association between the DAT positivity strength and the detection of anti-A/anti-B by heat elution ($p = 0.045$). However, in six neonates, a 'negative' elution result could be due to the inherent limitation of the technique itself.

Our study had following limitations: (1) The number of group O mothers recruited for study was low, as the study was conducted during the ongoing COVID-19 pandemic and there were periodic lockdowns and travel restrictions, so the overall number of pregnant women presenting to our institute for delivery were quite limited as compared to pre-pandemic times; (2) For the out-born neonates, the clinical presentation in the first few hours or upto 2–3 days could not be recorded and analysed and (3) The follow-up of neonates was conducted telephonically, which could have affected the actual evaluation.

In conclusion, the frequency of ABO incompatibility in neonates born to group O mothers was 67.69% (176/260), reflecting that a significant number of neonates are at risk of developing ABO-HDFN. The maternal IgG titre of anti-A/anti-B of 64 or more could be a good

predictor for identifying the neonates at risk, and combining it with neonatal DAT has a significant role in determining the at-risk neonates for developing hyperbilirubinaemia requiring further management.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Demand and usage of unrelated donor products for allogeneic haematopoietic cell transplantation during the COVID-19 pandemic: A Canadian Blood Services Stem Cell Registry analysis

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Abstract

Background and Objectives: Understanding changes in the demand and usage of unrelated allogeneic haematopoietic cell transplantation (HCT) donors during the COVID-19 pandemic is needed to optimize pandemic preparedness of registry and donor collection services. The aim of this study was to understand the extent to which the pandemic has impacted the demand and usage of unrelated donors and cord blood units (CBUs) at Canadian Blood Services (CBS).

Materials and Methods: Data regarding stem cell donor interest and product usage for unrelated allogeneic HCT were retrieved from the database at CBS using de-identified anonymous information.

Results: Unrelated donor searches for Canadian patients remained unchanged by the pandemic, reflecting stable demand. The number of unrelated allogeneic transplants performed within Canada also remained stable, while the number of cord blood transplants increased, chiefly for paediatric patients. Requests for donor verification typing, a first signal of potential interest, increased from domestic centres during the first 6 months of the pandemic and decreased from international centres, before returning to baseline levels. The proportion of transplants for Canadian patients who used stem cell products procured from Canadian donors increased between 3 and 6 months after the start of the pandemic before returning to baseline and appears to be increasing again more than 1 year after the start of the pandemic. Use of CBUs for Canadian paediatric patients increased and remains elevated.

Conclusion: Demand for unrelated adult HCT donors has remained stable despite the evolving pandemic with a transient and recurring increased interest and usage of domestic adult donors. Use of CBUs for paediatric patients has increased and remains elevated. Registries and donor collection centres should maintain the capacity to expand services for domestic donor collection during pandemics to offset threats to international donor usage.

KEYWORDS
cord blood, COVID-19, donor usage, haematopoietic cell transplantation, pandemic, unrelated donor

Highlights

- Transient and recurring increased interest and usage of domestic donors for Canadian patients has occurred during the pandemic.
- Use of cord blood units from the Canadian Blood Services Cord Blood Bank has increased.
- Capacity for domestic donor evaluation and collection is needed during this pandemic to offset potential threats to international donor usage.

INTRODUCTION

COVID-19 has impacted the procurement of unrelated haematopoietic stem cells for allogeneic transplantation, and transplant centres have undergone significant changes in their approach to selecting donors for haematopoietic cell transplantation (HCT) [1, 2]. Aligning with many other countries [3], transplant centres in Canada initially deferred or delayed transplantation for some patients with non-urgent diagnoses to minimize the potential demand on intensive care units and to avoid hospital capacity crises. In many cases, however, deferring life-saving therapy was not possible, and many centres continued to perform HCT. Indeed, a recent report indicates greater mortality among patients whose transplants were delayed because of the pandemic [4]. Patients with aggressive leukaemias who could not be maintained with additional cycles of chemotherapy represented situations wherein the transplant could not be safely deferred. Moreover, recommendations from the World Marrow Donor Association [5], Cellular Therapy and Transplant Canada [6] and Canadian Blood Services (CBS) Stem Cell Registry [7] strongly encouraged transplant centres to cryopreserve all haematopoietic cell products prior to initiating the preparative regimen before transplant. This allowed the centres to continue with unrelated donor collections and defer the infusion of the cells until a later date, if necessary. Although the impact of the pandemic on adult registries and cord blood banks have been reported [8–10], the impact of COVID-19 on the demand and usage of unrelated donors for Canadian centres has not been described. Given the regional differences in public health measures during the pandemic, understanding the changes in donor usage in Canada is important to optimize services that support patients and transplant centres as the pandemic evolves and to better prepare for possible future threats.

METHODS

Data were retrieved from the Stem Cell National System Solution, which houses prospectively collected data related to the management of donor searches in Canada (excluding the province of Quebec), the unrelated donor Stem Cell Registry, and the public Cord Blood Bank for CBS. Results were analysed in a before-and-after approach using descriptive statistics and considering 15 March 2020 as the initial date of the COVID-19 pandemic. All data were aggregated and anonymized before analysis.

RESULTS

Following the declaration of the global pandemic, new searches for unrelated donors from Canadian transplant centres remained comparable to levels before the pandemic (data not shown), suggesting

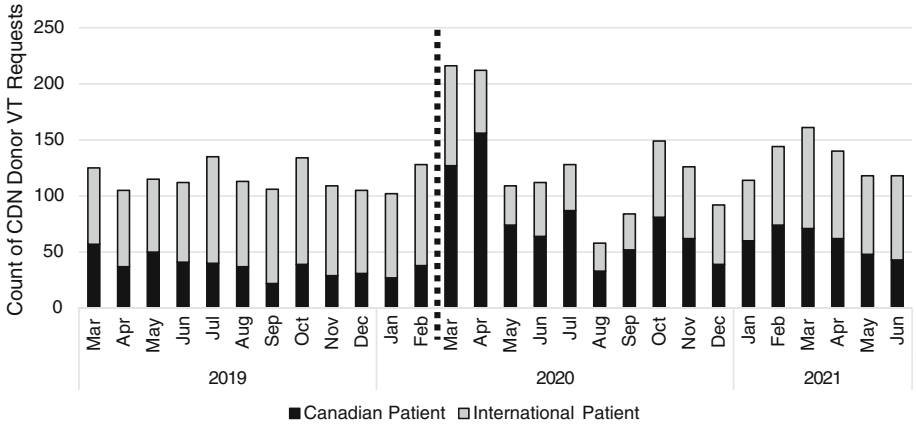

FIGURE 1 Efforts to identify donors on the Canadian Blood Services Stem Cell Registry by Canadian transplant centres, as reflected by increased requests for verification typing (VT), which increased during the initial 3–6 months after the start of the pandemic (black), with reduced requests from international centres (grey). Overall VT requests appeared to return to pre-pandemic levels approximately 6 months into the pandemic

stable demand for unrelated donors. Requests for verification typing (VT), which is the first step in potential donor selection and signals interest in potential donors, also remained stable with an increased proportion of VT requests arising from domestic transplant centres during the first 6 months of the pandemic and reduced levels of VT requests from international centres during the same time period (see Figure 1). VT requests from Canadian and international centres returned to pre-pandemic levels by 6 months after the onset of the pandemic. Work-up requests for Canadian donors, whether initiated by Canadian or international transplant centres, remained stable in the first 6 months of the pandemic (14.8/month) compared to the 6-month period before the pandemic (13.2/month, $p = 0.48$). However, the proportion of Canadian donor work-ups for Canadian patients increased significantly during the first 6 months of the pandemic (29% before the pandemic vs. 52% post pandemic, respectively, $p = 0.003$). Moreover, collections occurred in a timely manner following the request to initiate a donor work-up. The mean number of days from work-up initiation to product collection over the 6 months before the pandemic was 45 days (range, 18–172) compared with 37 days (14–139) over the first 6 months of the pandemic. The total number of allogeneic transplants performed in Canada has remained stable at a mean rate of 33 unrelated donor transplants per month throughout the pandemic (data not shown). However, a greater proportion of transplants that used a domestic donor from the CBS Stem Cell Registry increased transiently between 3 and 6 months after the start of the pandemic, with decreased usage of international donors (see Figure 2). Usage of Canadian and international donors for Canadian patients returned to baseline levels after the 6-month mark

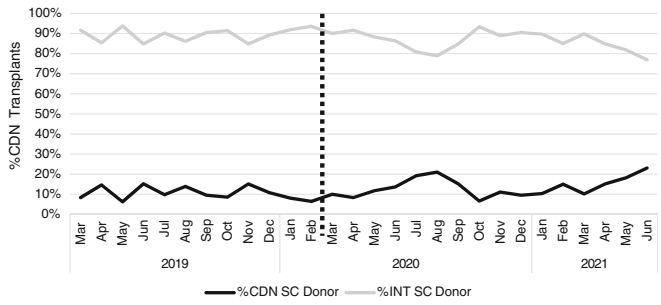

FIGURE 2 Proportional use of Canadian donors, as opposed to international donors, for Canadian patients, plotted over time. An apparent increase was observed for a brief period of several months starting from 3 months after the onset of the pandemic and may be increasing again in the most recent 3-month period

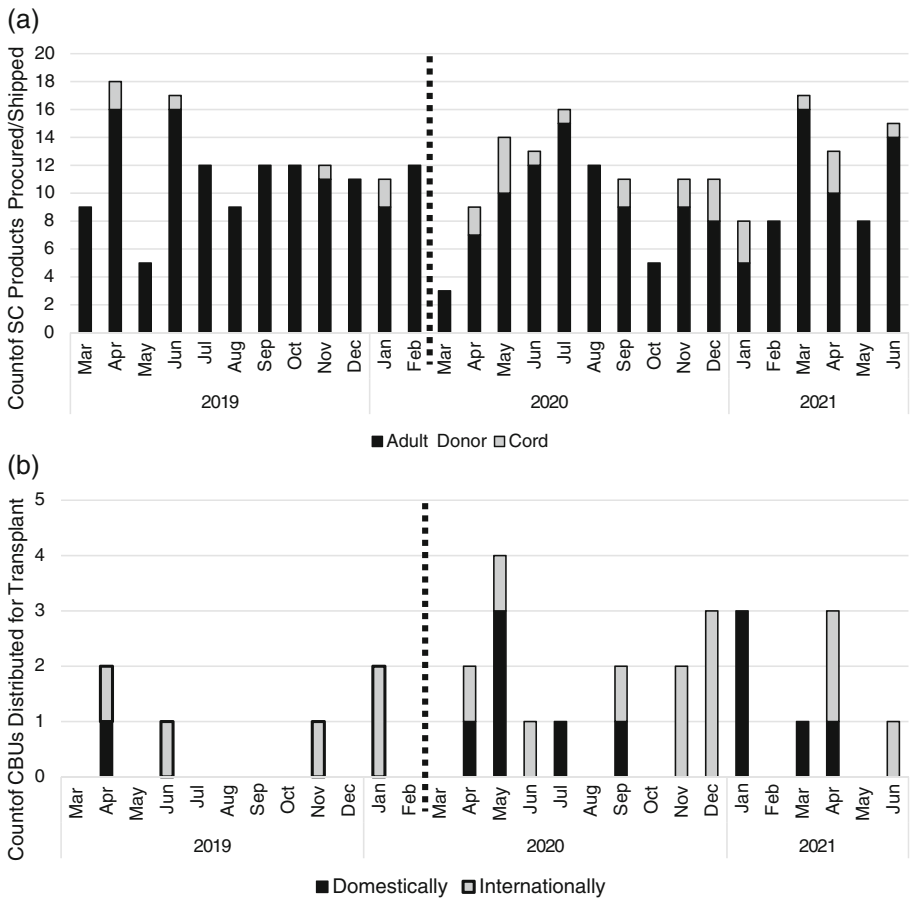

FIGURE 3 Usage of stem cell products from Canadian donors plotted over time. (a) Total number of unrelated donor products collected (black) and number of cord blood units (CBUs) distributed (grey) from Canadian donors. (b) CBUs distributed from the Canadian Blood Services cord blood bank over time to both Canadian (black) and international (grey) transplant centres

of the pandemic but may be increasing again in the second quarter of 2021 (see Figure 3).

Regarding the procurement of stem cell products from Canadian donors, we observed stable rates of donor collections after the onset of the pandemic compared to pre-pandemic levels (see Figure 3a), except for the first month of the pandemic. The use of cord blood units (CBUs) from the CBS's Cord Blood Bank for domestic transplant centres (exclusively for paediatric patients) has increased and remained elevated compared to baseline levels (see Figure 3b).

In response to the recommendations from national and international bodies, the majority of products collected from unrelated donors have been cryopreserved (68% of products collected from Canadian donors from March 2020 to June 2021) before infusion in comparison to 4% in the 12 months before the pandemic and approximately 7% noted in a historical cohort that was previously described [11]. With regard to adverse events related to cryopreservation, none of eight adverse event reports that were filed by CBS to the World Marrow Donor Association between January 2019 and March 2020 was related to cryopreservation, whereas between March 2020 and June 2021, a total of four adverse events were reported, all of which described impacts related to cryopreservation.

DISCUSSION

Transplant centres in Canada have continued to search for unrelated donors and CBUs for their patients during the COVID-19 pandemic. A shift to increased interest in donors from the CBS Stem Cell Registry and a significant increase in usage of units from the CBS's Cord Blood Bank have occurred, likely reflecting perceived advantages in the availability of domestic donors/CBUs and simplified logistics related to the transportation of a product procured within the country. Cryopreservation of adult unrelated donor products during the first 6 months and beyond by Canadian transplant centres was used to mitigate against potential unforeseen logistical barriers and scheduling challenges related to the pandemic. These data support the substantial value to HCT centres and their patients of maintaining a national registry of adult unrelated donors and a public inventory of high-quality CBUs during this pandemic.

Although the continued demand for new searches for adult unrelated donors highlights the ongoing needs of patients despite the pandemic, it underscores the perceived importance of human leucocyte antigen (HLA) matching on patient outcomes. Other recent reports outline the steps taken by adult registries to meet the ongoing demand for unrelated donors during the pandemic and describe a reduction in the use of bone marrow, an increased usage of domestic donors, more work-up requests in case backup donations were needed, and more donor cancellations attributed to COVID-19 [8, 9]. The increased focus on domestic donors reflects considerations related to constraints on donor travel to hospitals, collection centre staff, courier arrangements, and cryopreservation requirements. In cases where multiple potential donors were identified from the CBS Stem Cell Registry, information regarding donor proximity to donor centres was provided to transplant centres as a means of reducing risks related to donor travel. While it is possible that unrelated donor products may be collected, cryopreserved, and then stored until a time when transplants can be safely scheduled, most transplants occurred within a short time after cryopreservation was completed. Adverse events related to cryopreservation were uncommon, but did occur. Taken together, this highlights the balance between the advantages of cryopreservation and potential negative issues related to the process of cryopreservation and its impact on transplantation outcomes as well as the risk that cryopreserved products may pose [12–15].

The ability to continue with unrelated donor collections during this pandemic reflects the urgent nature of many transplants and the high level of commitment of donors and collection centres to the process of donation. Interest and usage of CBUs from the CBS bank increased substantially, likely reflecting interest in a product that was collected before the pandemic and which is already stored and ready for distribution without the need for a personal courier. Transplant centres may have preferred to organize transplants using cord blood given the increased certainties associated with delivery and scheduling and the predictable high quality of the units [16]. A 7% increase in the usage of CBUs was reported by one European network of banks while banking efforts were stalled during the early stages of the pandemic [10].

Although new collections and banking of CBUs and registration of potential donors at in-person swabbing events were halted during the initial stages of the pandemic, resuming these activities carries increased urgency given our observations of increasing demand. With adaptations to the pandemic, collection and banking of CBUs resumed in Canada within a period of 4 months. Shifting the registration of potential donors to a completely on-line registration process has occurred, which may enhance donor availability at the time of donor activation [17]; however, the effectiveness of this method remains a challenge.

Unrelated donor registries will need to continue to track the trends and challenges related to the ongoing COVID-19 pandemic in order to ensure continued access to high-quality CBUs and unrelated donor products within Canada and abroad. In addition, registries will need to ensure that relevant services such as donor collection facilities and cryopreservation services at cell processing labs remain safely available.

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D.S.A. conceived the study design. M.G., G.M., and J.W. performed data extraction and data analysis. D.S.A. and M.S. were responsible for the initial drafting of the manuscript. All authors were involved in manuscript revisions before final approval.

CONFLICT OF INTEREST

All authors are employed and/or paid consultants at Canadian Blood Services. There are no other conflicts of interest.

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Vox Sanguinis ISBT International Society of Blood Transfusion

LETTER TO THE EDITOR

Unlikely influence of ABO blood group polymorphism on antibody response to COVID-19 mRNA vaccine against SARS-CoV-2 spike protein

We [1] and others proposed two possible mechanisms of the association between ABO blood groups and SARS-CoV-2 susceptibility/COVID-19 severity. The consensus is higher and lower risk for people in groups A and O, respectively. First, viruses can express A/B glycans on the spike protein, reflecting the ABO phenotype of the host cells in which they are produced, and natural antibodies can react to corresponding antigens and inhibit interpersonal infection, at least partially, resembling 'ABO-matched' and 'ABO-unmatched' transfusion (Figure 1). Second, lower serum levels of von Willebrand factor and factor VIII, essential for blood clot formation, could explain a lower risk of thrombosis, pulmonary embolism and venous thromboembolism in group O individuals. After reviewing the literature, Le Pendu et al. [2] concluded that the ABO polymorphism could play a role in the COVID-19 pandemic at the population level despite modest differences in risk between ABO groups.

In the recent issue of Vox Sang, Vicentini et al. [3] published a letter to the editor entitled 'Does ABO blood group influence antibody response to SARS-CoV-2 vaccination?'. The authors examined IgG titre against spike protein in 85 medical students who completed a full two-dose Pfizer/BNT162b2 mRNA vaccination and found no significant difference between ABO groups.

There are four main categories of COVID-19 vaccines: whole virus, protein subunit, viral vector and nucleic acid (Figure 2). An immune

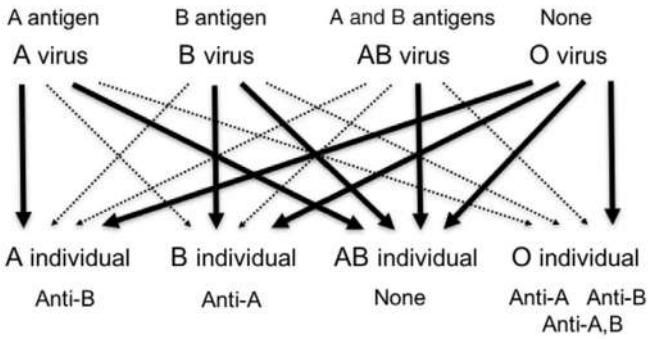

FIGURE 1 ABO-dependent inhibition of infection between SARS-CoV-2 viruses presenting different ABO phenotypes and individuals from groups A, B, AB and O. Solid and dotted arrows indicate infectivity without and with ABO-dependent inhibition, respectively. Inhibition is directional. Newly produced SARS-CoV-2 viruses exhibit the same ABO phenotype as the infected individual and are no longer neutralized (reproduced from Reference [1]).

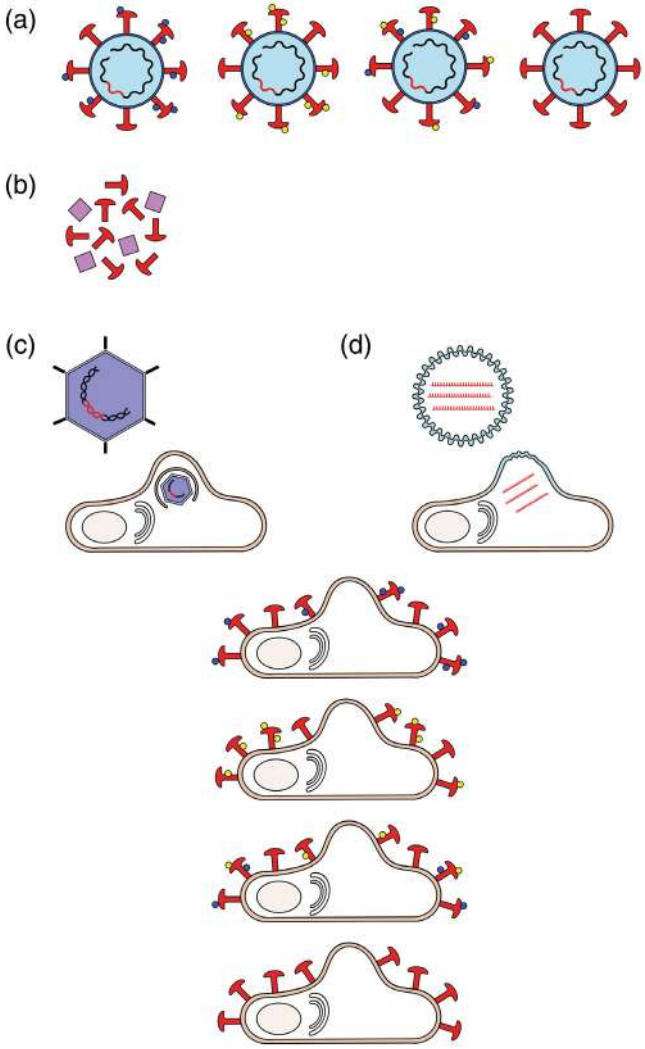

FIGURE 2 Four main categories of COVID-19 vaccines and the expected presence or absence of ABO blood group association. (a) *Whole virus*: A virus, B virus, AB virus and O virus are shown from left to right with the A and B glycans in blue and yellow circles. Natural antibodies can neutralize vaccinated viruses in an ABO-dependent manner. (b) *Protein subunit*: Protein subunit vaccines produced in bacteria or yeast do not carry the A/B glycan antigens. As a result, no ABO association is expected. (c) *Viral vector*: A harmless virus, such as the adenovirus, is used to introduce genetic instructions into the cells to produce the spike proteins. The cells can express the A/B-glycosylated proteins in the vaccinated individuals (A cell, B cell, AB cell and O cell are shown from top to bottom). No ABO-dependent inhibition is expected. (d) *Nucleic acid*: The mRNA case is shown, where mRNA molecules are embedded in lipid nanoparticles. No ABO-dependent inhibition is expected.

response is elicited, largely to the spike protein that viruses use to invade cells. When whole viruses are produced in human epithelial cells, they can be A/B-glycosylated and thus subject to ABO-dependent inhibition by natural immunity. However, those produced in cells with O phenotype can be used for universal vaccination, bypassing the inhibition. Protein subunits prepared for COVID-19 vaccination typically lack A/B glycosylation. Viral vectors, as well as nucleic acids, such as DNA and mRNA, induce spike protein expression on the cell surface. Although muscle cells do not express A/B glycans, the proteins can be A/B-glycosylated in other cell types depending on the ABO phenotype of the vaccinated individuals. However, these glycans do not cause ABO-related immune reactions because they are compatible. Consequently, ABO groups are unlikely to affect the anti-spike protein immune response. In contrast, the negative results of Vicentini et al. fit well with the natural immunity model to explain the ABO association with SARS-CoV-2 infectivity.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

Response to 'Unlikely influence of ABO blood group polymorphism on antibody response to COVID-19 mRNA vaccine against SARS-CoV-2 spike protein'

We, the authors, want to thank the working group of Yamamoto et al., which provided interesting insights into the interpretation of our results [1, 2]; in our study, we failed to identify a significant difference in antibody titres among patients of different blood groups who underwent a full mRNA vaccination course (ComiRNAty PFIZER-BioNTech).

We did not assess the influence of ABO type on natural SARS-CoV-2 infection. Our work was based on the hypothesis that there could be differences in the antibody response elicited by the vaccine, regardless of the A/B glycosylation of the spike protein.

Other authors have also hypothesized a difference in the behaviour of the immune system in response to COVID-19 vaccines in patients with different blood types. In a letter from Sgherza et al., the authors start from the same hypothesis and describe how, even in their sample, there are no differences in response to the ComiRNAty vaccine based on the patient's ABO group [3].

These data are consistent with the assertion by Yamamoto et al. that the absence of pre-existing glycosylation in mRNA-induced spike proteins avoids an ABO-based difference in immune response following vaccination.

Yamamoto et al. also provide an overview of the effect that the glycosylation of the spike protein of SARS-CoV-2, induced by the blood group of the host in which the virus is replicating, can have both on infectivity and clinical outcome.

These statements add to a growing body of literature, which, similarly to SARS-CoV, suggests that patients with blood group A are at greater risk of infection than group O patients. Amoroso et al. found, in a study conducted on transplant patients or on transplant wait-listed individuals, that blood group A patients had a higher incidence of COVID-19, possibly due to a lack of protection induced by the absence of anti-A agglutinins.

If the fact that group O patients are at lower risk of infection fits well with the protection afforded by agglutinins against viruses generated in ABO individuals not identical to the host, protection from disease progression once the individual is infected (and therefore produces viruses with ABO identical glycosylation) could instead be explained by a lower propensity to thrombosis in group O patients.

In a recent paper, Sardu et al. compared the incidence of cardiac injury, pro-thrombotic index levels and death among group O versus non-O hypertensive and COVID-19 patients; in the non-O group, the incidence of these pathologies was significantly increased [4]. As this study is focalized on O versus non-O patients, applying the considerations discussed by Yamamoto et al., the results could also be attributed to the reduced levels of von Willebrand factor and factor VIII in group O patients [5].

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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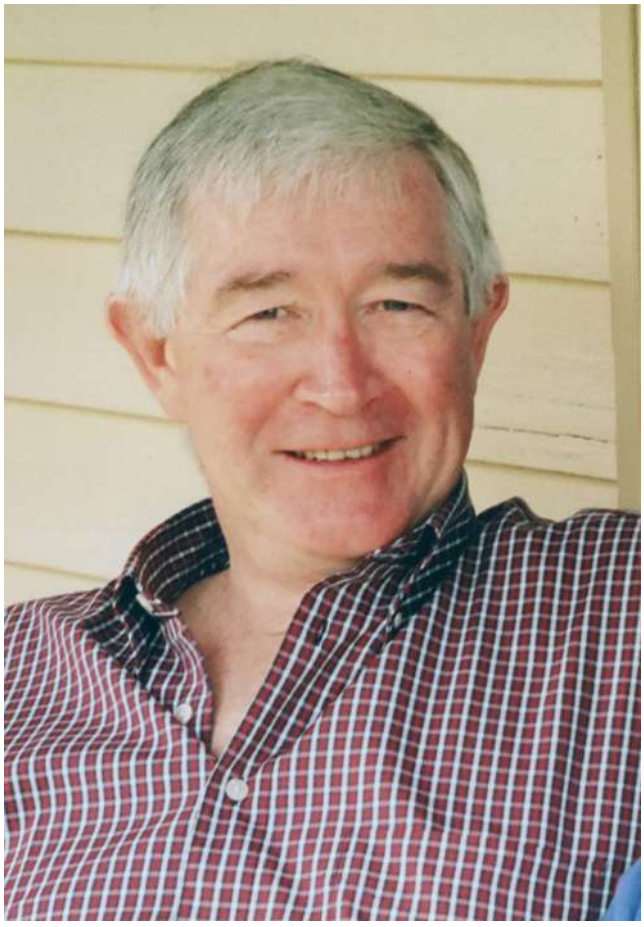
OBITUARY

Vox Sanguinis ISBT International Society of Blood Transfusion

Gordon Stuart Whyte, 1943–2022



Dr Gordon S. Whyte, a pioneer in transfusion medicine, passed away on 20th April 2022 after a long illness. Gordon was a vital and passionate man with great vision and an insatiably curious intellect. He also had an enormous drive and a great sense of humour. He was a politically astute leader and always focussed on achieving desirable outcomes. Here, he is recognized primarily for his outstanding contributions to transfusion medicine, although he was truly a man for all seasons. Gordon was born in India where he received much of his schooling before returning to his parents' hometown, Sydney, Australia, where he qualified as a physician.

In 1982, Gordon became the Regional Director of the Canterbury Transfusion Services in New Zealand, where he developed effective strategies for managing the issues associated with HIV and sparking his interest in risk and quality management systems. In 1987, Gordon took on the role of Director of the Victorian Red Cross Blood Transfusion Service in Melbourne, Australia, which collected blood and provided blood products for the state of Victoria and later the state of Tasmania. At that time, the organization was confronted with the major challenges of inadequate governance, chronic underfunding, managing quality and risk during the HIV/AIDS pandemic and mitigation of the transfusion-transmission risk of non-A, non-B hepatitis (later identified as HCV). He found an organization in serious need of radical reform. He developed a vision to modernize and create a financially independent and professionally managed blood service that would meet public healthcare needs well into the future.

From 1990, Gordon set about establishing a competent senior leadership team with complementary skills and experience to drive the change. Together, the team established a reform agenda which was implemented over several tough years. He led a successful implementation of the first Australian Code of Good Manufacturing Practice for Therapeutic Products—Blood and Blood Components, and the Code was finally published in July 1992. Throughout this period of rapid change, Gordon never lost sight of the need to adequately support his staff through the major elements of this extensive change plan.

Gordon succeeded in establishing an effective governance structure in which management and administrative functions were moved from the Red Cross to a dedicated, professional team, following an overseas practice. By the mid-1990s, by many measures, the Victorian Red Cross Blood Bank had become the most professionally managed state blood service in Australia and provided one model for establishing a new national organization. In November 1995, the Australian Red Cross Society, after a number of reviews, resolved to unify all state blood transfusion services into a single blood service, the Australian Red Cross Blood Service. Implementation of this change commenced early in 1996.

Gordon encouraged innovation and research and hoped to establish a broad collaboration of investigators in Melbourne, but he was ahead of his time in this objective. He also implemented an annual scientific meeting addressing current transfusion science and medicine and attracting speakers and attendees from all parts of the world. Gordon was himself very much engaged in training and was the council member of the Australian and New Zealand Society of Blood Transfusion (ANZSBT) and was tasked in 1997 with managing educational issues and initiatives. He became the inaugural Chair of the Education Subcommittee of the ANZSBT (1998–2000). He was actively supportive of training blood bankers from developing

Patrick Coghlan is retired.

countries in Melbourne and was an active member of ISBT with a particular focus on international education in transfusion medicine.

Despite his success in establishing the model for blood collection in Australia and in working on the establishment of a nationally focussed blood system, by 1998, Gordon felt it was time to pursue some of his other intellectual passions. He subsequently focussed his considerable talents on a PhD in systems engineering, followed by studies in rural medicine, Arabic history and the history of early medicine in Italy (which included learning some Latin!). He loved nature, music, good food and robust discussion. Throughout an extraordinary life, Gordon cherished his family, delighted in life, never stopped learning and was a wonderful friend to many. He will be missed by all who knew him, were influenced by him and learned from him. His many accomplishments live on.

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18.08.2022	Haemovigilance Webinars: Sharing international haemovigilance experiences. Session 1: International Programs
25.08.2022	Haemovigilance Webinars: Sharing international haemovigilance experiences. Session 2: Consumer Engagement
01.09.2022	Haemovigilance Webinars: Sharing international haemovigilance experiences. Session 3: Data
06.09.2022	Haemovigilance Webinars: Sharing international haemovigilance experiences. Session 4: Donors

