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Vox Sanguinis

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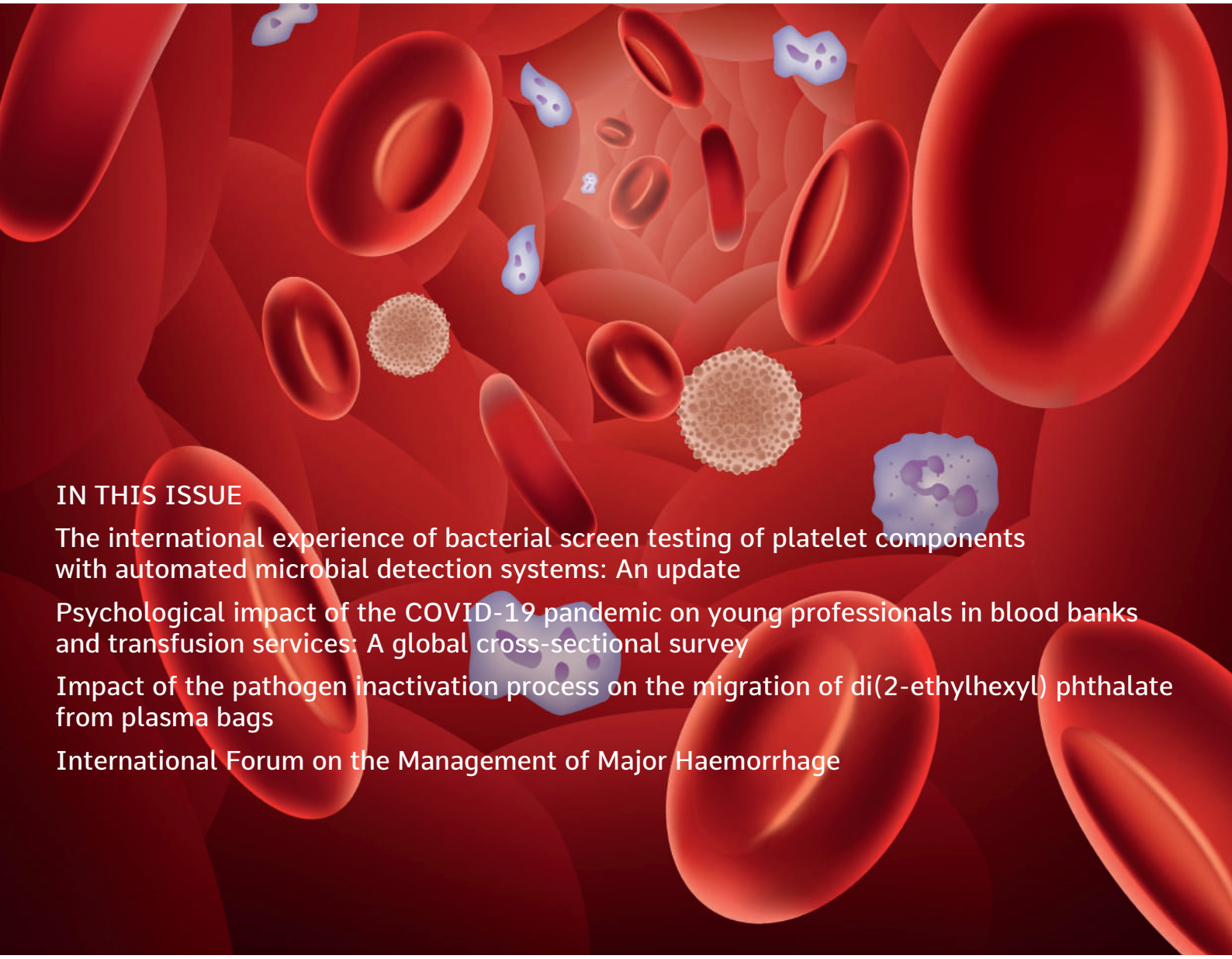

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ISBT International Society of Blood Transfusion

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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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REVIEW ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Incidence of anti-D alloimmunization in D-negative individuals receiving D-positive red blood cell transfusion: A systematic review and meta-analysis

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Abstract

Background and Objectives: The transfusion of D-negative red blood cells (RBCs) to D-negative patients has been widely adopted to prevent anti-D alloimmunization, especially in women of childbearing age. Still, transfusion of D-positive RBCs to D-negative recipients is occasionally inevitable in practice, and the resulting incidence of anti-D in different D-negative groups of patients has not been well summarized.

Materials and Methods: We searched the relevant literature using PubMed, Cochrane Library, and Embase databases from inception date to 30 September 2021. We looked for studies of anti-D occurring in D-negative recipients who received D-positive RBC transfusions. The anti-D incidence was summarized with 95% confidence intervals (CIs). Data with similar characteristics were combined using a random-effects model.

Results: About 42 studies (2226 cases), which found anti-D, the exact volume of D-positive RBC transfused, and the follow-up time for anti-D detection, met the inclusion criteria. The pooled anti-D incidence was 64% (95% CI, range 55%–74%) in volunteers receiving small volumes of D-positive RBCs, 84% (95% CI, 74%–94%) in those receiving whole units, 26% (95% CI, 19%–32%) in mixed patients, 12% (95% CI, 8%–16%) in oncology patients, 27% (95% CI, 13%–40%) in trauma patients, 4% (95% CI, 0%–8%) in immune-compromised transplant patients, and 6% (95% CI, 1%–39%) in those with AIDS.

Conclusion: Compared with the high frequency of anti-D in healthy D-negative volunteers given D-positive RBCs, we found a lower rate of anti-D immunization in various D-negative patients and almost none in transplant and AIDS patients.

KEYWORDS
anti-D alloimmunization, D-negative blood type, D-positive RBCs transfusion

Highlights

- Compared with the high frequency of anti-D in healthy D-negative volunteers given D-positive RBCs, a lower rate of anti-D immunization was observed in various D-negative patients including oncology and trauma patients, and almost absence in transplant and AIDS patients.
- For D-negative oncology patients, the diagnosed disease was the main factor significantly associated with anti-D production, such as a higher anti-D rate in solid malignancies (22.6%),

and myelodysplastic syndrome (MDS, 23%) patients, while a lower rate in leukemia patients (6-10%).

- For D-negative trauma patients, there was no significant difference of incidence of anti-D formation from hospitalized mixed non-trauma patients after D-positive RBC transfusion.

INTRODUCTION

Alloimmunization against the D blood group antigen in D-negative individuals may lead to a subsequent haemolytic transfusion reaction or to haemolytic disease of the foetus and newborn in later pregnancy [1]. Therefore, routine D typing and D-negative-matched transfusion have been generally applied worldwide. Although the D-negative phenotype is common in Caucasians (15%–17%) and not rare in Africans (3%–5%), in clinical practice, it is difficult to assure a supply of D-negative blood in certain regions [1]. In Asia, the rarity of D-negative donors (0.3%–0.4%) leads to common shortages of D-negative blood [1]. Thus, transfusion of D-positive RBCs to D-negative patients is unavoidable, particularly in massive transfusion in an emergency or multiple transfusions when there is a shortage of D-negative RBC. When D-positive RBC transfusion is given to D-negative patients, the occurrence of anti-D after transfusion is one of the most important clinical concerns.

The incidence of Rh alloimmunization, that is, the formation of anti-D when D-negative recipients receive D-positive RBC transfusion, varies with the volume of infused D-positive RBCs and the recipient's immunocompetence [2]. In the 1970s, at least 13 studies in healthy volunteers explored the effect of anti-D immunoglobulin in preventing anti-D immunization and assessed the most efficient immune stimulation strategy for anti-D immunoglobulin production [3–13]. A high incidence of primary immunization (>80%) occurred in such volunteers with whole units of D-positive RBC transfusion [14, 15]. However, other studies, on D-negative patients, found an unexpected lower incidence of anti-D or even none at all [16–28].

In this study, we conducted a meta-analysis including a subgroup analysis to assess the overall incidence of anti-D in D-negative recipients after D-positive RBC transfusion. The resulting data may assist physicians in predicting the outcome when D-negative recipients require D-positive RBC transfusion as well as deciding appropriate transfusion treatment for patients. Besides, immunosuppressive protocols administered to transplant patients may be applied to reduce or even avoid anti-D immunization [2].

MATERIALS AND METHODS

Methods

This meta-analysis followed that in the *Cochrane Handbook for Systematic Reviews* [29] and is presented based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines [30].

Search strategy

We searched the PubMed, Cochrane library, and Embase databases from inception date to 30 September 2021, using the keywords RhD-negative blood group, erythrocytes, blood transfusion, and anti-D, to identify related meta-analysis and observation studies, both retrospective and prospective, and to analyse the rate of anti-D immunization in D-negative individuals after receiving D-positive RBCs. We also manually checked the cited references of the retrieved studies and previous reviews to identify any additional eligible studies.

Inclusion criteria

Transfusion of D-positive RBCs to D-negative patients is not common in clinical settings, only in massive transfusion in an emergency, multiple transfusions, or when insufficient D-negative RBCs are in stock. So we included all retrospective or prospective studies assessing the incidence of anti-D immunization in D-negative individuals after D-positive RBC transfusion or re-exposure to D-positive RBC. We excluded studies on D-negative patients who only received D-positive platelet concentrate or apheresis platelets and lacked estimation of exact RBC contamination volume. The D-negative recipients were classified into several subgroups for anti-D incidence analysis according to the type of disease and immune status.

Data extraction

Two researchers (YL Ji and GP Luo) independently extracted the following data from each study that fulfilled the inclusion criteria: lead author; publication year; country of origin; occurrence rate of anti-D; characteristics of D-negative recipients, such as physical condition or disease diagnosis; D-positive RBC transfusion volume; follow-up time; the interval between D-positive RBC transfusion and time of anti-D appearance and recipient age.

The incidence of anti-D immunization was the main outcome of this study to determine the difference between D-negative subgroups.

Statistical analysis

We performed a meta-analysis to calculate the occurrence rate of anti-D in each study and the exact 95% confidence interval (CI) using

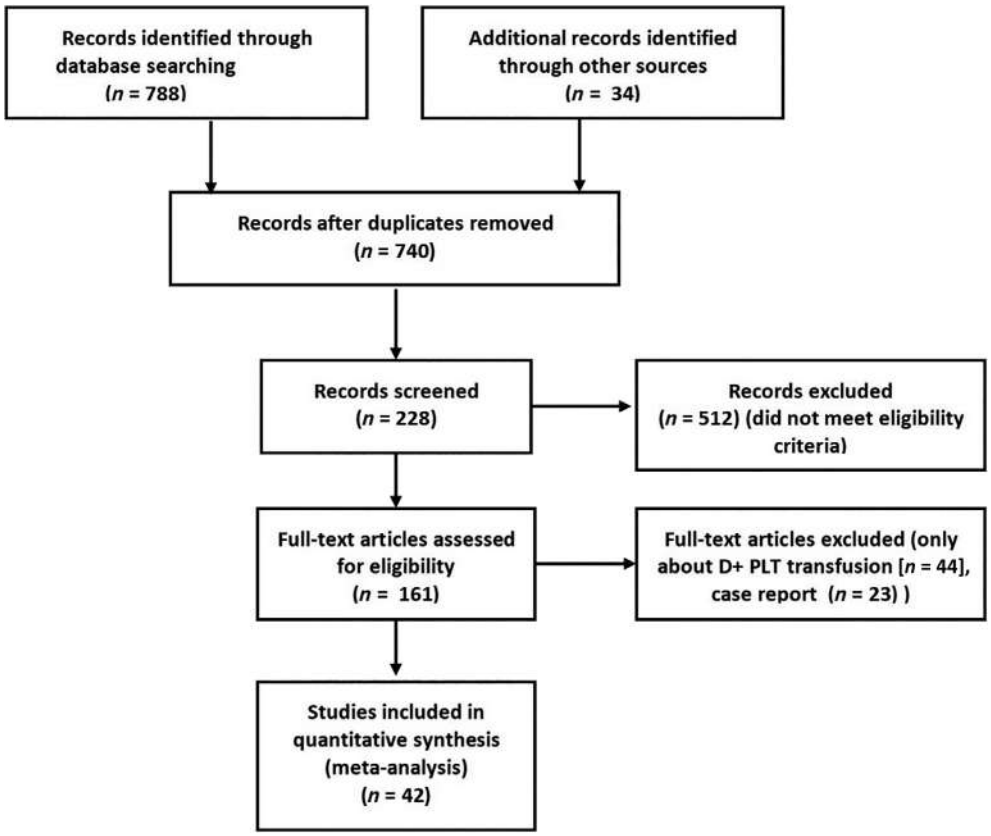

FIGURE 1 Literature search and screening process

the Clopper–Pearson method. If no events were reported for the number of anti-D occurrences, a value of 0.5 was added to both the number of events and the total number of observations in such a study in the meta-analysis.

Then, a random-effects model was used to pool the data based on seven subgroups of D-negative people (healthy volunteers with a small volume of D-positive RBCs, healthy volunteers with whole units of D-positive RBCs, pooled in-patients with various types of mixed diseases, oncology patients, trauma patients, patients with solid organ or haematopoietic cell transplantation and AIDS patients). Overall and subgroup statistical heterogeneity were evaluated using I^2 statistics.

All meta-analyses were performed using Stata version 15.1 (StataCorp LLC, College Station, TX, USA). All tests were two-tailed, and differences with $p < 0.05$ were considered statistically significant.

RESULTS

Retrieved studies

From our searches, we identified 740 potentially eligible records. The titles and abstracts of all records were screened for inclusion. Then, we read the full texts of 161 records. Of these, 42 studies met the inclusion criteria for review (Figure 1).

Small-volume of D-positive RBC transfusion in D-negative healthy volunteers

Table S1 shows a series of trials with a small volume of D-positive RBC transfusion (0.01–10 ml) in healthy volunteers ($n = 265$), particularly males. These trials assured regular follow-up with predefined interval times ranging from 2 weeks to 12 months. Thus, we obtained not only the incidence of anti-D but also the exact time of its appearance after the infusion of D-positive RBCs.

Figure 2 shows 13 trials assessing the incidence of anti-D after small-volume D-positive RBC transfusion to healthy volunteers included in the meta-analysis. The overall incidence of anti-D in this subgroup was 64% (95% CI, 55%–74%). Most studies had a small sample size, <15 cases (10/13 studies), and the anti-D incidence varied from 35% to 92% to show a high degree of heterogeneity between studies ($I^2 = 61.74\%$, $p = 0.00$). A well-run follow-up showed the appearance of anti-D in most volunteers (114/159, 72%), commonly between 1 and 6 months after transfusion. These results suggested that an unusually rapid response within less than 1 month indicated a secondary response than primary response. Re-exposure to a small volume of D-positive RBCs could increase the anti-D immunization rate up to 90%. However, there was a certain percentage of nonresponders (Table S1), even with re-exposure of D-positive RBCs (median, three times), who did not exhibit an immune response against D-positive RBCs.

Study or subgroup	No. with alloanti-D after D-positive RBCs transfusion	Total No. receiving D-positive RBCs transfusion	Incidence of alloanti-D (95% CI)	% Weight
Healthy volunteers with small volume of D-positive RBCs				
Freda et al., 1966	8	13	0.62 (0.36–0.82)	1.97
Freda et al.,1966	4	5	0.80 (0.38–0.96)	1.58
Woodrow et al., 1969	7	11	0.64 (0.35–0.85)	1.87
Mollison et al., 1969	7	14	0.50 (0.37–0.73)	1.98
Mollison et al., 1970	7	13	0.54 (0.29–0.77)	1.94
Gunson et al., 1970	9	11	0.82 (0.52–0.95)	2.15
Cook et al., 1971	23	34	0.68 (0.51–0.81)	2.49
Gunson et al., 1971	11	12	0.92 (0.65–0.99)	2.50
Pollack et al., 1971	49	86	0.57 (0.46–0.67)	2.71
Jokobowicz et al., 1972	8	11	0.73 (0.43–0.90)	1.97
Woodrow et al., 1975	11	31	0.35 (0.21–0.53)	2.44
Samson et al., 1975	9	12	0.75 (0.47–0.91)	2.08
Contreras et al., 1981	6	12	0.50 (0.25–0.75)	1.88
Total	159	265	0.64 (0.55–0.74)	27.55
Heterogeneity: I^2 = 61.74%, p = 0.00				
Healthy volunteers with whole units of D-positive RBCs				
Pollack et al., 1971	18	22	0.82 (0.61–0.93)	2.48
Urbaniak et al., 1981	24	28	0.86 (0.69–0.94)	2.62
Total	42	50	0.84 (0.74–0.94)	5.09
Heterogeneity: NA				
Mixed patients				
Frohn et al., 2003	16	78	0.21 (0.13–0.31)	2.77
Yazer et al., 2007	22	98	0.22 (0.15–0.32)	2.79
Gonzalez-Porras et al., 2008	34	159	0.21 (0.16–0.28)	2.84
Brien et al., 2014	14	50	0.28 (0.17–0.42)	2.64
Yazer et al., 2021	117	335	0.35 (0.30–0.40)	2.87
Total	203	720	0.26 (0.19–0.32)	13.90
Heterogeneity: I^2 = 74.06%, p = 0.00				
Oncology patients				
Goldfinger et al., 1971	8	102	0.08 (0.04–0.15)	2.87
Balldwin et al., 1988	9	73	0.12 (0.07–0.22)	2.81
Arora et al., 2017	76	545	0.14 (0.11–0.17)	2.90
Total	93	720	0.12 (0.08–0.16)	8.58
Heterogeneity: NA				
Trauma patients				
Dutton et al., 2005	1	10	0.1 (0.02–0.40)	2.36
Miraflor et al., 2011	1	3	0.33 (0.06–0.79)	0.98
Tchakarov et al., 2014	3	15	0.20 (0.07–0.45)	2.28
Meyer et al., 2015	1	8	0.13 (0.02–0.47)	2.14
Selleng et al., 2017	14	31	0.45 (0.29–0.62)	2.41
Flommersfeld et al., 2018	9	15	0.60 (0.36–0.80)	2.05
Williams et al., 2019	10	59	0.17 (0.09–0.28)	2.75
Raval et al., 2021	10	129	0.08(0.04–0.14)	2.88
Yazer et al., 2021	41	93	0.44 (0.34–0.54)	2.73
Total	90	363	0.27 (0.13–0.40)	20.57
Heterogeneity: I^2 = 87.82%, p = 0.00				
Transplant patients				
Ramsey et al., 1989	1	19	0.05 (0.01–0.25)	2.73
Blomquist et al., 1991	0	3	0.14 (0.02–0.64)	1.51
Casanueva et al., 1994	0	17	0.03 (0.00–0.22)	2.80
Cummins et al., 1995	0	6	0.08 (0.01–0.46)	2.26
Yuan et al., 2008	0	15	0.03 (0.00–0.25)	2.77
Solves et al., 2015	0	5	0.09 (0.01–0.51)	2.09
Burin des Roziers et al., 2016	0	20	0.02 (0.00–0.20)	2.83
Mijovic et al., 2002	0	9	0.05 (0.01–0.36)	2.56
Asfour et al., 2004	0	6	0.08 (0.01–0.46)	2.26
Total	1	100	0.04 (0.00–0.08)	21.82
Heterogeneity: I^2 = 0.00%, p = 1.00				
AIDS patients				
Boctor et al., 2003	0	8	0.06 (0.01–0.39)	2.49
Overall heterogeneity between groups: I^2 = 93.74%, p = 0.00			0.33 (0.27–0.40)	100.00

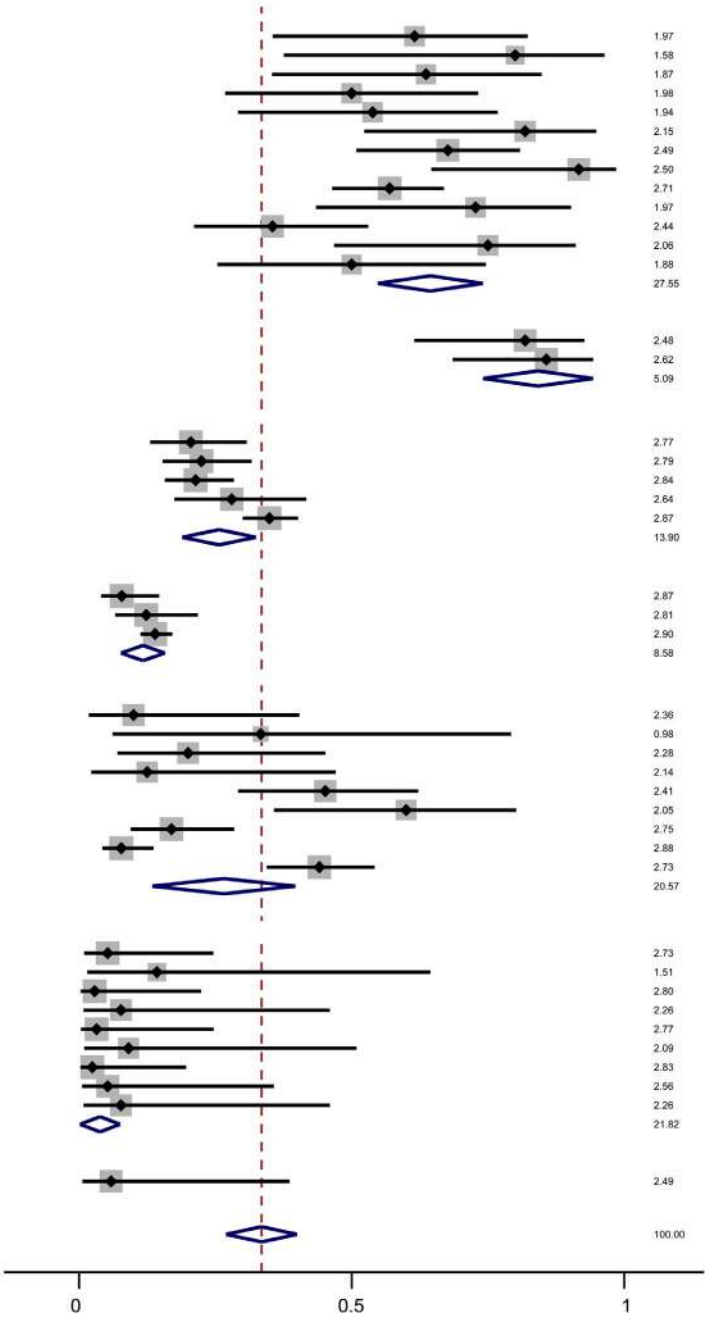

FIGURE 2 Meta-analysis results of anti-D incidence in D-negative recipients after D-positive RBC transfusion. NA, not available when calculated using Stata software

Whole units of D-positive RBC transfusion in D-negative healthy volunteers

There were two trials in which whole units of RBCs (200 and 500 ml) were transfused to healthy volunteers (n = 50). The anti-D incidence was consistent and the overall rate was 84% (95% CI, 74%–94%). The appearance of anti-D occurred in 2–5 months in one trial (n = 18) [14] and in 2–9 months (mean 17 weeks) in another trial (n = 24) [15], which was similar to the subjects who underwent small-volume D-positive RBC transfusion.

D-positive RBC transfusion in D-negative patients with mixed diseases

There were four retrospective studies (n = 561) [16, 18, 28] and one prospective (n = 159) [17, 31] (Table S1) to observe anti-D in

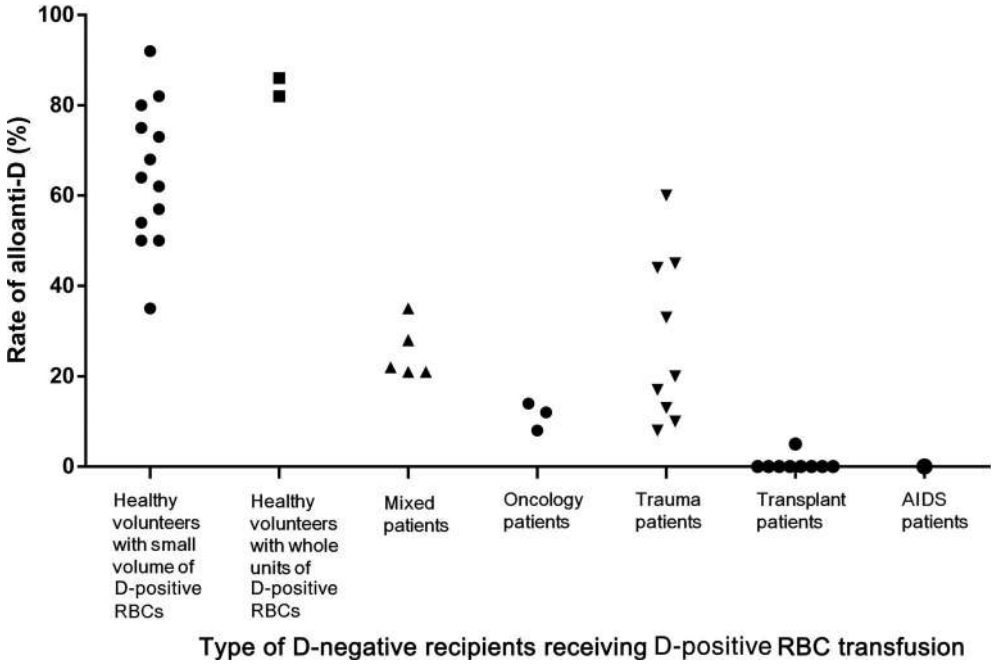

FIGURE 3 The diagram of incidence of anti-D immunization in D-negative recipients after D-positive RBC transfusion

D-negative in-patients with various diseases, after D-positive RBC transfusion in the clinic. Unfortunately, the type of disease could not be ascertained due to insufficient details or classification. Meta-analysis showed that the overall anti-D immunization rate was 26% (95% CI, 19%–32%) with heterogeneity ($I^2 = 74.06\%$, $p = 0.00$; Figure 2).

In retrospective studies, most of the recipients were bleeding, requiring urgent RBC transfusions. In many cases, D-positive RBCs were administered before typing and screening could be completed, while others received D-positive RBCs due to insufficient D-negative RBCs in stock. Thus, some patients received several sessions of D-positive RBC transfusion and also received D-negative RBCs when the D-negative blood type was confirmed or the supply of D-negative blood was replenished. In the study of Yazer et al. [27] with mixed disease patients, 80.9% patients (277/335) received a total of 2727 units of D-negative RBC-containing components in the period after the index D-positive product to "wash out" the transfused D-positive RBCs, but there were still 34.9% patients (117/335) who became immunized. A similar situation occurred among trauma patients in the study of Tchakarov et al [32].

Commonly, antibody screening is performed during hospitalization or follow-up visit. Thus, regular and longer-term follow-up could not be guaranteed in all patients.

D-positive RBC transfusion in D-negative oncology patients

Three studies investigated the anti-D incidence in immunosuppressed D-negative oncology patients. One followed D-positive RBC transfusion and the other two studied the transfusion of D-positive platelet and granulocyte concentrates with the estimated contaminating D-positive RBCs volume. Among these, more than half were patients with haematologic malignancies (Table S1). Meta-analysis showed that the overall anti-D immunization rate was 12% (95% CI, 8%–16%; Figure 2), which was significantly lower than in patients with mixed diseases ($\chi^2 = 51.455$, $p = 0.000$). In the study by Arora et al., including a large number of D-negative oncology patients ($n = 545$) [32], logistic regression analysis indicated that the diagnosed disease was the only factor significantly associated with anti-D production. Patients who had myelodysplastic syndrome (MDS, 23%) or solid malignancies (22.6%) were more likely to be responders with anti-D than those who had other haematologic malignancies (7%) such as acute leukaemia.

D-positive RBC transfusion in D-negative trauma patients

Nine studies focused on the anti-D incidence in D-negative trauma patients after D-positive RBC transfusion, and these showed it to be similar to that of mixed D-negative in-patients: 27% (95% CI, 13%–40%; Figure 2, Table S1). No statistically significant differences occurred ($\chi^2 = 1.414$, $p = 0.234$). Because trauma patients are associated with a high mortality rate (around 40% in these two studies [31, 33]), part of patients did not have follow-up data. When patients had died, it would make the incidence of anti-D development seem lower, since these patients still count in the denominator in calculating the anti-D rate. Thus, we included only the anti-D rate of surviving trauma patients with follow-up data.

D-positive RBC transfusion in D-negative immunocompromised patients with solid-organ and haematopoietic stem cell transplantation

There were 9 studies in which a total of 100 D-negative recipients with solid-organ or haematopoietic stem cell transplantation were transfused with D-positive RBCs and one of them (1%, 1/100) developed anti-D. Meta-analysis showed that the overall statistics of anti-D immunization rate was 4% (95% CI, 0%–8%) without any apparent heterogeneity ($I^2 = 0.00\%$, $p = 1.00$; Figure 2). It indicated that the immunosuppressive protocols adopted to prevent transplantation rejection (such as using cyclosporin A and mycophenolate mofetil [MMF]) also prevented alloimmunization against the D antigen.

D-positive RBC transfusion in D-negative AIDS patients

The absence of anti-D in D-negative AIDS patients after D-positive RBC transfusion was only observed in one study that had a limited sample size (0/8).

DISCUSSION

More than 80% of D-negative healthy volunteers produce anti-D after whole units of D-positive RBC transfusion. But it is not well known that, compared with healthy volunteers, patients are not as likely to become immunized to D, and the likelihood of anti-D in patients with different immune states varies. This may often lead to avoidance of the most suitable transfusion treatment for D-negative patients. It also makes it difficult for the doctor to discuss the risks of transfusion of D-positive RBC to D-negative patients with patients and their families. For these reasons, we conducted a meta-analysis to provide support to the practice of transfusing D-positive RBC to D-negative patients when the situation warrants it. Figure 3 shows a brief diagram to illustrate the incidence of anti-D immunization in seven different D-negative subgroups of patients.

In the healthy D-negative volunteers, a variable rate (35%–92%) occurred with small-volume D-positive RBC transfusion (0.01–10 ml) [7–13], while a very high anti-D rate (>80%) took place after they received whole units of D-positive RBCs [14, 15]. The volunteers were healthy adults with competent immune responses and usually ranged in age from 30 to 50 years. The huge heterogeneity among these studies after small-volume D-positive RBC transfusion may be mainly attributable to different transfusion volumes of D-positive RBCs, the Rh genotype of the donor RBCs, and the interval of restimulation. *DcE*/*DcE* RBCs are more immunogenic than *DCe*/*Ce* or *DCe*/*Dce* RBCs, which is consistent with a higher mean concentration of D antigen on *DcE*/*DcE* RBCs (473 pmol/ml) than *DCe*/*Ce* RBCs (240 pmol/ml) [4, 34, 35]. Even with repeated stimulation by D-positive RBCs, there are still 10%–20% volunteers who do not have an immune response. The immune tolerance resulting from transfused D-positive RBCs surviving in the circulation for several weeks [36, 37] might partly account for this.

In about 70% of the healthy volunteers, it took 1 to 6 months after transfusion for the primary response to produce anti-D, while the other 30% needed 6 to 12 months (Table S1). Production of anti-D within 1 month or 28 days is generally considered an anamnestic response, although such an extreme primary response has also occurred within 28 days in few male recipients who lacked transfusion or other known immune history [10, 23, 38].

In D-negative hospitalized patients with mixed diseases after D-positive RBC transfusion, a lower anti-D rate (26%, 95% CI 19%–32%) was noted compared with the historical volunteer studies. Among the five studies involved, the comparatively higher rate (34.9%) observed by Yazer et al. might partly be associated with the involvement of immune-competent younger patients, which also contribute to the heterogeneity of this group. Besides, fewer blood transfusions and immunosuppression [39] in severely ill patients may partly explain the observed lower immunization rate. In multivariate analysis, the age of less than 77 years was the only predictor for alloimmunization [17] rather than sex, or the presence of a haematological disorder, surgery, or solid tumour. A similar alloanti-D rate (2/10, 20%) was also observed in a limited number of D-negative Chinese patients with mixed diseases (data not shown).

In addition, we saw an inverse correlation between anti-D formation and the number of units of RBCs, and no increase in anti-D rate after re-exposure to D-positive RBCs [16, 18]. Furthermore, in the study of Yazer et al. [27], although there was no significant dosage effect in the alloimmunization rates with exposure to D-positive units, the immunization rate was 30.6% in the 1-unit group, 43.6% in 3–5 units group, and lowest rate of 18.4% in 11–20 units group [27]. It is consistent with the study of Gonzalez-Porras et al. [17], in which most patients who developed anti-D did so within the first two or four RBCs transfused (64% after the first two RBCs transfused and 88% after the first four). Besides, in another exploratory analysis, patients who received 100% of their D-positive transfusions within 72 h of the index transfusion had a significantly higher rate of immunization compared with those who were transfused over a longer time (42.3% vs. 21.4%) [27].

In D-negative oncology patients, lymphocyte impairment caused by many kinds of haematologic diseases and reduced IgG and IgM levels due to immunosuppressive chemotherapy [40] might account for the lower anti-D incidence. However, heterogeneity in the immune response against D-positive RBC was detected in D-negative patients with different types of diagnostic haematologic malignancies, such as a higher anti-D rate in MDS (23%) [32] and multiple myeloma (3/6, 50%) patients [41], while a lower rate was observed in leukaemia (6%–10%) patients [32, 41, 42]. Such differences might partly relate to differences in immune status and degree of immune suppression caused by variations in drug combinations, dosages and schedule of administration.

In D-negative trauma patients, we saw a lower incidence of anti-D formation, but no significant difference from hospitalized mixed

nontrauma patients [31, 33, 38, 43]. The lower rate might be related to an immunomodulatory effect [44] and stress-related immune suppression caused by traumatic injury [45]. When evaluating the strategy to replace O D-negative RBC units with O D-positive in emergency recipients, we found no adverse reactions, many D-negative RBCs saved and a low risk of inducing anti-D (as low as 4%, 17/437, in the study of Selleng et al. [31]). However, when focusing on the D-negative patients and excluding the high percentage of those who died (around 40% did not survive within 1 week) which prevented follow-up, around 10% to 60% of D-negative patients produce anti-D with great heterogeneity [31, 33, 46]. Besides, a higher anti-D rate was observed in the patients with longer follow-up time [33].

In D-negative patients who underwent liver, heart and heart-lung transplants, haematopoietic cell transplants, or those receiving immunosuppression treatment including cyclosporin A or MMF [20, 23, 24], we found a very low anti-D immunization rate (4%, 95% CI 0%–8%) [19–25]. In D-negative patients with haematopoietic stem cell transplants, two studies reported that none of the total 15 D-negative recipients with nonmyeloablative or myeloablative haematopoietic stem cell transplants from D-positive donors developed anti-D after D-positive RBCs transfusion and regular and long-term follow-up [19, 22]. Thus, it appears that these immunosuppressive protocols for transplantation and certain haematologic malignancies can prevent primary immune responses to the D antigen [2].

Boctor et al. reported that none of the eight D-negative AIDS patients, who were transfused with D-positive RBCs, developed anti-D [26]. This could be attributable to the decrease in CD4+ T helper lymphocytes that could not effectively assist B cell activation to produce the anti-D.

Another problem is the D variant that includes partial D and weak D, which were not fully described until the 1990s. In the Caucasian population, weak D occurs at a frequency of 1%–3% and a majority of them are weak D 1, 2 and 3, which could not produce anti-D against D-positive RBCs [47–49]. Individuals with weak D 1, 2 and 3 are possibly included in these studies, especially before the 1990s, due to mistyping as the D-negative phenotype and regarded as nonresponders.

This study also has several limitations that have to be addressed. First, most of the studies are observational and not systematic. In addition, most of the studies have a limited sample size as D-positive RBC transfusion in D-negative patients is not a common clinical practice. The type of diseases of D-negative patients classified to have mixed diseases, and the subtype of trauma with burns or not, having different immunosuppressive states, could not be determined, which might have contributed to the heterogeneity among studies. Furthermore, several studies had short follow-up times, particularly trauma patients, thereby resulting in the loss of the cases of anti-D responders, which may also contribute to the heterogeneity in results.

On this basis, more systematic studies are needed to establish more precise and feasible policies for transfusion of D-positive RBCs to D-negative patients, as well as to optimize the use of scarce D-negative RBC component resources.

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CONFLICT OF INTEREST

The authors declare no conflicts 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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REVIEW ARTICLE

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Are convalescent plasma stocks collected during former COVID-19 waves still effective against current SARS-CoV-2 variants?

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Abstract

COVID-19 convalescent plasma (CCP) was among the few frontline therapies used to treat COVID-19. After large randomized controlled trials (RCTs) relying on late use in hospitalized patients and/or low antibody titres failed to meet their predefined primary endpoint, the infectious disease community reduced usage of CCP in favour of monoclonal antibodies. Consequently, there are CCP stocks at most transfusion centres worldwide, although scattered usage continues. Further, better designed RCTs are also being launched. The urgent question here is: should we use CCP units collected months before given the largely changed viral variant landscape? We review here in vitro evidence that discourages usage of such CCP units against Delta and other variants of concern. CCP collections should be continued in order to update the armamentarium of therapeutics against vaccine breakthrough infections or in unvaccinated patients and is especially relevant in next-generation RCTs.

KEYWORDS
COVID-19 convalescent plasma, SARS-CoV-2, variants of concern

Highlights

- COVID-19 convalescent plasma (CCP) stockpiles remain after massive collection campaigns in 2020
- Dominant SARS-CoV-2 variants of concern (VOC) have largely changed during the last year of the pandemic
- In vitro evidence shows that CCP collected in 2020 is unlikely to be effective against current VOCs

Massive vaccination campaigns worldwide have largely reduced hospitalization rates after SARS-CoV-2 infection, but current vaccines allow asymptomatic infection and transmission, making herd immunity currently unreachable. Hence COVID-19 is still widespread in the form of infection in unvaccinated patients or alternatively as vaccine breakthrough infection. One year and a half after the pandemic began and many published clinical trials, COVID-19 convalescent plasma (CCP) is still among the few treatments for COVID-19 in low-to-middle income countries that cannot afford monoclonal antibodies or antivirals. In the United States, CCP remains under FDA Emergency Use Authorization and several hundred units continue to be used weekly (Figure 1).

The lifespan of fresh frozen plasma is 2 years when kept at temperatures below −20°C, and CCP makes no exception to this rule of thumb. The combination of long shelf-life and suddenly reduced usage has translated into unused stockpiles at many blood banks across the world, with regulatory authorities recommending preservation rather than shipping to plasma manufacturers. But meanwhile, the SARS-CoV-2 variant landscape has changed dramatically across

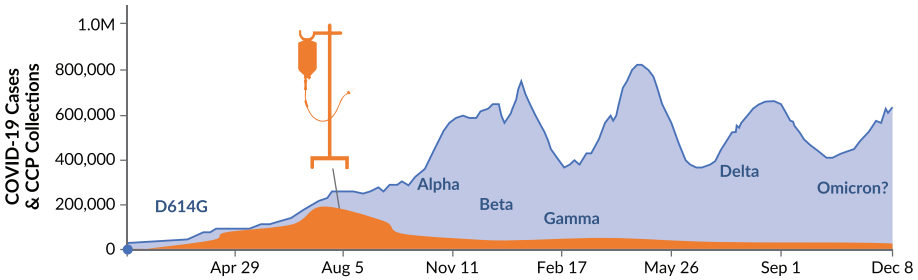

FIGURE 1 Kinetics of COVID-19 convalescent plasma (CCP) collections across the COVID-19 pandemic waves, driven by different variants of concern. Collections (and usage) declined after the first waves, making current bulks poorly useful and requiring a restart of collections from convalescent vaccines to counteract the upcoming Omicron variant

successive waves, with most countries observing major antigenic changes in the predominant strain. This raises the question of whether these CCP stockpiles are still useful. Analysis of the geographic provenance of CCP in relation to usage locales provided the surprising observation the efficacy diminished with distance even during a single wave, which probably reflected local evolution of the virus [1]. Hence, it is reasonable to assume that such differences are also maintained or more dramatic across subsequent waves.

Published clinical trials are not informative on the question of CCP efficacy against new variants since these mostly recruited patients at times when D614 strains were predominant, or, at their best, during Alpha predominance. As of 27 December 2021, the WHO had identified four variants of concern (VOCs) (dubbed Alpha, Beta, Gamma, and Delta), five variants of interest (VOIs) (dubbed Eta, Iota, Kappa, Lambda and Mu, with the former three later declassified) and issued many more alerts for further monitoring [2]: among them, Delta (which actually accounts for more than 200 different AY sublineages [3]) is largely the predominant clade worldwide. Most of these variants emerged at times CCP trials had already been discontinued, and none has been reported after emergence of Delta, although several are proceeding during this time.

In the absence of clinical evidence, we are left with in vitro evidence as the major pillar for inferring the efficacy of CCP against novel variants. On 9 September 2021, we mined PubMed (which is also indexing the medRxiv and bioRxiv pre-publishing servers) for keywords related to in vitro neutralization of specific strains by CCP. The viral neutralization test was varyingly conceived as plaque reduction neutralization test (PRNT) with authentic or pseudotyped virus, or RBD-ACE2 competition assay [4]. The results are summarized in Table 1. No data from animal models (especially nonhuman primates) are available yet.

Most studies reported neutralization as folds-reduction in geometric mean titer (GMT) compared with a wild-type D614G SARS-CoV-2 strain (e.g., Wuhan-Hu-1, USA-WA1/2020, B.1, or other reference strains) circulating in early 2020. The fold-reduction unit of each measurement normalizes for different neutralizing antibody (nAb) titres or time elapsed between infection and CCP collection. To simplify interpretation, after looking at the data, we arbitrarily grouped fold-reductions into four categories: no reduction, onefold–threefold reduction, threefold–fivefold reduction and >fivefold reduction. Nevertheless, differences in viral reference strain, PRNT methodology (viral inoculum dose, incubation time, reading threshold) create discrepancies when classifying fold reduction for a given strain (most evident for Delta and Kappa studies). Given clinical data associating CCP *in vitro* nAb titre with in vivo efficacy [5, 6] and vaccine-elicited *in vitro* nAb titre with vaccine efficacy [7, 8], it is logical to assume that *in vivo* efficacy could be similarly reduced: higher nAb titres in CCP could counteract such failure, but this remains unproven, and such very high nAb titres are hardly retrieved.

Alpha, which drove the early 2021 pandemic, was still very sensitive to CCP collected during early 2020, with neutralization reduced less than threefold. Delta, the currently predominating clade worldwide, is neutralized by CCP collected during early 2020 threefold–fivefold less than wild-type D614G strain. Beta and Gamma perform far worse, escaping CCP neutralization more than fivefold. Many VOIs have neutralization reduced by more than fivefold. Among them Mu, a recently classified VOI, seems the most CCP resistant variant, being 12.4-fold less susceptible to sera of 8 COVID-19 convalescents, who were infected during the early pandemic (April–September 2020), than the parental virus [9]. Delta sublineages such as Delta+N501S or variants under monitoring such as C.1.2 are largely resistant to CCP from previous waves [10]. Of interest, BNT162b2-elicited sera from donors who had a history of COVID-19 showed an overall higher neutralizing titre against all of the variants compared with unexperienced donors [10].

Currently, no differences have been reported about median nAb titres in convalescents affected by different SARS-CoV-2 variants. Of interest, the polyclonal nature of CCP has ameliorated the fold-reduction, when compared with the total loss of activity manifested by several currently approved monoclonal antibodies, which have their efficacy zeroed by Spike variants [11]. A single study from the United States investigated the efficacy of a (yet unlicensed) CCP-derived polyclonal antiserum formulation, which was preserved against Alpha and Epsilon, and largely reduced against Beta and Gamma [12]. Given the batch manufacturing of hyperimmune sera, it is likely that lot-to-lot or inter-vendor variation can occur according to geographical provenance of the source CCP.

An additional reason to reinitiate CCP collections is provided by the increased potential of the so-called Vax-CCP, that is, CCP

TABLE 1 Summary of in vitro evidences of efficacy from COVID-19 convalescent plasma (CCP) collected during early 2020 against currently circulating SARS-COV-2 VOCs and VOIs

	Variants of concern (VOC)					Variants of interest (VOI)		Former VOI/variants under monitoring (VUM)					
Pangolin name	B.1.1.7	B.1.351	P.1	B.1.617.2 AY.1-AY.39	B.1.1.529	B.1.621	C.37	P.2	B.1.427 B.1.429	P.3	B.1.525	B.1.526 with E484K or S477N	B.1.617.1
NextStrain name	20I/S:501Y.V1	20H/S:501Y.V2	20J/S:501Y.V3	21A/S:478K	21K	21H	20D	20B/S.484K	20C/S.452R	20B/S:265C	20A/S484K	20C/S.484K	21A/S:154K
PHE name	VOC-20DEC-01	VOC-20DEC-02	VOC-21JAN-02	VUI-21APR02	VUI-21NOV-01	VUI-21JUL-01	VUI-21JUN-01	VUI-21JAN-01 VUI-202101/01	-	VUI-21MAR-02	VUI-21FEB-03	-	VUI-21APR-01
WHO name	Alpha	Beta	Gamma	Delta	Omicron	Mu	Lambda	Zeta	Epsilon	Theta	Eta	Iota	Kappa
GISAID name	GRY (formerly GR/501Y.V1)	GH/501Y.V2	GR/501Y.V3	G/452R.V3	GR/484A	GH	GR/452Q.V1	GR	GH/452R.V1	GR	G/484K.V3	GH	G/452R.V3
Local name	VUI/VOC 202012/01, UK variant	501Y.V2 VOC 202012/02	B.1.1.28.1 B.1.1.248 VOC 202101/02	Indian variant	novel South African variant	-	-	B.1.1.28.2 B.1.1.28(E484K)	CAL.20C/ L452R, West Coast variant	-	Nigerian variant	-	Indian variant
CCP (sera) from previous waves	↓ [23–38] ↓ RBD [39]	↓↓↓ [24, 26, 28, 29, 32, 33, 36–38, 40–48] ↓↓↓ RBD [39]	↓↓↓ [32, 35, 36, 49] = [50] ↓↓↓ RBD [39]	= [51] ↓ [33, 36, 52–55] ↓↓ [45, 48, 56] ↓↓↓ [57] ↓ RBD [39]	?	↓↓↓ [9, 10]	↓↓ [47, 48] ↓↓ RBD [39]	= [58] ↓↓↓ [59] (hamsters)	↓↓ [46, 60] ↓ RBD [39]	↓ RBD [39]	↓↓↓ RBD [39]	↓↓ [61, 62]	↓↓↓ [56] ↓↓ RBD [39] ↓ [55]

Note: Arrows indicate fold-reductions in in vitro neutralizing activity compared with wild-type D614G strain (= no reduction; ↓: onefold–threefold reduction; ↓↓: threefold–fivefold reduction; ↓↓↓: >fivefold reduction; ?: data not available). References mined from the Stanford University Coronavirus Antiviral & Resistance Database (accessed online at https://covdb.stanford.edu/search-drdb/?cp=any&host=human on 6 December 2021).
Abbreviation: RBD, ACE2-RBD competition assay.

harvested from patients who have been vaccinated after infection. Romon et al have shown that a single dose of mRNA vaccine in convalescents actually leads to Ortho Vitros® antibody levels predictive of high nAb content [13]. After two vaccine doses, Vax-CCP can have extremely high nAb titres. Vickers et al. have shown that ~50-fold increases in Spike-specific antibody levels: although the majority of mRNA vaccine-induced antibodies do not have neutralizing activity [14], vaccination nevertheless translated in at least a 20-fold increase in the IC_{50} nAb titre based on PRNT [15]. Furthermore, the polyclonal antibodies Vax-CCP have been shown heterologous and active against many of the variants [16–18]. Vax-CCP is also less likely to benefit from expensive pathogen inactivation technologies since collections do not stem from recently hospitalized subjects, and this makes product manufacturing simpler and cheaper. Transfusion of Vax-CCP has resulted in recipient nAb titres as high as seen in convalescents [19] and, together with emergence of novel VOCs with Spike mutations, could re-launch the declining CCP demand over monoclonal antibodies. As more recovered patients are vaccinated, Vax-CCP may emerge as a source of both high nAb titre and "broad spectrum" CCP during the next phases of the pandemic.

As humanity approaches the second anniversary of the COVID-19 pandemic, CCP remains a viable therapy when used in accordance with the principles of antibody therapy, namely that it must contain specific nAb in sufficient amount to mediate a therapeutic effect and must be used early in the course of disease [20]. We argue for reinitiating CCP collection rather than relying over old CCP stockpiles. Certainly, new RCTs should not be initiated with older CCP as this is a recipe for failure. Furthermore, we suggest that great attention be given to matching collections with use regionally given the observation that efficacy drops markedly after 150 miles. The time when Delta prevalence has become higher than 90% seems a reasonable cut-off for convalescent donor eligibility in countries where Delta is now predominating.

CCP is the only antibody therapy that keeps up with the variants since every variant elicits a response that neutralizes it during convalescence: Not by chance the fold-reductions in neutralization ability of CCP are usually below 10-fold, while for monoclonal antibodies are often higher than 100-fold. In particular, the recently recognized Omicron VOC, with its huge mutation load disrupting Spike conformation and evading most monoclonal antibody therapies developed to date [21, 22], is a serious quest for polyclonal therapies.

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CONFLICT OF INTEREST

We declare we have no conflict of interest to disclose.

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REVIEW ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

The international experience of bacterial screen testing of platelet components with automated microbial detection systems: An update

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Abstract

In 2014, the bacterial subgroup of the Transfusion-Transmitted Infectious Diseases working party of ISBT published a review on the International Experience of Bacterial Screen Testing of Platelet Components (PCs) with an Automated Microbial Detection System. The purpose of this review, which is focused on publications on or after 2014, is to summarize recent experiences related to bacterial contamination of PCs and the use of an automated culture method to safeguard the blood supply. We first reviewed septic transfusion reactions after PC transfusion as reported in national haemovigilance systems along with a few reports from various countries on bacterial contamination of blood products. Next, we reviewed PC automated culture protocols employed by national blood services in the United Kingdom, Australia, Canada and large blood collection organization and hospital transfusion services in the United States. Then, we acknowledged the limitations of currently available culture methodologies in abating the risks of transfusion-transmitted bacterial infection, through a review of case reports. This review was neither meant to be critical of the literature reviewed nor meant to identify or recommend a best practice. We concluded that significant risk reduction can be achieved by one or a combination of more than one strategy. No one approach is feasible for all institutions worldwide. In selecting strategies, institutions should consider the possible impact on platelet components availability and entertain a risk-based decision-making approach that accounts for operational, logistical and financial factors.

KEYWORDS
bacteria, culture-based methods, platelets

Highlights

- Significant advances in safety of platelets transfusion therapy were achieved by introduction of multiple strategies to mitigate the risks of bacterial contamination of platelet components.
- The contributions of bacterial screen testing of platelet components with automated microbial detection systems are reviewed here.
- This is not a substitute for vigilant monitoring of transfusion recipients to detect and report septic transfusion reactions.

INTRODUCTION

Bacterial contamination of platelet components (PCs) represents one of the highest risks for transfusion-transmitted bacterial infection (TTBI), infrequently causing septic transfusion reactions (STRs) in recipients. The storage conditions for PCs support bacterial proliferation from low contamination levels to high numbers. Before the implementation of routine bacterial culture testing of PCs, the incidence of bacterial contamination in units varied widely in published studies. In contradistinction to the available data on the bacterial contamination of PCs, the frequency of clinically apparent septic transfusion events due to such contaminated products was considerably lower [1]. Over the past 15 years, the safety of PC transfusion therapy has demonstrably improved with a marked reduction in reported fatalities. This has been the result of a combination of interventions, but primarily from the implementation of culture-based methods [2]. Several reviews discussed the issues related to bacterial contamination of PCs [3–5]. In 2014, Benjamin and McDonald described the international experience of bacterial screen testing of PCs with an automated microbial detection system calling for consensus testing and reporting guidelines [6]. The aim of this review is to share recent experiences in the field of improving bacterial safety through enhancements of culture methods to detect bacterially contaminated components with focus on publications on or after 2014.

THE PROBLEM: STRS

Periodically, STRs are reported in several national haemovigilance systems. Between 2010 and 2019, the United Kingdom's Serious Hazards of Transfusion (SHOT) programme documented one confirmed STR involving PCs contaminated with *Staphylococcus aureus* [7]. From 2015 to 2019, the US Food and Drug Administration (FDA) reported 20 fatalities caused by blood components contaminated with a range of bacterial species [8]. The National Healthcare Safety Network Haemovigilance Module reported 54 transfusion-transmitted infections (TTI) over a 7-year period (2010–2016). Of the 54 cases, 37 (69%) were bacterial [9]. In Canada, the programme report of the Transfusion-Transmitted Injuries Surveillance System for 2011–2015 described seven cases related to bacterially contaminated blood components [10]. From 2000 to 2017, the Québec Haemovigilance System received 23 reports of adverse transfusion reactions involving bacterially contaminated PCs [11].

Between 2003 and 2014, a total of 14 cases of TTBIs were reported to transfusion and transplantation reactions in patients; the Dutch competent authority to which all transfusion reactions must be reported. Of which, 57.1% received a PC stored in platelet additive solution (PAS) and 42.9% a PC stored in plasma. Of all produced PCs, 22.3% were stored in PAS and 77.7% in plasma. The relative risk of TTBI after transfusion of a PAS-stored PC was 4.63 (95% confidence interval [CI], 1.4–16.2) compared to transfusion of a plasma-stored PC. The incidence of TTBIs was 22.2 per million (95% CI, 12.1–37.2 per million) transfused buffy coat derived pooled platelet components [12].

BOX 1 Strategies to reduce septic transfusion reactions

- Interventions to reduce contaminations at time of collection:
  - Improved disinfection of the skin
  - Diversion of the first 20–40 ml of blood collected.
- Shelf-life reduction
- Interventions to detect contaminated components:
  - Culture methods
  - Rapid methods
- PR

Reports on blood components contamination with bacteria from several countries and institutions emerged. Hume et al. reviewed three studies of bacterial contamination in 63 tested PC units in Africa. All studies found alarmingly high rates of bacterial contamination—an average of 8.8% of units tested with a range of 3.1% (Zimbabwe) to 17.5% (Ghana). In a prospective observational study, 337 platelet-rich-plasma were evaluated for bacterial contamination at a cancer centre in Uganda. A total of 323 units were transfused in 151 transfusion episodes to 50 patients. Acute reactions occurred in 11 transfusion episodes, involving 13 whole blood-derived platelet units, for a rate of 7.3% (95% CI, 3.7–12.7%) per transfusion episode. All recipients of units with positive bacterial cultures were receiving antibiotics at the time of transfusion; none experienced a reaction. While the rate of bacterial contamination observed in this study is lower than previously reported in Sub Saharan Africa (SSA), they concluded that methods alternate to Gram stain should be explored to prevent transfusing bacterially contaminated PCs in SSA [13]. Bacterial contamination of PCs in Africa was addressed in a recent report [14]. A survey of members of the African Society of Blood Transfusion revealed that regional preventative measures have not been widely adopted. Skin disinfection and conversion pouches were reported at 91 and 31 percent of respondents, respectively. Bacterial culture, pathogen reduction (PR) and point of release testing were methods being considered for implementation by members. Several other small studies reported the prevalence of bacterial contamination of blood and blood components using cultures on different media, in which isolates were identified using standard biochemical and bacteriological methods [15, 16]. Research, surveillance and implementation of interventions in lower-income countries at present lags that of high-income countries (Box 1).

THE GOOD—PURPORTED SOLUTIONS

Detection of contaminated PCs by automated culture systems depends on the sampled volume tested and the time of sampling of the product. Lacking a gold standard protocol, blood collection establishments developed various approaches that evolved over the years.

In the United States, blood collection organizations implemented primary culture of PCs, initially with a 4- to 6-ml sample volume, moving to 8- to 10-ml sample volume, inoculating an aerobic culture bottle only, at least 24 h after collection. Most recently, large volume delayed sampling (LVDS) protocol was introduced that incorporates inoculation of aerobic and anaerobic culture bottles. Additional measures, for example, secondary testing and PR are being introduced at some institutions. In Europe, significant variations of strategies are in place across the continent. Early sampling (<36 h) culture based in Denmark, Portugal, Netherlands, Ireland, UK and late sampling (>36 h) culture based in the UK. PR is employed nationally in Belgium, Switzerland and France and shelf-life reduction is the practice in Germany [17]. Limitations of performing primary cultures to detect all bacterial contamination near the time of collection and strategies to enhance PC safety through bacterial culture methods are listed in Table 1.

Several blood collectors reported on their experiences with variations on the single-step LVDS primary bacterial culture strategies using the Bact/ALERT-3D (BTA-3D) Automated Microbiology Detection System (Table 2). In the NHS Blood and Transplant (NHSBT), PC was held for 36–48 h post donation before sampling [18]. A 16-ml sample was taken into a sampling pouch. An 8-ml volume was inoculated into each of aerobic and anaerobic blood product culture bottles of the BTA-3D. All component splits, that is, single, double and triple, were treated in the same manner. Bottles were incubated after inoculation for the remainder of the 7-day shelf life of the component and units were held for 6 h before release to hospitals. All bottles were unloaded after the first positive BTA-3D signal. Unused time-expired PCs were tested as per the initial screening protocol. Initial reactive cultures were confirmed by repeat testing of the unit. From February 2011 to September 2015, a total of 1,239,029 PCs were screened. Confirmed-positive rates were 300 per million: 278,559 buffy coat derived pooled platelet components (BCPPC) and 960,470 apheresis platelet components (APCs) were screened. Confirmed positive results were obtained in 195 (700 per million) and 208 (200 per million) for BCPPC and APC units, respectively. False-negative cultures, all with *S. aureus*, occurred on four occasions; three were visually detected before transfusion and one confirmed transmission resulted in patient morbidity. Testing of 4515 time-expired PCs after screening revealed no positives. The NHSBT screening protocol effectively reduced the number of clinically adverse transfusion transmissions by 90% in this reporting period, compared to a similar period before implementation (Figure 1).

Blood Systems, Inc. (now Vitalant) implemented a minimal proportional sample volume (MPSV) of at least 3.8% of mother bag volume into one to three aerobic culture bottles (7–10 ml per bottle) [19]. Trima leukoreduced APCs were collected during two study periods (45 and 31 months) using standard procedures. Primary testing for bacterial contamination was performed using BTA-3D with sampling from the mother bag 24 to 36 h after collection. Components released as negative to date after 12 h minimum incubation period. Two culture approaches were compared: in Period A, an 8-ml sample in one

TABLE 1 Counter measures to overcome limitations of primary cultures

Why primary culture fails to detect all bacterial contamination near the time of collection?	How to decrease the likelihood of primary culture failure?
• Uneven distribution of bacteria at low concentrations in PCs units, • Slow-growing (generally Gram-positive) organisms failing to reach detection level by the time of sampling and • Formation of surface-attached bacterial communities, known as biofilms. • Anaerobic organism that does not grow in aerobic culture medium.	• Increasing the sensitivity of primary culture through ○ Increasing sample volume, ○ Delaying sampling time and/or ○ Incorporating an anaerobic culture bottle or • Performing secondary bacterial testing of previously cultured PCs closer to the time of transfusion.

Abbreviation: PCs, platelet components.

TABLE 2 Methods employed to screen apheresis platelet component (APC) for bacteria[a]

Study (reference)	Delay before sampling (h)	Sample volume (ml)	Aerobic (ml)	Anaerobic (ml)	Hold time (h)	Shelf-life (days)
NHSBT [17]	≥36	16[b]	8	8	6	7
Vitalant [18]	≥24	10–28[c]	10–28		12	5
Australian Red Cross [19]	≥24	15–20[c]	7–10	7–10	See footnote[d]	5
Héma-Québec [20]	≥48	20[c]	10	10	12	7
Canadian Blood Services [21]	≥36	20[ce]	8–10	8–10	6	7
John Hopkins Hospital [22][f]	≥24	8[c]	8		Variable	5

[a]All used the BACT/ALERT 3D system.
[b]Sampling of the split unit.
[c]Sampling of the mother bag.
[d]Platelet component is released to inventory when culture begins as culture-negative to date.
[e]For double APC, 40 ml volume, with three aerobic and one anaerobic bottle.
[f]Five millilitre secondary culture on day 3 using BTA-3D. Shelf life remains 5 days.

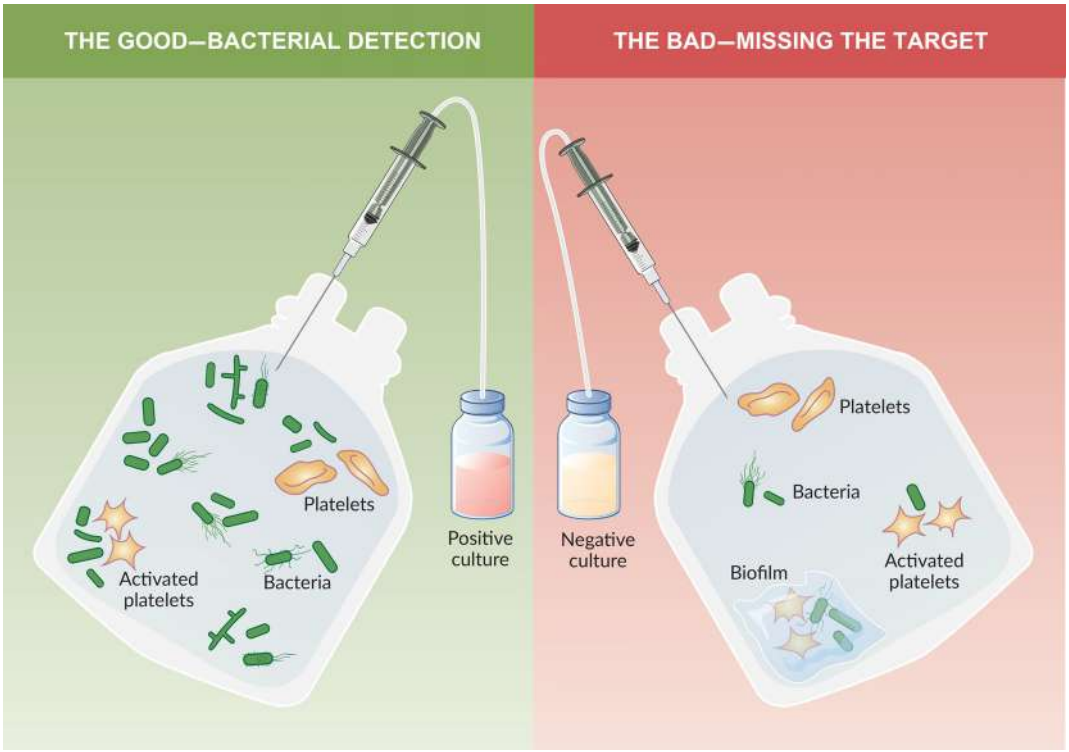

FIGURE 1 Bacterial detection of platelet components with automated culture screening is successful when bacterial concentration is above the limit of detection of the testing method (the good). Bacteria, however, could be missed during sampling of the platelet components if the concentration is too low or if bacteria are aggregated as biofilms (the bad)

aerobic culture bottle, and in Period B an MPSV of at least 3.8% of mother bag volume into one to three aerobic culture bottles (7–10 ml per bottle). In Periods A and B, 188,389 and 159,098 APC collections were tested, respectively. The true-positive (TP) rate in Period A was 90 per million collections increasing to 183 per million collections in Period B ($p < 0.05$). In Period B, 12 of 29 (41%) TP results had discrepant culture bottle results (at least one of multiple bottles without growth). One contaminated collection resulting in STR(s) was reported in each study period. MPSV improved the sensitivity of primary testing and identified collections that could have escaped detection had only a single bottle with 8- to 10-ml volume been used. The authors proposed MPSV as an approach to enhance PCs safety for 5-day storage without a requirement for secondary testing [19].

Australian Red Cross Blood Service implemented bacterial contamination screening in April 2008 [20]. Testing used the BTA-3D automated microbial detection system. PCs manufactured included BCPPCs from four donations suspended in PAS (SSP+) sampled as a pool, and APC suspended in plasma (Trima Accel) sampled individually or pooled if a double collection. All PCs were sampled for bacterial contamination screening. Closed sampling of 15–20 ml from each PC occurred at a minimum time of 24 h post-phlebotomy or 24 h post-phlebotomy of the 'youngest' buffy coat in a platelet pool. Samples of 7–10 ml each were aseptically inoculated into both aerobic and anaerobic culture bottles and incubated with continuous monitoring for 7 days (8 days post-phlebotomy). PCs were released into inventory when culture began as 'culture-negative to date' and had a shelf life of 5 days from phlebotomy. Between 2010 and 2012, 1.1% of PC donations tested initial machine positive (IMP); since 2013, this rate has fallen to 0.6% through improved instrument management, reducing false-positive IMPs, but maintaining sensitivity for cultures yielding bacterial growth. On average, 66% of confirmed positive and indeterminate PC units had been transfused at the time of detection. The majority (95%) of these grew *Cutibacterium* sp., a slow-growing organism that rarely causes sepsis in the transfusion setting. The incidence of reported TTBI has fallen since the introduction of bacterial contamination screening, with a 4.2-fold [0.5, 28.2] lower rate from PCs [20].

In Héma-Québec, sample volume was increased from 10 to 20 ml: 10 ml each was inoculated into one aerobic and one anaerobic blood culture bottle and delayed inoculation from 24 to 48 h and a 12-h holding period before labelling and distribution were introduced [21]. The outdated components were reduced by 71%. The rate of positive cultures went from 0.011% to 0.044%. None of the 908 PC cultures done at outdate were positive. The modal age (most frequent age) of PCs at transfusion went from 4 to 6 days. There has not been to date any case reported to the provincial haemovigilance system of an STR following the transfusion of a contaminated platelet component.

In August 2017, Canadian Blood Services (CBS) implemented a new testing algorithm modelled on the NHSBT screening protocol for extension of PC shelf life from 5 to 7 days [22]. Screening involved testing at least 36 h post collection, inoculation of aerobic and anaerobic culture bottles (three aerobic and one anaerobic bottle for double APC, with samples taken from the mother bag) and post sampling

quarantine for at least 6 h. A total of 223,156 BCPPC and 39,725 AP units were screened. Confirmed positive results were obtained in 201 (900 per million) and 16 (400 per million) for BCPPC and APC units, respectively, predominantly with *Cutibacterium acnes*. Outdated units (2954 BCPPC and 2356 APC) were screened and confirmed positive results were reported in four BCPPC and two APC units. One nonfatal reaction involving a 7-day BCPPC contaminated with *S. epidermidis* occurred. Combined PC outdating at CBS and hospitals was reduced from 18.9% to 13.1%.

To contend with residual risk of bacterial contamination, the Johns Hopkins Hospital in October 2016 implemented secondary bacterial culture (SBC) on screened APC received from the blood collection facility [23]. On day 3 post collection, a 5 ml sample was inoculated aseptically into an aerobic culture bottle and loaded into the BTA-3D incubator modules and incubated for 3 days or until positive. PCs were not subjected to any quarantine period following inoculation. During the 13-month study period, eight positive cultures (one in 2881 PCs products) were obtained; five were confirmed positive on re-culture while in three cases, the units had been transfused without adverse events before positive culture results were obtained. As expected, the bacteria were slow growers such as coagulase-negative staphylococci. There were no STRs during the study period, compared to three definite reactions, including one fatality, in the 13 months before the study period. The authors concluded that their protocol was logistically simple and cost-effective and decreased STRs. No STRs reported during an extended 3-year analysis during which higher rates of false positivity were observed using a 10-ml volume inocula [24].

The FDA published a guidance for bacterial risk control strategies related to PCs in September 2019 [25]. In this guidance, the FDA listed nine viable non-PR methods for APC testing. Walker et al. used simulation to compare safety of the nine risk control strategies involving APC testing [26]. The primary outcome was the risk of exposure. An exposure event occurred if a patient received PCs exceeding a specific contamination threshold (>0, 10^3 and 10^5 colony-forming units [CFU/ml]). They generated a range of bacterial contamination scenarios (inoculum size, doubling time, lag time) and compared risk of exposure for each policy in each contamination scenario, then they computed the average risk difference over all scenarios. Their findings were that at the 0 CFU/ml exposure threshold, two-step policies that used secondary culture ranked best (all top three), while single-step 24-h culture with 3-day expiration ranked last (ninth). This latter policy performed well (median rank of 1) at both the 10^3 and 10^5 CFU/ml thresholds, but 48-h culture with 7-day expiration performed relatively poorly. At these higher thresholds, median ranks of two-step policies that used secondary culture were again top three. Two-step policies that used rapid testing improved at the higher (10^5 CFU/ml) harm threshold, with median rankings between 1 and 5. They concluded that two-step policies that used secondary culture were generally safer than single-step policies. Failure of single-step methods is usually attributed to low inoculum size, long lag time or long doubling time, each is mitigated by secondary culture.

Another modelling analysis of financial and clinical impact of implementing all variants of LVDS, PR, point of release testing and SBC described in FDA guidance from a hospital perspective was performed [27]. With some limitations, the analysis showed LVDS as cost-competitive approach when compared to other proposed strategies.

In summary, despite significant variability in methods among the studies reviewed, there has been improvement in detection of contaminated PCs as well as improvement in PC availability when shelf life is extended to 7 days [28]. However, it is difficult to compare residual risk for each strategy, for a variety of reasons: most published reports compare results before and after implementation of a given approach and randomized studies comparing different strategies are lacking. Contamination is a low-frequency event, and for this reason, large sample sizes are required to identify differences in detection rates. For example, a sample size of 500,000 (250,000 in each arm) would be required to reliably detect a difference of 1 in 10,000 in the bacterial detection rate. These types of sample sizes make it difficult, if not impossible, to compare multiple policies [26]. It is assuring that simulation study and meta-analysis of published reports supported the notion that larger sample volumes increased sensitivity, and that bacterial contamination rates have decreased over time [29, 30].

Detection systems

A brief review of instrument-based continuous monitoring automated blood culture systems used by blood collection facilities to screen PCs for bacteria is warranted. All use aerobic and anaerobic culture media capable of detecting a wide variety of microorganisms, including bacteria, moulds and yeasts [4]. The most widely used BacT/ALERT system (bioMerieux, Durham, NC) and the BACTEC System (BD Microbiology, Cockeysville, MD), both with analytical sensitivity of 1–10 CFU/ml, have been cleared for quality control testing of leucocyte reduced PCs by the US FDA. Both monitor carbon dioxide production due to bacterial growth. The BacT/ALERT uses colorimetric sensor while the BACTEC uses a fluorometric detection principle. The VersaTREK (Thermo Fisher Scientific, Waltham, MA), with analytical sensitivity of 10–20 CFU/ml, monitors bacterial growth by detecting pressure changes in the headspace of the blood culture bottle secondary to gas consumption–production PCs samples are inoculated into aerobic–anaerobic culture media and incubated in the corresponding instrument system, which monitor the change in colour, increase in fluorescence or change in pressure every 10 min. A positive reading indicates the presumptive presence of viable microorganisms [31, 32].

Newer systems are introduced offering more advanced features. Of note, the BACTEC FX and BACT/ALERT VIRTUO. The VIRTUO, employing the same colorimetric technology, offers a new instrument design to improve temperature stability, automatic loading and unloading of culture bottles to reduce manual processes and an enhanced proprietary algorithm to shorten time to detection of positive cultures [33–35].

THE UGLY—MISSING THE TARGET

Detection of contaminated PCs by automated culture systems depends on the sampled volume tested and the time of sampling of

the product. False-negative testing results are documented during the investigation of STRs or through testing of outdated PCs and are associated with low initial bacterial loads, slow-growing organisms and formation of surface-attached bacterial communities, known as biofilms [36]. These factors may result in the absence of bacteria in the sampled PC contributing to missed bacterial detection. The following findings highlight that even when following current procedures, the risk for transfusion-related infection and fatality persists. Clinicians need to be vigilant in monitoring for transfusion-transmitted bacterial infection and report adverse reactions to blood suppliers and haemovigilance systems. Blood suppliers and hospitals could consider additional evidence-based bacterial contamination risk mitigation strategies, including PR systems, rapid detection devices and modified screening of bacterial culture protocols. However, as reviewed below, STR was confirmed after the transfusion of units treated with PR systems and after the transfusion of units negative by a secondary rapid test 5 h before transfusion.

S. epidermidis and other common PC contaminants form biofilms during PCs storage, by either adhering to platelets or the inner surface of PC containers. Importantly, biofilm-negative bacteria convert to a biofilm-positive phenotype during PCs storage. Furthermore, structural modifications of the cell surface of *S. epidermidis* cells have been observed, which may be responsible for resistance to immune factors present in PCs and enhanced pathogenicity. Biofilm formation in PCs results in reduced numbers of bacterial cells in suspension, likely contributing to missed detection during PCs screening. Transfusing biofilm-forming bacteria has an additional safety risk to PC recipients since biofilms can colonize biomedical devices and display increased resistance to patient antibiotic treatments and clearance by the immune system [36].

An STR reported in a 73-year-old female patient 2.5 h after the transfusion of an irradiated 4-day-old BCPPC was interrupted following obstruction of the infusion line by a large fibrous clot. The implicated BCPPC had been tested for bacterial contamination during routine screening yielding negative results. This change in appearance was not noticed in the blood bank at the time of issuing the BCPPC or at the bedside before the transfusion started. Phenotypic and genetic analyses demonstrated that the same *S. aureus* strain was isolated from the patient samples and BCPPC. Investigation on the ability of this isolate to form biofilms was performed and the results showed that this isolate is a strong biofilm former in PCs [37].

A fatal false-negative transfusion infection involving a BCPPC contaminated with biofilm-positive *S. epidermidis* was reported in a splenectomized elderly male patient, suffering from leukaemia, who was transfused with two 5-day-old BCPPC pools. The patient returned to emergency on the same day with a low-grade fever. He was bacteraemic and died the next day. Microbiology and molecular testing of a blood sample from the patient and one of the BCPPC yielded the same *S. epidermidis* strain. Further analysis demonstrated that this *S. epidermidis* isolate displays a biofilm-positive phenotype in the BCPPC [38].

With over 1·6 million PC units who underwent bacterial screening prior to issue, *S. aureus* accounted for 21 cases of contamination. This included one confirmed TTBI and three 'near-miss' incidents detected on visual inspection, which was negative on screening. Follow-up investigations of 16 donors for skin carriage of *S. aureus* and molecular characterization of donor and pack isolates showed that donors of 10 APCs and two pooled packs screen positive for *S. aureus* were confirmed as the source of contamination; seven had a history of skin conditions, predominantly eczema; 11 were nasal carriers. The 'near-miss' incidents were associated with apheresis donors, two donors harboured strains indistinguishable from the pack strain. The TTBI was due to a screen-negative pooled unit, and a nasal isolate of one donor was indistinguishable from that in the unit. Donor isolates showed almost universal correspondence in molecular type with pack isolates, thus confirming the source of contamination [39].

Similarly, the Northern Ireland Blood Transfusion Service routinely extends PC shelf life to 7 days. Components are sampled and screened for bacterial contamination using an automated microbial detection system, the BACT/ALERT 3D Signature System. They reported three near-miss serious adverse events due to false-negative BACT/ALERT 3D results in PCs with confirmed bacterial contamination. In all three cases, *S. aureus* was isolated from the PC residue and confirmed on terminal sub-culture using BacT/ALERT 3D. In two cases, *S. aureus* with similar genetic makeup was isolated from the donors [40].

The first case of transfusion-transmitted *Lactococcus garvieae* sepsis caused by a contaminated PC from a donor who consumed raw octopus before blood donation was reported [41]. Retrospective examination of the laboratory results of the index donor revealed that his haemoglobin levels had been steadily decreasing, which led to the detection of a latent colon cancer. Another report of a case of contamination with *L. garvieae* that was interdicted by SBC was performed on day 3 after collection on three APC products collected from the same donor [42]. The primary aerobic and anaerobic cultures at the blood centre remained negative throughout. The index donor, a 64-year-old man, had donated 122 APC and 21 whole blood products since 1997. *Streptococcus* group C was detected during routine primary PC culture in 2010 and the donor was put under surveillance at the blood centre at that time. The donor was recalled and questioned regarding risk factors for exposure, including consumption of raw fish or dairy products. The donor reported consumption of Mahi Mahi in the week prior to donation, which he had caught in the Florida Keys. The fish had been frozen for 8 months and had been thawed for 2 days in the refrigerator prior to cooking. The donor denied obvious spoiling of the fish and remained asymptomatic following consumption. *L. garvieae* is a Gram-positive coccus previously assigned to the *Streptococcus* genus. It is a known fish pathogen. Although of low virulence and only rarely implicated in human infection, it is a recognized cause of endocarditis, liver abscess and osteomyelitis. *L. garvieae* can grow at 10°C and can contaminate both red blood cell concentrates and PCs; thus, this species should be listed as a cryophilic bacterium that could threaten blood safety [43].

Twenty of 51,440 PC units transfused (0.004%; 389 per million) were bacterially contaminated by active surveillance—a small (1–2 ml) aliquot of every PC is screened at time of issue for bacteria using an

aerobic plate culture technique. Any positive culture is investigated by clinical examination of the transfused patient and by medical record review. The 20 contaminated units resulted in five STRs occurring 9 to 24 h posttransfusion; none of these STRs had been reported by passive surveillance. STR occurred only in neutropenic patients transfused with high bacterial loads. The APC and pre-pooled whole-blood-derived PC units were released by the blood collection facility as negative to date after sampling at 24 h post collection [44].

In August 2017, two separate clusters of PC transfusion-associated bacterial sepsis were reported. In Utah, two patients died after PC transfusions from the same donation. *Clostridium perfringens* isolates from one patient's blood, the other patient's PC bag and donor skin swabs were highly related by whole genome sequencing (WGS). In California, one patient died after PC transfusion; *Klebsiella pneumoniae* isolates from the patient's blood and PC bag residuals and a non-transfused PC unit were matched using WGS. In both incidents, routine inoculation for aerobic culture, performed 24 h after donation, was negative for bacterial growth through 5 days [45].

During May–October 2018, four patients from three states experienced sepsis after transfusion of APs contaminated with *Acinetobacter calcoaceticus-baumannii* complex (ACBC) and *S. saprophyticus* [46]. Three APC donations, each yielding two units. The first donation was treated by PR technology. Septic shock developed in the recipient of one of the units. ACBC was isolated from posttransfusion patient blood cultures and from the PC bag residual. GS and culture of the second unit (not transfused) were negative. Fever and hypotension developed after the transfusion of one unit of the second donation that was negative on primary culture. ACBC was isolated by culture from PC bag residuals and posttransfusion blood samples from the patient. The patient died of septic shock. GS and culture of the second unit (not transfused) were negative. Fever and hypotension developed in two patients; each received a unit of PCs prepared from the third donation that was negative on primary culture. ACBC and *S. saprophyticus* were isolated by culture from post-transfusion blood samples from both patients and from both PC bag residuals. Within 5 h before transfusion, the hospital screened both PC units of the third donation with a rapid bacterial detection device; all tests were negative. Extensive multistate investigation of donors and environments, including WGS analysis—indicated that the 14 ACBC isolates from these sources were highly related, indicating a potential common source (that remained unidentified) of bacterial contamination among the four cases of STRs reported. In response, the FDA issued a notice informing blood establishments of recent reports of PC contamination with *Acinetobacter* species bacteria and encourage blood establishments and transfusion services to contact FDA concerning cases [47].

The possibility of exogenous contamination, that is, post-processing environmental contamination of a PC via a non-visible, acquired storage container leak was described [48]. A fatal STR occurred in a 63-year-old patient who received a pathogen-reduced PC transfusion. The residual PC was cultured, with the detected microorganisms undergoing 16S genotype sequencing. Gram-negative rods were detected in the PC unit and cultures of both PC and the patient's blood grew *A. baumannii* complex, *Leclercia adecarboxylata* and *S. saprophyticus*. These strains were effectively inactivated with >7.2, 7.7 and >7.1 log10 kill, respectively. The PC storage container revealed a leak visible only on pressure testing. Hospital environmental cultures were negative, and the contamination source remained unknown. *A. baumannii* complex and *S. saprophyticus* 16S genotyping sequences were identical to those implicated in the reported STR reviewed above.

REALITY—CONCLUSIONS

Despite every effort, bacterial contamination of PCs and risk of STR remains. For every mitigation strategy implemented, break-through TTBI was reported. Zero risk is unattainable with current technologies. This is not to despair. Significant risk reduction was achieved by one or a combination of more than one strategy. No one approach is feasible for all institutions. In selecting strategies, institutions should consider the possible impact on PC availability and entertain a risk-based decision-making approach that accounts for operational, logistical and financial factors.

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CONFLICT OF INTEREST

All authors declare that there are no conflicts of interest with any of the manufacturers included in this study or any other conflicts of interest relevant to the following manuscript.

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ORIGINAL ARTICLE

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Impact of COVID-19 on blood donor deferral patterns during the COVID-19 pandemic: A retrospective analysis

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Abstract

Background and Objectives: Blood donor deferral is an essential tool for blood safety. The ongoing COVID-19 pandemic has adversely affected blood transfusion services all over the world. But its impact on donor deferral rate and the pattern is unclear in light of the new donor deferral policy due to the COVID-19 pandemic.

Materials and Methods: This retrospective study was divided into pre-COVID and COVID (15 March 2019–14 March 2021). All the deferred donors were divided into six different categories: (1) medical causes, (2) surgical causes, (3) drugs and vaccination, (4) risk of transfusion-transmitted diseases, (5) miscellaneous causes and (6) flu-like symptoms. In addition, COVID-related deferrals were also incorporated. All these above categories along with the donor demography were analysed by SPSS software version 25.

Results: The donor deferral rate was 17.03% and 12.74% during the pre-COVID and COVID periods, respectively. During the pre-COVID period, Category 3 deferrals and during COVID period, Category 6 deferrals were significantly higher. A reversal in pattern with increased blood pressure (40.2% vs. 24.04%) over-riding low haemoglobin (34.77% vs. 55.5%) was noted in the Category 1 deferral during the COVID period. Category 1 deferral was more in middle-aged adults as compared to young and old adults ($p < 0.05$). Among middle-aged adults, deferral due to flu-like symptoms was also significantly more during the COVID period ($p < 0.05$).

Conclusion: COVID-19 significantly affected the donor pool and changed the pattern of donor deferral. Understanding donor deferral patterns may help in identifying targeted donor populations and planning donor recruitment strategies in future pandemic crises.

KEYWORDS
COVID 19, donor selection, temporary deferrals

Highlights

- This study observed medical causes as the most predominant reason for deferrals during both pre-COVID and COVID periods.
- There was a change in pattern with hypertension overriding low haemoglobin in the COVID period. Middle-aged adults were most commonly deferred group individuals.
- The deferral due to drugs and vaccination was significantly increased in the pre-COVID period, whereas flu-like symptoms were substantially more during the COVID period.

INTRODUCTION

Coronavirus disease caused by novel Severe acute respiratory syndrome coronavirus 2 (SARSCoV2) virus was first detected at Wuhan, China, on 31 December 2019. It was declared as a global pandemic on 11 March 2020, by World Health Organization (WHO). This pandemic has resulted in the collapsed health care system in many countries and has created a worldwide blood crisis [1]. Many nations imposed lockdown to decrease infection rate, further adding to decreased supply of blood products [2,3]. India also implemented a nationwide lockdown at different phases from 25 March 2020 despite reporting its first case on 27 January 2020, at Thrissur, Kerala. There has been no evidence suggesting the transmission of these viruses through blood products [1,4,5]. However, some studies have detected the presence of viral RNA in the plasma or serum of COVID-19 patients [6]. So, in the presence of unknown facts to ensure recipients and donor's safety, new recommendations relating to blood donation and deferral strategies were issued by various governing bodies like WHO, Association for the Advancement of Blood & Biotherapies (AABB), Food and Drug Administration (FDA), European Center for Disease Prevention and Control and Joint United Kingdom Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee [7–9]. Similarly, a stringent donor screening procedure with new donor deferral guidelines came into effect by the National Blood Transfusion Council (NBTC), ministry of health and family welfare, Government of India [10].

New deferral guidelines might have affected the donor pool and supply chain directly. Many works of literature have stated the effect of the pandemic on the blood transfusion services (BTS) from different corners of the world, focusing on blood donation and mitigation strategies [1,11,12]. We could hardly find any literature on the pandemic effect on the donor deferral pattern in light of the new deferral guidelines in India. So, this study was planned to assess the impact of COVID-19 on deferral patterns among blood donors and the impact of COVID-19 related deferrals on the BTS.

MATERIALS AND METHODS

Study period

This is a retrospective observational study conducted in the Department of Transfusion Medicine, AIIMS, Bhubaneswar, Odisha, India, on the blood donor deferral pattern over 2 years from 15 March 2019 to 14 March 2021. The total period was divided into pre-COVID (15 March 2019–14 March 2020) and COVID (15 March 2020–14 March 2021). The study was approved by Institutional Ethical Committee with the registration no: T/IM-NF/Trans.Med/21/02.

Donor deferral policy

NBTC deferral guidelines 2017 were followed during the pre-COVID period [13]. In light of the COVID 19 pandemics in March 2020, the latest NBTC guideline was implemented, which increased the donor deferral period from 7 to 28 days for donors with flu-like symptoms. In addition, donors with a history of travel/close contact or resident of hot spot/red-zone/containment zone or a history of COVID vaccination were also deferred for 28 days [10]. We included all the registered blood donors unfit for donation as per the latest NBTC guideline.

Data collection

The demographic details of the deferred donors were retrieved from the paper-based donor deferral record. The reason for deferral was broadly divided into six categories as follows.

Category 1: Medical causes: low haemoglobin as per donor selection guideline in Indian setting (Hb < 12.5 g/dl) [13], suspected polycythaemia (Hb > 16.5 g/dl in males and 16 g/dl in females), asthmatics on steroid therapy, uncontrolled hypertension/diabetes mellitus, thyroid disorder, epilepsy, liver disease, heart disease and infectious diseases.
Category 2: Surgical causes: any minor or major surgical procedure within 6 months or 1 year, respectively. Dental procedures and abortion were also included under this category [13].
Category 3: Drugs and vaccinations: donors on antithyroid, antiepileptics, antibiotics, anticoagulants, immunosuppressives, sedatives, non-steroidal anti-inflammatory drugs, aspirin, insulin, finasteride, dutasteride or with any history of vaccination, including rabies were deferred as per the latest guidelines [10].
Category 4: Transfusion transmitted diseases: donors with a history suggestive of high-risk behaviour, tattoo, ear piercing, jaundice, syphilis and gonorrhoea.
Category 5: Miscellaneous causes include donation interval less than 3 months, underage, history of alcohol, ongoing menstrual cycles, missed menstrual cycle, anxiety and previous vaso-vagal reactions.
Category 6: Flu-like symptom; symptomatic donors with a history of fever, flu-like symptoms or acute respiratory tract infections.

In addition, the donor deferral pattern during COVID time was classified as COVID-related deferral, which was further subdivided into four subclasses:

Subclass 1: Donors who were known COVID positive (within 28 days of recovery) or had history suggestive of flu-like symptoms, acute respiratory tract infections, and fever.
Subclass 2: Close contact with COVID positive patient or on quarantine period.
Subclass 3: History of travel from other countries or other states within the country.
Subclass 4: Donors from containment zone/red-zone/hot spot as declared by the Government of Odisha (as our Institute is under the Odisha State of Jurisdiction) were deferred for 28 days [14].

Initially, from March 2020 to June 2020, donors were deferred from the containment zone or red zone.

From June 2020 to August 2020, donors were deferred from the hot spot region (within 5 km of detection of cases) to increase the donor pool.

From September 2020 to November 2020, donors were deferred, if cases were detected within 500 m (microcontinent zone).

November 2020 onwards, donors were deferred if only there is a history of contact with a known COVID positive patient (within 28 days).

Definition of regular donor and repeat donor

Regular donor: A voluntary non-remunerated blood donor who has donated at least three times, the last donation being within the previous year, and continues to donate regularly at least once per year [15].

Repeat donor: A donor with a history of previous blood donation but the donation interval between two donations being more than 1 year is called repeat donor [15].

Statistical analysis

The data were collected from paper-based donor deferral record and was entered in excel sheet. Data analysis was done using SPSS version 25, IBM Corporation (Armonk, NY). Categorical variables were represented as frequencies and percentages. The chi-square analysis was performed for qualitative comparison among different donor deferral categories during the COVID period with the pre-COVID time and odds ratio (OR) was calculated. A *p*-value less than 0.05 was considered significant at a confidence interval (CI) of 95%. As age and sex were known to affect the deferral rate, logistic regression analysis was performed to assess the confounding effect.

RESULTS

During the pre-COVID and COVID period, a total of 10,568 and 9136 blood donors have been registered, respectively. The total deferral rate over 2 years in our institution is 15.04%. Out of these registered donors, 1800 (17.03%) and 1164 (12.74%) donors were deferred during the pre-COVID and COVID periods, respectively. Deferral patterns based on type, donation status, gender and different age group are depicted in Table 1. The deferral rate among the female donors and first-time donor was more than male and repeat donors. An increase in deferral rate was observed with increasing age of the donors during both pre-COVID and COVID period.

A similar trend of deferral pattern among the deferred donors was observed during both periods, with deferred male being substantially more than deferred females. The deferred repeat donors were slightly more as compared to first-time donors, although the difference was not significant. Among the deferred individuals, middle-age adults (25–44 years) were more than the young adults (<24 years) and older age group adults (>44 years). The temporary deferral was the most predominant type of deferral during both periods. Logistic regression analysis of all reasons for deferral showed middle-age

TABLE 1 Donor demographic profile and frequency distribution (percentage of the donor pool of that same domain) during the pre-COVID and COVID periods

	Pre-COVID (*n*) (%)[a]	Denominator (attempted to donate)[b]	COVID (*n*) (%)	Denominator (attempted to donate)[b]	Odds ratio (*p*-value)	Confidence interval
Total deferral	1800 (**17.03**)	**10,568**	1164 (**12.74**)	**9136**	0.71 (**<0.05**)	0.65–0.77
Type of deferral						
I. Permanent deferral	60 (**0.56**)	**10,568**	30 (**0.32**)	**9136**	1.30 (0.273)	0.82–2.10
II. Temporary deferral	1740 (**16.47**)		1134 (**12.42**)			
Gender						
I. Male	1419 (**14.09**)	**10,066**	1034 (**11.68**)	**8876**	2.03 (**<0.05**)	1.71–2.66
II. Female	381 (**75.89**)	**502**	130 (**50**)	**260**		
Number of donation						
I. First time donor	813 (**18.76**)	**4333**	555 (**16.96**)	**3271**	0.90 (0.186)	0.77–1.05
II. Repeat donor	987 (**15.82**)	**6235**	609 (**10.38**)	**5865**		
Age group (years)						
<24	501 (**15.38**)	**3257**	272 (**10.53**)	**2583**	**(p<0.05)**	
25–44	1100 (**16.60**)	**6623**	770 (**12.79**)	**6019**		
>44	199 (**28.92**)	**688**	122 (**22.84**)	**534**		

Note: Significance *p* value is boldfaced.

[a]Percentage is calculated by the denominator being the total number of donors of the same domain. Ex: Male donor registered in pre-COVID: 10,066, deferred donor: 1419 (total number of male donor donated = 10,066–1419 = 8647), percentage 1419/10,066 = 75.89%.

[b]Total number of donors registered (attempted to donate) in that particular domain.

adults and females are independent risk factors predicting the deferral rate as shown in Table 2. Applying 10% rule, age and sex were not found to be confounders in this study.

Deferral due to medical causes (Category 1) was higher during both periods, but no significant association was observed either with the pre-COVID or COVID periods. Category 3 deferral (deferral due to drugs and vaccines) was significantly higher during pre-COVID time than the COVID period (OR: 0.61, CI: 0.48–0.76, $p < 0.05$). In contrast, Category 6 deferral (deferral due to flu-like symptoms) was significantly associated with the COVID period with a much higher number of donor deferral during COVID time (OR: 4.47, CI: 3.04–6.58, $p < 0.05$) (Table 3, Figure 1a). Low haemoglobin (55.5% of 1123) followed by increased blood pressure (24.04% of 1123) accounts for most category 1 related deferral during the pre-COVID period. A reversal in the pattern where increased blood pressure (40.2% of 696) followed by low haemoglobin (34.77% of 696) was observed in medical causes during the COVID period though it was not statistically significant (Figure 1b). Females (52.4% of 618) contributed to a major share of low haemoglobin related deferral during the pre-COVID period, whereas males were predominant during the COVID period (56.01% of 241). The age group comparison showed significantly increased deferrals among middle-aged adults due to medical reasons than other age groups (Table 4). Among the middle age group donor deferral, the deferral due to drugs and vaccines, and miscellaneous deferral were much higher during the pre-COVID period. However, deferral due to flu-like symptoms during COVID time was significantly higher during the COVID period than pre-COVID time in this age group (Table 5).

Although the deferral due to drugs and vaccinations (Category 3) was significantly more during the pre-COVID period, still the deferral due to vaccination only was more during the COVID period (44.54% vs. 19.47%) (Figure 1c) There were more donor deferrals in Category 4 and 5 during the pre-COVID period but no significant difference observed (Table 3).

TABLE 2 Logistic regression analysis of effect of age and sex on donor deferral during pre-COVID and COVID period

Variables	Estimate	SE	*z* Value	*p*-Value
Intercept	−0.45998	0.07849	−5.861	<0.05
Age 25–44 years	0.21353	0.08972	2.380	**<0.05**
Age > 44 years	0.05404	0.13848	0.390	0.696
Sex (female)	−0.74781	0.10991	−6.804	**<0.05**

TABLE 3 Comparison of total deferral, different categories of deferral between pre-COVID and COVID periods (chi-square test)

	Pre-COVID	COVID	Odds ratio	*p*-Value	Confidence interval
Total registration	10,568	9136	0.71	**<0.05**	0.65–0.77
Total deferral	1800	1164			
CAT 1	1123	696	0.89	0.164	0.76–1.04
CAT 2	94	67	1.1	0.562	0.79–1.54
CAT 3	303	128	0.61	**<0.05**	0.48–0.76
CAT 4	115	65	0.86	0.387	0.62–1.19
CAT 5	128	64	0.76	0.093	0.54–1.04
CAT 6	37	100	4.47	**<0.05**	3.04–6.58

Note: CAT 1, CAT 2, CAT 3, CAT 4, CAT 5 and CAT 6 represents deferral due to medical causes, surgical causes, drugs and vaccination, transfusion-transmitted infections, miscellaneous causes and flu-like symptoms, respectively. Significance *p* value is boldfaced.

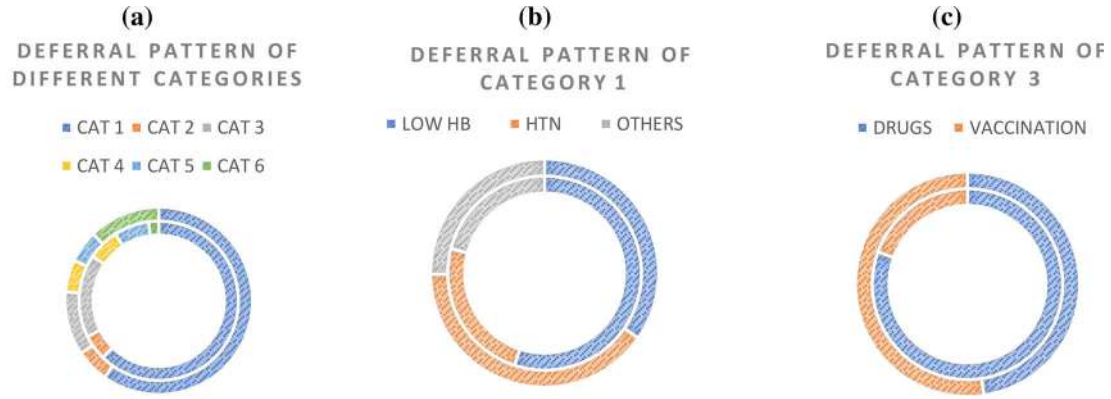

FIGURE 1 Different deferral patterns during pre-COVID and COVID period. (a) Different categories (b) Category 1 and (c) Category 3. The inner-circle represents the pre-COVID period. The outer circle represents COVID period

TABLE 4 Analysis of deferral pattern (six categories) in three different age groups of donor during pre-COVID and COVID periods

	15–24 years (V1)			25–44 years (V2)			>44 years (V3)		
Category	Pre-COVID	COVID	*p*-Value (V1 vs. others) Odds ratio CI	Pre-COVID	COVID	*p*-Value (V2 vs. others) Odds ratio CI	Pre-COVID	COVID	*p*-Value (V3 vs. others) Odds ratio CI
1	267	115	**<0.05**[a]	704	493	**<0.05**[b]	152	88	0.618
			0.63			1.44			0.92
			0.49–0.80			1.17–1.77			0.69–1.22
2	24	19	0.720	61	43	1.000	09	05	0.780
			1.15			0.96			0.76
			0.57–2.33			0.52–1.86			0.24–2.38
3	99	38	0.573	180	83	0.331	24	07	0.421
			0.87			1.26			0.67
			0.55–1.36			0.82–1.93			0.28–1.60
4	52	33	0.535	62	31	0.441	01	01	1.000
			1.24			0.77			1.70
			0.67–2.29			0.42–1.43			0.11–28.96
5	51	29	0.535	66	29	0.446	11	06	1.000
			1.25			0.77			1.10
			0.68–2.29			0.42–1.42			0.38–3.12
6	08	30	0.394	27	60	0.230	02	10	0.513
			1.55			0.55			1.94
			0.63–3.79			0.24–1.27			0.40–9.32

Note: Significance *p* value is boldfaced.
[a]Chi-square test was done between one age group (<24 year) versus remaining (24–44 years and >44 years) and so on.
[b]The significant difference of Category 1 deferral rate is because of the middle-age adults (25–44 years).

TABLE 5 Showing deferral pattern in 25–44 year age group during COVID and pre-COVID periods

	COVID period (n = count)	Pre-COVID period (n = count)	Odds ratio (p value)	Confidence interval
Medical deferral	493	704	1.00 (1.000)	0.82–1.21
Surgical deferral	43	61	1.00 (1.000)	0.67–1.50
Drugs and vaccine	83	180	0.61 (0.001)	0.46–0.81
High risk behavioural deferral	31	62	0.70 (0.130)	0.45–1.09
Miscellaneous deferral	29	66	0.61 (**<0.05**)	0.39–0.95
Deferral due to flu like symptom[a]	60	27	3.35 (**<0.05**)	2.11–5.34
Total deferral	739	1100		

Note: Significance *p* value has been bold faced.
[a]Deferral due to reason specific to COVID (total: 31) has been excluded.

COVID-related deferral comprised 12.6% of temporary deferrals during the COVID period. Subclass 1 was the prominent cause (100 cases out of 144, 69.44%) of COVID-related deferral. First-time donors (n = 71) and repeat donors (n = 73) constituted equally to COVID-related deferrals. The COVID-related deferral was more (n = 84) during the first 6 months than the subsequent 6 months (n = 60). Middle-aged adults (25–44 years) were twice more deferred as compared to young adults (15–24 years) and this difference was noteworthy but not significant in subclass 1 and 2 (Figure 2). The deferral pattern of the first time and repeat donors among the subclasses of COVID-related deferral were of similar distribution (Figure 2). There was no significant difference in the deferral pattern of various subclasses observed between first-time and repeat blood donors.

DISCUSSION

This study highlighted medical causes as the most predominant reason for deferrals during both pre-COVID and COVID periods. We noticed a change in pattern with hypertension overriding low haemoglobin in the COVID period. Middle-aged adults were most commonly deferred group individuals. The deferral due to drugs and vaccination was

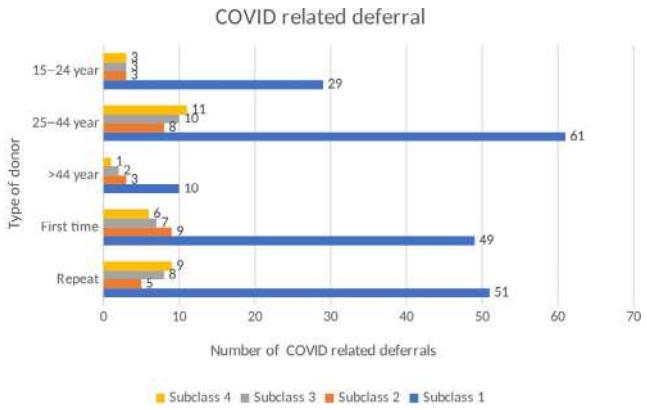

FIGURE 2 COVID-related deferral in different age group and type (first-time/repeat) of donor during the COVID period

significantly increased in the pre-COVID period, whereas flu-like symptoms were substantially more during the COVID period.

In this study, the overall deferral rate among blood donors is 15.04% more than the median rate of deferral of 12% as per the global WHO report, 2017 [16]. Donor deferral rate varies widely from region to region (1%–37%) depending on the pre-existing donor selection criteria, medical and endemic conditions [16,17]. Socioeconomic differences among the countries may be an added factor. Low prevalence of infectious diseases and good nutritional status among blood donors in high and middle-income countries are attributed to lower deferral rates than in low-income countries. On the other hand, stringent donor screening with better donor counselling on the high-risk behaviours of the donors may account for an increased rate of deferral [18]. Here, the deferral rate among first-time and female donors was higher than repeat and male donors. A low turnover of donors leads to a significant decrease in the deferral rate during the COVID period (Table 2). Mobility constraints during the lockdown and increased awareness about deferral criteria among regular donors through various social media platforms or telephonic consultation with blood centres might have contributed to the low turnover of donors.

A similar distribution of deferral pattern was found among the age group, gender, donation status (first-time vs. repeat donors) and type (permanent vs. temporary) throughout the study period. Though there was a significant reduction in deferral rate (12.74% vs. 17.03%) during the COVID period, a significant increase of deferred middle-aged young adults (66.15% of 1164) and males (88.83% of 1164) was observed. It might be due to our donor population characteristics, with the majority of donors being middle-aged adults [18]. Apprehension, anxiety about blood donation and fear of needles among young adults and females act as barriers to donor recruitment [19]. Studies from other parts of India also reported fewer female donors. Deferral rate among males outnumbered females in different studies from India [20]. Mobility restriction, closure of the college, universities during the pandemic might have resulted in the confinement of females and young adult males to home. On the contrary, a study from Iran reported an increase in female donors during the pandemic [21]. Though a rise in first-time donors is generally seen during disasters, we observed a decrease (41.00% of 10,568 vs. 35.80% of 9136) of first-time donors during the COVID periods [22].

We found medical causes to be the most common cause of deferral, with low haemoglobin accounting for most cases during the pre-COVID period and hypertension during the COVID period. Deferral due to low haemoglobin was more in females (52.4%) and males (56.01%) during the pre-COVID and COVID periods, respectively. It is the major cause for deferral in different parts of India and many countries due to the high prevalence of iron deficiency anaemia in repeat donors and childbearing females [13–25]. Low haemoglobin in males is generally associated with increased age, low body weight and regular blood donation [26]. A relative increase in anaemia among male donors during the pandemic may not be the true reflection of its prevalence as the number of female donors was less than the pre-COVID period. The prevalence of hypertension among adults is mostly underestimated [27]. About 31% of urban Indians are hypertensive. Stressful lifestyle, distorted sleep cycles, body mass index and habits like alcohol, smoking plays a major role in young hypertensives [28–30]. With the implementation of Joint National Committee 7 guidelines for hypertension (140/90 mm Hg) as a cut-off for blood donor selection (previously 160/100 mm Hg), hypertension is also emerging as a critical factor in donor deferral [31,32]. We noticed a reversal of pattern during the pandemic with more hypertension-related deferral. The reason for this is far-fetched. Reversal may be due to mental stress, anxiety and uncertainty, which prevail in the whole society because of the ongoing pandemic. The increased mobility among middle-aged adults may be the reason for higher deferral in this age group. With the introduction of COVID-19 vaccination in India from 16 January 2021, we noticed an increase in vaccine-related deferral in the latter half of the pandemic (Figure 1c). Till 15 March 2021, more than 30 million individuals were vaccinated for COVID-19 since its initiation. Nevertheless, deferrals due to drugs and vaccine are significantly more during pre COVID period. Easy accessibility of medications over the counter in India may increase drug use among adults for common ailments. Outdoor facilities were closed in many hospitals with increasing infections during different pandemic phases, limiting prescriptions. Strict drug control regulatory policies and extensive social media awareness regarding not to use any drugs until prescribed by the physician during a pandemic may be the reason for the decrease of donors on medications [33].

Unlike the study from the United States, where an increase in the rate of high-risk behaviour donors during disasters occurs, we observed a decline by less than 1% among deferrals due to factors associated with transfusion transmitted infection (TTI) reactivity [22]. Vassallo et al. also reported decreased behavioural risk and infectious disease rate in pandemic COVID 19 donors [34]. A newer perspective of risk behaviour and social distancing during pandemic might result in decreased TTI associated factors [35].

During this pandemic, increased donor deferral period from 7 to 28 days for flu-like symptoms and donors from containment zone, hot spot and contact history added to the burden of the donor pool. So there was a significant increase in the donor deferral rate during the

COVID period due to COVID-related reasons. At the peak of infection, the possibility of getting a disease is more. So the deferral related to flu-like symptoms was more during the strict lockdown period than the unlock period. Moreover, most of the first-time donors presented to our blood centre during COVID were relative to the admitted COVID-19 positive patients.

To the best of our knowledge, this is the first study that highlighted changing donor deferral during the pandemic in the Indian scenario. We hope that our study will help in better understanding the donor deferral pattern in the donor population due to the COVID-19 pandemic. This study will help plan donor recruitment and formulate new strategies on blood inventory management for future disaster or pandemic preparedness. However, it is a single-centre study, and it was a retrospective analysis, thus could not be generalized for all conditions and different settings. Many family members of COVID-19 patients were deferred without registering for blood donation to avoid any possible infection to the blood bank staff. Many donors from containment zone/hot spots without any flu symptoms or contact history were also deferred, which could have been included in the donor pool.

In conclusion, the donor deferral rate and the pattern have significantly changed in the ongoing pandemic due to COVID-19. There is an increased burden on BTS by reducing the donor pool. The increased deferral among middle-aged adults due to medical causes, especially hypertension and vaccination strategy needs to be evaluated and could increase the donor pool. In addition, implementing a prioritization policy of blood components for patients anticipating the blood shortage during pandemic and a stringent application of patient blood management principles will help mitigate the impact of the pandemic.

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CONFLICT OF INTEREST

The authors disclose no conflicts of interest.

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ORIGINAL ARTICLE

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It is about who you know (and how you help them): Insights from staff and donors about how to recruit and retain a panel of committed anti-D donors

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Abstract

Background and Objectives: Maintaining a panel of committed anti-D donors is crucial for the production of anti-D immunoglobulin to prevent haemolytic disease of the foetus and newborn. Given low numbers of donors in the Australian panel, there is a need to better understand motivators and barriers specific to anti-D donors.

Materials and Methods: A qualitative approach was used to gather perspectives of staff and current anti-D donors in Australia. Focus groups were held with staff involved with the anti-D programme. An asynchronous online discussion forum and interviews were conducted with donors. All data were coded using deductive and inductive thematic analysis.

Results: Staff stressed the importance of recruiting donors who met their own informal criteria as well as the formal selection criteria in order to maximize the chances of donors committing to making regular plasma donations. In contrast, donors were motivated by having a personal connection to anti-D, the recipient group and being eligible to join the programme. Support from staff and understanding the value of their donations also helped donors overcome concerns about the risks of joining the programme and reduced barriers to remaining in the programme.

Conclusion: Anti-D donors in Australia are motivated by multiple factors, including knowing who the recipient is, and dedicated staff are integral to building donors' commitment through education and support. Findings suggest the current approach to recruitment could be broadened to include all donors who meet formal selection criteria, with retention enhanced by reinforcing and rewarding the motives identified by donors for donating.

KEYWORDS
donor motivation, donor recruitment, plasma

Highlights

- Knowing who the recipient was motivated donors to overcome barriers.
- Staff were key to building donors' commitment through education and support.
- Blood Collection Agencies should consider how the identified motives of anti-D donors can be continually reinforced and rewarded to maintain their commitment to the programme.

INTRODUCTION

Since 1967, pregnant RhD negative Australian women have received anti-D immunoglobulin (anti-D) to prevent haemolytic disease of the foetus and newborn (HDFN) [1, 2]. HDFN can have serious health outcomes for the foetus or newborn, including anaemia, jaundice, brain damage or death [2–4]. Since the introduction of the anti-D donation programme, an estimated 2 million pregnant women in Australia have received anti-D [2] and mortality from HDFN has significantly reduced [4].

Australian Red Cross Lifeblood (Lifeblood) is responsible for ensuring the supply of anti-D in Australia through non-renumerated donors. A donor can be recruited into the Lifeblood anti-D programme if they fulfil these selection criteria: are RhD-negative and already have anti-D antibodies in their blood (due to immunization as a result of a previous blood transfusion or during pregnancy), or if they are RhD-negative and eligible to be intentionally immunized by administration of Rh-positive red blood cells[1] [2, 5]. All donors are given infusions of Rh-positive red blood cells to 'boost' their anti-D titre. The anti-D is then collected by apheresis donation, at a maximum interval of fortnightly. Side-effects associated with immunization and boosting include chills, fever, muscle and joint aches, and headaches. In addition, immunized donors requiring blood transfusions will need to receive Rh(D) negative blood, which can be difficult to obtain in some countries. Internationally, anti-D is collected in a variety of ways. In the Netherlands, anti-D is obtained through Rh-D immunized donors and imported products [6] while in the United States anti-D is collected from boosted, remunerated plasma donors. In the UK, most of the anti-D is imported from the United States [7].

It is difficult to determine how 'successful' a new anti-D donor will be in terms of the response to immunization and boosting and the donor's commitment to donating. Due to the time and cost invested in recruiting, immunizing and boosting individual donors, those who produce sufficient anti-D are encouraged by Lifeblood to donate plasma at least monthly and to make a long-term commitment. Lifeblood employs staff specifically to manage the anti-D programme who are in close contact with donors. In recent years, the stock of anti-D in Australia has dropped below target due to deferrals, and donors ending their donation careers. With only 128 active (donated plasma in the last 6 months) anti-D donors [8] there is an urgent need to recruit more donors into Lifeblood's programme. Standard recruitment methods have been employed but were insufficient to match the loss of donors from the panel. Further measures have not yet been explored as little is known about what encourages someone to become and remain an anti-D donor.

Slootweg and colleagues [6] conducted one analysis of the motivators, barriers and predictors of Rh-D immunized Dutch women becoming and remaining anti-D donors. They found the main barrier to recruitment was lack of information about the programme, while wanting to give back, wanting to prevent the incidence of HDFN and knowing that anti-D was needed were the strongest motivators to join and remain in the programme. While these findings are valuable, participation in this study was limited to naturally Rh-D immunized women and not all participants were blood donors. As such, there is still a gap in the knowledge around motivators and barriers for anti-D donors in the Australian context.

One motivator of anti-D donors may be knowledge that only a minority of donors (<20%) can provide this product. Jensen and colleagues [9] showed that O-negative whole-blood donors were aware of their rare status and felt obligated to donate. Motivations stemming from possessing a rare blood type [10] or providing a product that most donors did or could not [11] have also been more broadly documented in the whole-blood and plasma literatures.

Knowledge of the need for anti-D and being asked to donate frequently may also motivate donors. Canadian researchers found that plasma donors gained a sense of pride from donating plasma because it was in demand [12]. Further, Thorpe and colleagues [13] showed that this sense is enhanced in frequent plasma donors (6–10 donations per year) who identified few barriers to donating. Even first-time plasma donors, while they were aware that donating plasma is a 'bigger ask' than whole-blood, perceived it as an opportunity to enhance their self-concept as an altruistic donor through donating more frequently [14].

While these motivators may be relevant to anti-D donors, being in the anti-D programme requires a greater commitment, and donors are more likely to have a personal connection to anti-D recipients, providing opportunities for development of unique motivators and (potentially) barriers. Given these additional factors, social capital may be a useful concept for understanding the behaviour of anti-D donors. In the context of blood donation, social capital has been defined as benefits or resources 'arising from networks and interactions that accrue to individuals, groups of individuals and communities' [15], (pp 47). Blood donation is theorized as being associated with having, and accumulating, social capital through providing opportunities for individuals to engage in an activity that is meaningful, gives back to the community and is valued by their social networks, with staff playing a key role in these processes [15–17]. These elements may also be relevant to anti-D donors in Australia.

Given the limited knowledge of motivators and barriers specific to anti-D donors in the Australian context, there is a need for further research. In this paper, we discuss findings of a study on motivators and barriers to joining and remaining in an anti-D donation programme in Australia. We focus on the perspectives of both staff and donors to address the question from the organizational and donor perspectives.

MATERIALS AND METHODS

A qualitative approach was used to allow for the generation of new insights from the perspective of participants; staff working in the anti-D programme and current anti-D donors. We used online focus groups hosted on Microsoft Teams to gather anti-D co-ordinators' (co-ordinators) and medical officers' (MOs) perspectives on recruitment and retention of anti-D donors. These staffs are involved in identifying eligible donors, recruitment, scheduling appointments, boosting and safety management of anti-D donors.

Donors participated in either an asynchronous, online discussion group or a semi-structured interview. An online asynchronous discussion group is a focus group that uses an online written discussion board to post discussion topics that participants respond to in their own time [18]. This method was used to overcome barriers to research participation such as location or time commitments for the small, geographically dispersed panel of donors [18]. The discussion group was held over a two-week period using the online platform Blackboard CourseSites (Blackboard Learn, 2018). As some people are less comfortable with using technology or with group interaction, donors could opt to participate in a one-on-one telephone interview instead. This study was approved by Lifeblood's Human Research Ethics Committee (approval number 2020#6) and all data collection took place in July–August 2020. All participants gave informed consent before participating in the research.

Recruitment/participant selection

All five co-ordinators and five MOs involved with the anti-D programme were invited to participate. Donor participants were those who had donated anti-D via plasma in the past 6 months. Forty-four donors were sent an email with study information, 37 were reached by telephone and invited to participate, with seven unable to be reached.

Data-collection procedure

Staff focus groups

One focus group was conducted with the co-ordinators who manage the anti-D programme and one with MOs who provide medical oversight for anti-D donors. For each focus group, one researcher guided the discussion using a semi-structured topic guide (Data S1) and a second took notes. The focus groups took 90 min, were audio-recorded and transcribed verbatim. Transcripts were reviewed against the audio recording for inconsistencies.

Online asynchronous discussion group

Two moderators posted questions using a semi-structured question schedule (Data S2). Participants were able to post responses to the moderator's posts and those of the other participants at any time over a 2-week period. They were also able to post their own questions to participants. Responses were visible to all and could not be edited. At the end of the two weeks, the discussion data were downloaded, and deleted from the online platform.

Interviews

Telephone interviews were conducted by one of two researchers and varied in length from 30 to 75 min. The interview guide was based on the semi-structured question guide used for the discussion forum (Data S3). Interviews were audio-recorded, transcribed verbatim and reviewed against the audio recording for inconsistencies.

Analysis

The researchers developed initial coding frameworks for each data set from the literature and research aims [10, 11]. The donor interviews and staff focus groups were coded by one of two researchers, and both researchers coded the online forum. For both the donor and staff interviews, coding involved both deductive and inductive thematic analysis. The researchers came together regularly to agree on new codes and any changes to existing codes before adding these to the codebooks. Transcripts were then coded in NVivo 11 (QSR International). Following this initial process, the researchers met to identify higher-level themes relating to the research question that captured the codes; named and described each theme in a memo; and mapped out how the staff and donor data fitted into each of the themes [19].

RESULTS

In total, five anti-D co-ordinators and four MOs participated (response rate = 90.0%). A total of 23 donors participated in the study (response rate = 62.0%); 13 in the discussion forum (DF) and 10 in an interview (Int) (refer to Tables 1 and 2 for demographics).

Staff perspectives on recruiting and retaining a committed anti-D donor

Identifying eligible and suitable donors

Staff stressed the importance of identifying donors who met the formal selection criteria and had a good chance of committing to making regular plasma donations. From their collective experiences working in the programme, staff (MOs and co-ordinators) believed that donors with certain lifestyle and demographic characteristics were most likely to become committed anti-D donors. These included: living close to a donor centre, being older, being in good health, having the right lifestyle, ability to understand information about the programme and having time:

> That's a really important bit with recruitment is that there might be someone who on paper looks perfect, but unless they are able to come in regularly, unless they have a lifestyle which complements our requirements, there's not much point in recruiting them. (MO 3)

Given the expectation that anti-D donors commit to donating plasma monthly over a number of years, staff preferred prospective donors to already be frequent plasma donors. Staff did not think that

TABLE 1 Participant demographics

Demographics	Participated in online discussion forum ($n = 13$)	Participated in one-on-one interview ($n = 10$)	Total ($n = 23$)
Age			
Less than 30	0 (0%)	1 (10.0%)	1 (4.3%)
30–45	3 (23.1%)	0 (0%)	3 (13.0%)
46–55	5 (38.5%)	0 (0%)	5 (21.7%)
56–65	4 (30.8%)	4 (40.0%)	8 (34.8%)
More than 65	1 (7.7%)	5 (50.0%)	6 (26.1%)
Sex			
Female	2 (15.4%)	3 (30.0%)	5 (21.7%)
Male	11 (84.6%)	7 (70.0%)	18 (78.3%)
Total donation count Mean (SD)	263.0 (115.9)	252.9 (169.8)	258.6 (134.8)

TABLE 2 Staff demographics

Demographics	Anti-D co-ordinators ($n = 5$)	Medical officers ($n = 4$)	Total ($n = 9$)
Sex			
Female	5 (100%)	3 (75.0%)	8 (88.9)
Male	0 (0%)	1 (25.0%)	1 (11.1%)
Time in role working with anti-D donors (years)	9.2 (5.0)	14.5 (10.5)	11.6 (7.8)

educating lower frequency donors, or non-plasma donors, about the importance of anti-D would motivate them to donate plasma frequently if they had not already demonstrated this behaviour, even if donors already had anti-D antibodies:

> You want a history of them coming in off their own bat (by themselves). So, it's all well to say anti-D is special, but you don't want them to go, 'Oh, yeah, I'll donate every month', when they don't have that previous history of showing that commitment. You really do need them to show that. (Co-ordinator 1)

Donor perspectives on reasons for joining the anti-D programme

All donor participants were blood or plasma donors prior to joining the anti-D programme. Thirteen mentioned being invited to join the programme, while nine had enquired about the programme themselves. In contrast to staff perspectives on who should be recruited into the anti-D programme, a number of participants had not donated plasma prior to being invited to join, or enquiring about, the anti-D programme and others were not regular plasma donors.

Having a personal connection to anti-D

When asked why they wanted to become an anti-D donor a number of participants talked about understanding the value of anti-D either through their own experiences of receiving anti-D during pregnancy or because of a family member who was a recipient or prospective recipient. Those who had received anti-D viewed their participation as an opportunity to give back to a programme that had helped them. They talked about the decision to donate anti-D as a given due to the gratitude they felt for the programme:

> I had an injection after my daughter was born… and I thought I owed the world this because somebody was doing it for me way back then. (Int 1)

Participants also spoke about having personal connections to anti-D through their daughters, mothers or other family members who were Rh-negative and had either received anti-D or who they assumed would need it in the future. These participants talked about how being close to an anti-D recipient motivated them to join and to remain in the programme:

> Because…my niece had to get the Anti D antibody and she got through and that was around the time that I was donating. So…I felt really good about that. Because there was somebody I actually knew. (Int 4)

Donating for mothers and babies

All participants, regardless of their personal experiences with anti-D, talked about feeling strongly motivated to join the anti-D programme when they understood they would be helping mothers and newborn

babies. Participants thought that knowing who the recipient group was made their donations more meaningful, and some spoke of how they felt special to be able to help that specific group of recipients:

> Being chosen to participate on the program is like winning the lottery for me. I have been given the opportunity to help dozens/hundreds of mums and their baby. It is a pretty special gift to give someone and very few people in Australia get the chance to participate. I'm the lucky one! (DF4)

Happy to help, wanting to do more and feeling special

A small number of participants mentioned joining the programme because they viewed donating anti-D as an opportunity to make a bigger contribution; were asked to by a staff member or because they were eligible to join. These donors perceived the anti-D programme to have value, with little additional effort required from them and they felt special at being able to do so because they had Rh(D)-negative blood.

Balancing benefits and risks during the recruitment process

Informing and educating donors

Staff considered it important for prospective donors to demonstrate engagement with, and understanding of, information given to them during the recruitment process by asking questions and discussing concerns they might have about the risks of immunization and red cell boosts with friends and family. Staff described themselves as 'the donor's advocate' in that they felt responsible for ensuring that donors were able to consent fully to joining the programme:

> I'll ask them what their understanding of the program is, I'll ask if they understand the potential risks that we discuss in the first interview and if they've spoken with family, relatives, their doctor…and if they've got any questions. (MO 2)

While staff placed emphasis on donors understanding the risks of joining the programme, the majority of participants did not recall having concerns about the risks. The small number of donors who did, explained that staff had addressed their concerns during the recruitment process and they did not perceive the potential risks to outweigh the benefits of joining the programme, such as helping the recipient group:

> My main concerns were long term health effects. After discussions with my wife and reading the literature that I was sent it was a pretty easy decision to join the program. Pretty hard to say no to all those mums and bubs. After all I was already donating plasma every fortnight. (DF11)

Other donors indicated that the information given to them by staff reassured them that the anti-D programme was well established and, as such, not risky. Moreover, they trusted the process, the staff and the organization.

Remaining a donor

We asked donors about their intentions to remain in the anti-D programme, and what would influence these intentions, and staff their perspectives on why donors remained in the programme. Staff believed that the current panel of anti-D donors were dedicated to the programme, demonstrated through donating frequently and regularly and committing to receiving red cell boosts despite potential side effects. Staff also noted that very few donors leave the anti-D programme voluntarily and that donor attrition was usually due to deferrals for health reasons.

Donors also expressed their dedication to the programme. They aimed to donate anti-D frequently, wanted to remain in the programme for as long as possible and took actions to avoid missing donations and to maximize the length of their donation careers. For example, some scheduled holidays around donation appointments, while others described eating certain foods and exercising to stay healthy so they could donate anti-D for as long as possible and avoid deferrals (such as for low haemoglobin). They expressed disappointment when they were unable to donate, either through temporary deferrals or illness. As this donor indicates, the participants desired to be the best donor they could while in the anti-D programme, and believed that other anti-D donors shared this goal:

> So that's my goal, to stay fit and healthy and eat well and exercise and give them those years. That's what I want. All the other people that are in my program …, that's exactly what they want as well. (Int 1)

Donors explained their dedication to the programme derived from their awareness of how few anti-D donors were in the panel, the importance of their donations for recipients and concerns about a shortage of anti-D if they did not donate. Some described feeling obliged to donate as often as possible. While information about the small number of donors in the anti-D panel initially came from staff, donors did not indicate that they felt any pressure from staff to donate frequently. Rather they positioned the sense of obligation as emerging from their own desire to contribute to the programme:

> I do feel a sense of responsibility. I'm aware that producing Anti-D is a resource-intensive process and I think I need to be worth the effort. I feel a little guilty about not donating fortnightly. This is a feeling that originates within me, though. There has been nothing but gratitude and encouragement expressed by staff. (DF10)

Making it 'easier' to donate and overcoming barriers

Both staff and donors talked about the systems that existed at Lifeblood to help anti-D donors overcome some of the typical barriers to donation, such as booking appointments at suitable times or changing appointment times. Co-ordinators observed that the anti-D donors were able to contact them directly if they had questions or needed help to make or change appointments. Donors appreciated having access to staff and agreed it made their experience of donating easier. Donors also discussed receiving contact and support from staff to manage any side-effects from boosts.

Overall, donors did not raise many barriers to being in the anti-D programme. None of the donors suggested that it was difficult for them to commit to frequent and regular donations. In contrast, most were able to make frequent donations because of working or living close to a donor centre, being retired or being able to take time off work to donate. The barriers that were discussed related to being a frequent donor, such as difficult phlebotomy because of scar tissue, and wait times in donor centres.

When asked under what circumstances they would stop donating anti-D, the majority of donors said they would only stop when advised they were no longer eligible. Most believed this would likely be due to illnesses related to advanced age. A few donors thought that they might leave if they moved far away from a donor centre during their retirement.

DISCUSSION

Maintaining a panel of committed anti-D donors is crucial for the production of anti-D immunoglobulin to prevent HDFN in Australia and internationally. In this paper, we present perspectives from staff and donors on motivators and barriers to becoming and remaining an anti-D donor. We suggest that donors are motivated largely through understanding the value of the product they donate and that the dedicated staff in the anti-D programme are integral to building donors' commitment through the provision of education and supporting donors through physiological, social and practical aspects of anti-D donation. Further, while there is an interesting interplay between staff and donor perspectives on the requirements for recruitment, our data suggest that the current approach to recruitment could be broadened to include all donors who meet formal selection criteria to help meet the ongoing need for more anti-D donors.

Donors positioned their motivation to join the programme as derived from personal experiences and understanding who they were helping. The identification of personal experiences as important is consistent with Slootweg and colleagues [6] in their research on anti-D donors in the Netherlands, and with the identification in the broader donor literature of donors being motivated to donate to repay the Blood Collection Agency (BCA) for products they (indirect reciprocity [self]) or loved ones (upstream [friends and family] indirect reciprocity) received [10, 11]. Given our findings, and with Slootweg and colleagues, we suggest raising awareness of anti-D donor programmes among anti-D recipients and their families in order to attract new donors to the programme [6]. Knowing who their donations would help is consistent with the literature on the identifiable victim effect. This literature shows that identified victims, in this case, mothers and babies, elicit powerful emotional responses resulting in a greater willingness to help them, [20]. In this study, despite not all participants being plasma donors, or having demonstrated an ability to donate frequently prior to joining the anti-D programme, participants expressed pride and gratitude at being able to help this recipient group. This overcame any concerns they may have had about risks to their health and the behaviour desired of them.

Consistent with Slootweg and colleagues [6], a small number of participants viewed being an anti-D donor as an opportunity to make a bigger contribution as a donor because of who the recipient group was and the organizational need for anti-D. This motivation may be similar to that identified in frequent plasma donors [13].

Donors' experience in the anti-D programme was shaped and enhanced by the staff in that programme. Staff noted that they play an integral role in selecting donors most likely to be able to donate at the frequency required and commit to the programme in the longer term. They believed the most efficient way to recruit committed donors was by identifying those who already possessed characteristics and behaviours thought to be important to success in the anti-D programme. This approach was informed by the belief that the organizational 'ask' of anti-D donors was high in terms of the required commitment, and that donor knowledge of who they were helping would not be sufficiently motivating for donors to commit to donate plasma frequently for a number of years. However this view of how to optimally recruit contrasted with the perspectives of, and motivations, noted by donors. When recruited, many of the existing donors did not meet the staff's selection criteria (i.e., being frequent plasma donors) and viewed their engagement with the anti-D programme as being self-initiated. The importance of donor initiation to conversion success has been shown in the broader plasma literature [21]. As such, providing in-centre and online collateral that both educates in terms of desirable attributes given the demands of the role (i.e., living close to a centre, healthy lifestyle, capacity to donate regularly) and stimulates interest and engagement from those motivated [22] may be an effective way to generate a donor led expansion of the anti-D donor panel.

Despite this contrasting view on recruitment, staff and donors both agreed that donors were dedicated to the programme. Donors expressed a sense of responsibility to remain in the programme due to their awareness of their importance to its ongoing success. This awareness is accumulated largely through the efforts of staff. We suggest that these interactions between donors and staff across the anti-D donor career build trust and community between donors and the other actors involved in the anti-D programme—recipients, staff, donors and the BCA, which leads to donor dedication to the programme. These interactions between donors and staff build social relationships and social capital as donors come to view themselves as a valued and integral part of a process that helps others [17].

While our analysis is limited by its focus only on current committed donors and staff already involved in an anti-D programme in one

BCA in Australia, the analysis suggests that the distinct characteristics of anti-D donation and the product that results are intrinsically motivating for donors who commit to the programme. In the Australian context, these motives are guided into an ongoing commitment to frequent donation through dedicated staff who reduce identified barriers [23], reinforce the ongoing need for anti-D and help these donors build social capital. While BCAs should always strive to minimize barriers for those donors most needed, such as by giving them priority in donor centres and assigning experienced phlebotomists to them, other BCAs wishing to build a committed anti-D panel should consider how the identified motives of anti-D donors can be continually reinforced and rewarded to maintain these donors' involvement with and commitment to the programme.

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R.T. designed and conducted the study, analysed the data, co-wrote the first draft of the manuscript and significantly revised and edited subsequent drafts. S.K. conducted the study, analysed the data, co-wrote the first draft and edited the manuscript. B.M. advised on the study design and reviewed and edited the manuscript. The Authors would like to acknowledge Perfecto Diaz and Glen Shuttleworth for data management. We would also like to thank the donors and staff who participated in the study for their time.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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ENDNOTE

[1]To be eligible, women must be postmenopausal or have had a hysterectomy.

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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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Evaluation of platelet concentrates prepared from whole blood donations with collection times between 12 and 15 min: The BEST Collaborative study

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Abstract

Background and Objectives: In many countries, whole blood (WB) donations with collection times between 12 and 15 min are not allowed to be used for platelet concentrates (PC). Since the development of guidelines, many process-related changes have been introduced. We aimed to determine the effect of WB with long collection times on PC quality.

Materials and Methods: Five participating centres tested buffy coat (BC)-derived PC in platelet additive solution type E prepared from only WB collections lasting <12 min (control) versus similar PC including one BC from a collection lasting >12 min (study group, $n = 8$). One centre produced platelet-rich plasma (PRP)-derived PC from single donations (<10 or >12 min). All PC were stored at 22 ± 2°C and sampled on Days 1, 6 and 8 post-collection for in vitro quality determination.

Results: Average collection time was significantly longer in the study group compared to controls (8.9 ± 2.6 vs. 7.3 ± 1.3 min, $p < 0.001$). There were no differences in volume, platelet concentration, basal CD62P expression, soluble-CD62P and CCL5 levels, or nucleotide content between the groups. Stimulation with TRAP-6 resulted in comparable levels of cell surface CD62P. On Day 8, all PC fulfilled requirements for pH. The findings from single PRP-derived PC centre were similar.

Conclusion: PC with one BC and single PRP derived from collections lasting >12 min had equivalent in vitro quality to controls during storage. This study provides evidence that 12–15 min donations should not be excluded for PC preparation and justifies to readdress the guidelines to <15 min instead of <12 min of collection in line with current practice in some countries.

KEYWORDS
buffy coat platelets, long collection time, platelet concentrates, whole blood collections

Highlights

- Platelet concentrates with one buffy coat from collections lasting >12 min had equivalent in vitro quality to controls during storage.

- Also 'worst case' pooled platelet concentrates with three long collection time buffy coats or single PRP derived from collections lasting >12 min had equivalent in vitro quality to controls during storage.

INTRODUCTION

Platelet concentrates (PC) for transfusion are collected by plateletpheresis or produced from whole blood (WB) via buffy coat (BC) or platelet-rich plasma (PRP) methods. When produced from WB, there are some specific time constraints regarding duration of the blood collection. The Council of Europe Guidelines [1] stipulate that for WB collections, the blood should not be used for the preparation of platelets if the duration of the collection is longer than 12 min, and that the plasma should not be used for direct transfusion or for the preparation of coagulation factors if the duration of the collection is longer than 15 min. The same rules are applied for blood collection in Australia, where Council of Europe guidelines are mandated, but not for those in the United States [2], Canada [3] or the United Kingdom [4]. There is little evidence for the exclusion of WB with a collection time between 12 and 15 min to be used for platelet component production. Previous publications have demonstrated that donations between 8 and 12 min for PRP-derived platelet production are acceptable, but this study did not provide data to suggest slower collections should be excluded [5]. Similarly, minor differences between PRP-derived platelets manufactured from donations with a 5- to 10-min or from 10- to 15-min collection time have been demonstrated [6]. However, a significant decrease in platelet recovery was observed in components prepared from collections lasting longer than 15 min.

Moreover, since the development of these guidelines, improved blood mixers and new production methods, including bottom-and-top separation and PC production from pooled BC, have been introduced. Therefore, re-evaluation of the quality of PC from donations of longer durations is justified.

Under current collection conditions, the number of donations taking between 12 and 15 min varies from 1% to 9% at the participating centres. In the absence of any time constraints, it would be easier to fulfil the need for BCs to produce pooled PCs, especially with the current trend for reduced erythrocyte usage resulting in reduced WB collection. Preliminary studies with single BC-derived PCs from long collection time WBs were published [7], showing minimal in vitro quality differences between platelets derived from WB with collection times <10 min or >12 min. To collect further evidence that BCs prepared from WB donations with a collection time no longer than 15 min can be used in platelet pooling, a multi-centre study was conducted through the BEST Collaborative (www.bestcollaborative.org).

MATERIALS AND METHODS

Pooled BC-derived PC

Five centres (Sites A, B, C, D and E; Table 1) each produced two groups of 8 BC-derived PC from pools of 4 or 5 BCs, one with BC from WB collections of ≤12 min duration (control group), and the other included 1 BC from a collection of >12 min duration (study group). WB collections were in citrate-phosphate-dextrose (CPD) and each centre used their own BC production process, followed by production of leukoreduced PC from a BC pool. The BC pools were prepared by adding 250–300 ml platelet additive solution type E (PAS-E, SSP+, MacoPharma, Mouvaux, France) to 4 BC, or 300 ml PAS-E (T-PAS+, Terumo BCT, Lakewood, CO) to 5 BC to produce suspensions in 60%–70% v/v PAS-E with 30%–40% plasma v/v. After soft spin centrifugation, the platelet-rich supernatant was expressed through an in-line filter into a standard storage bag, either manually or using a semi-automatic press. One centre (Site D) used the fully automated TACSI system and protocol (Terumo BCT). One centre (Site F) produced PRP-derived PC in plasma from single donations ($n = 8$), either lasting <10 min or >12 min. In all centres, PC were stored for 8 days at 22 ± 2°C with agitation.

Worst case study

WB donations (500 ml) anticoagulated with CPD were centrifuged and separated after overnight hold into red cell concentrates, plasma

TABLE 1 Summary of studies by participating centres

Site	Country	PC production method	Additive solution	Repeats
A	Australia	4 BC-pooling	PAS-E	8
B	Czech Republic	4 BC-pooling	PAS-E	8
C	Germany	4 BC-pooling	PAS-E	8
D	Ireland	4 BC-pooling	PAS-E	8
E	Netherlands	5 BC-pooling	PAS-E	8 (5)[a]
F	Brazil	Single PRP	None	8

Abbreviations: BC, buffy coat; PAS-E, platelet additive solution type E; PC, platelet concentrates; PRP, platelet-rich plasma.
[a]Worst case study.

and BCs. To obtain a 'worst case' PC ($n = 5$), three long collection time BCs were pooled with two standard BCs, diluted with 300 ml PAS-E (SSP+) and after a soft spin, the supernatant PC was leukodepleted via an in-line filter into a polyvinyl chloride container with butyryl trihexyl citrate plasticizer (C5000, Fresenius, Bad Homburg, Germany).

PC in vitro tests

All PCs were sampled on Day 1 or 2, 6 and 8 post-collection for measurement of platelet count, mean platelet volume (MPV), pH, glucose, lactate, CD62P expression and annexin-V binding, thrombin-receptor activated protein (TRAP)-6 induced activation, morphology/swirling, hypotonic shock response, nucleotide concentrations and supernatant levels of soluble CD62P (sCD62P), CCL5 (RANTES) and microparticles. Not all laboratories were able to perform all measurements.

Platelet counts and MPV were determined using haematology analysers (CELL DYN, Abbott, Chicago, IL; Sysmex 2000i or Sysmex XS1000i, Sysmex, Kobe, Japan). pH was measured at 22°C using pH meters (MP230 or SevenMulti, Mettler Toledo, Giessen, Germany, converted to 37°C value) or at 37°C using blood gas analysers (ABL90, Radiometer, Copenhagen, Denmark; cobas b221 OMNI system, Roche, Basel, Switzerland). Glucose and lactate were measured on blood gas analyser, on i-STAT (Abbott Point of Care, Princeton, NJ) or cobas 701 (Roche).

Flow cytometry

Platelet CD62P expression was determined at baseline and after stimulation with serial dilutions of TRAP-6 (Sigma-Aldrich), principally after the method described by Middelburg et al. [8], although different concentration ranges of TRAP-6 were used in different centres (Sites B, D, E: 0.038–625 μM, Site A: 0.31–40 μM, Site C: 3.3–16.6 μM). Platelets were stained with anti-CD62P-PE or anti-CD62P-FITC (BD Biosciences, San Jose, CA; EXBIO, Vestec, Czech Republic) and FACSCalibur or FACSCanto II flow cytometers (Becton Dickinson, Franklin Lakes, NJ) were used to assess fluorescence for determination of the percentage positive platelets.

Phosphatidylserine (PS) exposure was measured by annexin-V-FITC binding (Biolegend, San Diego, CA; EXBIO; VPS Diagnostics, Hoeven, The Netherlands) using the same flow cytometers. Details of flow cytometry assays have been previously described [9–11].

Morphological and functional properties

Morphology was determined according to the method of Kunicki [12]. Swirling effect of platelets was judged as present or absent, or ranked from very good (3 or 2) to absent (0).

Hypotonic shock response was measured using 250 or 300 $\times$ 10^9/L platelets diluted in freshly thawed plasma and a light transmission aggregometer (Chronolog, Havertown, PA; Helena Laboratories, Beaumont, TX) [13].

Nucleotide concentrations

To check the energy status of the platelets, nucleotides like adenosine triphosphate (ATP), adenosine diphosphate and adenosine monophosphate (AMP) were extracted from platelets with perchloric acid, as previously described [14]. All extracts were shipped to centre E, where the nucleotide concentrations were determined using high-performance liquid chromatography with a Partisphere SAX column and $NaH_2PO_4.H_2O$/acetonitrile gradients as running buffer at 254 nm extinction [15].

Supernatant samples

Each centre prepared supernatants of PC samples by centrifugation at 1600*g* for 20 min at room temperature. Supernatant samples were aliquoted and stored at −80°C. Site B assessed CCL5 and sCD62P levels using commercially available ELISA kits (EVOLIS system, Bio-Rad, Hercules, CA) according to the manufacturer's instructions. At Site A, centralized procoagulant phospholipids (PPL) and thrombin generation assays were performed as measurements of supernatant microparticles. Procoagulant activity of microparticles in the supernatant was determined using a STA-Procoag-PPL kit (Diagnostica Stago Ltd, Asnieres, France), measuring clot formation in seconds using an automated coagulometer (STACompact, Diagnostica Stago Ltd), as previously described [16]. Thrombin generation by the platelet microparticles was also measured using a Calibrated Automated Thrombogram (CAT; Thrombinoscope BV, Maastricht, The Netherlands). Thrombin generation was measured using a PRP reagent (Thrombinoscope BV), which contains 1 pmol/L Tissue Factor to assess the presence of phospholipid in the sample, as previously described [16].

Statistical analysis

Data are presented as mean ± SD, unless stated otherwise. Statistical analyses of the controls versus long collection time PCs, the PRP-derived PC and the worst-case PCs were performed using unpaired *t*-tests (Microsoft Excel for Office 365 MSO, Microsoft Corp, Redmond, WA) comparing each study group with its corresponding control group, $p < 0.05$ was considered significant. Combined data from PCs in PAS-E were analysed using SPSS version 23.0 (IBM Corporation, Armonk, NY). Results from controls and long collection time PCs were compared across the storage period using a repeated measures two-way analysis of variance, where $p < 0.05$ was considered to be significant.

TABLE 2 In vitro quality of pooled platelet concentrates in PAS-E, prepared from whole bloods including one with long collection time (study group, S) compared with controls (C, results per site, $n = 8$)

		Site A		Site B		Site C		Site D		Site E	
	Day	S	C	S	C	S	C	S	C	S	C
Volume (ml)	1	387 ± 26	407 ± 8	253 ± 19	259 ± 17	309 ± 22	290 ± 30	320 ± 17	314 ± 12	369 ± 7	368 ± 8
Platelets ($\times 10^9$)	1	331 ± 46	321 ± 49	202 ± 35	222 ± 23	357 ± 47	337 ± 21	396 ± 59	416 ± 58	357 ± 40	367 ± 47
pH (37°C)	1	7.04 ± 0.01	7.04 ± 0.07	7.04 ± 0.01	6.99 ± 0.02***	6.95 ± 0.06	7.97 ± 0.04	7.20 ± 0.03	7.18 ± 0.04	7.10 ± 0.03	7.11 ± 0.01
	8	7.21 ± 0.05	7.17 ± 0.09	7.04 ± 0.07	7.04 ± 0.05	7.02 ± 0.08	7.04 ± 0.05	7.15 ± 0.13	7.11 ± 0.13	7.15 ± 0.05	7.16 ± 0.02
Glucose (mmol/L)	1	6.2 ± 0.6	6.0 ± 0.8	5.6 ± 0.9	5.6 ± 0.3	7.3 ± 0.8	7.2 ± 0.6	7.7 ± 0.4	7.6 ± 0.2	7.3 ± 0.2	7.4 ± 0.1
	8	3.5 ± 0.9	3.6 ± 1.2	1.4 ± 0.8	1.2 ± 0.6	3.7 ± 1.5	3.8 ± 0.9	2.7 ± 1.6	1.9 ± 1.6	4.0 ± 0.6	4.2 ± 0.4
Lactate (mmol/L)	1	3.8 ± 0.4	5.2 ± 1.5*	7.6 ± 1.3	9.2 ± 0.6**	5.6 ± 0.8	5.3 ± 0.8	5.3 ± 0.6	5.5 ± 0.7	3.7 ± 0.6	3.6 ± 0.5
	8	9.0 ± 1.0	9.9 ± 2.1	16.5 ± 1.9	19.0 ± 1.1*	12.1 ± 2.1	11.7 ± 1.8	18.9 ± 3.9	20.7 ± 4.6	9.4 ± 0.8	9.5 ± 1.0
CD62P (% pos.cells)	1	3.4 ± 1.2	6.5 ± 6.2	32.0 ± 9.1	30.9 ± 3.7	18.3 ± 5.8	18.0 ± 6.0	19.4 ± 7.6	22.2 ± 2.2	14.8 ± 2.0	15.5 ± 3.9
	8	19.8 ± 5.4	15.5 ± 3.7	56.8 ± 3.9	51.3 ± 3.9*	32.5 ± 3.4	37.9 ± 6.9	43.1 ± 7.5	39.8 ± 5.6	35.7 ± 4.1	34.5 ± 2.5
sCD62P (ng/ml)	1	16 ± 3	15 ± 3	46 ± 23	41 ± 8	23 ± 5	23 ± 7	31 ± 16	27 ± 9	21 ± 7	24 ± 7
	8	38 ± 8	31 ± 6	129 ± 26	144 ± 25	51 ± 18	56 ± 17	82 ± 28	80 ± 18	75 ± 18	91 ± 23
Annexin V (% pos.cells)	1	0.4 ± 0.2	0.7 ± 0.4*	ND	ND	ND	ND	2.1 ± 1.1	2.5 ± 1.1	6.5 ± 3.7	5.7 ± 3.6
	8	1.5 ± 0.5	1.5 ± 0.8	ND	ND	ND	ND	8.5 ± 6.3	6.1 ± 1.7	14.4 ± 4.9	13.8 ± 4.5
MPV (fl)	1	ND	ND	9.0 ± 0.4	9.9 ± 0.6**	9.5 ± 0.4	9.5 ± 0.4	ND	ND	9.3 ± 0.4	9.2 ± 0.3
	8	ND	ND	9.3 ± 0.5	9.6 ± 0.7	9.2 ± 0.2	9.3 ± 0.4	ND	ND	9.2 ± 0.3	9.2 ± 0.2
HSR (%)	1	73 ± 7	75 ± 6	ND	ND	87 ± 9	83 ± 8	ND	ND	63 ± 9	65 ± 10
	8	62 ± 10	65 ± 7	ND	ND	72 ± 6	71 ± 7	ND	ND	62 ± 7	57 ± 9
CCL5 (ng/ml)	1	9 ± 3	13 ± 6	24 ± 15	32 ± 6	33 ± 19	29 ± 19	30 ± 10	24 ± 7	33 ± 20	37 ± 21
	8	57 ± 13	68 ± 15	122 ± 64	118 ± 23	95 ± 23	101 ± 32	163 ± 43	164 ± 52	132 ± 33	130 ± 34
PPL activity (s)	1	50.8 ± 5.6	58.7 ± 6.3*	36.7 ± 4.0	36.6 ± 4.8	ND	ND	ND	ND	ND	ND
	8	50.1 ± 6.0	49.3 ± 3.2	32.9 ± 1.9	32.9 ± 1.7	ND	ND	ND	ND	ND	ND
TGA lag time (s)	1	7.5 ± 1.1	8.4 ± 1.0	5.0 ± 0.5	5.0 ± 0.5	ND	ND	ND	ND	ND	ND
	8	6.8 ± 0.8	6.8 ± 0.3	5.4 ± 0.3	5.7 ± 0.5	ND	ND	ND	ND	ND	ND
TGA peak thrombin (nM)	1	60.6 ± 14.0	56.5 ± 5.6	108 ± 11.6	110 ± 4.7	ND	ND	ND	ND	ND	ND
	8	64.0 ± 6.1	74.7 ± 8.1*	98.9 ± 10.6	102 ± 6.8	ND	ND	ND	ND	ND	ND

Note: Data represent mean ± standard deviation and were analysed using a *t*-test for each site separately.

Abbreviations: CCL5, C-C motif chemokine ligand 5; HSR, hypotonic shock response; MPV, mean platelet volume; ND, not determined; PAS-E, platelet additive solution type E; PPL, procoagulant phospholipid; TGA, thrombin generation assay.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ versus corresponding test group.

RESULTS

Pooled BC-derived PC

Each centre produced 8 PC from pooled BC (four centres with 4 BC and one centre with 5 BC per pool) from WB with standard collection times (control group) and 8 PC containing 1 BC from a long collection time WB (study group). The WB units of the control group had a mean collection time of 7.3 ± 1.3 min ($n = 168$), whereas the collection times in the study group were on average longer: 8.9 ± 2.6 min ($n = 168$, $p < 0.001$), due to inclusion of one long collection time WB per PC with a collection time between 12 and 15 min (average 13.1 ± 0.8 min).

The PCs in the control and study groups had similar volumes and platelet concentrations, when compared per centre (Table 2). Similarly, no significant differences were detected in product composition between the two groups when all $n = 40$ PCs were pooled (see Table S1). There was a broad range in the characteristics of both products, with relatively large overall standard deviations due to the different preparation methods, but there were no differences between the groups.

The in vitro quality parameters during storage are also shown in Table 2 and Table S1, with average values for 2–5 centres, as not all centres performed all of the tests. Per centre, very small but clinically not meaningful differences were observed over storage time (Table 2). Combined results showed no significant differences between the two groups over the 8-day storage period (Table S1).

No differences between the groups were found in expression of activation marker CD62P (combined results), or for supernatant levels of sCD62P and CCL5 at the various time points. Furthermore, comparable levels of activation were observed following stimulation of platelets with a range of TRAP-6 concentrations on each sampling day at each concentration for the different time points, as measured by the percentage of CD62P-positive cells (Figure 1a). In Figure 1b, CD62P expression of platelets is depicted after stimulation with the maximal TRAP-6 concentration. On Day 8, pH (mean: 7.14 ± 0.11) fulfilled requirements in all units, and all units had a swirling effect and/or morphology score >200. Over storage time the glucose consumption

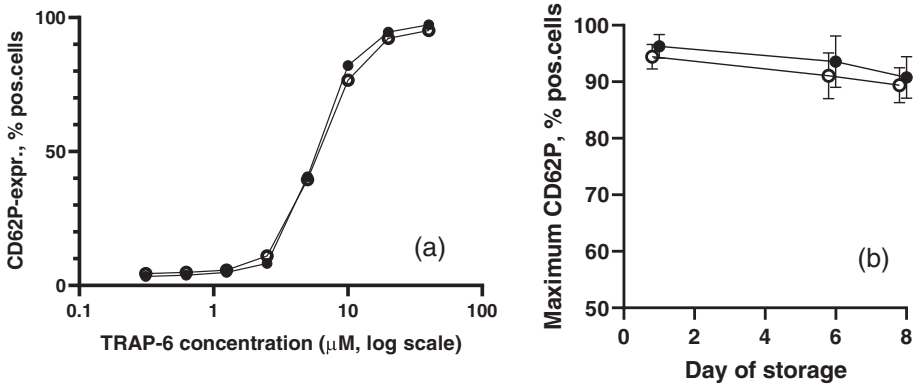

FIGURE 1 (a) Representative curve for TRAP-6 stimulated CD62P-expression on Day 1 ($n = 1$); (b) representative maximum CD62P-expression following TRAP-6 stimulation at maximum concentration ($n = 8$, ● study group, ○ control group, Site A). Data represent mean ± standard deviation

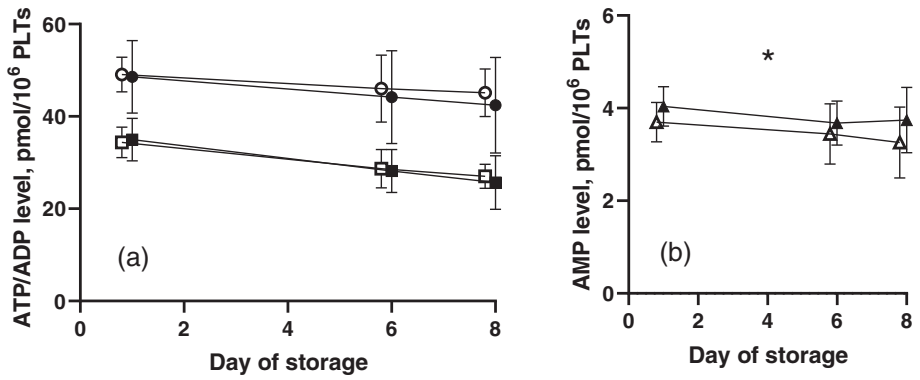

FIGURE 2 Nucleotide levels of study group (filled symbols) and control group (open symbols): (a) ATP (●, ○) and ADP (■, □); (b) AMP (▲, △); pooled data from Sites A, C and E; data represent mean ± standard deviation; *$p < 0.05$ (two-way analysis of variance). ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; PLT, platelets

was 0.06 ± 0.02 mmol/day/10^{11} platelets, with concomitant lactate production of 0.13 ± 0.05 mmol/day/10^{11} platelets.

The combined nucleotide levels of the PCs in the control group (Figure 2) were similar to that of the study group, with the exception of AMP, which was (statistically) significantly higher across the entire storage period ($p = 0.016$).

Single PRP-derived PC

Results obtained from the single PRP-derived PC in plasma are given in Table S2. The trend in the findings from this centre was similar to those in pooled BC-derived PC: no significant differences between the control and study PC were observed in the majority of variables. Only a small significant difference in AMP level on Day 1 was observed (Table S2).

Worst case study

As a worst-case situation, centre E performed an additional study with PC in PAS-E ($n = 5$) derived from 5 pooled BCs, with 3 BC from WB with long collection times per PC. No significant differences were found compared to standard PC (Table S3).

DISCUSSION

Several jurisdictions, including Europe and Australia, do not allow production of platelets from WB units with a collection time longer than 12 min [1]. As there is a lack of data to justify or refute this rule, a multi-centre study was undertaken to determine whether WB donations with a collection time longer than 12 min could be used for platelet production without affecting the in vitro quality of the final product. This study demonstrated that BC-derived PCs that included one BC from a long collection time WB have equivalent in vitro quality to controls during 8-day storage. A pilot study suggests that this is also the case for PRP-derived PC. Despite differences in production methods between the participating centres, it was possible to compare the results, due to the use of standardized methods and centralized testing.

Measures of platelet activation, including cell surface CD62P, sCD62P and annexin-V binding demonstrate that including long collection time BC in PC preparation does not lead to increased platelet activation in the resultant products. This was further evidenced by procoagulant activity (supernatant phospholipid and thrombin generation), which was not different in the two groups. The similarity in responses to TRAP-6 in the two groups indicates that longer phlebotomy times did not diminish the capacity of platelets to respond to agonist stimulation. The only significant difference between the two groups with respect to quality was an increase in AMP for the study group. This suggests minor degradation of ATP, as even a 1% decrease in the ATP concentration will lead to a doubling of the AMP concentration. Similar effects have been found in studies examining the effects of various processing or storage conditions for erythrocytes (D. de Korte, personal communication).

A limitation of this study is that only one long collection time BC was included in the pooled PC from the study group. The chance of having two long collection time BC in 1 PC is around 1 in 1000 PC with a frequency of 1% long collection time and around 1 in 23 PC for the centres with 9% long collection times. Moreover, the findings in the PRP study (which can be considered as a worst-case situation, Table S2), the preliminary study with single BC-derived PCs [7] and the worst-case study with three long collection time BC in a pool of 5 (Table S3), although limited, suggest that more than one long collection time BC in one PC would not reduce the quality of the PC.

An argument against use of BCs from long collection time WB donations might be that these units are more likely to contain blood clots. Data from two participating centres showed that indeed long collection time WBs have a higher frequency of clots, but all BCs are inspected for presence of clots and therefore likely to be identified and discarded.

Overall, this study provides evidence that BC from WB donations lasting 12–15 min can be included in PC preparation, and justifies a change in Council of Europe guidelines, as well as current practice in some countries.

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CONFLICT OF INTEREST

The authors declare no conflicts 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

Assessment of bacterial growth in leukoreduced cold-stored whole blood supports overnight hold at room temperature prior to filtration: A pilot study

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Abstract

Background and Objectives: Whole blood (WB) transfusion has regained attention to treat trauma patients. We reported no significant changes in in vitro quality through 21 days of cold storage for leukoreduced WB (LCWB) when time to filtration was extended from 8 to 24 h from collection. This study evaluated the impact of extended WB-hold at room temperature (RT) prior to leukoreduction on proliferation of transfusion-relevant bacteria.

Materials and Methods: WB units were spiked with suspensions of *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Listeria monocytogenes* prepared in saline solution (SS) or trypticase soy broth (TSB) to a concentration of ~0.2 CFU/ml ($N = 6$). Spiked units were held at RT for 18–24 h before leukoreduction and cold-stored for 21 days. Bacterial growth was determined on days 2, 7, 14 and 21. In vitro quality of WB inoculated with unspiked diluents was assessed.

Results: *K. pneumoniae* and *S. pyogenes* proliferated in WB prior to leukoreduction reaching concentrations ≤10^2 CFU/ml. These bacteria, however, did not proliferate during the subsequent cold storage. *S. aureus* did not survive in WB while *L. monocytogenes* reached a concentration of ~10^2 CFU/ml by day 21. LCWB in vitro quality was not affected by SS or TSB.

Conclusion: Extended WB-hold prior to leukoreduction allowed proliferation of bacteria able to resist immune clearance, although they did not grow to clinically significant levels. While *L. monocytogenes* proliferated in LCWB, clinically relevant concentrations were not reached by day 21. These data suggest that transfusing LCWB may not pose a significant bacterial contamination safety risk to transfusion patients.

KEYWORDS
bacterial contamination, cold storage, whole blood

The views expressed in this article do not necessarily represent those of the Canadian Federal government.

Highlights

- Bacteria able to resist immune clearance can grow in whole blood (WB) hold at room temperature prior to leukoreduction by filtration.
- Bacteria that grow in cold-stored WB do not reach clinically significant levels after 21 days of storage.
- Transfusing cold-stored leukoreduced WB may not pose a major bacterial contamination safety risk to transfusion patients.

INTRODUCTION

Transfusion of fresh whole blood (WB) was a widespread practice prior to the development of modern technologies for blood component manufacturing and is still a customary practice in some countries [1]. Interestingly, transfusion of low-titre group O WB has received renewed attention to treat actively bleeding patients in both military and civilian trauma care [2, 3]. WB transfusion possess clinical and logistical advantages over component transfusion, with improved rates of patient survival in prehospital and emergency settings [4–6].

A dual-centre case-match study of trauma patients who were treated with either cold-stored WB (CWB) or red blood cell concentrates (RBCC) showed a higher mean haemoglobin and haematocrit in patients who received CWB at 24 h posttransfusion and had less trauma bay mortality although 30-day mortality was not different between the two groups of patients [5]. Williams et al. described their experience of transfusing uncrossmatched, group O CWB in comparison to plasma or RBCC for civilian trauma resuscitation [7]. In their study, patients who received CWB had lower postemergency department transfusion and an increased prospect of survival. Similarly, Shea et al. reported improved outcome in patients who were transfused with low-titre group O WB [8]. More recently, a single-centre retrospective study showed no differences in major clinical outcomes in patients receiving CWB compared to recipients of blood component therapy [9].

Despite the benefits of treating trauma patients with CWB, introduction of this blood product poses logistical challenges to blood suppliers. Outstanding questions that need to be addressed include: leukoreduction requirements, type of anticoagulant used, evaluation of shelf-life, defining in vitro quality control criteria, compatibility with pathogen reduction technologies and inventory management [10].

Canadian Blood Services is considering the (re)introduction of CWB as a leukoreduced transfusion product (LCWB). As per manufacturer's instructions, leukoreduction should be performed within 8 h of blood collection. However, we have recently demonstrated that extending the time to WB filtration from 8 to 24 h, had no significant effect on LCWB in vitro quality and functionality during 21 days of refrigerated storage [11]. A similar approach of extending the time prior to leukoreduction has been adopted in this study to evaluate the impact of WB hold at room temperature (RT) on bacterial survival.

Hold of WB at RT prior to leukoreduction may affect survival of contaminant bacteria introduced during venipuncture. Bactericidal and bacteriostatic action of WB during RT hold prior to leukoreduction has been reported by us and others. Gravemann et al. showed that bacterial survival in WB and distribution into blood components is strain-dependent [12]. We have also demonstrated that overnight hold of bacterially inoculated-WB resulted in the elimination of some bacteria while others were able to survive or proliferate [13]. Similar findings have been documented by Mohr et al. following 8 h of WB storage at RT [14].

Bacteria that resist immune clearance prior to WB leukoreduction, may not necessarily proliferate during cold storage as recently shown by Braathen et al. [15]. In their study, *Escherichia coli*, which was not fully eliminated before WB leukoreduction, did not replicate during storage at 2–6°C. However, the safety risk posed by bacteria able to proliferate in CWB (i.e., psychrotrophic) was described by James and Stocks in 1957 [16]. In this study, we evaluated the impact of WB hold at RT for 18–24 h prior to leukoreduction on proliferation of transfusion-relevant bacteria, including a psychrotrophic species, during cold storage for 21 days.

MATERIALS AND METHODS

Bacterial strains and inoculation of WB storage bags

Four transfusion-relevant bacteria were used in this study: *Klebsiella pneumoniae* PEI-B-P-08 and *Streptococcus pyogenes* PEI-B-P-20, which are able to proliferate in WB [12]; *Staphylococcus aureus* PEI-B-P-63, representing a species often involved in transfusion reactions [17] and *Listeria monocytogenes* PEI-A-199, which grows in RBCC [18]. Five-ml suspensions containing 100–200 colony forming units (CFU) prepared in 0.9% saline solution (SS) or trypticase soy broth (TSB) supplemented with 15% glycerol were inoculated into transfer bags, which were covered with absorbent paper and bubble wrap with the port facing up and stored at −30°C for approximately 24 h in the Canadian Blood Service Microbiology Laboratory in Ottawa (Ontario, Canada) (Microlab). The bags were shipped to the Canadian Blood Services netCAD Blood4Research Facility in Vancouver (British Columbia, Canada) (netCAD), where they were stored at −30°C until they were used for WB spiking. The diluents SS and TSB were used to ensure bacterial survival during shipping from Ottawa to Vancouver under freezing conditions.

These two diluents were tested in preliminary assays and showed to support bacterial viability in transfer packs stored at −30°C; however, it was not clear if one of the two diluents would better support bacterial viability during shipping from Ottawa to Vancouver, and therefore, suspensions were prepared in both diluents to optimize WB spiking.

WB: Donation, bacterial spiking and leukoreduction by filtration

At netCAD, the transfer bags containing the bacterial suspensions were allowed to thaw for 30 min at RT. Thirty WB units were collected in citrate–phosphate-dextrose from research consented donors. Within 6-h of collection, WB units were mixed and transferred into the spiked transfer bags to reach initial bacterial concentrations of about 0.2 CFU/ml. Six WB units were spiked with each bacterial species, three with a suspension prepared in SS and three with a suspension prepared in TSB. Spiked WB units were leukoreduced (IMUFLEX WB SP filtration sets, TerumoBCT, Lakewood, CO) between 18 and 24 h post-stop bleed time as described before [11] providing >18 h for bacterial proliferation at RT. The resulting leukoreduced WB units were stored at 1–6°C for 1-2 h and shipped back to the Microlab in refrigerated boxes where they were stored at 1–6°C for 21 days.

Determination of bacterial concentration

On days 2, 7, 14 and 21 of storage, WB samples were serially diluted and plated on blood agar (BA). Colony counts were obtained after BA plate incubation at 37°C for 18–24 h. Units with negative growth on BA by day 21 of cold storage, were tested with BACT/ALERT cultures (bioMérieux, St. Laurent, QC, Canada). Three repetitions were performed per diluent (SS and TSB) for a total of six repetitions per bacterium.

In vitro quality assessment of LCWB

The potential impact of SS or TSB on in vitro LCWB quality was determined using published protocols [11]. Briefly, each three LCWB units containing 5 ml of the unspiked diluents SS or TSB were warmed at RT for 30 min, manually mixed and sampled. Leukocyte count was measured on day 1 only, while other parameters were monitored throughout 21 days of storage. WB volume was determined by weight, residual leukocytes by flow cytometry (Beckman Coulter FC500, Mississauga, ON, Canada), metabolic activities with a blood gas analyser (GEM5000, Instrumentation Laboratories, Bedford, MA), RBC and platelet counts as well as haemoglobin and haematocrit with a haematology analyser (Sysmex XN1000, Mississauga, ON, Canada), haemolysis with a HemoCue device (HemoCue, Brea, CA) and platelet activation and ADP response to degranulation by flow cytometry FACSCanto II (BD, Franklin Lakes, NJ).

Statistical analyses

Bacteria concentrations were Log10 transformed with zero values adjusted to 1. Data were summarized as mean with SD. Analysis of variance was applied to test the difference between diluents, and analyses were performed using Statistical Analysis System [19]; $p < 0.05$ was considered statistically significant.

RESULTS

In vitro quality and bacterial growth of LCWB is comparable in both diluents

While only 5 ml of SS or TSB (representing ∼1% of the WB volume) was used, the potential impact of these diluents on in vitro LCWB quality was determined. Quality parameters collected throughout 21-day storage were compared to previous data [11]. No impact on in vitro quality by either diluent was observed (Table 1). Similarly, no significant differences in bacterial viability were found between SS and TSB ($p > 0.05$).

S. aureus loses viability in LCWB

S. aureus' initial concentration in WB was 0.09 ± 0.02 Log10 CFU/ml. Except for one of the six repetitions, this bacterium was quickly eliminated when in contact with WB (Figure 1). All repetitions yielded negative BACT/ALERT culture results on day 21 of cold storage.

K. pneumoniae and *S. pyogenes* proliferated in WB held at RT prior to leukoreduction but not in LCWB

The initial concentrations of *K. pneumoniae* and *S. pyogenes* in the WB units were 0.10 ± 0.04, and 0.08 ± 0.01 Log10 CFU/ml, respectively. These bacteria grew during the hold of WB at RT prior to leukoreduction reaching concentrations of 1.80 ± 1.13 and 1.00 ± 0.42 Log10 CFU/ml, respectively. While *K. pneumoniae* did not survive during subsequent WB cold storage, which was confirmed with negative BACT/ALERT culture results on day 21, *S. pyogenes* remained viable yielding a concentration of 0.62 ± 0.45 Log10 CFU/ml by day 21 (Figure 1).

L. monocytogenes proliferated in LCWB

L. monocytogenes' initial concentration (0.09 ± 0.02 Log10 CFU/ml) in WB was maintained during the RT hold prior to leukoreduction and it slowly proliferated during subsequent cold storage reaching 1.95 ± 1.86 Log10 CFU/ml on day 21 (Figure 1).

TABLE 1 In vitro quality of WB inoculated with unspiked SS and TSB and subsequent cold storage

In vitro WB quality parameters (units)		Day 0		Day 1		Day 7		Day 14		Day 21		Day 21
		SS	TSB	SS	TSB	SS	TSB	SS	TSB	SS	TSB	Comparison
Process related	WB volume (ml)	n/d	n/d	471 ± 8	476 ± 6	459 ± 8	467 ± 6	451 ± 8	459 ± 6	444 ± 8	451 ± 6	448 ± 9
WBC related	WBC count ($\times 10^6$/unit)	n/d	n/d	0.200 ± 0.092	0.282 ± 0.201	n/d	n/d	n/d	n/d	n/d	n/d	0.400 ± 0.312[a]
Metabolism related	Glucose (mM)	19.4 ± 0.5	20.9 ± 1.6	15.4 ± 0.1	16.4 ± 1.5	14.0 ± 0.1	14.1 ± 1.6	12.1 ± 0.1	12.2 ± 1.7	10.8 ± 0.1	10.5 ± 1.7	12.0 ± 5.5
	Lactate (mM)	1.5 ± 0.5	1.4 ± 0.2	7.2 ± 0.3	8.8 ± 2.1	10.9 ± 0.2	12.7 ± 2.5	15.1 ± 0.4	16.2 ± 1.4	19.6 ± 0.3	19.8 ± 1.8	17.9 ± 2.0
	K^+ (mM)	2.8 ± 0.1	3.1 ± 0.1	3.7 ± 0.2	3.9 ± 0.8	9.2 ± 0.5	9.6 ± 1.2	13.8 ± 0.9	14.3 ± 1.6	18.2 ± 0.9	18.7 ± 3.3	16.5 ± 2.1
	pH (@25°C)	7.46 ± 0.02	7.46 ± 0.01	7.35 ± 0.01	7.37 ± 0.02	7.29 ± 0.03	7.28 ± 0.02	7.24 ± 0.06	7.18 ± 0.03	7.10 ± 0.03	7.12 ± 0.01	7.11 ± 0.02
RBC related	RBC count ($\times 10^{12}$/unit)	3.91 ± 0.28	412 ± 0.30	3.98 ± 0.31	4.20 ± 0.15	3.99 ± 0.32	4.31 ± 0.20	3.95 ± 0.31	4.26 ± 0.24	4.02 ± 0.19	4.20 ± 0.17	4.21 ± 0.42
	Haematocrit (L/L)	0.362 ± 0.015	0.376 ± 0.012	0.390 ± 0.027	0.404 ± 0.006	0.393 ± 0.028	0.416 ± 0.020	0.391 ± 0.021	0.414 ± 0.006	0.406 ± 0.013	0.411 ± 0.005	0.428 ± 0.031
	Haemoglobin (g/unit)	56 ± 4	67 ± 2	58 ± 6	61 ± 1	56 ± 6	61 ± 1	55 ± 4	59 ± 1	55 ± 3	56 ± 1	57 ± 7
	Haemolysis (%)	0.002 ± 0.03	0.05 ± 0.00	0.30 ± 0.17	0.20 ± 0.10	0.39 ± 0.22	0.22 ± 0.12	0.35 ± 0.17	0.22 ± 0.13	0.43 ± 0.20	0.28 ± 0.14	0.23 ± 0.15
PLT related	PLT yield ($\times 10^9$/unit)	82 ± 6	102 ± 20	55 ± 9	68 ± 8	49 ± 11	64 ± 8	41 ± 14	54 ± 7	32 ± 15	44 ± 8	52 ± 12
	CD62P (%)	n/d	n/d	33.9 ± 2.2	13.1 ± 3.1	68.8 ± 1.8	57.2 ± 3.7	72.3 ± 1.9	70.6 ± 4.7	68.3 ± 14.2	73.8 ± 8.6	76.4 ± 11.0
	ADP response (% CD62P)	n/d	n/d	50.5 ± 7.2	60.1 ± 8.6	12.1 ± 3.9	23.3 ± 4.3	−0.6 ± 4.7	9.2 ± 0.9	6.5 ± 21.9	6.8 ± 5.3	2.2 ± 9.9

Note: In vitro quality parameters (average ± SD; $n = 3$ for each diluent) determined of WB containing only the diluent of the bacteria, either SS or TSB upon leukoreduction filtration on day 0 and throughout cold storage for 21 days. As control data, corresponding data obtained from a previous study with WB that did not include diluents [11] is displayed in the right column shaded in light grey.
Abbreviation: N/d, not determined; PLT, platelet; SS, saline solution; TSB, trypticase soy broth; WB, whole blood; WBC, white blood cell.
[a]Day 1 data.

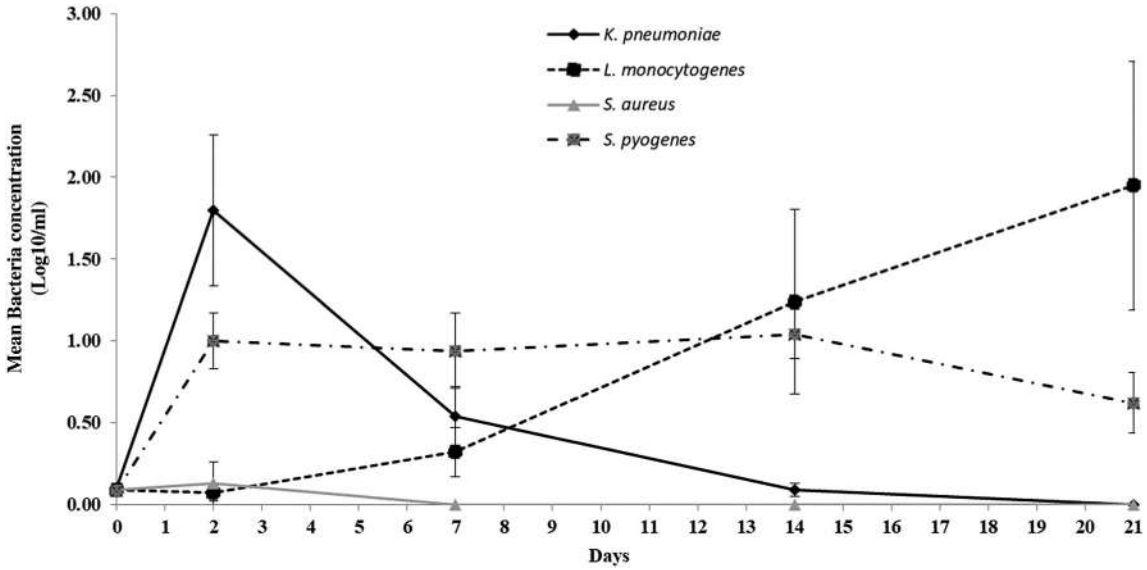

FIGURE 1 Bacterial growth during cold storage of leukoreduced whole blood (WB). Growth curves of *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Streptococcus pyogenes* are depicted over 21 days of storage of LCWB at 1–6°C. Each data point represents mean bacterial concentration ± SD ($N = 6$)

DISCUSSION

We demonstrated that WB hold at RT prior to leukoreduction allows proliferation of bacterial species able to resist clearance by the immune factors present in the collected blood. These findings confirm results previously published by us and others showing that bactericidal and bacteriostatic effects of WB depend on the bacterial species and strain [12, 13]. More specifically, we have shown that bacterial survival in WB depends on the strain resistance to plasma factors and the killing action of neutrophils [20]. In order to verify that the diluents used to prepare bacterial suspensions (SS and TSB) had no impact on in vitro WB quality, and subsequent effect on bacterial survival and/or proliferation, unspiked WB units with only the respective diluent volumes were analysed.

In our study, three out of the four tested bacteria, *K. pneumoniae*, *L. monocytogenes* and *S. pyogenes*, were able to survive or proliferate during holding of WB at RT prior to leukoreduction. An interesting study by Graham et al., has shown that gene expression in *S. pyogenes* changes rapidly upon exposure to human blood [21]. Virulence genes such as those encoding for superantigens and host-evasion proteins were upregulated indicating that these genes are important for bacterial survival in the hostile blood environment. Similarly, Franca et al. showed that exposure of *S. epidermidis* biofilms to human blood resulted in upregulation of several genes, including those involved in iron utilization, indicating that this change was important for bacterial survival [22]. Gram-negative bacteria such as *K. pneumoniae* are more likely to resist immune clearance if they are equipped with mechanisms to resist the action of complement-mediated killing and phagocytosis. It has been shown that the outer membrane protein A of *E. coli* prevents the formation of the Complement Membrane Attack Complex [23]. In addition, the capsule of *K. pneumoniae* poses a physical barrier that may prevent phagocytosis [24].

Despite the survival of *K. pneumoniae*, *L. monocytogenes* and *S. pyogenes*, in WB at RT, each of these species showed different abilities to grow during subsequent cold storage. Surprisingly, refrigeration had a bactericidal effect on the *K. pneumoniae* strain used in our study. This species has been shown to resist refrigeration conditions [25], and thus we speculate that the slow decline of viability observed in our assays is the result of strain-dependent sensitivity to the killing action of blood factors released during cold storage. *L. monocytogenes*, on the other hand, is recognized by its characteristic psychrotrophic growth. This bacterium is a common contaminant of refrigerated food and has also been shown to proliferate in RBCC [18, 25]. Importantly, none of the three bacteria, *K. pneumoniae*, *L. monocytogenes* and *S. pyogenes*, reached clinically relevant bacterial concentrations (i.e., ≥10^5 CFU/ml) [26] either during WB hold at RT or during cold storage for up to 21 days.

As *K. pneumoniae* grew to approximately 10^2 CFU/ml during the RT hold of WB, it is unlikely that harmful endotoxin levels were accumulated before cold storage. Jacobs et al have shown that endotoxin concentrations >11,000 EU are associated with septic shock symptoms [26], and we have demonstrated that these levels of endotoxin are linked to >10^3 CFU/ml of common RBC Gram-negative contaminants such as *Yersinia enterocolitica* and *Serratia marcescens* [27].

We showed that *S. aureus* was quickly eliminated when in contact with WB. The natural habitat of this species is the human mucosa although it is often found colonizing human skin. The strain used in our study was chosen for its proven ability to grow in platelet concentrates [28]. The results obtained herein, therefore, indicate that this

specific isolate is highly susceptible to immune factors present in WB that are eliminated during manufacturing of platelet concentrates. Other *S. aureus* isolates involved in bloodstream infections adapt successfully to evade immune clearance. Malachowa and DeLeo showed that when a methicillin-resistant strain of *S. aureus* was cultured in human blood, genes involved in iron uptake and haemolysis were upregulated, indicating that these factors contribute to the ability of this isolate to become a bloodstream pathogen [29].

This pilot study could be complemented by testing other bacterial species and isolates. Although a comparison of WB filtration times was not performed, our data suggest that extending the time to leukoreduction from 8 to 24 h, may not pose a significant bacterial contamination safety risk to transfusion patients.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

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Psychological impact of the COVID-19 pandemic on young professionals in blood banks and transfusion services: A global cross-sectional survey

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Abstract

Background and Objectives: The COVID-19 pandemic brought about changes to daily life as measures to contain the spread of the virus increased across the world. The aim of this survey was to assess the psychological impact of the pandemic on young professionals (YPs) in transfusion medicine.

Materials and Methods: A cross-sectional web-based survey was distributed electronically to ISBT members inviting YPs (≤40 years) to participate. Statistical analysis was performed using SPSS software.

Results: Two hundred and fifty-nine YPs completed the survey, including 107 clinicians/physicians and/or nurses. Almost half of the YPs (52.5%) indicated increased stress levels and 15.4% indicated symptoms of depression. YPs highlighted the loss of social engagement (59.1%) and increased pressure from information seen on media (35.5%) as factors negatively impacting their psychological wellbeing. Further, 20.8% expressed increased economic stress resulting from concerns about job security. Almost half of the YPs indicated that their organization provided moderate/occasional holistic support to them and their families. Sixty percent and 74.4% of YPs reported increased workload and staff absence due to COVID-19 infection, respectively. Only half of clinicians/physicians and/or nurses indicated that they often had sufficient personal protective equipment. The majority of these (76.6%) had family/household members living with them, and 61% indicated that they were significantly worried about infecting them because of the nature of their work.

Conclusion: COVID-19 had a major impact on the well-being of YPs working in transfusion medicine. Measures are required to ensure that YPs are protected and mentally supported while undertaking their duties in current and future pandemics.

KEYWORDS
COVID-19, transfusion, young professionals

Highlights

- Young professionals experience a range of negative psychological impacts during the COVID-19 pandemic, including increased stress levels.
- Different sources of stress were reported, including being exposed to COVID-19 at work, increased workload, social isolation and restriction of movement.
- Having a clear understanding of the mental health and well-being of the young professionals during the pandemic would ensure that those affected receive support from their organizations.

INTRODUCTION

On 11 March 2020, the World Health Organization (WHO) declared a novel coronavirus disease 2019 (COVID-19) pandemic [1]. As cases increased and the death toll surged, measures to contain the spread of the virus escalated, primarily relying on compliance with public health recommendations including the quarantine of infected and exposed individuals, closure of businesses and public spaces and travel restrictions. These restrictions caused immediate disruptions to daily routine, schooling, finances and individual well-being with the additional fear of long-term impacts. For example, different stressors were identified during quarantine, including fears for personal and family health, inadequate basic supplies and inadequate information from public health authorities [2]. It has been suggested that the prolonged quarantine experienced by many may result in post-traumatic stress symptoms, depression, insomnia, confusion and anger [2], while the social and economic consequences of COVID-19 may lead to longer term loss of social connections and loneliness [3].

Young people are some of those most affected by the pandemic's socioeconomic and psychological impact [4]. Studies on the psychological impact of the COVID-19 pandemic showed that many young adults experienced increased anxiety and depression symptoms provoked by lockdown and social distancing [4, 5]. Healthcare workers are potentially more vulnerable to the negative psychological impact of the COVID-19 pandemic through their risk of increased exposure, resulting in high rates of stress-related symptoms, depression, generalized anxiety and insomnia [6–9].

This survey aims to assess the impact of COVID-19 pandemic on the psychological well-being of young professionals (YPs) working in blood bank and transfusion services around the world. Assessing this impact is important to provide data that will enable health authorities to allocate resources to support YPs.

METHODS

A cross-sectional electronic survey administered in English was designed by the ISBT Young Professional Council after a review of the literature on the impact of COVID-19 pandemic on YPs. The survey included questions that assessed the pandemic's psychological impact on YPs using a previously published validated scale to assess COVID-19-related anxiety [10]. In addition, the survey included questions that assessed changes in the work routine for YPs and the degree of organizational support received by YPs. Survey development took place between July and August 2020, with input obtained from an expert in behavioural medicine and psychology. The survey was reviewed by the council members for face and content validity to assess if the content was suitable to capture the information needed. The test was programmed electronically via Survey Monkey by an expert on questionnaire construction who further checked the survey for common errors. After multiple rounds of testing and editing the electronic survey, the survey was piloted among 30 YPs (aged 40 years or younger), 5 from each WHO region as part of its validation to assess for any ambiguity, vagueness, errors or duplications. The survey was then distributed to 1481 ISBT members from 95 countries from all WHO regions via the ISBT office, inviting YPs (aged 40 years or younger) to participate. Participation was voluntary and anonymous. The YPs reviewed an informed consent page and provided consent through completing the questionnaire. The survey was made available between 9 September 2020 and 7 January 2021.

Data from participants who completed ≥75% of the survey were included in analyses. Descriptive statistics were calculated to describe responses on the demographic measures, and responses to knowledge and attitude questions. Likert scales were reported as frequencies with percent for each point on the scale. Analytical statistics were applied to investigate the association between self-knowledge and attitude with the demographic variables. Categorical variables were presented as proportions. Analyses with responses stratified by WHO region and chi-square tests were used to assess for statistical significance. We derived a minimum sample size of 50 YPs from each WHO region to enable comparison between results based on a normal approximation to a binomial distribution, assuming an observed proportion of YPs selecting a specific response option of 50%, a 95% confidence level and a margin of error of 5%. A p-value of <0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS software (version 25.0, IBM Corp, 2017). Ethical approval was obtained from the Research Ethics Committee at the College of Medicine and Health Sciences at the Sultan Qaboos University.

RESULTS

A total of 402 YPs participated, of which 259 YPs completed the 75% or more of the survey. Participants with less than 75% completed

TABLE 1 Demographics of the participants who completed all measures ($n = 259$)

Variables	N	%
Age (years)		
20–25	15	5.8
26–30	71	27.4
31–35	85	32.8
>35	88	34
Gender		
Male	78	30.1
Female	181	69.9
WHO region		
South East Asia	55	21.2
Western Pacific	54	20.8
Africa	49	18.9
Eastern Mediterranean	38	14.7
Europe	23	8.9
North America	21	8.1
South America	19	7.3
Workplace		
Hospital based blood bank	128	49.4
National/regional blood centre	66	25.5
A hospital	48	18.5
A research centre	9	3.5
Others	8	3.1
Nature of work		
University/college undergraduate student	3	1.2
Postgraduate student (e.g., master, PhD)	17	6.6
Researcher	17	6.6
Clinician/physician	64	24.7
Medical biologist	11	4.2
Allied healthcare professional (technologist)	68	26.3
Nurse	43	16.6
Industry professional	6	2.3
Others	30	11.6
COVID-19 related healthcare services (multi-choice)		
Clinical bedside care	32	12.4
Immunohaematological/biological testing	76	29.3
Blood bank inventory management	120	46.3
Developing COVID-19 molecular tests	3	1.2
Developing COVID -19 antibody/titre tests	20	7.7
Developing COVID-19 vaccines	5	1.9
CCP donor recruitment and/or retention	67	25.9
CCP production, use and/or related research	66	25.5
Not involved	43	16.6
Others	24	9.3
Status of COVID-19 pandemic[a]		
First wave with increasing cases	49	18.9
First wave with decreasing cases	70	27.0
Second wave with increasing cases	77	29.7
Second wave with decreasing cases	63	24.3

[a]At the time of undertaking the survey.

responses were excluded ($n = 143$). Representation was obtained from all WHO regions, with three regions meeting the required sample size to allow for inter-region comparisons; Africa (Af), South East Asia (SEA) and Western Pacific (WP) (Table 1, Figure 1). The number of COVID-19 cases reported by the WHO during the period of the survey had increased from 27,486,960 confirmed cases and 894,983 confirmed deaths in 9 September 2020 [11] to 88,828,387 confirmed cases and 1,926,625 confirmed deaths in 11 January 2021 [12]. The majority of participating YPs were aged 36–40 years (34%), female (69.9%) and worked in a hospital-based setting (67.9%). One hundred and seven YPs (41.3%) were clinicians/physicians and/or nurses. Within the sample were 85 YPs who were undertaking undergrade or post-graduate studies, such as a residency, Masters or PhD.

Participating YPs were involved in different healthcare services during the pandemic including blood bank inventory management (46.3%) and bedside care of COVID-19 patients (12.4%). More than half (62.2%) were involved in specific COVID-19 laboratory and research services including convalescent plasma research, COVID-19 molecular and serological testing and vaccine development. Participants indicated they were in different phases of the COVID-19 pandemic: first wave inclining (47.3% of YPs from SEA), first wave declining (57% from Af, 52.6% from SA), second wave inclining (81% from North America [NA], 73.9% from Europe [EU], 34.2% from Eastern Mediterranean region and second wave declining (70.4% from WP).

Psychosocial impact

Half of the YPs (52.5%) reported increased stress levels, and 15.4% indicated experiencing symptoms of depression (Table 2). More than half of the YPs indicated loss of social engagement, and 46.3% indicated that they were spending little in-person time with friends, extended family members, teachers and neighbours. Thirty-five percent of the respondents indicated that information seen on media negatively impacted their psychological well-being. Most of their concerns were related to the high transmissibility of the virus, related fatalities and the disturbed/disordered life caused by the pandemic.

A proportion of the YPs expressed increased economic stress resulting from concerns about job security (20.8%; 30.9% SEA, 22.4% Af, 9.3% WP; overall $p = 0.020$, SEA/AF vs. WP $p = 0.012$), and decreased income (19.7%), with this not differing significantly between the 3 WHO regions ($p = 0.871$) (*subgroup data not shown*). Difficulties in accessing basic supplies were reported by 21.6% of the

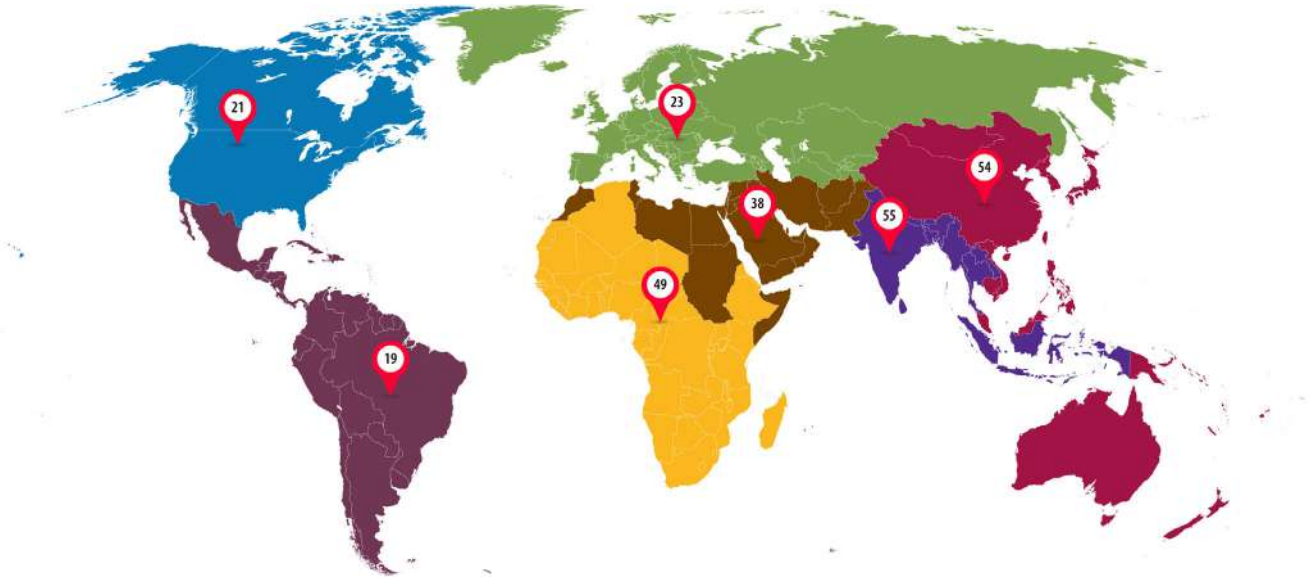

FIGURE 1 Distribution of young professionals who participated in the survey ($n = 259$)

TABLE 2 Psychological impact of COVID 19 pandemic on young professionals

Variables	Total *n* (%)	SEA	WP	Af	EMR	EU	NA	SA
What impact do you feel the COVID-19 pandemic has had on you? ($n = 259$, multi-choice)								
Increased stress levels	136 (52.5)	29 (52.7)	25 (46.3)	33 (67.3)	18 (47.4)	12 (52.2)	11 (52.4)	8 (42.1)
Depression	40 (15.4)	8 (14.5)	8 (14.8)	7 (14.3)	6 (15.8)	2 (8.7)	8 (38.1)	1 (5.3)
Loss of social engagement with peers and colleagues	153 (59.1)	34 (61.8)	34 (63)	30 (61.2)	20 (52.6)	16 (69.6)	13 (61.9)	6 (31.6)
Increased pressure from information I have seen on media (social media, TV, etc.)	92 (35.5)	22 (40)	15 (27.8)	22 (44.9)	13 (34.2)	7 (30.4)	9 (42.9)	4 (21.1)
Difficulties in accessing basic supplies	56 (21.6)	12 (21.8)	10 (18.5)	11 (22.4)	9 (23.7)	5 (21.7)	7 (33.3)	2 (10.5)
Increased economic pressure resulting from decreased income	51 (19.7)	12 (21.8)	12 (22.2)	9 (18.4)	6 (15.8)	3 (13.0)	7 (33.3)	2 (10.5)
Increased economic stress resulting from concerns about job security	54 (20.8)	17 (30.9)	5 (9.3)	11 (22.4)	10 (26.3)	4 (17.4)	5 (23.8)	2 (10.5)
Loss of a family member/friend/colleague	31 (12.0)	5 (9.1)	7 (13)	9 (18.4)	3 (7.9)	1 (4.3)	3 (14.3)	3 (15.8)
Others	15 (5.8)	1 (1.8)	1 (1.9)	4 (8.2)	3 (7.9)	3 (13.0)	1 (4.8)	2 (10.5)
In the past 7 days, how often have you spent time in person with friends, extended family, teachers and neighbours? ($n = 259$)								
Not at all	61 (23.6)	13 (23.6)	11 (20.4)	13 (26.5)	11 (28.9)	8 (34.8)	2 (9.5)	3 (15.8)
A little	120 (46.3)	27 (49.1)	25 (46.3)	25 (51.0)	14 (36.8)	9 (39.1)	11 (52.4)	9 (47.4)
Somewhat	60 (23.2)	12 (21.8)	15 (27.8)	8 (16.3)	11 (28.9)	5 (21.7)	5 (23.8)	4 (21.1)
A lot	18 (6.9)	3 (5.5)	3 (5.6)	3 (6.1)	2 (5.3)	1 (4.3)	3 (14.3)	3 (15.8)
Which fact about the COVID-19 pandemic bothers you the most? ($n = 219$)								
High transmissibility of the virus	57 (26.0)	8 (17.4)	14 (30.4)	13 (28.9)	9 (30)	4 (19.0)	5 (29.4)	4 (28.6)
Fatalities caused by the pandemic	41 (18.7)	14 (30.4)	8 (17.4)	7 (15.6)	6 (20)	3 (14.3)	3 (17.6)	-
No proven treatment available	23 (10.5)	5 (10.9)	4 (8.7)	5 (11.1)	4 (13.3)	3 (14.3)	1 (5.9)	1 (7.1)
No vaccine available	26 (11.9)	7 (15.2)	3 (6.5)	3 (6.7)	5 (16.7)	1 (4.8)	4 (23.5)	3 (21.4)
Disturbed and disordered life	36 (16.4)	7 (15.2)	7 (15.2)	7 (15.6)	2 (6.7)	5 (23.8)	3 (17.6)	5 (35.7)
Economic impact	22 (10.0)	3 (6.5)	7 (15.2)	4 (8.9)	3 (10)	4 (19.0)	-	1 (7.1)
Others	14 (6.4)	2 (4.3)	3 (6.5)	6 (45)	1 (3.3)	1 (4.8)	1 (5.9)	-

Abbreviations: Af; Africa; CCP, COVID-19 convalescent plasma; EMR, Eastern Mediterranean Region; EU, Europe; NA, North America; SA, South America; SEA, South East Asia; WP, Western Pacific.

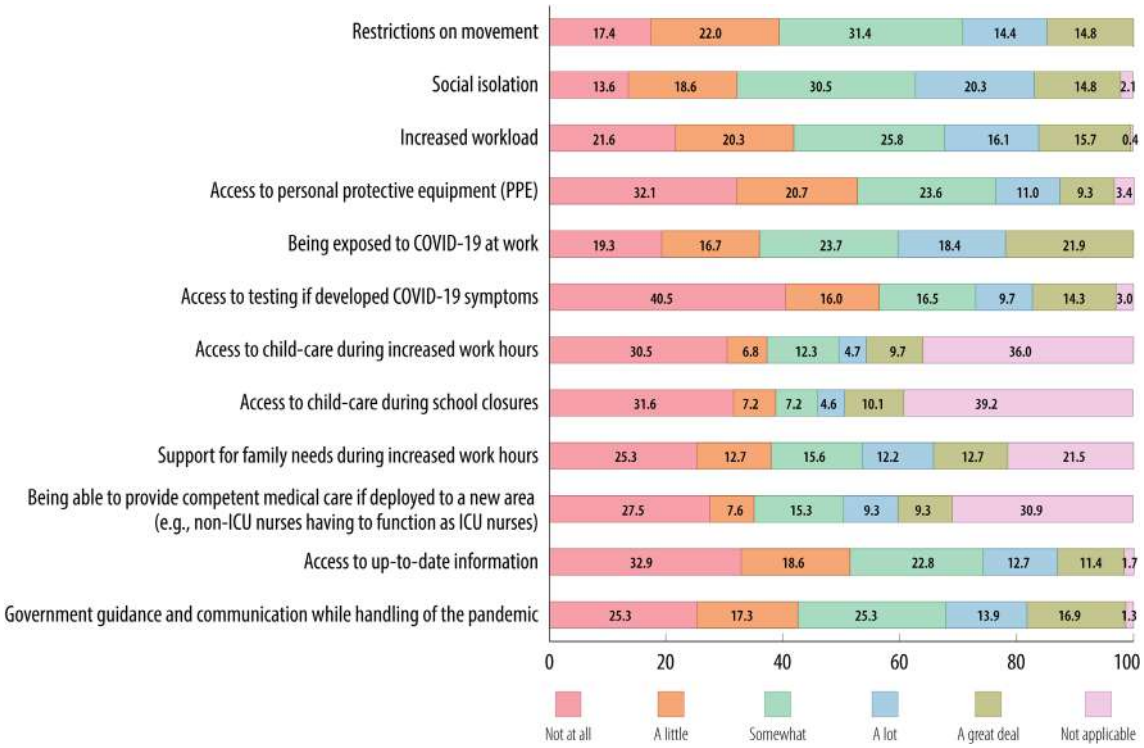

FIGURE 2 Source of stress for young professionals during the 2-week period prior to filling the survey ($n = 237$)

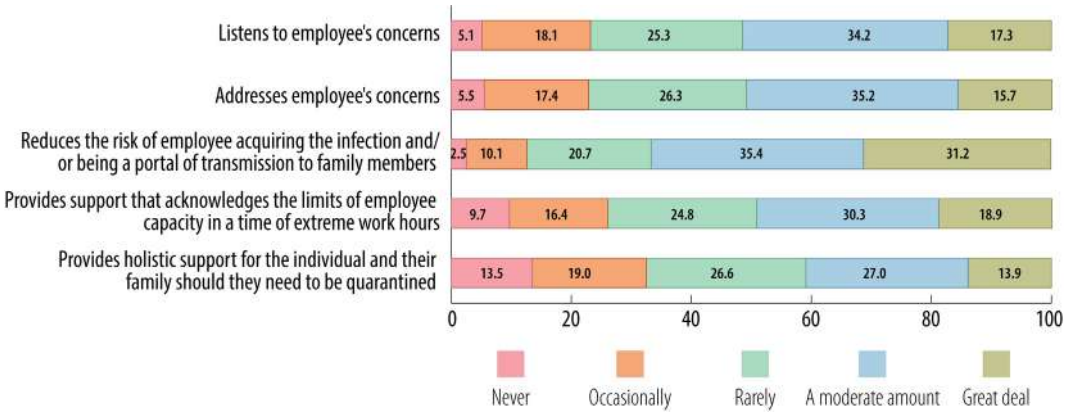

FIGURE 3 Participants' perceptions of support by their organization during the COVID-19 pandemic ($n = 237$). No statistically significant difference for all variables between the three WHO regions (SEA, AF, WP)

YPs to have an impact on their psychological wellbeing. There was no statistically significant difference in the responses obtained between participants undertaking studies and the rest of YPs in any of the factors assessed.

Figure 2 displays the magnitude of the different stressors experienced by YPs in the 2 weeks before completing the survey. Being exposed to COVID-19 at work, government guidance and communication while handling the pandemic, increased workload, social isolation and restriction of movement were reported as significant sources of stress. There was a statistically significant difference between the WHO regions with regards to concerns of having access to testing for COVID-19 (overall $p = 0.02$, SEA/AF vs. WP $p = 0.68$) and providing support to family needs during increased work hours as a source of stress (overall $p = 0.004$, SEA/AF vs. WP $p = 0.014$). Only 3.2% of YPs recorded dysfunctional levels of anxiety on the Coronavirus Anxiety Scale (CAS) (≥9) [10]. Eight percent (20/238) of YPs had trouble falling or staying asleep due to thinking about coronavirus. Moreover, 5.9% (14/237) felt nauseous or had stomach problems for several days when thinking about or being exposed to information about coronavirus. Treatment for anxiety was sought by 25.5% (54/212).

TABLE 3 Impact of COVID 19 pandemic on young professional clinicians/physicians and nurses ($n = 107$)

Variables	Total *n* (%)	SEA	WP	Af	EMR	EU	NA	SA
On average; how many patients do you take care of in a week? ($n = 64$ clinicians/physicians)								
1–5	1 (1.6)	-	-	-	1 (10)	-	-	-
5–10	13 (20.3)	5 (33.3)	3 (16.7)	-	1 (10)	1 (33.3)	3 (50.0)	-
10–15	13 (20.3)	4 (26.7)	2 (11.1)	2 (18.2)	3 (30)	-	2 (33.3)	-
>15	37 (57.8)	6 (40.0)	13 (72.2)	9 (81.8)	5 (50)	2 (66.7)	1 (16.7)	1 (100.0)
To what degree you feel protected in performing your duties? ($n = 107$)								
Not at all	4 (3.8)	1 (3)	-	1 (7.7)	1 (6.3)	1 (10.0)	-	-
Slightly	12 (11.3)	5 (15.2)	2 (9.1)	1 (7.7)	2 (12.5)	1 (10.0)	1 (10.0)	-
Moderately	45 (42.5)	15 (45.5)	9 (40.9)	5 (38.5)	9 (56.3)	3 (30.0)	3 (30.0)	1 (50.0)
Very	35 (32.7)	11 (33.3)	10 (45.5)	3 (23.1)	1 (6.3)	4 (40.0)	5 (50.0)	1 (50.0)
Extremely	10 (9.4)	1 (3)	1 (4.5)	3 (23.1)	3 (18.8)	1 (10.0)	-	-
How often do you feel that you have sufficient personal protective equipment to protect yourself from COVID-19 while completing your work? (n= 107)								
Never	5 (4.7)	1 (3)	1 (4.3)	-	2 (12.5)	1 (10.0)	-	-
Rarely	8 (7.5)	4 (12.1)	1 (4.3)	-	2 (12.5)	1 (10.0)	-	-
Sometimes	35 (32.7)	11 (33.3)	9 (39.1)	3 (23.1)	6 (37.5)	2 (20.0)	3 (30.0)	1 (50.0)
Often	59 (55.1)	17 (51.5)	12 (52.2)	10 (76.9)	6 (37.5)	6 (60.0)	7 (70.0)	1 (50.0)
Do you have family/household members living with you in your home (e.g., children, elderly)? (n= 107)								
Yes	82 (76.6)	28 (84.8)	18 (78.3)	10 (76.9)	13 (81.3)	7 (70.0)	5 (50.0)	1 (50.0)
No	25 (23.4)	5 (15.2)	5 (21.7)	3 (23.1)	3 (18.8)	3 (30.0)	5 (50.0)	1 (50.0)
If yes, to which extent are you worried about infecting your family/household members because of the nature of the work you do? ($n = 82$)								
Not at all	3 (3.7)	-	2 (11.1)	-	-	-	1 (20.0)	-
A little	16 (19.5)	6 (21.4)	7 (38.9)	2 (20)	1 (7.7)	-	-	-
Somewhat	13 (15.9)	4 (14.3)	2 (11.1)	3 (30)	2 (15.4)	2 (28.6)	-	-
A lot	30 (36.6)	11 (39.3)	5 (27.8)	3 (30)	7 (53.8)	3 (42.9)	1 (20.0)	-
A great deal	20 (24.4)	7 (25)	2 (11.1)	2 (20)	3 (23.1)	2 (28.6)	3 (60.0)	1 (100)

Abbreviations: Af, Africa; CCP, COVID-19 convalescent plasma; EMR, Eastern Mediterranean Region; EU, Europe; SA, South America; SEA, South East Asia; NA, North America; WP, Western Pacific.

Organizational support

Figure 3 displays YPs perceptions of being supported by their organizations during the COVID-19 pandemic. Although a third of the YPs indicated that their organizations engaged in significant efforts to reduce the risk of employees acquiring the infection through their work, the provision of holistic support for the individual and their family should they need to be quarantined was ranked as only moderate/occasional by almost half of the YPs.

Increased workload was reported by 60.3% of YPs (see Table S1). Only 29.3% (76/259) of YPs indicated that they were asked to work from home, if possible, to minimize the risk of COVID-19 infection. However, 50.4% (129/256) of YPs indicated that they were asked to work in shifts in their workplace to minimize the risk of COVID-19 infection. Staff absences due to COVID-19 infection were reported by 74.4% of YPs (163/219), while 39.4% (86/218) were tested for COVID-19 due to suspected exposure or unexplained illness.

Among the clinicians/physicians and nurses working in transfusion services, over half took care of >15 patients a week (Table 3). Most of the respondents felt moderately (42.5%) or very (33%) protected with 55.1% indicating that they often had sufficient PPE to protect themselves during work. However, 12.1% (13/107) indicated that they never or rarely had sufficient PPE. The majority (76%, 82/107) had family/household members living with them at home (e.g., children/elderly), and almost half indicated that they were worried about infecting them because of the nature of their work.

Lessons learned

The YPs shared lessons learned in relation to work and for everyday living (see Table S2). For safety and well-being, the need to advocate for personal and family safety and well-being support was highlighted. Measures suggested included seeking counselling and peer support. Considering the dynamics of the pandemic and its impact on daily life, the importance of adapting to rapid changes was highlighted. A call for work-home balance was also advocated

to avoid burnout. The YPs highlighted the importance of solidarity within society, and to find and support vulnerable individuals in their community.

DISCUSSION

This study sought to assess the psychological impact of the COVID-19 pandemic on YPs working in blood banks and transfusion services globally. Our study showed that YPs were heavily involved in COVID-19-related healthcare services, including laboratory and clinical care, community services and research. In addition, the reported participation in donor recruitment may reflect the significant challenges in ensuring a sufficient blood supply during the COVID-19 pandemic documented in Asian, EMR and Af regions [13–16].

This study showed that more than half of YPs reported increased stress levels due to multiple factors. This is consistent with research showing the broader impact of COVID-19. The COVID-19 pandemic brought about economic challenges, lifestyle disruption, loss of occupation, restriction of movement, lockdowns, social isolation and loneliness, which have been associated with negative mental health outcomes including anxiety and depression [17, 18]. Surveys conducted during the pandemic have shown youth adherence to social distancing measures driven by public recommendations and protecting the health of others [5, 19], with women being more likely to engage in these health behaviours than men [20]. As such, the negative impact of COVID-19 on our survey respondents is not unexpected, with general population studies showing higher rates of anxiety, depression and distress among young adults and females [21–24]. The impact of social media as a source of information on triggering stress among young individuals was previously described [24].

Considering the nature of YP's work, the majority were not offered the opportunity to work from home, but were instead offered shift-work. The reported involvement and increased workload with moderate support from the organizations raise concerns as to whether the YPs or their families were sufficiently supported, especially if they required quarantine. Moreover, the pandemic was reported to be associated with challenges in the working environment with staff training on new procedures, or transfer to other units that can increase anxiety levels [25].

The rate of YPs reporting significant levels of coronavirus anxiety as per the CAS was however low [10]. This implies that most of the YPs in our survey were managing the stresses related to the pandemic. However, it should be noted that our sample was self-selected and included only a small number of clinicians/physicians in direct contact with COVID-19 patients. A representative sample of YPs, including clinicians/physicians in direct contact with COVID-19 patients, may report higher rates of stress and anxiety [26].

Considering that only half of the clinicians/physicians and nurses who responded perceived they had sufficient PPE, it is perhaps not surprising to see some concern in the context of emotional stress and worries about infecting family members. This can drive YPs to isolate themselves from their families, and narrow their social support network [27]. COVID-19 has a considerable impact on the psychological well-being of front-line hospital staff, including depression, anxiety, stress and burnout, with a higher impact on females, staff with underlying medical illness or who are in close contact with COVID-19 patients, and in the presence of PPE shortages [18, 28]. Further, insights from previous pandemics suggest that quarantined healthcare workers may be more likely to perceive being stigmatized and rejected by people in their local neighbourhoods [2]. All these factors are likely to impact the safety and mental health status of YP healthcare workers.

Regarding the limitations of this survey, cross-sectional surveys capture relevant data only at a single moment in time, and causal or temporal interpretations cannot be made, particularly regarding the variable degree of spread of the virus between countries even within the same WHO region. As a consequence, we cannot be sure that what was observed was caused by the pandemic or was already present prior to the pandemic. Relying on social networks for voluntary recruitment of the survey and re-sharing could have introduced a vital selection bias, and the dependence on a self-report instrument could introduce a systematic bias. A subgroup analysis to assess the impact on different participants based on their professional background was not possible. Further, mental health was self-reported and not based on clinical diagnoses. The survey was conducted at different pandemic phases across different WHO regions, which could affect the results. Finally, the survey was only administered in English, which could have hindered non-English speaking YPs. Strengths of our approach, however, include this being the first study to assess the involvement of and the impact of the pandemic on YPs in blood banks and transfusion services. Furthermore, participation from all WHO regions provides a broad comparative snapshot of the impact on YPs worldwide. While descriptive, these results provide glimpses of the need for mental health interventions, such as improved coping strategies. These findings provide essential information to enable health authorities and executives to allocate health resources and support to YPs and their families during pandemics, including the provision of mental support.

In conclusion, this survey highlights the psychological impact of the COVID-19 pandemic on YPs working in blood banks and transfusion services. YPs play an important role in providing the clinical and supportive care during pandemics, but experience a range of negative psychological impacts. Having a clear understanding of mental health and well-being of the YPs during the pandemic would ensure that those affected receive support from their organizations. Further studies should assess the long-term effect of this pandemic on YPs.

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CONFLICT OF INTEREST

The authors declare no conflicts 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

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The transfusion of non-prophylactically RH-KEL1 antigen-matched red blood cells is feasible in selected myelodysplastic syndrome and acute myeloid leukaemia patients

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Abstract

Background and Objectives: Most myelodysplastic syndromes (MDS) patients become red blood cell (RBC) transfusion-dependent. Transfusing MDS patients with prophylactically RH-KEL1 antigen-matched (PAM) RBC units is recommended to avoid RBC allo-immunization. D+C−E−c+e+, D+C−E+c+e− and D+C+E−c−e+ phenotypes are infrequent among French blood donors. To preserve infrequent phenotype RBC units for patients other than MDS, and to manage frequent phenotype RBC unit stocks, we let, for 1 year, higher-risk non-immunized chronically transfused MDS and acute myeloid leukaemia (AML) patients receive RBC transfusions matched only for D. Our objectives were to evaluate the impact of non-PAM transfusions on the transfusion policy (which would be modified in case of RBC allo-immunization) for frequent and infrequent phenotypes patients and to estimate the number of infrequent phenotypes RBC units that could be redistributed to other patients.

Results: Ninety patients were enrolled. Thirty-five patients had infrequent phenotypes, nine received only PAM RBC (143 units) and 26 PAM and non-PAM RBC (415 and 532, respectively): none developed allo-immunization. Fifty-five patients had frequent RBC phenotypes, 34 received only PAM RBC (561 units) and three developed antibodies (2 non-RH-KEL1 and one anti-E); 21 received PAM and non-PAM RBC (436 and 109, respectively) and one developed allo-immunization (unknown specificity). Our strategy enabled us to preserve 532 infrequent phenotypes RBC units: 216 D+C−E−c+e+, 33 D+C−E+c+e− and 283 D+C+E−c−e+ units, representing 48.8% of the total number of RBC units received by infrequent phenotypes patients during the study period.

Conclusion: Allowing the transfusion of non-PAM RBC in selected chronically transfused MDS and AML patients was feasible and enabled to redistribute infrequent phenotypes RBC units to other patients in need.

KEYWORDS
myelodysplastic syndrome, red blood cell allo-immunization, transfusion

Highlights

- The transfusion of non-PAM RBC in selected chronically transfused MDS and AML patients was feasible without an apparent increase in RBC allo-immunization.

INTRODUCTION

Myelodysplastic syndromes (MDS) are a heterogeneous group of malignant myeloid stem cell disorders, characterized by ineffective haematopoiesis, peripheral blood cytopenias and a variable risk of disease progression into acute myeloid leukaemia (AML) [1]. Anaemia is the most frequent cytopenia in MDS patients. During the course of their disease, 30%–80% of MDS patients will not respond or will lose response to erythropoiesis stimulating agents, becoming dependent on red blood cell (RBC) transfusions [2]. Repeated transfusions will make these patients at risk of developing allo-immunization against RBC antigens, leading to delayed haemolytic transfusion reactions and difficulties finding compatible RBC units in case of poly-immunization.

Previous studies [2–6] in transfused MDS patients report a 15%–30% incidence rate of RBC allo-immunization. The median numbers of RBC received until antibody formation were 12 and 16 in two studies, respectively [2, 6], and the median time to allo-immunization was 5 months in a third study [5]. The cumulative incidence of allo-immunization increased with the number of transfusions, with a plateau at 19.4% after 130 RBC units and 12.3% after 59 units, in two studies, respectively [4, 5]. In 74% of cases, antibodies were directed against RH, K, Fy(a) and Jk(a) antigens [4, 6].

To avoid allo-immunization, experts agree on the indication for prophylactically RH-KEL1 antigen-matched (PAM) RBC transfusions for patients with chronic diseases for which prolonged survival is conditioned by iterative transfusions, such as MDS (Haute Autorité de Santé 2014 recommendations). However, Lin et al. [2] retrospectively compared, in 176 MDS patients, RBC transfusions matched only for D versus for D C, E and K: in the case of D, C, E and K matching policy, the rate of total RBC allo-immunization was not significantly different (11% vs. 23%, $p = 0.06$) but the rate of RH-KEL1 allo-immunization was significantly lower (7% vs. 22%, $p = 0.008$). Moreover, previous studies have suggested that azacytidine, a major treatment for MDS patients, could lead to immunomodulation [7] and possibly decrease the rate of allo-immunization against RBC antigens [5].

Blood resources available for transfusions are limited, especially for infrequent phenotypes, such as D+C−E−c+e+, D+C−E+c+e− and D+C+E−c−e+ representing respectively 2.3%, 2% and 19% of D+ blood donors in metropolitan France. The exposure to RH antigens through transfusion bears a risk of allo-immunization, because of the high immunogenicity of these antigens. In women of childbearing age, young patients and patients at high risk of RBC allo-immunization (sickle cell disease patients), RBC antibodies may be involved in post-transfusion haemolysis and haemolytic disease of the newborn. For frequent RBC phenotypes, blood resources available for transfusion are less limited; however, blood inventory management must be performed wisely to ensure the minimization of RBC units' wastage. To preserve infrequent phenotypes RBC units for patients other than MDS, and to manage frequent phenotypes RBC units stocks, we allowed, for 1 year, the transfusion of non-PAM RBC to higher-risk non-immunized chronically transfused MDS patients. The objectives of the study were to evaluate the impact of non-PAM transfusions on the transfusion policy (which would be modified in case of RBC allo-immunization) for frequent and infrequent phenotypes MDS and AML patients and to estimate the number of infrequent phenotypes RBC units that could be redistributed to other patients.

PATIENTS AND METHODS

From 1 January to 31 December 2017, we prospectively screened all patients with myeloid-lineage haematological disease that came for transfusion to the haematology outpatient department of Saint-Louis Hospital, Paris, France. Four patients with previous RBC allo-immunization were excluded. Ninety non-immunized patients were identified; including 67 MDS and 23 AML (primary AML, $n = 7$, AML secondary to previous haematological disorder, $n = 16$). AML patients had previously failed intensive remission-induction chemotherapy or were not eligible for intensive chemotherapy, and were thus receiving the same treatments as MDS patients, without any curative aim. All patients had short-life expectancy due to higher-risk MDS according to the IPSS-R classification or AML ineligible to curative treatment.

Patients were divided into two groups according to their RBC phenotypes: group 1 ($n = 35$) included patients of infrequent phenotypes D+C−E−c+e+, D+C−E+c+e− and D+C+E−c−e+, group 2 ($n = 55$) included all the remaining patients, as well as D− patients. Prophylactically antigen-matched (PAM) RBC units were matched for D, C, E, c, e and K, while non-prophylactically antigen-matched (non-PAM) RBC units were matched only for D. Physicians in charge of the patients gave their agreement for patients to receive non-PAM RBC transfusions each time it was possible. However, no D− patient received D+ RBC units, and the transfusion of the highly immunogenic E and K antigens was avoided whenever possible in E− and K− patients. To preserve uncommon D− RBC (representing only 15% of blood donors and used for emergency settings-transfusion), no D+C−E−c+e+ patient was transfused with D− RBC. No patient was transfused outside of our department during the study period. Patients in both groups could receive PAM and non-PAM RBC

TABLE 1 Repartition of patients according to RH phenotypes and transfusions

	Group 1			Group 2			
	D+C−E −c+e+	D+C−E +c+e−	D+C+E −c−e+	D−C−E −c+e+	D+C−E +c+e+	D+C+E −c+e+	D+C+E +c+e+
PAM and non-PAM	6	1	19	3	2 (1 AI = 50%)	16	0
PAM only	0	0	9	6 (2 AI = 33%)	7	14 (1 AI = 7%)	7

TABLE 2 Repartition of patients according to RH phenotypes and azacytidine treatment

	Group 1		Group 2	
	Azacytine	No azacytidine	Azacytine	No azacytidine
PAM and non-PAM	12	14	11	10 (1 AI = 10%)
PAM only	2	7	11 (1 AI = 9%)	23 (2 AI = 8.6%)

Abbreviations: AI, allo-immunization; PAM, prophylactically RH-KEL1 antigen-matched.

transfusions. Both groups were subdivided into patients receiving PAM and non-PAM, and patients receiving only PAM transfusions (Table 1).

RBC antibody screening on gel-filtration was performed within 3 days before each transfusion. Post-transfusion RBC antibody screening was performed according to patient's transfusion need, and considered interpretable if performed at least 1 week after the last 2017 transfusion (or at allogeneic HSCT, if performed before 31 December 2017).

Haematological disease and treatment data were collected from the patient chart, and transfusions data were collected from the Etablissement Français du Sang database. All patients gave written informed consent for data collection, and the study was conducted according to the Declaration of Helsinki.

RESULTS

Patient characteristics

Forty-seven patients were male (52%), median age was 76 (range: 55–93). Haematological diagnosis at first transfusion in 2017 was AML or MDS with a high or very high IPSS-R score in 47 patients (52%). Group 1 included 35 patients: six D+C−E−c+e+, one D+C−E+c+e− and 28 D+C+E−c−e+ patients, respectively. Group 2 included 55 patients: 9 D−C−E−c+e+, 9 D+C−E+c+e+, 30 D+C+E−c+e+ and 7 D+C+E+c+e+ patients, respectively (Table 1).

Fifty-nine (65.6%) patients had been transfused before the study started. In group 1, 25 patients (71.4%) had received a median of 35 RBC units (range: 2–249) transfusions before the study began, including a median of 10 (range: 1–31) non-PAM RBC units. In group 2, 34 patients (61.8%) had received a median of 10 RBC units (range:1–246) before the study began, including a median of 4 (range: 1–25) non-PAM RBC units.

Thirty-six patients received azacytidine during the study period or the preceding year, including 23 in the PAM and non-PAM cohort (12 patients in group 1 and 11 in group 2) and 13 in the PAM only cohort (two patients in group 1 and 11 in group 2), respectively (Tables 2 and 3).

In total, during the 1-year study period, patients received 2196 RBC units, including 1555 (71%) PAM and 641 (29%) non-PAM RBC units, respectively (Table 4). In group 1, 26 patients (74%) received 532 non-PAM (56%) and 415 PAM (44%) RBC units, while 9 patients (26%) received only PAM RBC (143 units). In group 2, 21 patients (38%) received 109 non-PAM (20%) and 436 PAM (80%) RBC units, while 34 patients (62%) received only PAM RBC (561 units).

Forty-eight patients out of 88 evaluable (two group 2 patients were censored at allogeneic haematopoietic stem cell transplantation) died (54.5%), including 20 patients (41.6%) during the study period and 24 patients (50%) within 1 year after the end of the study period. The most frequent causes of death were disease progression (60%) and infection (23%).

Patients with positive RBC antibodies screening

RBC allo-immunization was detected in four patients of frequent phenotype (group 2), including two D−C−E−c+e+, one D+C−E+c+e+ and one D+C+E−c+e+ patient, respectively. Specificities of the four RBC antibodies were: S, Jka, unknown, and E, respectively. Three patients had received only PAM RBC transfusions and one had received PAM and non-PAM RBC transfusions. No delayed haemolytic transfusion reaction was observed and no patient in group 1 developed RBC allo-immunization.

Patient 1 D−C−E−c+e+ (Figure 1a) was 53 years old in 2009 when diagnosed with MDS without blast excess and isolated trisomy 8. She received 10 PAM RBC units in 2013–2014, followed by two PAM RBC units early December 2017 (one S− unit, and one of

TABLE 3 Patient characteristics, post-transfusion red blood cell (RBC) screening and azacytidine treatment

		Group 1: infrequent RBC phenotypes				Group 2: frequent RBC phenotypes				
	Total	D+C−E−c+e+	D+C−E+c+e−	D+C+E−c−e+	Total (%)	D−C−E−c+e+	D+C−E+c+e+	D+C+E−c+e+	D+C+E+c+e+	Total (%)
n=	90	6	1	28	35	9	9	30	7	55
median age (years) (range)	76 (55–93)	79 (56–86)	63	76 (59–92)		82 (60–93)	76 (63–89)	77 (55–89)	74 (68–84)	
male (%)	46 (52)	2 (33)	0	12 (43)	14 (40)	4 (44)	4 (44)	19 (63)	5 (71)	32 (58)
AML or MDS high/very-high IPSS at first 2017 tf° (%)	50 (52)	4 (66)	0	17 (61)	22 (61)	5 (56)	3 (33)	17 (57)	3 (43)	28 (51)
Pts receiving PAM and non-PAM RBC tf° (%)	47 (52)	6 (100)	1	19 (68)	26 (74)	3 (27)	2 (25)	16 (53)	0	21 (38)
Pts dying <1 month after last tf° without RBC Ab screening	11 (23)	2 (33)	NA	3 (16)	5 (19)	1 (34)	0	5 (31)	NA	6 (29)
Pts without RBC Ab screening after last 2017 tf°	0	0	0	0	0	0	0	0	NA	0
Pts with RBC Ab screening after last 2017 tf°	36 (77)	4 (67)	1 (100)	16 (84)	21 (81)	2 (66)	2 (100)	11 (69)	NA	15 (71)
Negative post-transfusion RBC Ab screening	35 (37)	4 (100)	1 (100)	16 (100)	21 (100)	2 (100)	1 (50)	11 (100)	NA	14 (93)
Positive post-transfusion RBC Ab screening	1 (3)	0	0	0	0	0	1 (50)	0	NA	1 (7)
Pts treated with Aza in 2016–2017	23 (49)	1 (25)	0	11 (58)	12 (46)	2 (100)	1 (50)	8 (50)	NA	11 (52)
Number of Aza cycles (median and range)		(5)	NA	6 (2–18)		(1 and 2)	(4)	6.5 (4–15)	NA	
Pts receiving only PAM RBC tf° (%)	43 (48)	0	0	9 (32)	9 (26)	6 (67)	7 (78)	14 (47)	7 (100)	34 (62)
Pts dying <1 month after last tf° without RBC Ab screening	9 (21)	NA	NA	2 (22)	2 (22)	2 (33)	0	1 (7)	1 (14)	7 (21)
Pts without RBC Ab screening after last 2017 tf°	6 (14)	NA	NA	2 (22)	2 (22)	0	2 (29)	1 (7)	1 (14)	4 (12)
Pts with RBC Ab screening after last 2017 tf°	31 (73)	NA	NA	5 (55)	5 (55)	4 (67)	5 (71)	12 (86)	5 (71)	26 (76)
Negative post-transfusion RBC Ab screening	28 (90)	NA	NA	5 (100)	5 (100)	2 (50)	5 (100)	11 (92)	5 (100)	23 (88)
Positive post-transfusion RBC Ab screening	3 (10)	NA	NA	0	0	2 (50)	0	1 (8)	0	3 (12)
Pts treated with Aza in 2016–2017	13 (14)	NA	NA	2 (22)	2 (22)	2 (33)	1 (14)	6 (43)	2 (29)	11 (32)
Number of Aza cycles (median and range)		NA	NA	(6)		(2 and 11)	(6)	5 (1–14)	(10 and 21)	

Abbreviations: Ab, antibody; AML, acute myeloid leukaemia; Aza, azacytidine; IPSS, International Prognosis Scoring System; MDS, myelodysplastic syndrome; PAM, prophylactically antigen-matched; Pts, patients; RBC, red blood cell; Tf°, transfusions.

TABLE 4 RBC transfusions during the study period

		Group 1: infrequent RBC phenotypes				Group 2: frequent RBC phenotypes				
	Total	D+C−E−c+e+	D+C−E +c+e−	D+C +E−c−e+	Total (%)	D−C−E−c +e+	D+C−E+c+e+	D+C+E−c +e+	D+C+E+c +e+	Total (%)
Total RBC units	2196									
Pts receiving PAM and non-PAM RBC tf° (%)	47 (52)	6 (100)	1	19 (68)	26 (74)	3 (27)	2 (25)	16 (53)	0	21 (38)
Total number of PAM and non-PAM RBC units	1492	265	46	636	947	65	30	450	0	545
Median tf° time (days) (range)		277.5 (139–343)	356	324 (1–351)		256 (72–260)	(70 and 206)	173 (77–354)	NA	
Total number of non-PAM RBC units	641 (43)	216	33	283	532 (56)	9	6	94	0	109 (20)
Median number of non-PAM RBC units (range)		29 (10–82)		18 (2–30)		3 (2–4)	(2 and 4)	2.5 (1–19)	NA	
C+ RBC units										
Pts transfused	10 (83)	6 (100)	0	NA	6 (86)	2 (66)	2 (100)	NA	NA	4 (80)
Total number	213	204		NA	204	5	4	NA	NA	9
Median number (range)		28.5 (4–82)		NA		2 (2–3)	(2 and 2)	NA	NA	
E+ RBC units										
Pts transfused	32 (73)	5 (83)	NA	12 (63)	17 (68)	0	NA	15 (94)	NA	15 (79)
Total number	148	19	NA	43	62	NA	NA	86	NA	86
Median number (range)		3 (2–6)	NA	2 (1–12)		NA	NA	2 (1–17)	NA	
c+ RBC units										
Pts transfused	19 (100)	NA	NA	19 (100)	19 (100)	NA	NA	NA	NA	NA
Total number	279	NA	NA	279	279	NA	NA	NA	NA	
Median number (range)		NA	NA	18 (2–28)		NA	NA	NA	NA	
e+ RBC units										
Pts transfused	1 (100)	NA	1 (100)	NA	1 (100)	NA	NA	NA	NA	NA
Total number	33	NA	33	NA	33	NA	NA	NA	NA	
Median number (range)		NA		NA		NA	NA	NA	NA	
K+ RBC units transfused to K− pts	12/50 (24)	0/6	0/1	3/16 (19)	3/23 (13)	1/3 (33)	1/2 (50)	7/16 (43)	0/6	9/27 (33)
Total number of K+ RBC units	28	NA	NA	6	6	4	2	16	NA	22
Median number of K+ RBC units (range)		NA	NA	2		NA	NA	2 (1–4)	NA	

(Continues)

TABLE 4 (Continued)

	Total	Group 1: infrequent RBC phenotypes				Group 2: frequent RBC phenotypes			
		D+C−E−c+e+	D+C−E+c+e−	D+C+E−c−e+	Total (%)	D−C−E−c+e+	D+C−E+c+e+	D+C+E−c+e+	D+C+E+c+e+	Total (%)
PAM RBC units										
Pts	45 (96)	5 (83)	1 (100)	18 (95)	24 (92)	3 (100)	2 (100)	16 (100)	0	21 (100)
Total number	851	49	13	353	415	56	24	356	NA	436
Median number (range)		9 (2–23)		17 (1–47)		22 (7–27)	(2 and 22)	15 (2–122)	NA	
Pts receiving only PAM RBC tf° (%)	43 (48)	0	0	9 (32)	9 (26)	6 (67)	7 (78)	14 (47)	7 (100)	34 (62)
Median tf° time (days) (range)		NA	NA	116 (1–355)		35 (1–349)	78 (1–352)	146 (1–332)	76 (1–339)	
Total number of PAM RBC units	704	0	0	143	143	35	111	339	76	561
Median number of PAM RBC units (range)		NA	NA	14 (2–52)		6 (2–11)	8 (2–59)	22.5 (2–55)	6 (2–30)	

Abbreviations: PAM, prophylactically antigen-matched; Pts, patients; RBC, red blood cell; Tf°, transfusion.

unknown extended phenotype): RBC antibody screening became positive against S 3 weeks after this transfusion.

Patient 2 D−C−E−c+e+ (Figure 1b), without previous transfusion history, was diagnosed aged 82 in 2016 with chronic myelomonocytic leukaemia without blast excess and normal karyotype. In December 2016 and January 2017, he twice received two PAM RBC units, with one Jka+ and one Jka− unit at each transfusion episode. RBC antibody screening was positive against Jka in April 2017.

Patient 3 D+C−E+c+e+ (Figure 1c) was diagnosed aged 89 with AML in October 2016. She had previously been transfused in 2010 (2 non-PAM K+ RBC units). She refused anti-leukaemic treatment and received supportive care only: she was transfused with 8 RBC units from December 2016 to April 2017 (6 PAM and 2 non-PAM K+ RBC units). RBC antibody screening was positive in May 2017 (no specificity identified despite extensive biological work-up).

Patient 4 D+C+E−c+e+ (Figure 1d), without previous transfusion history, was diagnosed aged 69 in January 2017 with AML, with complex karyotype and TP53 mutation. Anti-E allo-immunization was identified in February 2017, after the patient had received 23 PAM E− RBC units, one E+ apheresis platelet concentrate and eight pooled platelet concentrates. Anti-E allo-immunization could also have occurred naturally.

None of these RBC allo-immunizations could have been avoided if patients had received only PAM RBC units: indeed, antibodies were directed outside of the RH-KEL1 antigenic systems in three cases, and the anti-E allo-immunization occurred without exposure to E+ RBC units, possibly triggered by platelet transfusions.

Number of preserved RBC units

Allowing these transfusions of non-PAM RBC units to some selected MDS patients enabled us to preserve a significant number of infrequent phenotype RBC units: 48.8% of the 1090 RBC units transfused to group 1 patients were non-PAM units. Altogether, our strategy enabled us to preserve a total of 532 RBC units, including 216 D+C−E−c+e+, 33 D+C−E+c+e− and 283 D+C+E−c−e+ RBC units, respectively.

DISCUSSION

The first aim of this report of real-life experience of blood inventory management was to evaluate the impact of non-PAM RBC transfusions on the transfusion policy (which would be modified in case of RBC allo-immunization) in selected MDS and AML patients. RBC antibody screening was positive in 4.4%. As previously described, antibodies were mainly directed against RH-KEL1 and Jka antigens [4, 6], although we also identified an anti-S antibody and one of unknown specificity. In our cohort, the allo-immunization rate was lower than previously reported (15%–30%) in haematology and MDS patients [4, 6]. Our study was conducted for only 1 year and two thirds of our

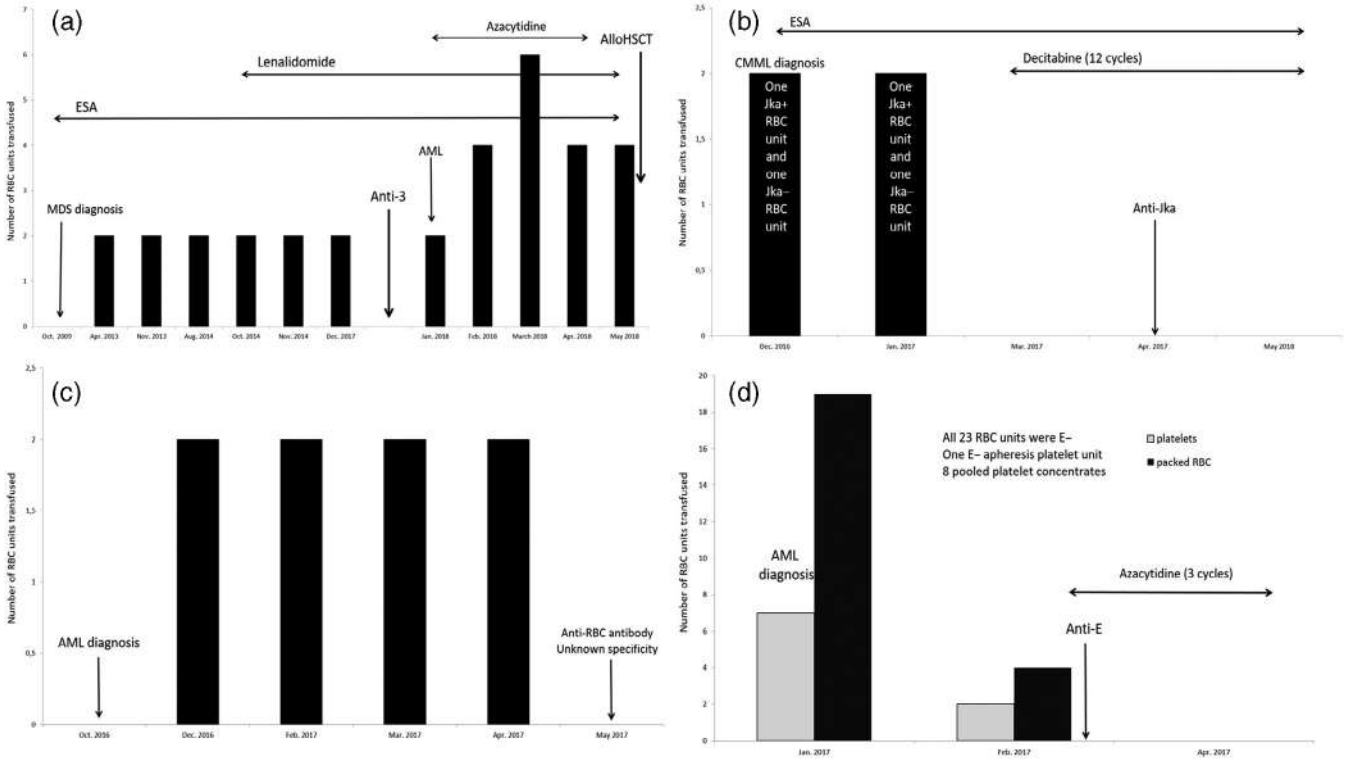

FIGURE 1 Patients with RBC allo-immunization. (a) Patient 1. (b) Patient 2. (c) Patient 3. (d) Patient 4. AlloHSCT, allogeneic haematopoietic stem cell transplantation; AML, acute myeloid leukaemia; CMML, chronic myelomonocytic leukaemia; ESA, erythropoiesis stimulating agents; RBCs, red blood cells

patients had already been transfused before the study started without RBC allo-immunization, thus representing a selection of our patients. Post-transfusion RBC antibody screening was not performed systematically, but according to patient's transfusion need. The high mortality rate observed in our study can be considered as a competing event of RBC allo-immunization. These two points could have partly explain the low allo-immunization rate observed in our study. It also highlights the correct selection of our higher-risk patients: more than half of the patients died, three-quarters of the deaths being related to disease progression or infection.

The impact of azacytidine on RBC allo-immunization in MDS patients has been reported in a retrospective study including 209 patients [5]: allo-immunization was detected in 2.3% of patients in the azacytidine group, versus 13.9% of the control group ($p = 0.033$). In multivariate logistic regression analysis, the number of transfused RBC units significantly increased the risk of allo-immunization, while increasing age and azacytidine therapy significantly decreased that risk. The authors suggested that the immunosuppression state induced by azacytidine therapy could create an immunological tolerance explaining the weak antibody response to incompatible transfusions. In our cohort, only one of the 36 azacytidine-treated patients (2.8%) developed RBC allo-immunization 3 days after the start of azacytidine: he was the PAM-only patient, with the anti-E allo-immunization possibly triggered by platelet transfusions. Three out of 54 patients (5.6%) who did not receive azacytidine developed RBC allo-immunization. The small number of patients in our cohort precludes any statistical analysis, however, we can suspect, as previously suggested, an immunomodulatory effect of azacytidine in the tolerance of non-PAM RBC transfusions, contributing to the absence of RBC allo-immunization despite incompatible transfusions.

The second objective of our study was to preserve RBC units of infrequent phenotypes D+C−E−c+e+, D+C−E+c+e− and D+C+E−c−e+, while not systematically applying the PAM transfusion policy to some selected MDS patients. In this prospective cohort of 90 patients, we were able to preserve 532 RBC units of these infrequent phenotypes, representing 48.8% of the total burden of RBC transfusions during the study period for these patients. This economy is significant, and could, for example, represent up to 4 or 5 years of monthly RBC exchanges for a medium-size patient with sickle cell disease.

Our study has limitations: the study was conducted in a single centre and the number of patients is low and included previously patients transfused, but our results show the feasibility of non-PAM RBC transfusions in some selected patients with MDS, without an apparent increased risk of RBC allo-immunization. This strategy enabled us to preserve a significant number of infrequent phenotype RBC units that can be used for young patients or women of childbearing age.

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the performance of the research; all authors critically revised the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

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Estimating the risks of prehospital transfusion of D-positive whole blood to trauma patients who are bleeding in England

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Abstract

Background and Objectives: D-negative red cells are transfused to D-negative females of childbearing potential (CBP) to prevent haemolytic disease of the foetus and newborn (HDFN). Transfusion of low-titre group O whole blood (LTOWB) prehospital is gaining interest, to potentially improve clinical outcomes and for logistical benefits compared to standard of care. Enhanced donor selection requirements and reduced shelf-life of LTOWB compared to red cells makes the provision of this product challenging.

Materials and Methods: A universal policy change to the use of D-positive LTOWB across England was modelled in terms of risk of three specific harms occurring: risk of haemolytic transfusion reaction now or in the future, and the risk of HDFN in future pregnancies for all recipients or D-negative females of CBP.

Results: The risk of any of the three harms occurring for all recipients was 1:14 $\times$ 10^3 transfusions (credibility interval [CI] 56 $\times$ 10^2–42 $\times$ 10^3) while for females of CBP it was 1:520 transfusions (CI 250–1700). The latter was dominated by HDFN risk, which would be expected to occur once every 5.7 years (CI 2.6–22.5). We estimated that a survival benefit of ≥1% using LTOWB would result in more life-years gained than lost if D-positive units were transfused exclusively. These risks would be lower, if D-positive blood were only transfused when D-negative units are unavailable.

Conclusion: These data suggest that the risk of transfusing RhD-positive blood is low in the prehospital setting and must be balanced against its potential benefits.

KEYWORDS
HDFN, low-titre group O whole blood, major haemorrhage, massive transfusion, red cells, trauma, whole blood

Highlights

- We modelled the risk of harm that would be predicted from a policy change from transfusion of RhD-negative to -positive whole blood in the prehospital setting.
- The risk of a haemolytic transfusion reaction due to an index D-positive transfusion was 1:27,000 transfusions for all recipients and 1:6660 for D-negative females under 50; one event occurring every 4 years and 65 years, respectively, in England.

- The main risk was that of haemolytic disease of the foetus and newborn, where for every 570 transfusions, one foetal death or disability would be predicted to occur, one event every 5.7 years in England.

INTRODUCTION

UK guidelines recommend that for females of childbearing age, group O D-negative red blood cells (RBC) should be administered if blood group is unknown, to prevent D alloimmunization, which can lead to haemolytic disease of the foetus and newborn (HDFN) in future pregnancies [1]. Group O D-negative RBC are frequently transfused in the prehospital phase of resuscitation, as it is not possible to predict in advance if the patient is going to be a male or a woman of childbearing age or determine their D type. In the last decade our improved understanding of the biology of acute traumatic coagulopathy has resulted in the development of damage control resuscitation [2–5], which advocates for the rapid and balanced administration of RBC, platelet and plasma as early as possible in the patient's resuscitation. This has improved outcomes for patients compared to the standard of care alone, especially in the prehospital phase of the resuscitation [6–9]. The need to provide early haemostatic resuscitation with plasma has awakened significant interest in the reintroduction of low-titre group O whole blood (LTOWB), due in part to logistical advantages for the clinical team, and the ability to transfuse RBC, plasma and platelets in a 1:1:1 ratio [10–12]. The demand for blood products to be used in the prehospital phase of the resuscitation is likely to grow and blood collectors will need to optimize their procedures to ensure sufficient blood is available for patients both in and out of hospital [13].

Currently, most air ambulances in the United Kingdom carry D-negative RBCs and either dried or thawed plasma. Supply of O D-negative RBCs is a challenge for most blood operators due to the limited numbers of donors (around 8% blood donors in the United Kingdom and United States are Group O D-negative). While in England demand for RBCs has fallen by 24% since 2014, this is not mirrored by a similar decrease in demand for O D-negative RBCs, as noted by other blood collectors [14]. Currently the demand for group O D-negative RBCs is 14% of total RBCs in England, many issued as the next best alternative when those of RhcDe phenotype are unavailable for the treatment of sickle cell disease [15]. The disparity between demand for group O-negative RBCs and the proportion of such donors in the population necessitates significant recruitment activity to enrich the donor pool to meet demand [16]. There is concern regarding the long-term sustainability of supplying group O D-negative RBCs, especially if the demand for prehospital and treatment of sickle cell disease transfusion increases. Due to limited availability, group O D-positive RBCs are sometimes used to resuscitate bleeding trauma patients especially in the prehospital phase of the resuscitation in some jurisdictions [17]. This balance of the risk of D-alloimmunization against providing early balanced resuscitation to injured patients is a particular consideration in relation to the use of LTOWB, which has a shorter shelf life than additive solution containing RBCs, may result in more wastage of the product, but may be more efficacious than current prehospital standard of care in England, that is, RBCs and plasma.

The risk of significant harm due to transfusion of D-positive blood to bleeding trauma patients of unknown D group is likely smaller than historically thought since (a) 75% to 80% of trauma patients are male [18, 19], (b) the proportion of trauma patients that become D-alloimmunized is lower than values for healthy individuals [20, 21] and (c) improvements in the diagnosis and treatment of HDFN have markedly reduced the foetal mortality rate where access to modern foetal-maternal care is available [22]. The aim of this paper was to model the risks of harm from transfusing group O D-positive RBC components to trauma recipients in England who are bleeding in the prehospital setting.

MATERIALS AND METHODS

Model of sequence of events required to cause harm and definition of harms

WE calculated the probability of causing immediate and future harm to a D-negative patient resuscitated with D-positive RBCs in the prehospital setting. The harms modelled are the same for RBCs or LTOWB, and therefore, apply to either. We modelled three specific harms:

1. Haemolytic transfusion reaction (HTR) associated with index transfusion of D-positive blood
   Major morbidity or mortality due to an HTR caused by pre-existing anti-D in the patient reacting with D-positive red cells that were transfused during haemorrhagic trauma resuscitation. This may be acute or delayed and excludes hyperhaemolysis and ABO incompatibility. Major morbidity was defined as per the Serious Hazards of Transfusion Scheme as: Intensive care or high dependency admission and/or ventilation, renal dialysis and/or renal impairment, evidence of acute intravascular haemolysis (e.g., haemoglobinaemia or severe haemoglobinuria) and life-threatening acute reaction requiring immediate medical intervention, reaction resulting in a low or high haemoglobin level of a degree sufficient to cause risk to life [23].
2. HTR associated with future transfusion of D-positive blood
   Major morbidity or mortality due to an HTR to a future D-positive transfusion caused by the anti-D that was formed following the transfusion of the index D-positive RBCs during the haemorrhagic trauma resuscitation.
3. HDFN
   Death or lifelong disability of a foetus or child in a future pregnancy caused by HDFN, following maternal anti-D alloimmunization

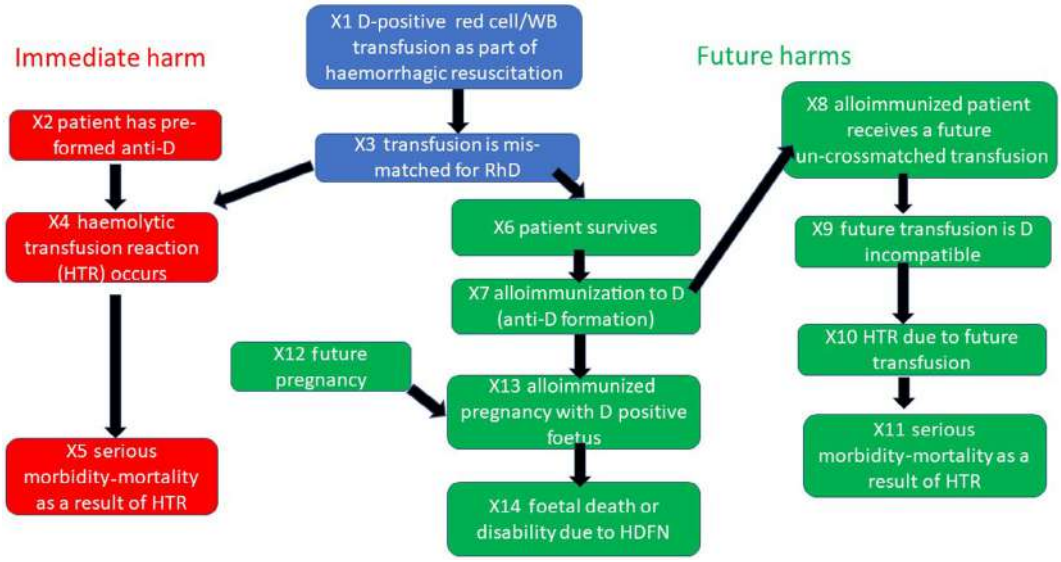

FIGURE 1 Sequence of events required to cause harm from the transfusion of D-positive red cells in the prehospital setting. HTR, haemolytic transfusion reaction; WB, whole blood

following the index transfusion of D-positive RBCs during the haemorrhagic trauma resuscitation.

These harmful events are dependent on the occurrence of a chain of contingent conditions and possibilities, for example, for an HTR to occur due to the index D-positive transfusion, the patient must have pre-existing anti-D. An estimate of the risk of resultant harm can thus be made from estimates of the probabilities of the necessary pre-requisites in the chain for harm to have occurred. Figure 1 presents the chain of events that are necessary for alloimmunization to occur, and for this alloimmunization to result in an HTR to the index transfusion of D-positive blood, future HTR or HDFN.

For the model, we assessed two patient populations: (a) all trauma patients of any sex or D type who require haemorrhagic resuscitation with D-positive RBCs or LTOWB components in the prehospital setting, and (b) the subset of patients who are D-negative females of childbearing potential (CBP, <50 years of age). The latter analysis was included to model the probability of causing harm to the patient population considered to be most at risk.

Model inputs and key assumptions used

We modelled the chain of upstream events that are necessary for the various harms to occur as a probabilistic graphical model (Figure 1). For each of the events (X1-14) that must happen for one of the three harms in Figure 1 to occur, we can estimate the expected number of events seen per recipient assuming the upstream events have occurred (model inputs N1-14, Table S1). The model inputs are specified in the form of probability distributions, to reflect the level of uncertainty regarding the true value of the input. Where possible, model inputs used published data from the United Kingdom, or if unavailable then published information from the literature was used as described in Table S1.

Estimation of risk of harm using Monte Carlo simulation

The model was calculated by Monte Carlo simulation using the probability distributions assigned as model inputs. The output allows the determination of credibility intervals (CIs) for the expected number of harmful events. The model was calculated over 1000 iterations, where a random value from the model input distribution is assigned to each event for each iteration. By repeating the process over multiple iterations, the frequency histogram for the event of interest converges to the probability distribution for the event and was used to calculate an estimated mean value and CI. The final expectation was expressed in a reciprocal form (number of transfusions expected to cause one harmful event). Calculations were performed using Wolfram Mathematica 12.0. Further details of the model and its calculation are given in Supplementary information.

In addition, for D-negative females under 50, we calculated the percentage reduction in 30-day mortality associated with the use of LTOWB (in terms of life-years gained) that would be equal to life years lost due to HDFN in future arising from the use of D-positive LTOWB for 10,000 bootstrap samples from the Monte Carlo simulation results. The survival data described above was used to calculate the probability that life-years gained would exceed life years lost if the percentage improvement in trauma survival was above a given level compared to current standard of care. For life years lost due to HDFN, we equated either permanent disability or death as 83 life years lost based on current data for UK life expectancy at birth.

Sensitivity analysis

A sensitivity analysis was undertaken to assess how the level of uncertainty in the individual model input assumptions contributes to

the overall uncertainty in the estimate of harm. This sensitivity analysis aimed to help prioritize future work to refine the risk estimate. Two complementary approaches were used. First, scatterplot analysis was used to demonstrate the relationship between the final estimate and individual input factors used in the Monte Carlo simulation. Second, a variance-based sensitivity analysis whereby the sensitivity index (S_i) for model input N_i represents the average proportional reduction in the variance of the final risk estimate if model input *Ni* could be fixed at an exact value [24]. Details of calculations are provided in Supplementary information.

RESULTS

Overall estimate of risk for all trauma recipients of D-positive RBCs

Results of the model showed that for every 14,000 D-positive transfusions that were administered to all trauma patients of any sex and age, one of the three specific harms (95% CI 5600–42,000) would occur. The frequency of occurrence of the three specific harms is shown in Table 1.

Risk estimate for D-negative females <50 years of age

For the highest risk subgroup (D-negative females under age 50), the model showed that for every 520 D-positive transfusions, one of any of the three specific harms would occur (CI 250–1700).

The main risk in this population was that of HDFN, where for every 570 transfusions, one foetal death or disability would occur (CI 260–2300), which is higher than the risk of the other two specific harms (contemporary and future HTR).

The data from Table 1 were used to calculate the expected length of time before a specific harm would be expected to occur if a policy of using group O-positive RBCs for prehospital transfusion is introduced universally across England. There are no UK data to estimate how often un-crossmatched blood is transfused prehospital for trauma patients, so we used the data from Stanworth et al. [18] as a surrogate, who reported the overall incidence of major haemorrhage in trauma as 83 per million patients per year (defined as receiving at least 4 RBC units in the first 24-h of hospital admission) in England, using 2020 population figures for England of 67 million. The results of the modelling demonstrated that the number of years required for any of the three specific harms to occur is 2.5 (CI 1.0–7.5) for all recipients, and 5.2 (CI 2.5–17.3) for D-negative females of CBP (Table 2). HDFN would be expected to occur every 5.7 years in the latter group. Based on the assumptions and data used in this model, in 95% of the simulations, a survival increase of at least 1.0% from the use of LTOWB compared to current standard of care in the United Kingdom, would lead to life years gained exceeding life years lost due to HDFN in the future if D-positive whole blood was transfused to D-negative females under 50. Thus, we can be 95% confident that if the trauma survival improvement was greater than 1%, then life-years gained associated with the use of LTOWB would exceed years lost due to HDFN for the latter group of patients.

The risk of an HTR due to an index D-positive transfusion was 1:27,000 transfusions for all recipients and 1:6600 for D-negative females under 50; this translates to one event occurring every 4 years and 65 years, respectively, in England.

Sensitivity analysis of the overall risk estimate to individual model inputs is shown in Figures S4 and S5. These Supplementary data show that for unselected recipients, uncertainty in the risk of HTRs dominates the uncertainty of the total risk. In contrast, for D-negative recipients under 50, the uncertainty of the total risk is dominated by uncertainty regarding the alloimmunization risk.

DISCUSSION

We modelled the three main risks associated with the transfusion of D-positive RBCs to trauma patients of unknown D type, as would be the case when transfusions are administered in the prehospital and early in-hospital phases of the resuscitation. In general, the risks of acute or future HTR and HDFN are low, especially when compared to the benefits of providing transfusions early in the resuscitation. The risk of HTR from future transfusions is several orders of magnitude lower than that of HDFN.

TABLE 1 Estimation of risk of harm from transfusion of D-positive red blood cells or low-titre group O whole blood in the prehospital treatment of major haemorrhage using model inputs and Monte Carlo simulation

Harm	All recipients One event every × transfusions (95% CI)	D-negative females of childbearing potential One event every × transfusions (95% CI)
Major morbidity or mortality due to HTR from index D-positive transfusion	2.7×10^4 (7.6×10^3–3.4×10^5)	6.6×10^3 (1.8×10^3–9.2×10^4)
Major morbidity or mortality due to future HTR	8.5×10^5 (1.8×10^5–2.1×10^7)	1.4×10^5 (3.1×10^4–3.7×10^6)
Foetal death or permanent disability due to anti-D HDFN in future pregnancy	2.9×10^4 (1.2×10^4–1.2×10^5)	570 (260–2300)
Any of above three harms	1.4×10^4 (5.6×10^3–4.2×10^4)	520 (250–1700)

Abbreviations: CI, credibility interval; HDFN, haemolytic disease of the foetus and newborn; HTR, haemolytic transfusion reaction.

TABLE 2 Predicted number of years to observation of harm in England based on model if a change in policy to use D-positive red blood cells or low-titre group O whole blood was implemented

	All recipients	D-negative females of childbearing potential
Prehospital transfusions per year	5561	100
Years to one HTR major morbidity or death due to index D-positive transfusion	4.8 (1.4–61)	66 (18–910)
Years to one future HTR major morbidity or death	150 (35–3700)	1400 (310–37,000)
Years to one future anti-D HDFN death or disability	5.2 (2.2–21.6)	5.7 (2.6–22.5)
Years to any of three above harms	2.5 (1.0–7.5)	5.2 (2.5–17.3)

Abbreviations: HDFN, haemolytic disease of the foetus and newborn; HTR, haemolytic transfusion reaction.

The most feared sequelae of transfusing D-positive RBCs to patients who are later found to be D-negative is foetal demise or the requirement for intensive, and often invasive, ante- and post-partum management of HDFN. These data predict that one foetal death or disability due to HDFN will occur for every 500 transfusions to females of CBP (once every 5 years in practice in England), if exclusively D-positive transfusions are provided in the prehospital setting to all patients. Our modelling assumed that D-positive blood would be exclusively transfused instead of RBC negative units, that is, the scenario with the highest potential for causing anti-D-related adverse events. If D-positive blood is provided as a 50:50 mix with D negative units, or only if D-negative units are unavailable, then these risks would be lower. At one large American trauma centre [25], the authors calculated that the overall risk of experiencing HDFN would be 1.2/100 transfused D-negative females, or approximately 1 HDFN case every 20 years. At another American trauma centre, it was estimated that it would take 250 years for 3–30 females, to become D-alloimmunized due to the relative infrequency at which these females are transfused at that centre [17]. During this time, 500 females of CBP would die from haemorrhage if prehospital transfusions were not available. We predicted that a case of HDFN would be expected to occur in England more frequently than at these American centres, likely due to the total higher number of females of CBP, approximately 20-fold that would be transfused throughout the country of England compared to the number transfused at a few regional trauma centres.

The strength of our study is that we have, for the first time, modelled the risk of a national policy change to the use of group O D-positive blood in the prehospital setting, based on the best available data and applied accepted mathematical modelling to illustrate the uncertainty in these estimates. This modelling permits the estimation of a credible range of frequencies for the potential risk, taking account of the uncertainty in the assumptions in parallel, allowing the use of conservative risk estimates in policy making. There is a high level of uncertainty regarding some of the underlying assumptions due to limited data on the frequency of occurrence of many of the events that were modelled. For example, the uncertainty surrounding the frequency of the overall risk of the three specific harms for females of CBP is dominated by uncertainty over the risk of alloimmunization. It can be seen from the factor analysis scatterplot that if the risk of immunization was 10%, then we would expect to see a specific harm every 7–17 years, whereas if the alloimmunization risk was 40%, we would expect to see a harmful event every 1–5 years. Nevertheless, the effect of uncertainty in the other variables means that the variance of the final risk estimate would only be reduced by 11% even if the value for risk of alloimmunization were certain. In other words, expending effort in performing studies to firm up the alloimmunization risk assumption would not have a large impact on final risk estimates.

While a rate of having any one of the three studied specific harms occurring once for every 14×10^3 D-positive transfusions in the overall population is nearly the same as the reported rate of transfusion-associated circulatory overload (1:25 $\times$ 10^3 transfusions) [23], the rate of anti-D-mediated HDFN among D-negative females of CBP is much higher at (one case of HDFN per 52×10^1 D-positive transfusions) in the prehospital setting. However, the survival benefits of early transfusion in trauma resuscitation must be balanced against these risk estimates. Based on the data used in our model, life years gained from a 1% reduction in mortality compared to current standard of care would be expected to exceed life years lost due to the risk of HDFN for D-negative females under 50 years of age. This value would be lower for all recipients of LTOWB prehospital since any survival benefit would be seen across the entire population with little increased risk of HDFN compared to that for D-negative females under 50. Compared to using only saline as the resuscitation fluid, several studies have demonstrated significantly better survival when blood products are administered during the patient's transport to the hospital [6–9]. Most of the air ambulance services in England already provide D-negative RBCs to their patients as well as some form of plasma, thus not conferring a risk of D-alloimmunization. So why change the current standard of care? Although the UK blood services have managed to supply D-negative RBC to prehospital services, if demand rises, further demand for D-negative RBCs may outstrip supply, and if this occurs, the resuscitation options would be either to revert to saline with its inherent risks and little benefit to the injured recipient [26], or to transfuse D-positive RBCs or LTOWB. Risk-assessments like this one, are vital to estimating the risks of harmful events associated with transfusion of D-positive units to D negative recipients. These need to be balanced against the benefits of either red cell or whole blood transfusion in the prehospital setting, which will depend upon the standard of care and availability of D-negative units in each jurisdiction. It would also be possible to mitigate the risk of HDFN by using D-positive blood only for males, and D-negative LTOWB for women of CBP. However, in practice, prehospital teams may find it challenging to store, transport and provide multiple types of blood.

The current philosophy of resuscitating trauma patients early with blood transfusion is shifting towards providing a balanced resuscitation that includes platelets in addition to RBCs and plasma, similar to whole blood [10–12]. Multiple donor qualifications are required in order to be eligible to donate LTOWB, which makes it challenging for blood providers to supply RhD-negative LTOWB to all trauma patients. As a result of these supply issues, the use of D-positive LTOWB and RBCs for injured patients early in their resuscitation is becoming a common practice at least in the United States; in an international survey of LTOWB practice (mainly from the United States), 27% of the respondents reported using D-positive LTOWB for females of any age [27], while a survey of American Level 1 trauma centres revealed that 51% would administer a D-positive LTOWB unit to a female of CBP whose D-type was unknown in a bleeding emergency [28].

Based on these risk estimates, a survival benefit of 1% or greater from transfusing red cells compared with no transfusion or whole blood versus red cells in the prehospital setting, outweigh the risks of harmful events associated with transfusion of D-positive units to D-negative recipients. In addition, reallocation of D-negative RBCs away from prehospital use would facilitate the provision of these scarce RBCs to patients who are already D-alloimmunized, and patients who require antigen-matched RBC such as sickle cell disease patients for whom alloimmunization could pose dire long term health consequences. A survey of transfusion and trauma services directors at the 30 largest children's specialty hospitals in the United States revealed some reluctance to enroll injured girls of unknown D-type in a study of D-positive LTOWB transfusion versus component therapy, consistent with the traditional practice of providing D-negative blood components in this clinical setting [29]. However, in a survey of staff at a large American university, 90% of the respondents who were females of CBP indicated that they would accept a lifesaving transfusion even with the knowledge that it could harm future pregnancies [30]. Further work is needed to understand the views of patients and the general public as to whether these risk are acceptable, so that their views can be taken into account when determining policy. In addition, protocols and surveillance for follow-up of D-negative females of CBP who receive D-positive whole blood or red cells are required.

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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

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Routine donor red cell antibody screening: Considering the alternate strategy

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Abstract

Background and Objectives: Australian Red Cross Lifeblood (Lifeblood) performs red blood cell (RBC) antibody screening on every whole blood donation. An alternate strategy has been proposed whereby an antibody screen is performed on the first donation and only repeated following pregnancy, transfusion or a significant break between donations (>2 years). We assess the blood safety risks associated with removing antibody screening for every whole blood donation.

Materials and Methods: A retrospective desktop analysis included all whole blood donations collected by Lifeblood between 01 May 2018 and 30 April 2019 to quantify the antibodies that would have been undetected with the alternate strategy. The strategy was further assessed using the Alliance of Blood Operators Risk-Based Decision-Making framework.

Results: One hundred and seventy-one routine donors had antibodies for the first time, but reported no sensitizing event since their last donation. Forty-seven of these had antibodies of a clinically significant specificity and titre that have the potential to cause a haemolytic transfusion reaction (HTR). The calculated risk of undetected antibodies being transfused to an incompatible recipient is 1 in 82,200.

Conclusion: The estimated risk of HTRs with the alternate strategy results in an increased risk. While the alternate strategy is identified as the most cost-effective option within the Australian setting, this additional residual risk was not deemed to be acceptable. Blood services would need to determine whether the increase in residual risk stemming from implementation of such a strategy is tolerable.

KEYWORDS
blood donation testing, blood safety, haemolytic transfusion reaction, RBC antigen and antibodies

Highlights

- The proposed alternate strategy to red cell antibody screening for whole blood donations is when an antibody screen is performed on the first blood donation and only repeated following pregnancy, transfusion or a significant break between donations (>2 years).
- The alternate strategy offers economic and operational benefits in the Australian setting, but is calculated to have an increased risk of acute and fatal haemolytic transfusion reactions.

INTRODUCTION

Red cell antibody screening is a critical step to identifying unexpected non-ABO antibodies in donor plasma. The reactivity of these antibodies can be variable and has the potential to cause haemolytic transfusion reactions (HTRs) or shortened red cell survival when transfused [1, 2]. This event can be severe and potentially fatal. In order for a non-ABO antibody to cause a HTR, it must be the correct isotype (IgG) and be present in a sufficient titre. The recipient must also express the antigen corresponding to the antibody [3]. The current rate of HTRs from non-ABO antibodies is low, ranging from 1 in 76,000 for acute reactions to 1 in 2500 for delayed reactions [4]. Rates of fatal HTR are much rarer and have had a recent downward trend; estimated at approximately 1 in 54 million [5–7].

It has been a long-standing practice in Australia to screen all whole blood donations for red cell antibodies. This strategy is the most conservative to maximize the detection of red cell antibodies in donor plasma, but involves substantial blood bank resources. While few changes have been made to this practice for decades, a modified test panel for apheresis plasma donations for fractionation was introduced in 2016. The modified panel meant that plasma for fractionation donations was no longer routinely screened and instead screening targeted donors who were most likely to have developed a non-ABO antibody. Criteria for screening includes:

1. A new donor
2. A returned donor (no donation in the previous 2 years)
3. Donor identified on screening questionnaire to have had a potentially sensitizing event since the last donation, including pregnancy or transfusion

This modified panel has shown notable economic benefits including productivity gains and a significant reduction in reagent expenditure. Consequently, this alternate targeted red cell antibody screening strategy had been proposed for extension to other blood donations. It was hypothesized that similar benefits would be seen by extrapolation of this strategy, although this must be weighed against any anticipated change in blood safety risk. The alternate strategy is heavily dependent on donor accuracy when answering screening questionnaires at every donation, to ensure that the collections that need further screening are appropriately identified. This study aimed to assess the blood safety risk associated with the alternate strategy by quantifying the number of antibodies that would have been undetected if this strategy were used as a result of a repeat donor failing to provide a history of a sensitizing event. Changes to blood safety risk were compared with a cost–benefit analysis to address if the cost-effectiveness and productivity of donor antibody screening could be improved while maintaining an acceptable risk profile.

The use of the alternate strategy whereby red cell antibody screening is targeted aligns with Australian regulations. Lifeblood must comply with the *Therapeutic Goods (Standard for Blood and Blood Components) Order 2019* (*TGO 102*). TGO 102 specifies that blood and blood components are subject to the requirements stated in the *Council of Europe Guidelines, 19th edition* (*CoE 19*) [8]. At present, the current or 'status quo' screening strategy for blood donations exceeds the requirements outlined in the *CoE 19* [9].

MATERIALS AND METHODS

A retrospective desktop analysis was performed for all whole blood donations collected by Lifeblood between 01 May 2018 and 30 April 2019, including a review of all antibody screening results. The primary outcome of the study assessed the number of antibodies that would have been undetected if the proposed alternate strategy were used. Antibody specificity and titre were recorded to estimate the overall anticipated increase in blood safety risk if these antibodies had not been detected.

The current and alternate antibody strategy were assessed for their suitability using the Alliance of Blood Operators (ABO) Risk-Based Decision-Making (RBDM) framework [10]. The RBDM framework offers a structured and systemic paradigm in evaluating risks to blood safety [11]. This framework was used to make several assessments including blood safety, health, economic and operational risk assessments.

RESULTS

A total of 824,917 samples were screened for red cell antibodies during the 12-month study period. A total of 942 (0.11%) donations from 430 unique donors had antibodies identified when the donor answered 'no' to being pregnant or receiving a transfusion (i.e., sensitizing event) since their last donation.

An overview of the study population is shown in Figure 1. One hundred and seventy-one of these donors were found to have non-ABO antibodies detected for the first time during the assessment period. Of these, 47 donors had antibodies of a clinically significant specificity and titre that had the potential to cause a HTR. Thirty-one donors were identified to have autoantibodies. The characteristics of the donor antibodies including specificities and titres are shown in Table 1.

Red cell autoantibodies

A total of 31 donors were found to have autoantibodies detected for the first time during the study period, all with a titre of neat. All donors were referred to their general practitioner (GP) for further assessment as per the current practice guidelines and the red cell component was discarded. Follow-up of the 31 donors showed the following:

1. Two donors confirmed a subsequent diagnosis of autoimmune haemolytic anaemia (AHA).

2. Two donors were assessed and cleared by their GP, and returned to donate. These donors were restricted to apheresis plasma for fractionation.

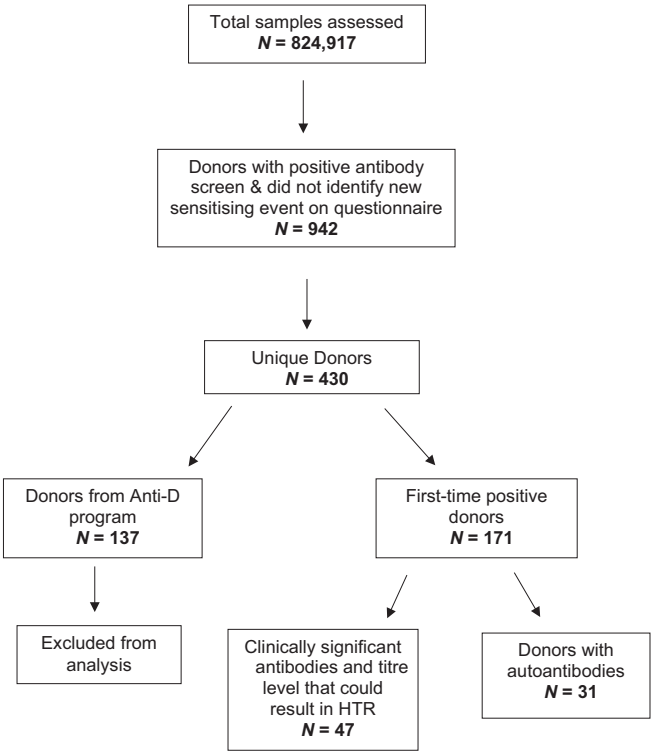

FIGURE 1 Flow chart displaying an overview of the study population and donor antibody screen findings. HTR, haemolytic transfusion reaction

3. The outcome of the remaining 27 donors is unknown. These donors did not return or notify Lifeblood of their outcome.

In Australia, each donor donates approximately 2.4 times per year. Consequently, it is predicted that 70 red cell components with autoantibodies would not be detected by the proposed alternative strategy.

Red cell alloantibodies

Antibody titre ≥64

An IgG antibody titre ≥64 is considered strongly clinically significant and has the potential to cause a HTR in a recipient. This finding is rare. Five donors were found to have non-ABO antibodies with a titre ≥64 during the assessment period. This included one donor with anti-D antibody, one donor with anti-K antibody and three donors with unknown antibody types.

These individual donors and their available histories were assessed further. The anti-D positive donor was found to have a history of previous medical episodes that required both surgery and transfusions. The donor-notified Lifeblood and donation deferral periods were applied. This deferral period did not exceed 2 years and consequently the donor did not meet the criteria of a 'Returned Donor' used in the screening questionnaire. Upon return, the donor reported 'no' on their questionnaire to having received a transfusion or blood products since their last donation. It is assumed the donor believed they had answered the question correctly given Lifeblood had already been notified of the previous sensitizing events. This

TABLE 1 Specificities and titres for antibodies detected for the first time during the assessment period

Specificity	Number with antibody type	Titre			
		Not clinically significant	Neat	8	64
Autoantibodies	31	N/A	31	0	0
Anti-D	11	0	10	0	1
Anti-c	1	0	1	0	0
Anti-C	1	0	1	0	0
Anti-Cw	4	0	3	1	0
Anti-E	11	0	9	2	0
Anti-Fya	2	0	1	1	0
Anti-Jka	7	0	7	0	0
Anti-K	6	0	4	1	1
Anti-Kpa	1	0	1	0	0
Anti-Lea, anti-Leb[a]	2	2	0	0	0
Anti-M[a]	10	5	2	2	1
Anti-N[a]	1	0	1	0	0
Non-specific antibodies	80	0	80	0	0
Unknown	3	0	0	0	3

[a]Antibody specificity or titre is not considered clinically significant.

TABLE 2 Risk estimations for antibodies with titre ≥64 if alternate strategy used

Antibody specificity	Antigen frequency in population [3]	Affected components	Number of components transfused to incompatible recipients	Donor sex
Anti-D	0.85	3	2.55	Male
Anti-K	0.09	2	0.18	Female
Unknown	1.0	5	5.0	2 Female 1 Male
Total	-	10	7.73	3 Female 2 Male

TABLE 3 Risk estimations for antibodies with titre of neat or 8 if alternate strategy used

Antibody specificity	Antigen frequency in population [3]	Male plasma products	Platelet and red cell components	Proportion of paediatric components	Number of components transfused to incompatible recipients
Anti-D	0.85	2	19	0.0022	1.7355
Anti-c	0.80	0	2	0.0022	0.0035
Anti-C	0.70	1	2	0.0022	0.7031
Anti-Cw	0.01	1	8	0.0022	0.0102
Anti-E	0.30	2	18	0.0022	0.6119
Anti-Fya	0.65	1	3	0.0022	0.6543
Anti-Jka	0.77	1	12	0.0022	0.7903
Anti-K	0.09	2	6	0.0022	0.1812
Anti-Kpa	0.02	1	3	0.0022	0.0201
Total	-	11	73	-	4.71

donor could have been detected if the strategy considered testing all donors following any deferral period despite its duration. However, the situation highlights the reliance of the alternate strategy on donors accurately answering the screening questionnaire at each donation.

The donor with anti-K had a history of pregnancy and required a transfusion in 2014. The appropriate deferral periods were applied and the donor made two donations following the expiration of the deferral, both donations had negative red cell antibody screens. The anti-K antibody was only detected subsequent to these donations and was detected on two separate donations. Most cases of anti-K are stimulated by pregnancy or transfusions, though there have been reports of the antibody occurring naturally [12]. Red blood cell (RBC) alloantibodies can also fall beneath the level of detection. This is a property known as antibody evanescence which has been found to occur with 35% of alloantibodies in healthy blood donors and can be considered in this case [13].

The identification of the remaining three antibodies were non-specific.

Antibody titre of neat or 8

A titre of neat is sufficient to cause a HTR in a neonate. Forty-two donors were found to have previously undetected IgG antibodies with a titre of neat or 8. Only six of these donors had comments reporting possible sensitizing events. This included one donor with a history of pregnancy, one with a history of both pregnancy and surgery and four with a history of surgery alone. Neither had an indication for transfusion. With the exception of one, all of these donors returned a negative antibody screen between the potential sensitizing event and these results.

Measurements of blood safety risks

The predicted number of blood components with an antibody titre ≥64 that would be transfused to incompatible recipients if the alternate strategy was used, are shown in Table 2. This risk was estimated by multiplying the number of affected components manufactured from these donations with the frequency of the corresponding antigen in the population. Plasma from female donors were excluded from calculations since only plasma from male donors are transfused due to the risk of transfusion-related acute lung injury (TRALI). For antibodies with an unknown specificity, a conservative assumption was made that the antibodies were clinically significant and all recipients express the corresponding antigen. It is estimated that a total of 7.73 components with a titre ≥64 would be transfused to incompatible recipients during this study period.

The predicted number of blood components with an antibody titre of neat or 8 that would be transfused to incompatible recipients

are shown in Table 3. Current procedure for donations with a titre of neat or 8 is to restrict the red cell and platelet transfusion from being used in neonates, and divert plasma for fractionation. Consequently, to estimate the number of components with an antibody titre of neat or 8 that would be transfused to any incompatible recipient, we assume that only paediatric red cell and platelet components, and plasma from male donors could result in a transfusion reaction. Of the total units issued between January and December 2018 (1,022,611 units), 0.22% were paediatric or neonatal units (2249 units).

It is estimated that a total of 4.71 components with a titre of neat or 8, would be transfused to an incompatible recipient during the 12-month period if the alternate strategy was used.

During the study period, a total of 1,022,611 clinical products were issued to approved health providers. The risk of undetected non-ABO antibodies in clinical products transfused to incompatible recipients was consequently measured at 1 in 82,200 (12.44/1,022,611).

DISCUSSION

Blood safety risk assessment

This study estimates a likelihood of HTRs due to donor non-ABO red cell antibodies with the proposed strategy at 1 in 82,200. This is considered a significant increase in risk that will be in addition to the current rate of HTRs (predominantly due to recipient red cell antibodies) at 1 in 76,000 [4]. This risk is estimated for over a 12-month period, and it can be presumed that the risk probability would be cumulative as additional routine donors present with undetected antibodies. Concerningly, the distribution of risk is not predicted to be equitable. The groups bearing the greatest risk will be the most vulnerable, namely, neonates who have the highest morbidity from haemolysis. The outcomes of a HTR are also broad, and can range from minor to catastrophic. However, as per the organizational risk framework, even relatively minor HTRs would be classified as moderately severe due to the need for hospitalization, investigations and treatment.

Several factors contribute to an increased risk of undetected non-ABO antibodies using the alternate strategy. This strategy heavily relies on the accuracy of donor questionnaires, though history may be incorrectly reported and there is a lack of validity with medical or other records. In a study by Karafin et al., a small percentage of donors were identified to have a positive antibody screen in the absence of risk factors such as pregnancy or previous transfusions [14]. Multiple possible causes included that donors may have been unknowingly transfused during past surgeries or transfusion histories may have been incorrectly answered on donor questionnaires [14]. Donors may also knowingly incorrectly answer the questionnaire should it disclose sensitive information such as a miscarriage or termination of pregnancy. Positive antibodies may also be in part due to non-specific reactivity, formed after environmental exposure or be 'naturally occurring' [14]. Our study results support that reliance on donor screening questionnaires is not sufficient to adequately identify all routine donors with non-ABO antibodies.

Outside of concerns around transfusion reactions, the presence of donor red cell antibodies can have further impacts for the recipient. Passively transferred antibodies can interfere with future antibody screens and impact on future transfusions for the recipient [14].

Other control measures can be identified and implemented to mitigate some of this risk associated with the alternate strategy. This includes the provision of the Blood Management System (BMS) to automatically order red cell antibody screening following expiry of selected deferral periods (i.e., surgery, pregnancy, miscarriage). This reduces the reliance on donor questionnaire accuracy. Strategies to reduce risk among vulnerable recipients can also be considered including the use of mandatory tests for neonatal components or antibody screening on all CMV-negative products. There is, however, no single measure that can address the many ways a donor may be sensitized, or effectively manage the manufacturing of neonatal components.

Health economic assessment

A comparison of the cost-effectiveness of the status quo and alternate red cell antibody screening strategies was assessed by the Centre for International Economics (CIE) [15]. Assessments considered testing volumes, test cost per sample (for the automated pooled cell screen, 2 cell screen and antibody identification assay), rate of antibody positive samples in the proposed untested population, probability of an acute or fatal HTR and consequent health impacts. The cost of the current strategy was approximated at $930,800/year compared to a cost of $252,800/year with the alternate strategy [15]. The alternate strategy was identified as the most cost-effective option, with an estimated savings of approximately $678,000 [15]. Though the alternate strategy had higher health impact costs compared to the status quo, this was outweighed by a reduction in overall testing costs.

The alternate strategy is expected to lead to a reduction in the number of donations requiring antibody screening from 824,917/year to as low as 156,298/year. The cost savings seen are largely from reduced reagent expenditure [15]. The alternate strategy not only reduces the number of antibody screens performed but also reduces the number of 2 cell screens completed in the setting of indeterminate primary screen results as well as antibody identification performed on both true and false positive antibody screens [15].

Health impact costs are expected to be between $55.96 to $147.80 per year [15]. These costs are related to the additional rare occurrence of acute and fatal HTRs, with an estimate value to life lost prematurely being approximately $2,575,000, with a statistical life year of approximately $198,000 [15].

Operational assessment

The different strategies also carry operational implications. Overall operational risks are measured to be small and manageable with existing control measures. The greatest operational impact will likely be

seen from an increase in system and process control in an attempt to mitigate the relatively increased blood safety risk.

There is otherwise likely to be operational benefit from the alternate strategy with an increase in productivity for operators and the fleet of immunohematology analysers. It is predicted that following a reduction in additional blood safety controls, the immunohematology analysers would have increased capacity to perform other required assays.

Comparison with other countries

Internationally, there is a varied approach to red cell antibody screening, ranging from not routinely screening blood donations for red cell antibodies at all, to a targeted approach based on donor history or routine screening of all donations.

Our understanding is that Australia's combined approach of mandatory testing of donations for direct transfusion, and targeted testing of apheresis plasma for fractionation is unique. The American Association of Blood Banks (AABB) Standards require that plasma or serum from blood donors are routinely tested for non-ABO antibodies [16]. If not completed, the FDA Code of Federal Regulations requires completion of a minor crossmatch to ensure compatibility [17]. Mandatory and routine antibody screening for all donations is also practiced by other nations including the Canadian Blood Services, National Health Service Blood and Transplant (NHSBT) and Irish Blood Transfusion Service [18–20].

There are little data available from nations that have adopted a similar approach to the alternate strategy assessed in this paper. There is unpublished data gathered as part of the ABO Benchmarking Working Group Report [21] that has identified Denmark, Croatia and Germany as having adopted a targeted red cell antibody screening approach though further published details are not available.

In contrast, some countries elect not to routinely screen blood donations for red cell antibodies. In mainland China, there is no regulatory requirement to screen blood donations for non-ABO red cell antibodies [2]. A 2018 study estimated that 0.152% of the Chinese donor population had non-ABO antibodies, with most cases among married women. The study concluded that given the homogenized donor population and predominate presence of IgM antibodies, the clinical benefits of routine antibody testing did not outweigh the resultant economic cost [2].

Other countries such as India, estimate a prevalence of non-ABO red cell antibodies in their donor population between 0.05% and 0.09% [22, 23]. While red cell antibody screening is required as part of the National Blood Policy in India, it is not a mandatory procedure. Consequently, routine red cell screening is not practiced in many centres due to the de-centralized natures of local blood services [1].

In conclusion, the proposed alternate strategy for whole blood donations is when an antibody screen is performed on the first blood donation and only repeated following pregnancy, transfusion or a significant break between donations (>2 years). The strategy meets current regulatory guidelines in Australia and is likely to offer economic and operational benefits. However, this is outweighed by the measured additional blood safety risk of acute and fatal HTRs from donor non-ABO antibodies. The consequent increase in risk is not equitable and is likely to predominately affect our most vulnerable population groups, namely, neonates. Given this increased risk, the proposal would be considered unacceptable by our current organizational risk framework. However, this risk assessment is specific to Lifeblood practices and context, and it is recognized that international practices vary widely. The adoption of an antibody screening strategy by other blood services would depend on the prevalence of red cell antibodies, available funding and the level of residual risk that is considered tolerable by the institution.

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S.H. performed the research and prepared a discussion paper. A.S. drafted the initial manuscript. J.D. and T.P. reviewed and edited the manuscript. All authors approved the submitted version.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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SHORT REPORT

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Impact of the pathogen inactivation process on the migration of di(2-ethylhexyl) phthalate from plasma bags

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Abstract

Background and Objectives: Di(2-ethylhexyl) phthalate (DEHP) is a toxic plasticizer that is commonly used in the manufacture of polyvinyl chloride (PVC) blood bags. It is well known that DEHP can migrate from a medical device into the blood plasma. For safety reasons, pathogens in plasma must be inactivated; however, this process may increase DEHP migration. Here, we assessed the impact of illumination-based pathogen inactivation on the migration of DEHP from PVC bags into plasma.

Materials and Methods: Pairs of native PVC-DEHP plasma bags were pooled. Each pool was then split into a pathogen-inactivated bag and a control bag. After illumination, the plasma concentrations of DEHP and its main metabolite (mono (2-ethylhexyl) phthalate, MEHP) in each bag were assayed and compared using liquid chromatography–tandem mass spectrometry. Concentrations were evaluated in repeated-measures, two-way analyses of variance.

Results: The MEHP concentration was significantly associated with storage but not with illumination ($p = 0.0001$). The DEHP concentration stayed constant throughout the storage period. The DEHP equivalent concentration (corresponding to the overall plasticizer migration rate into plasma) was not significantly associated with illumination ($p = 0.3$) or storage ($p = 0.09$; mean ± standard deviation of the mean DEHP concentration for all conditions: 147.9 ± 11.3 μg/ml).

Conclusion: Illumination-based inactivation of pathogens in plasma did not increase the DEHP equivalent concentration, relative to control (non-inactivated) plasma.

KEYWORDS
DEHP, di(2-ethylhexyl) phthalate, metabolite, pathogen inactivation process, plasma

Highlights

- We studied the impact of illumination-based pathogen inactivation on di(2-ethylhexyl) phthalate migration from a polyvinyl chloride bag into the blood plasma.
- The illumination process promoted the degradation of di(2-ethylhexyl) phthalate into mono (2-ethylhexyl) phthalate.
- The illumination process did not increase the di(2-ethylhexyl) phthalate equivalent concentration, relative to non-inactivated plasma.

INTRODUCTION

The plasticizer di(2-ethylhexyl) phthalate (DEHP) is widely used in the manufacture of polyvinyl chloride (PVC) medical devices for transfusion. It renders the bags and tubing used for blood collection more flexible and facilitates the preparation and storage of labile blood products (LBPs). As DEHP is not chemically bonded to the PVC, it can migrate into LBPs during storage. Since DEHP is classified as being toxic for reproduction (category 1B) and is an endocrine disruptor, its use is subject to particular attention [1]. In the intestine, DEHP is hydrolysed by lipases to form mono(2-ethylhexyl) phthalate (MEHP). Given that MEHP is thought to be responsible for much of DEHP's toxicity, it is also very important to monitor levels of this metabolite [2].

The release of plasticizers from a medical device depends on the type of LBP and the storage conditions (temperature and duration) [3–9]; the higher the temperature and the longer the storage period, the greater the release of DEHP [5, 8–10]. The quantity of DEHP released by the medical device also depends on the nature of the LBP [1, 4, 5, 7, 9, 11]. For example, after 1 day of storage, the mean DEHP concentration was higher in plasma and whole blood (18.7 μg/ml) than in red blood cell and platelet concentrates (6.6 μg/ml) [3].

Plasma is widely used in the treatment of trauma patients and burn victims [12]. It can be transfused directly or processed into blood-derived medicines. To ensure the safety of plasma transfusion with regard to the potential for virus transmission, pathogens can be inactivated using either solvent–detergent methods or an illumination process involving a DNA intercalant (e.g., amotosalen and methylene blue [MB] dye). MB interacts with viral nucleic acid; after the dye has been dissolved in the plasma, activation with visible light blocks viral replication. Finally, the MB is removed from the plasma using a specific filter. The preparation of inactivated, filtered plasma requires the use of additional plasticizer-containing PVC bags and thus further exposes the patient to a potential source of DEHP.

In the present study, we investigated the impact of the pathogen inactivation process on the DEHP exposure rate. To this end, we assayed the plasma concentrations of DEHP and its main metabolite MEHP in illuminated bags and non illuminated (control) bags.

MATERIALS AND METHODS

Preparation of plasma samples

Pooling and splitting of plasma samples

Plasma from whole blood was supplied in PVC-DEHP bags (Px, $n = 12$) by the French Blood Establishment (Lille, France). Six pairs of ABO- and Rh- matched bags were pooled. For each pair, the pooled contents were then split into equivalent volumes in the original two bags (Figure 1a). Hence, each pooled pair gave rise to a pathogen-inactivated bag (Px_i, $n = 6$) and a control, non-inactivated bag (Px_C, $n = 6$). Px_C bags were frozen at −40°C immediately after preparation, and Px_i bags were frozen at −40°C immediately after the pathogen inactivation process.

The pathogen inactivation process

Each Px_i bag was connected to a specific device (THERAFLEX MB-Plasma®, Macopharma, Tourcoing, France) for pathogen inactivation (Figure 1b). Before illumination, the plasma was filtered to remove leukocytes, red blood cells, platelets and aggregates. Solid MB was then dissolved in the plasma to a final concentration of 1 μM. The dissolved, intercalated MB was immediately activated with visible light ($\lambda = 627 \pm 10$ nm), for exactly 15 min (MacoTronic B2, Macopharma), to block viral replication. Finally, the illuminated plasma was filtered to remove the MB and any photoproducts.

Plasma sampling

To determine the extent of DEHP migration into plasma, a plasma sample was taken from each Px_i bag and each Px_C bag before the illumination process (D0) and then 1 and 30 days (D1 and D30, respectively) after the illumination process. The samples were collected in light-protected polypropylene tubes, frozen at −80°C immediately, and then assayed for DEHP and MEHP using liquid chromatography–tandem mass spectrometry (LC–MS/MS, see below).

Determination of DEHP and MEHP using LC–MS/MS

After a liquid–liquid extraction (LLE) step, described previously, DEHP and MEHP were assayed using LC–MS/MS [3]. Briefly, LC–MS/MS was performed on a UFLC-XR system (Shimadzu, Kyoto, Japan) coupled to a QTRAP® 5500 MS/MS hybrid triple quadrupole/linear ion trap mass spectrometer (Sciex, Foster City, CA, USA), equipped with a Turbo VTM ion source operating in positive ion mode. The analytes were extracted thrice from plasma via LLE in an n-heptane/ethyl acetate (1/2 v/v) mixture (Merck, Guyancourt, France). DEHP-d_4 was used as an internal standard (Sigma-Aldrich, Saint-Quentin Fallavier, France). The plasma DEHP and MEHP concentrations were determined using an internal calibration method. The concentration ranges were 10–120 ng/ml for DEHP and 3–30 ng/ml for MEHP, and each calibration standard was supplemented with 5 ng/ml of DEHP-d_4. DEHP and MEHP were quantified using 1/x weighted linear and quadratic models, respectively. Each sample was analysed in triplicate.

Calculations and statistics

The DEHP equivalent concentration (corresponding to the total plasticizer migration rate in the blood) was calculated using the following equation [13]:

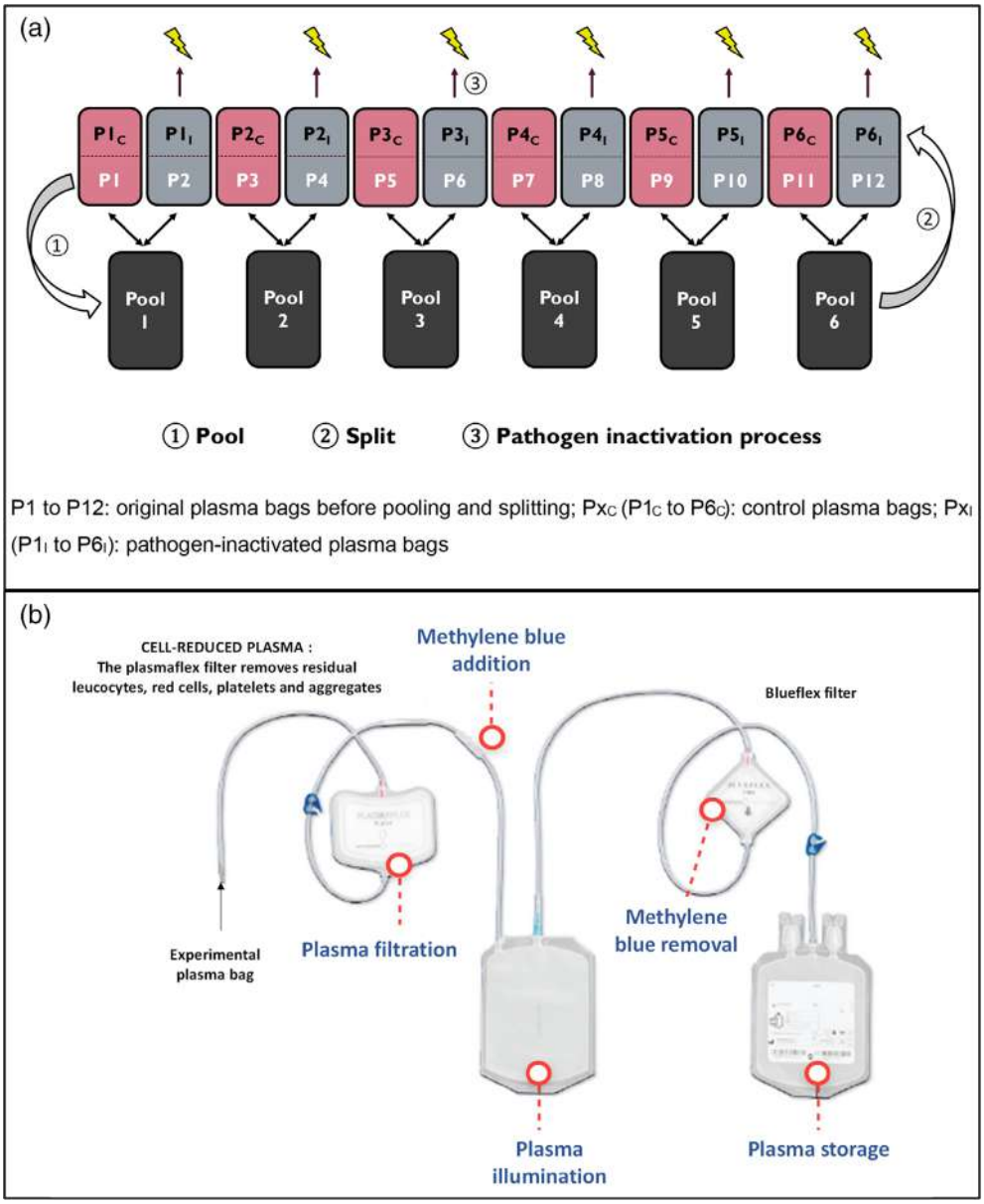

FIGURE 1 (a) Study design: evaluation of the impact of the pathogen inactivation process on plasticizer exposure in plasma. (b) The THERAFLEX MB-Plasma device for the illumination-based inactivation of pathogens

$$[\mathrm{DEHP}]_{\mathrm{equivalent}} = [\mathrm{DEHP}] + \frac{[\mathrm{MEHP}]}{M_{\mathrm{MEHP}}} \times M_{\mathrm{DEHP}},$$

where $[\mathrm{DEHP}]_{\mathrm{equivalent}}$ is the equivalent concentration of DEHP in blood (in μg/ml), [DEHP] and [MEHP] are the respective DEHP and MEHP concentrations in blood (in μg/ml), and M is the molecular mass (in g/mol).

Intergroup differences in plasticizer concentrations were analysed using a repeated-measures, two-way analysis of variance (ANOVA). If the ANOVA result was statistically significant, post hoc pairwise comparisons with Bonferroni correction were performed. All statistical tests were performed using GraphPad Prism version 7.0a for MacOS (GraphPad Software, La Jolla, CA). The data are presented as the mean ± standard deviation (SD). For all analyses, the threshold for statistical significance was set to $p < 0.05$.

RESULTS AND DISCUSSION

To determine the impact of the illumination process on the migration of DEHP into plasma, we determined DEHP and MEHP concentrations in the Px_I and Px_C bags on D1 and D30 after plasma illumination, using an internal calibration. The initial (D0) contamination of each analyte was subtracted from the D1 and D30 values (Figure 2a).

We found that the MEHP concentration was significantly associated with storage but not with illumination ($p = 0.0001$). In contrast to DEHP (the concentration of which remained stable throughout the storage period, with a mean ± SD of 115.3 ± 8.6 μg/ml), the MEHP concentration increased significantly from D1 to D30 in both the Px_C bags (from 25.0 ± 2.2 to 38.9 ± 3.7 μg/ml, respectively; $p = 0.0005$)

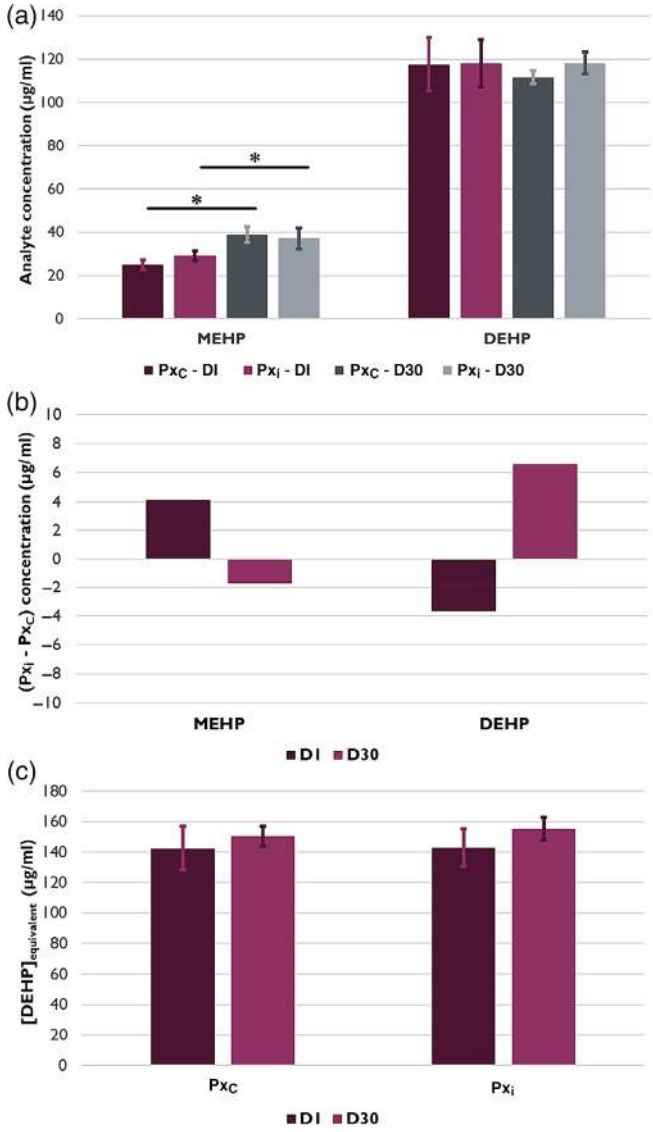

FIGURE 2 (a) MEHP and DEHP concentrations, (b) differences in MEHP and DEHP concentrations, and (c) DEHP equivalent concentrations in control plasma bags and pathogen-inactivated plasma bags on D1 and D30. DEHP, di(2-ethylhexyl) phthalate; MEHP, mono(2-ethylhexyl) phthalate; Px_C, non-inactivated (control) plasma bags; Px_i, pathogen-inactivated plasma bag. *$p < 0.05$

and the Px_i bags (from 29.1 ± 2.3 to 37.2 ± 4.8 µg/ml, respectively; $p = 0.02$).

To understand these phenomena, we subtracted the MEHP and DEHP concentrations in the Px_C bags from those in the Px_i bags (Figure 2b). On D1, the change (95% confidence interval [CI]) in the mean ± SD DEHP concentration (−3.7 ± 3.3 µg/ml [−7.2 to −0.2 µg/ml]) was correlated with the increase in the MEHP concentration (4.1 ± 1.7 µg/ml [2.3–5.9 µg/ml]). We suspected that the physical–chemical degradation of DEHP to MEHP had been induced by the light during the illumination process [14, 15]. This phenomenon has already been described (with greater intensity) during ultraviolet-A illumination under oxidative conditions [16]. On D30, the DEHP concentration was lower in the Px_C bags than in the Px_i bags (by 6.6 ± 4.3 µg/ml [2.2–11.1 µg/ml]), whereas the MEHP was higher in the Px_C bags than in the Px_i bags (by 1.7 ± 1.5 µg/ml [−3.2 to −0.2 µg/ml]). As expected, these values suggested that the migration of DEHP from the bag to the plasma during storage [3, 14, 15] was accentuated by the illumination process.

The DEHP equivalent concentrations were not significantly related to the illumination process ($p = 0.3$) or storage ($p = 0.09$; Figure 2c). On D1, the results were not statistically different between Px_C and Px_i bags (142.6 ± 14.3 µg/ml vs. 143.0 ± 12.3 µg/ml; $p > 0.9$) and were in-line with the literature data [2]. Hence, the greater inner surface area of plasticized PVC (9.68 dm^2) in the pathogen inactivation device (Figure 1b) did not accentuate the DEHP exposure, relative to control plasma bags. On D30, the DEHP equivalent concentration was slightly but not significantly higher in the Px_i bags than in the Px_C bags (155.4 ± 7.3 µg/ml vs. 150.5 ± 6.4 µg/ml, respectively; $p = 0.9$). This might have been due to the illumination process, the impact of which on DEHP migration became slightly more visible with storage.

In conclusion, our results demonstrate that illumination-based pathogen inactivation prompted the degradation of DEHP to MEHP but did not influence the DEHP exposure dose (relative to untreated plasma bags) on D1. The pathogen inactivation process did contribute to DEHP leachability, though, as evidenced by the slight increase in the DEHP equivalent concentration measured on D30. However, the differences in DEHP equivalent concentrations were small. We conclude that patients transfused with illumination-based pathogen-inactivated plasma are not significantly more exposed to plasticizers than patients transfused with non-inactivated plasma.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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SHORT REPORT

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The impact of the COVID-19 outbreak on activation of the massive transfusion protocol in the emergency department

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Abstract

Background and Objectives: An outbreak of coronavirus disease 2019 (COVID-19) occurred in mid-May of 2021 in Taiwan. After 2 months of hard work, transmissions were successfully prevented and the number of newly confirmed COVID-19 cases fell remarkably. We evaluated the impact of this outbreak on the massive transfusion protocol (MTP) in the emergency department (ED) of a trauma centre.

Materials and Methods: We retrospectively compared the activation and efficacy of MTP before, during and after the outbreak by analysing the clinical data relevant to MTP activations.

Results: There was no remarkable change in the average number of MTP triggers per month during the outbreak. The interval from an MTP trigger to the first unit of blood transfused at bedside was significantly increased during the outbreak compared to that before the outbreak (22.4 min vs. 13.9 min, $p < 0.001$); while the 24-h survival rate decreased (57.1% vs. 71.1%, $p = 0.938$). There were no remarkable changes in blood unit return or wastage during the outbreak.

Conclusion: The COVID-19 outbreak limitedly affected MTP activation and waste of blood products, but significantly increased the interval from an MTP trigger to the first unit of blood transfused at bedside.

KEYWORDS
COVID-19, massive transfusion protocol, patient blood management, trauma

Highlights

- The COVID-19 outbreak limitedly affected the number of MTP activation and waste of blood products.
- The COVID-19 outbreak significantly increased the interval from an MTP trigger to the first unit of blood transfused at bedside.

INTRODUCTION

The massive transfusion protocol (MTP) is a predefined algorithm to rescue patients with major haemorrhage from deoxygenation and coagulopathy, as suggested by the National Blood Authority, Australia guidelines for patient blood management (PBM) [1]. The clinical practice of MTP was introduced in 2018 and has been applied since that time in the Far Eastern Memorial Hospital (FEMH), a tertiary trauma centre in New Taipei City, Taiwan. Although a series of anti-pandemic policies were implemented in the FEMH during the coronavirus

disease 2019 (COVID-19) pandemic, an outbreak occurred in mid-May 2021. Fortunately, a reduction in transmissions was successfully achieved, and the number of newly confirmed COVID-19 cases remarkably decreased after 2 months of hard work. It has been reported that certain anti-pandemic policies may affect the efficacy of the healthcare system and health-providing behaviour [2], as well as the health-seeking behaviour of patients [3, 4], leading to worse outcomes. However, little is known about the influence of COVID-19 on the efficacy and activation of MTP, especially in Asian countries. Hence, we aimed to investigate the impact of the COVID-19 outbreak on the MTP trigger in the emergency department (ED).

METHODS

Patient selection and study design

A retrospective study was conducted on patients who were admitted to the ED in FEMH due to major haemorrhage from 14 November 2020 to 13 August 2021. MTP was activated based on emergency physician assessment, as well as assessment of the blood consumption (ABC) score. The ABC score was calculated and provided by the computerized provider order entry (CPOE), and the clinical decision support system (CDSS). The blood bank, located on the 3rd floor, would send blood components in an established ratio (round 1 and round 2) as MTP was triggered. Round 1 includes 6 units of leukocyte-depleted packed red blood cell (RBC) and 1 unit of leukocyte-depleted apheresis platelet (PLT), and round 2 includes 6 units of leukocyte-depleted packed RBC and 12 units of fresh frozen plasma (FFP). Patients who had MTP triggers in the ED were categorized into groups depending on the date of MTP activation: before the outbreak (from 14 November 2020, to 13 May 2021), during the outbreak (from 14 May to 13 July 2021) and after the outbreak (from 14 July to 13 August 2021). Epidemiological data were obtained via a chart review of the electronic medical records. Clinical data were recorded, including patient age, sex, causes of MTP activation, ABC score and shock index at the time of the MTP trigger, the interval from the MTP trigger to the first unit of blood transfused at bedside, 24-h survival, the unit number of blood return and waste in blood products of red cells, PLTs and FFP. The study was approved by the institutional review board of FEMH (110165-E).

TABLE 1 Demographic data of patients who had MTP triggers in the emergency department (ED) before, during and after COVID-19 outbreak

Variables	Before outbreak (mid-Nov, 2020 to mid-May, 2021) $n = 45$	During outbreak (mid-May to mid-Jul, 2021) $n = 14$	After outbreak (mid-Jul to mid-Aug, 2021) $n = 7$	p value (before vs. during outbreak)	p value (during vs. after outbreak)	p value (before vs. during outbreak)
Age (years)	45 (30–61)	41 (25–66)	64 (38–81)	1.000	0.481	0.264
Sex (female/male)	12/33	3/11	2/5	1.000	1.000	1.000
The average number of MTP trigger in ED per month	7.5	7	7			
Causes for MTP trigger (n)						
Trauma	32 (71.1%)	11 (78.6%)	3 (42.9%)	1.000	0.278	0.306
Non-Trauma	13 (28.9%)	3 (21.4%)	4 (57.1%)			
Cardiovascular haemorrhage	5 (11.1%)	1 (7.1%)	2 (28.6%)			
Gastrointestinal haemorrhage	3 (6.7%)	2 (14.3%)	1 (14.3%)			
Miscellaneous	5 (11.1%)	0	1 (14.3%)			
ABC score at MTP trigger	1 (1–2)	1 (1–2)	2 (1–3)	1.000	0.551	0.451
Shock index at MTP trigger	1.4 (1.3–2.0)[a]	1.8 (1.2–1.8)[b]	2.0 (1.4–2.5)	1.000	1.000	0.615
Interval from MTP trigger to first blood unit transfused at bedside (min)	13.9	22.4	16.0	<0.001	0.056	1.000
24-h survival rate	71.1%	57.1%	100%	0.938	0.129	0.354

[a]Shock index was unavailable in 10 patients with a systolic blood pressure of 0 mmHg.
[b]Shock index was unavailable in three patients with a systolic blood pressure of 0 mmHg.

Statistics

Continuous variables are presented as the mean with standard deviation or median with interquartile range, as appropriate. Categorical variables are expressed as counts with percentages. To identify whether there was a statistical difference between groups, the Kruskal–Wallis test with Bonferroni correction was performed using version 19.0 of the Statistical Package for the Social Sciences (SPSS), (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $p < 0.05$.

RESULTS

During the study period, 66 patients with MTP activated in the ED were enrolled in the study. Among these, MTP was activated in 45 patients before the outbreak, 14 patients during the outbreak and 7 patients after the outbreak. Epidemiological data are presented in Table 1. We found no remarkable change in the average number of MTP triggers in the ED per month during the outbreak. However, the interval from the MTP trigger to the first unit of blood transfused at bedside was significantly increased during the outbreak compared with that before the outbreak (22.4 min vs. 13.9 min, $p < 0.001$). After the outbreak, the interval appeared to be reduced (when compared with that during the outbreak), but the difference was not statistically significant (16.0 min vs. 22.4 min, $p = 0.056$). The 24-h survival rate decreased during the outbreak compared with the rates before and after the outbreak, however, the differences were not statistically significant. The average monthly usage of blood components in the ED was also decreased during the outbreak compared with that before the outbreak (RBC: 581.5 ± 109.6 units vs. 1022.8 ± 62.5 units, $p = 0.001$; PLT: 33.0 ± 5.7 units vs. 76.0 ± 11.2 units, $p = 0.004$; FFP: 158.5 ± 41.7 units vs. 336.7 ± 59.8 units, $p = 0.014$; Figure 1). Furthermore, there were no remarkable changes in blood unit return or wastage during the outbreak (Table 2). On average, 6 units of FFP were returned per month, and no blood was wasted from the MTP during the outbreak.

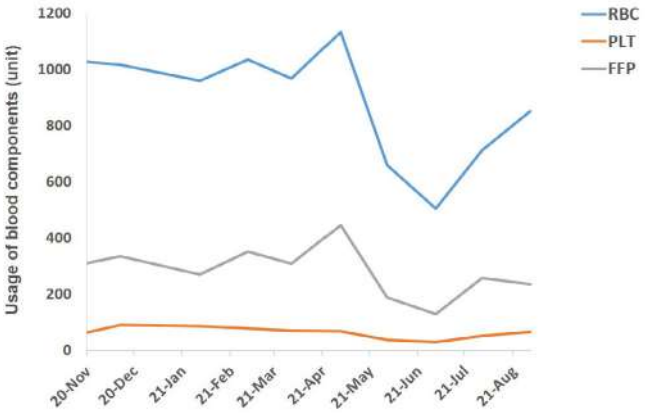

FIGURE 1 The average monthly usage of blood components in the emergency department from November, 2020 to August, 2021. FFP, fresh frozen plasma; PLT, apheresis platelet; RBC, red blood cell

DISCUSSION

In this study, our results indicated that the COVID-19 outbreak limitedly affected MTP activation as well as the return and wastage of blood products. Furthermore, the COVID-19 outbreak significantly increased the interval from an MTP trigger to the first unit of blood transfused at bedside. To the best of our knowledge, this is the first study to assess the impact of the COVID-19 outbreak on the clinical practice of MTP in Asia.

Reports have shown that blood donations dropped precipitously during the COVID-19 outbreak [5]. A similar phenomenon was observed in the present study. Hence, it is crucial to manage inventory

TABLE 2 Blood unit return and waste in MTP triggered in the emergency department (ED) before, during and after COVID-19 outbreak

Variables	Before outbreak (mid-Nov, 2020 to mid-May, 2021)	During outbreak (mid-May to mid-Jul, 2021)	After outbreak (mid-Jul to mid-Aug, 2021)	*p* value (before vs. during outbreak)	*p* value (during vs. after outbreak)	*p* value (before vs. after outbreak)
Blood unit return in MTP (unit per month)						
RBC	9.0 ± 2.4	3.0 ± 1.6	0	0.740	1.000	0.526
PLT	0	0	0	1.000	1.000	1.000
FFP	17.8 ± 4.4	6.0 ± 3.2	0	0.651	1.000	0.441
Blood waste in MTP (unit per month)						
RBC	0	0	0	1.000	1.000	1.000
PLT	0	0	0	1.000	1.000	1.000
FFP	3.3 ± 1.6	0	0	0.822	1.000	1.000

Abbreviations: FFP, fresh frozen plasma; PLT, apheresis platelet; RBC, red blood cell.

adequately during a blood shortage due to COVID-19. Currently, during the COVID-19 outbreak, daily communication between the blood bank, key clinical teams and blood centres has played a vital role in ensuring and predicting the sustainable inventory of blood products to meet patient needs, especially for MTP. A restrictive transfusion strategy was also applied in our hospital using a threshold for transfusion based on a drop in the haemoglobin level below 7 g/dl in patients with stable haemodynamics [6]. In addition, the provision of blood products to satellite clinics was temporarily withheld until the outbreak was controlled. In compliance with these transfusion strategies, blood components were successfully provided in MTP during the outbreak.

Recently, Braun et al. reported that the number of MTP activations significantly increased because of violent trauma during the COVID-19 pandemic in the United States of America [7]. In contrast, in our study population, most traumatic cases with an MTP trigger were due to motor vehicle ($n = 26$, 56.5%) and falling ($n = 13$, 28.3%) accidents, and no notable difference in traumatic cases with an MTP trigger was observed during the COVID-19 outbreak. In particular, we found that the interval from an MTP trigger to the first unit of blood transfused at the bedside was significantly increased during the outbreak, which may be attributed to changing into and wearing of personal protective equipment (such as N95 or P100 respirator masks, disposable coverall gowns, hoods, gloves and shoe protection and so on) for entry into the quarantine zones when delivering the blood products during an MTP. To summarize, these experiences may provide insight for coping with similar future events in transfusion practice and blood management during the COVID-19 outbreak in different geographic regions.

There were some major limitations in our study. This was a retrospective study in a single centre. The case number was also limited and the study interval was not sufficient, which could lead to certain bias in the etiological distribution of MTP trigger.

In conclusion, the COVID-19 outbreak had limited influence on the number of MTP activation and wastage of blood products, but significantly increased the interval from an MTP trigger to the first unit of blood transfused at bedside.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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SHORT REPORT

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Effects of dual chelation therapy with deferasirox and deferoxamine in patients with beta thalassaemia major

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Abstract

Background and Objectives: Patients with thalassaemia experience complications related to iron overload. In Australia currently, the two main options for iron chelation are deferasirox and deferoxamine. Optimal iron chelation using monotherapy can be limited due to toxicity or tolerability. Dual chelation therapy (DCT) may provide more aggressive iron chelation.

Material and Methods: A retrospective, observational study was performed on a statewide referral centre for patients receiving red cell transfusions for haemoglobinopathies (Monash Health, Australia). All patients prescribed DCT were identified using a local pharmacy dispensing database and were included in the study. Pre-DCT initiation and post-DCT completion were correlated with serum ferritin, cardiac iron loading (based on MRI T2* measurements) and liver iron content (LIC) using Wilcoxon signed-rank test.

Results: A total of 18 patients (12 adults, 6 children) were identified as receiving DCT. All patients received a combination of deferasirox and deferoxamine. The median duration of therapy was 23 months (range 2–73). Median serum ferritin reduced by 42% ($p = 0.004$) and there was a 76% reduction in LIC ($p = 0.062$). No significant changes were seen in cardiac iron loading.

Conclusion: DCT over a prolonged period is effective at reducing serum ferritin and may contribute to improvement in liver iron loading.

KEYWORDS
chelation, iron overload, thalassaemia, transfusions

Highlights

- Dual iron chelation therapy with deferasirox and deferoxamine is an effective way to reduce serum ferritin levels in patients with beta thalassaemia major.
- Dual chelation therapy may also reduce liver iron load.
- Efficacy was seen in both adults and children.

INTRODUCTION

Thalassaemia and other haemoglobinopathies are chronic diseases with therapeutic challenges. Chronic red cell transfusions remain the mainstay of treatment. However, with approximately 250 mg of iron in each unit of red cell, effective iron chelation is central to avoid the life-threatening complications of chronic iron overload, including cardiomyopathies and liver injury [1].

Iron chelation therapy effectively reduces exogenous iron load in patients on regular transfusion programmes and has markedly improved

life expectancy in patients with thalassaemia, other transfusion-dependent haemoglobinopathies and anaemias [2–4]. This must be balanced against the relatively common inherent toxicities associated with these therapies. In Australia, the two most commonly used iron chelation agents are deferasirox (DFX) followed by deferoxamine (DFO), with both available as first-line agents [5]. Deferiprone (DFP) is another available oral iron chelator but in Australia access requires DFO failure; and due to adverse effects of neutropenia, is seldom used [5, 6].

Each of these agents have their own risk–benefit profile which needs to be tailored to the individual patient. DFO is an effective iron chelator that eliminates iron primarily via the kidneys, but requires parenteral administration [7]. It is recognised to cause local skin irritation and visual/auditory neurotoxicity. In contrast, excretion of iron with DFX occurs primarily via the faecal route. It causes gastrointestinal upsets, with potential for osteoporosis, liver and renal toxicities [8, 9].

Despite availability of iron chelation, ferritin control may not be achieved with monotherapy [10]. This may occur for a myriad of reasons including poor tolerance, degree of transfusional iron intake or pharmacokinetic variability between patients [11, 12]. Dual chelation therapy (DCT) is an attractive option to counteract this. Utilising the combination of DFX and DFO is of interest given its non-additive side-effect profile and the varying mode of action by reducing iron from different storage pools [13, 14]. Combining therapies may provide improved iron excretion in patients in whom maintaining acceptable ferritin levels with monotherapy remains a challenge, and offers an alternative for patients intolerant to or plagued by adverse effects at adequate dosing with monotherapy.

We aimed to describe the effects of DCT on serum ferritin, cardiac and liver iron load in a reference haemoglobinopathy centre.

TABLE 1 Characteristics of patients receiving DCT

Characteristics	Adults $n = 12$	Children $n = 6$	Total $n = 18$
Age for initiating DCT (y), median (range)	39 (18–58)	7.5 (4–12)	29 (4–58)
Male sex (%)	5 (42%)	5 (83%)	11 (61%)
Diagnosis (%)			
Beta thalassaemia major	12 (100%)	6 (100%)	18 (100%)
Indication for DCT (%)			
Inadequate iron chelation with DFX monotherapy	4 (33%)	3 (50%)	7 (39%)
Inadequate iron chelation with DFO monotherapy	2 (15%)	3 (50%)	5 (26%)
Adverse effect limiting DFX	4 (31%)		4 (21%)
Adverse effects limiting DFO	2 (15%)		2 (11%)
Duration on DCT (m), median (range)	31 (2–73)	7 (3–15)	23 (2–73)
Reason for cessation (%)			
Improved iron loading/ferritin	1 (8%)	2 (33%)	3 (16%)
Adverse effect from DFX due to:			
RTA		1 (16%)	1 (5%)
Adverse effect from DFO due to:			
Tinnitus	1 (8%)		1 (5%)
Injection site irritation	2 (15%)		2 (11%)
Other	1 (8%)		1 (5%)
Modality of DCT (%)			
Daily DFX and intermittent DFO	8 (67%)	4 (67%)	12 (67%)
Daily DFO and intermittent DFX		2 (33%)	2 (11%)
Alternating DFX and DFO	4 (31%)		4 (21%)
Dose of DCT (mg/kg/day), median (range)			
DFX	16 (3–25)	18.5 (9–25)	16 (3–25)
DFO	43 (24–63)	30 (16–42)	41 (16–63)
Number of days per week on DFO, median (range)	5 (2–6)	5.5 (3–7)	5 (2–7)
Number of Days per week on DFX, median (range)	7 (4–7)	7 (4–7)	7 (4–7)
Baseline Ferritin (ng/L), median (range)	1589 (495–6440)	1936 (1458–4743)	1766 (495–6440)
Baseline Cardiac T2* (ms), median (range)	26.7 (3.9–37.4)	40.2 (23.4–44.4)	28.4 (3.9–44.4)
Baseline LIC (mg/g), median (range)	4 (<1–11)	4 (2–23)	4 (<1–23)

Abbreviations: DCT, dual chelation therapy; DFO, deferoxamine; DFX, deferasirox; LIC, liver iron content; RTA, renal tubular acidosis.

MATERIALS AND METHODS

A local thalassaemia database at Monash Health, Australia (a state-wide haemoglobinopathy referral site) was reviewed to identify patients currently receiving regular transfusion support for haemoglobinopathies. Patients were identified as receiving DCT by referencing the local hospital pharmacy dispensing database. As all chelation therapy is dispensed locally for our haemoglobinopathy patients, this was expected to capture all potential patients on DCT. All patients on DCT were included in the analysis. Baseline characteristics that were analysed include age, sex, underlying haemoglobinopathy, initiation/duration of therapy, dose, indication for therapy and reason for cessation. Due to the transition of DFX branding in Australia from Exjade dispersible tablets to Jadenu film coated tablets, a bioequivalent dose of DFX was calculated to enable comparison for applicable patients in this study making this transition. Calculations for conversion are based on the Jadenu product information and results in Exjade 125, 250 and 500 mg doses being converted to 90, 180 and 360 mg of Jadenu, respectively.

Ferritin levels prior to initiation of DCT were collected and if multiple levels were performed, the lowest was taken with the assumption that higher levels related to an acute phase response. Imaging reports were gathered from a local database to assess cardiac and liver iron loading. Previously validated cardiac MRI with volumetric analysis followed by myocardial and liver T2* sequences were performed using Thalassaemia Tools Software to assess iron loading [15, 16]. Degree of loading was based on MRI T2* measurements and were stratified into nil, mild, moderate and severe; corresponding to >20 ms, 14–20 ms, 10–14 ms and <10 ms for myocardial iron loading and >6.3 ms, 2.7–6.3 ms, 1.4–2.7 ms and <1.4 ms for liver iron loading, respectively [16]. Liver iron loading results were converted to liver iron content (LIC) mg/g by using the formula Fe (mg/g) = [0.028 × R2* − 0.454], since magnetic resonance imaging was obtained at 3 Tesla, results were divided by 2 to accurately represent the LIC [17, 18].

Comparisons of serum ferritin, cardiac and liver iron loading were based on most recent investigation results prior to start and post completion of DCT or most recent results at time of analysis. Due to the non-normal distribution of results, univariate analyses were performed using Wilcoxon signed-rank tests for ferritin, cardiac and liver iron. Analysis was performed using SPSS version 20.0 (IBM, Chicago, IL). Local ethics approval was obtained.

RESULTS

A total of 137 adults and 24 children were identified as receiving iron chelation with 18 patients (12 adults and 6 children) commencing DCT using DFX and DFO. No patients were receiving DCT with DFP. Ten patients (9 adults and 1 child) made the transition from Exjade to Jadenu. See Table 1 for a full breakdown of patient characteristics.

Twelve patients (10 adults and 2 children) had an available repeat MRI T2* report for assessment. From these 12 patients, 9 (7 adults and 2 children) were still on DCT at the time of the scan and 3 recently ceased DFO (2 due to local irritation and 1 non documented reason) before the scan was performed at 2, 3 and 6 respectively. For patients with available imaging, the modality of therapy was daily DFX and intermittent DFO in 9 patients (7 adults and 2 children) and alternating therapy in the 3 remaining adults. The median dose for intermittent DFO in these patients was 26 mg/kg/day (range 11–39) and for alternating DFO was 16 mg/kg (range 3–30).

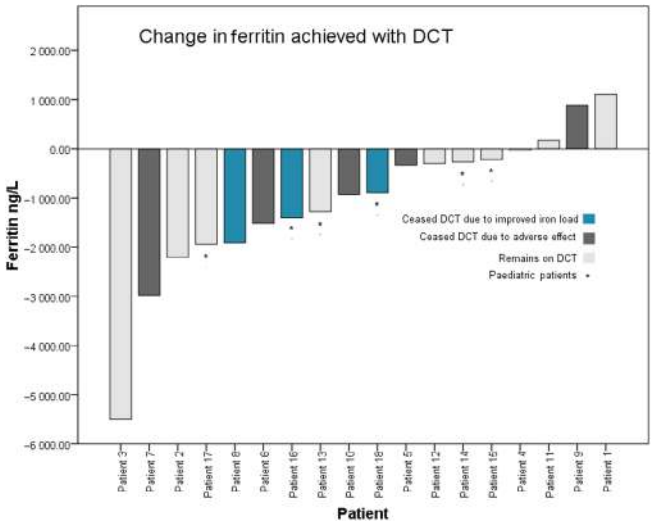

FIGURE 1 Waterfall plot demonstrating absolute reduction in serum ferritin within the cohort. DCT, dual chelation therapy

There was a significant reduction in median serum ferritin in the total cohort by 42%, with a reduction from baseline ferritin of 1766 ng/L (range 495–6440) to 1033 ng/L (range 278–2805, $p = 0.004$). In adults the reduction from baseline of 1589 ng/L (range 495–6440) to 966 ng/L (range 278–1954) was also significant ($p = 0.050$). Children also had a significant reduction from baseline ferritin of 1936 ng/L (1458–4743) to 1196 ng/L (range 559–2802, $p = 0.018$). Individual changes in serum ferritin are presented in Figure 1.

While on DCT there was a 76% reduction ($p = 0.062$) in median LIC from 4 mg/g (range < 1–23) to 2 mg/g (range < 1–11). The LIC for adults reduced to 2 mg/g (<1–11) and for children reduced to 1 mg/g (range < 1–1). There was an 8% deterioration in median cardiac iron load based on T2* results from 28.4 ms (range 3.9–44.4) to 24.3 ms (range 3.4–36.7) which was not statistically significant ($p = 0.657$). The cardiac T2* for adults reduced to 23.9 ms (3.4–35.1) and for children reduced to 30.5 ms (24.3–36.7).

Median baseline categorical liver iron loading was mild for adults and children based on LIC, while the baseline cardiac iron load was not increased in both adults and children (Table 1). Categorical liver iron overload improved in 7 patients (5 adults 2 children), remained stable in 3 adults and increased in 2 adults ($p = 0.031$). Two patients demonstrated reduced categorical cardiac iron levels, 1 patient showed increased levels and 9 patients remained the same ($p = 0.564$).

DISCUSSION

These data demonstrate the real-world efficacy of managing patients in a specialist haemoglobinopathy unit with DCT. Prior studies have shown efficacy of DCT using overlapping DFO and DFX in thalassaemia patients demonstrating reductions in ferritin and LIC by 24% and 31%, respectively; and to a lesser degree mild improvement in cardiac iron loading [19]. Alternating therapy with DFX/DFO has also shown reductions in serum ferritin in a small paediatric cohort [20]. Combining DFO and DFP has been particularly effective in reducing cardiac iron load and this may be partly to do with the lipophilic nature of DFP allowing access to the intracellular iron pool [21, 22]. Combination of oral chelation with DFX and DFP has also shown similar improvement in cardiac iron loading [23]. However, these improvements may not necessarily reflect real-world findings especially in the thalassaemia cohort where compliance with therapy can at times be difficult to maintain in a non-trial environment [24].

Despite having 18 patients in the analysis, a statistically significant reduction of serum ferritin was demonstrated. A limitation of this retrospective analysis is that patients were on DCT for variable periods ranging from 2 months to 6 years. Direct comparisons between patients should be made with caution; nevertheless, patients receiving long-term DCT in this cohort benefited from a reduction in ferritin levels. In fact, when comparing ferritin at the 1-year mark for the 11 applicable patients, the median ferritin level reduced by 43% from 1797 ng/L (range 495–6440) to 768 ng/L (range 410–5936, $p = 0.033$).

Radiological findings of improved cardiac iron status were inconsistent in this small cohort. This may have occurred as some patients started DCT due to adverse effects rather than iron overload. Other limitations that may explain this include the timing of scans compared to initiating/ceasing DCT. As this is a retrospective study, it was not mandated for patients to undergo an MRI T2* once DCT was ceased or at time of analysis. This same limitation does not extend to serum ferritin because this biochemical parameter is measured in all patients prior to transfusions (3–5 weekly) in our haemoglobinopathy unit and thus prompt comparisons were possible. LIC was reduced in our cohort, although this was not statistically significant, a majority of patients did improve their categorical liver iron load. This may be because DCT has been shown to be a means to quickly reduce LIC compared to cardiac iron [25].

In conclusion, this study benefits from analysing the long duration that patients received DCT. The authors are unaware of other studies in patients receiving DCT with DFX and DFO for up to 6 years or showing efficacy in paediatric patients as young as 4 years. DCT over a prolonged period was observed as an effective method to reduce serum ferritin and possibly liver iron load in patients with beta thalassaemia major in the real-world hospital-based setting.

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A.Z. designed the research, collected data, analysed data, wrote the paper. S.W. designed the research, collected data, edited paper. A.G. supervised, edited paper. K.C. supervised, edited paper. Z.K. supervised, edited paper.

CONFLICT OF INTEREST

A.G. received consulting fees from Novartis for prior work with the Therapeutic Goods Administration.

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ORIGINAL ARTICLE

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Characterization of alternatively spliced transcript variants of glycophorin A and glycophorin B genes in Chinese blood donors

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Abstract

Background and Objectives: The molecular basis of MNS blood group variants is not fully clear yet. In this study, we have characterized mRNA variants of *GYPA* and *GYPB* genes to reveal whether alternative RNA splicing may cause antigenic diversity of the MNS system.

Materials and Methods: Total RNA was extracted from peripheral blood of Chinese blood donors and full-length cDNA products were generated. A nested polymerase chain reaction (PCR)-based method was established for fragment amplification and Sanger sequencing. Resulted full-length mRNA sequences were aligned with *GYPA* or *GYPB* genomic sequences respectively for exon identification. Amino acid (AA) sequences of GPA and GPB proteins were extrapolated and *GYPA-EGFP*, *GYPB-EGFP* fusion genes were generated to monitor subcellular distribution of the encoded glycophorin (GP) proteins.

Results: Totally 10 blood samples were analysed. *GYPB* mRNAs of all the subjects demonstrated frequent exon insertion or deletion whereas this kind of variation was only observed in 3 of 10 *GYPA* mRNA samples. None of the reported Miltenberger hybrids was detected in any of the mRNA samples. The alternative splicing resulted in changes of AA sequences in N-terminal domains where the MNS antigenic motifs resided; however, subcellular localizations of GP-EGFP fusion proteins showed that the above-mentioned AA changes did not affect cell surface distribution of the encoded GP proteins.

Conclusions: Alternative RNA splicing may influence the antigenic features of GP proteins but not their cell surface distribution. Therefore, *GYPA* and *GYPB* mRNA characterization might be an invaluable supplement to serological phenotyping and DNA-based genotyping in MNS blood grouping.

KEYWORDS
alternative RNA splicing, antigenic diversity, *GYPA* mRNA, *GYPB* mRNA, MNS blood group system

Yanlian Liang, Jianwei Ren and Fuling Zhong contributed equally to this work.

Highlights

- Methods were established for full-length sequencing of *GYPA* and *GYPB* mRNAs, including intact RNA extraction from blood, full-length cDNA synthesis and a nested PCR-based method to specifically amplify the fragments covering full-length sequences of GYPA and GYPB transcripts.
- Alternative RNA splicing caused frequent exon expunction or addition in *GYPA* and *GYPB* mRNAs of the research subjects.
- Alternative RNA splicing usually caused amino acid changes in N-terminal domains where the MNS antigenic motifs resided but did not influence cell surface distribution of GPA and GPB proteins.

INTRODUCTION

MNS is the second blood group system discovered in human history after ABO [1]. It is also the second most complex system, next to the Rh system, for grouping blood types [2]. Usually, MNS antibodies are rarely considered to be clinically significant [3]; however, in some cases, they will cause dangerous and possibly fatal reaction, for example, hemolytic transfusion reaction (HTR) [4] and hemolytic diseases of the foetus and newborn (HDFN) [5].

MNS antigens are carried on two sugar-bearing proteins, glycophorin A (GPA) and glycophorin B (GPB), which are encoded by *GYPA* and *GYPB* genes respectively. At present, 49 MNS antigens have been recognized [6]. Among them, only M, N, S and s antigens, of which the composition and structure are determined by the sequences in original *GYPA* and *GYPB* genes, are regarded as regular antigens, whereas the others are MNS-related variant antigens as their features cannot be extrapolated on the basis of standard sequences of *GYPA* and *GYPB* genes. Actually, a single nucleotide variation may be enough already to create a novel MNS antigen [7]. These unpredictable antigens are often associated with HTR and HDFN, however, became conspicuous only when a special clinical case was encountered [8], possibly due to the limitations of traditional serological tests, such as scarcity of stable and specific antibodies [9]. The application of molecular genotyping in blood typing has overcome many of the drawbacks [10] and greatly facilitates the identification of novel MNS variant antigens [7, 11].

The molecular basis of MNS blood group variants has been intensively investigated. *GYPA* and *GYPB* genes are highly homologous and they are sharing ~97% identity between each other and with another homologous gene, pseudogene *GYPE*. These three closely linked paralogous genes locate in tandem on the long arm of chromosome 4q28–31 and are known to undergo extensive copy number variation, gene conversion and rearrangements that shuffle the coding regions to generate rare MNS antigens [12, 13].

Currently, novel MNS-related blood group antigens are identified mainly via genotype-based screening of genomic DNA for hybrid glycophorin genes, for example, *GYP(A-B)*, *GYP(B-A-B)* etc. [6, 14], whereas glycophorin transcripts (mRNA) are seldom examined, possibly due to the instability of RNA and technical challenges in RNA manipulation. However, it was reported that accuracy rate of DNA sequencing-based diagnosis, either by whole exome sequencing or whole genome sequencing, is less than 50% for Mendelian diseases whereas RNA sequencing provided a substantial increase in diagnosis rate, indicating information provided by RNA analysis was necessary for phenotype prediction in addition to DNA analysis [15]. The superiority of RNA characterization over DNA sequencing in molecular diagnosis is obvious as mRNA is the direct template for protein synthesis. Moreover, it is also understandable that data collected via DNA sequencing is not enough for fully understanding the complexity of RNA variations that may be affected by alternative splicing [16], biased allelic expression [17], transcription error [18] etc.

Actually, MNS variant antigens may also be created due to RNA structure changes occurring during transcription and maturation of mRNA, especially alternative RNA splicing [19] that allows multiple transcripts to be produced from a single gene. Pre-mRNA splicing is a very complex process and may be regulated by various factors, including extracellular and intracellular signalling [20] and chromatin structures [21]. When looking through the *GYPA* and *GYPB* genes, we can see that start codons of both open reading frames (ORFs) are located in their first exons which are usually 20 kb or 18 kb away from their second exons respectively. Hence, alternative splicing may be easy to happen due to any changes occurring within the large gaps. In fact, the influence of alternative RNA splicing on the MNS antigenic features has been noticed for a long time [19, 22] but the underlying mechanisms and subsequent consequences have not been well investigated yet. Therefore RNA analysis is a helpful and efficient approach for identification of novel MNS antigenic variants, especially when RNA-Seq with next-generation sequencing (NGS) is becoming increasingly popular for researchers studying the transcriptome [23].

In this report, we have examined the structures of *GYPA* and *GYPB* transcripts, via Sanger sequencing-based approach, in blood cells of Chinese blood donors in order to interpret the complexity of MNS antigenic features.

MATERIALS AND METHODS

Blood donor samples and MNS typing

Blood samples from volunteer Chinese blood donors, aged 18–55 in Shenzhen Blood Center, were randomly selected for this study

TABLE 1 Clinical information of research subjects and summary of mRNA exon composition

			Total exons		Recombination		ORF span[a]		MNS encoding	
Blood donors	Gender	MNS typing	GYPA	GYPB	GYPA	GYPB	GYPA	GYPB	GYPA	GYPB
Subject-1	Male	MN, ss, Mia(−)	7	6	No	No	E1–E7	E2–E6	N	s
Subject-2	Male	MM, ss, Mia(4+)	8	4	No	No	E3–E8	E1–E4	-	s
Subject-3	Male	MM, ss, Mia(1+)	7	4	No	No	E1–E7	E1–E4	M	s
Subject-4	Male	MN, Ss, Mia(−)	7	6	No	No	E1–E7	E2–E6	N	s
Subject-5	Male	MN, Ss, Mia(−)	6	4	No	No	E1–E6	E1–E4	M	s
Subject-6	Male	MM, Ss, Mia(2+)	6	4	No	No	E1–E6	E1–E4	M	S
Subject-7	Male	MN, ss, Mia(−)	7	4	No	No	E1–E7	E1–E4	N	s
Subject-8	Female	NN, ss, Mia(−)	8	4	No	No	E2–E7	E1–E4	-	s
Subject-9	Male	NN, ss, Mia(2+)	7	6	No	No	E1–E7	E3–E5	N	s
Subject-10	Male	MM, ss, Mia(−)	7	6	No	Yes	E1–E7	E3–E6	M	s

Abbreviation: ORF, open reading frame.

[a]The exon numbering was determined individually in each research subject according to the sequential order of exons identified in the mRNA.

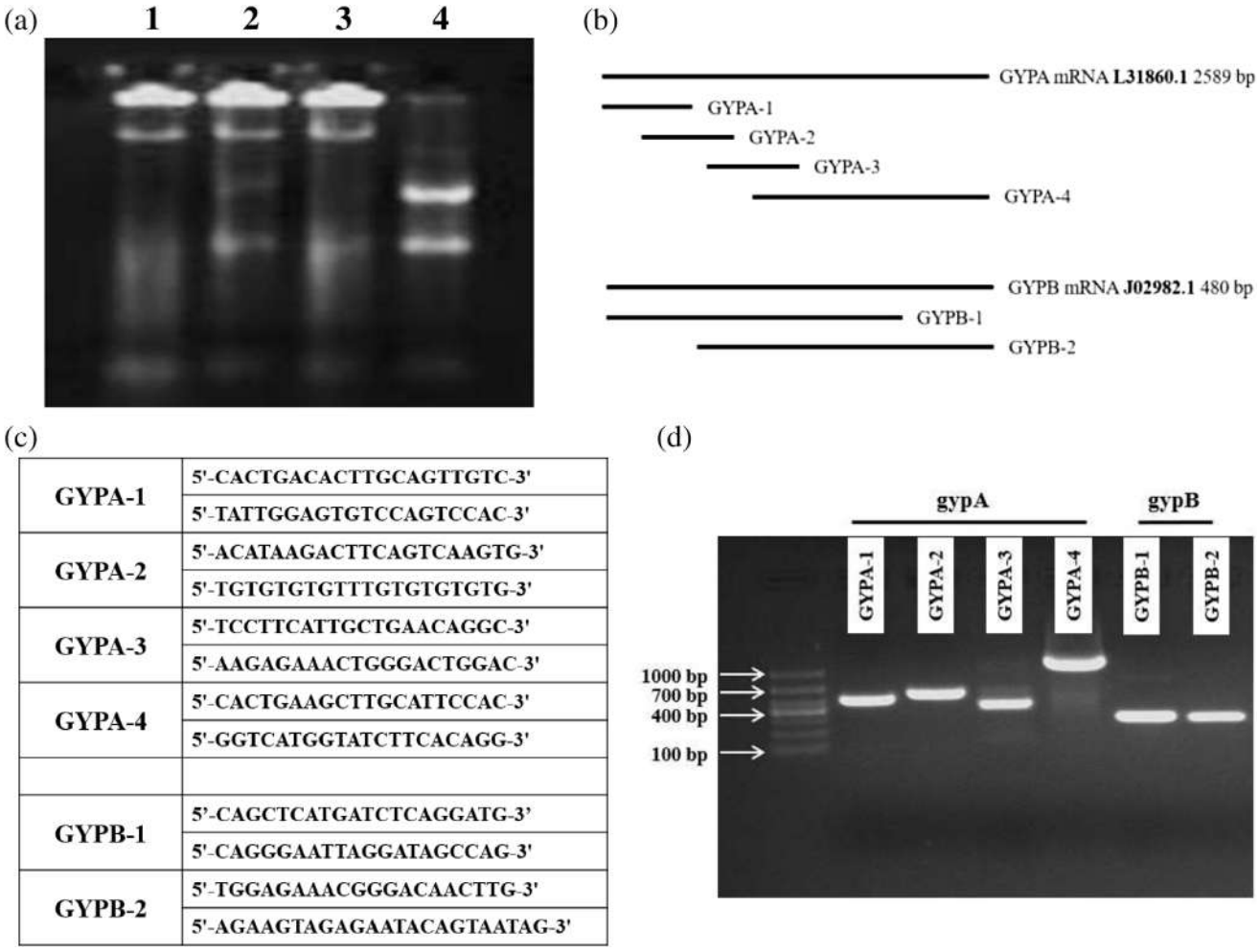


FIGURE 1 Fragment amplification for *GYPA* and *GYPB* mRNA sequencing. (a) Total RNA extracted from whole blood (lane 1), pelleted cells after erythrocyte lysis (lane 2), pelleted cells from whole blood (lane 3), isolated nucleated cells (lane 4). (b) Strategy for fragment amplification from *GYPA* and *GYPB* cDNAs. (c) Primers used in fragment amplification. (d) Representative gel image for amplification of *GYPA* and *GYPB* fragments

(Table 1). All the blood samples were collected after obtaining the written informed consent, and the use of the samples in this study was approved by Research Ethics Committee of Shenzhen Blood Center. Standard MN and Ss serological test was performed using following monoclonal antibody products of CE-Immundiagnostika GmbH (Germany): mouse anti-M IgG from clone 11H2, mouse anti-N IgM from clone 1422C7, human anti-S IgM from clone MS94 and human anti-s IgM from clone P3BER.

Total RNA extraction and reverse transcription

Nucleated cells were first isolated from fresh blood samples by density gradient centrifugation method using Ficoll–Hypaque (Merk/Sigma-Aldrich) and then total RNA was extracted with Trizol reagent (Takara) according to the manufacturer's protocol.

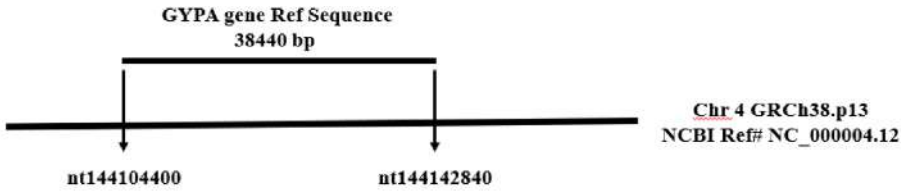
In reverse transcription, 500 ng total RNA was used in a 20-μl reaction with Oligo(dT)$_{20}$ primer and SuperScript™ III Reverse Transcriptase (Thermo/Invitrogen) following manufacturer's instructions.

PCR amplification and sequencing

DNA fragments were amplified via a two-round nested polymerase chain reaction (PCR) with EmeraldAmp® PCR Master Mix (Takara). In the first-round PCR (pre-amplification), 1 μl of cDNA was used in a 25-μl reaction. The primers used for *GYPA* pre-amplification are forward 5′-GCAG GCTAAGGTCAGACAC-3′ and reverse 5′-TTTTTTTTTTGGGGTCATG GTA-3′. The primers used for *GYPB* pre-amplification are forward 5′-GCACTAACTTCAGGAACCAG-3′ and reverse 5′-ATCTATCCTACTGT AATGGGC-3′. The PCR product was then subjected to a second-round PCR for amplification of individual fragments with respective primers according to the experimental strategy described in Figure 1.

The products of above nested PCR were purified and subjected for Sanger sequencing.

(a)



(b)

	Exon–1	Exon–2	Exon–3	Exon–4	Exon–5	Exon–6	Exon–7	Exon–8
L31860.1	5061-5153		25161-25262	25970-26067	26999-27038	28811-28898	30983-31063	34337-36438
NM_001308187.2	5034-5153		25161-25262	25970-26066		28812-28898	30983-31063	34337-36439
NM_001308190.2	5034-5153			25962-26067	26999-27038	28811-28898	30983-31063	34337-36439
NM_002099.8	5034-5153		25161-25262	25970-26067	26999-27038	28811-28898	30983-31063	34337-36439
Subject-1	**5062-5153**		**25161-25262**	**25970-26067**	**26999-27038**	**28811-28898**	**30983-31063**	**34337-36424**
Subject-2	**5048-5154**	**7507-7591**	**25161-26262**	**25970-26067**	**26999-27038**	**28811-28878**	**30983-31063**	**34337-36426**
Subject-3	**5048-5153**		**25161-25262**	**25970-26067**	**26999-27038**	**28811-28898**	**30983-31062**	**34337-36426**
Subject-4	**5048-5154**		**25161-25262**	**25970-26067**	**26999-27038**	**28811-28898**	**30983-31063**	**34337-36425**
Subject-5	**5048-5153**		**25161-25262**	**25970-26067**	**26999-27038**	**28811-28898**		**34340-36425**
Subject-6	**5048-5153**		**25161-25262**	**25970-26067**	**26999-27038**	**28811-28898**		**34340-36423**
Subject-7	**5049-5153**		**25161-25262**	**25970-26067**	**26999-27038**	**28811-28898**	**30983-31063**	**34337-36424**
Subject-8	**5048-5154**	**7507-7591**	**25161-25262**	**25970-26067**	**26999-27038**	**28811-28898**	**30983-31063**	**34337-36426**
Subject-9	**5048-5153**		**25161-25262**	**25970-26067**	**26999-27038**	**28811-28898**	**30983-31063**	**34337-36424**
Subject-10	**5048-5153**		**25161-25262**	**25970-26067**	**26999-27038**	**28811-28898**	**30983-31063**	**34337-36424**

(c)

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L31860.1    MYGKIIFVLLLSAIVSISASSTTGVAMHTSTSSSVTKSYISSQTNDTHKRDTYAATPRAHEVSEISVRTVYPPEEETGERVQLAHHFSEPEITLIIFGVMAGVIGTILLISYGIRRLIKKSPSDVKPLPSPDTDVPLSSVEIENPETSDQ   150
Subject-1   MYGKIIFVLLLSAIVSISALSTTEVAMHTSTSSSVTKSYISSQTNDTHKRDTYAATPRAHEVSEISVRTVYPPEEETGERVQLAHHFSEPEITLIIFGVMAGVIGTILLISYGIRRLIKKSPSDVKPLPSPDTDVPLSSVEIENPETSDQ   150
Subject-2   ---------------------------MHTSTSSSVTKSYISSQTNDTHKRDTYAATPRAHEVSEISVRTVYPPEEETGERVQLAHHFSEPEITLIIFGVMAGVIGTILLISYGIRRLIKKSPSDVKPLPSPDTDVPLSSVEIENPETSDQ   124
Subject-3   MYGKIIFVLLLSAIVSISASSTTGVAMHTSTSSSVTKSYISSQTNDTHKRDTYAATPRAHEVSEISVRTVYPPEEETGERVQLAHHFSEPEITLIIFGVMAGVIGTILLISYGIRRLIKKSPSDVKPLPSPDTDVPLSSVEIENPETSDQ   150
Subject-4   MYGKIIFVLLLSAIVSISALSTTEVAMHTSTSSSVTKSYISSQTNDTHKRDTYAATPRAHEVSEISVRTVYPPEEETGERVQLAHHFSEPEITLIIFGVMAGVIGTILLISYGIRRLIKKSPSDVKPLPSPDTDVPLSSVEIENPETSDQ   150
Subject-5   MYGKIIFVLLLSAIVSISASSTTGVAMHTSTSSSVTKSYISSQTNDTHKRDTYAATPRAHEVSEISVRTVYPPEEETGERVQLAHHFSEPEITLIIFGVMAGVIGTILLISYGIRRLIKRQVINENLFTKPNVERTQRRHKTSVK-----   145
Subject-6   MYGKIIFVLLLSAIVSISASSTTGVAMHTSTSSSVTKSYISSQTNDTHKRDTYAATPRAHEVSEISVRTVYPPEEETGERVQLAHHFSEPEITLIIFGVMAGVIGTILLISYGIRRLIKRQVINENLFTKPNVERTQRRHKTSVK-----   145
Subject-7   MYGKIIFVLLLSAIVSISALSTTEVAMHTSTSSSVTKSYISSQTNDTHKRDTYAATPRAHEVSEISVRTVYPPEEETGERVQLAHHFSEPEITLIIFGVMAGVIGTILLISYGIRRLIKKSPSDVKPLPSPDTDVPLSSVEIENPETSDQ   150
Subject-8   ---------------------------MHTSTSSSVTKSYISSQTNDTHKRDTYAATPRAHEVSEISVRTVYPPEEETGERVQLAHHFSEPEITLIIFGVMAGVIGTILLISYGIRRLIKKSPSDVKPLPSPDTDVPLSSVEIENPETSDQ   124
Subject-9   MYGKIIFVLLLSAIVSISALSTTEVAMHTSTSSSVTKSYISSQTNDTHKRDTYAATPRAHEVSEISVRTVYPPEEETGERVQLAHHFSEPEITLIIFGVMAGVIGTILLISYGIRRLIKKSPSDVKPLPSPDTDVPLSSVEIENPETSDQ   150
Subject-10  MYGKIIFVLLLSAIVSISASSTTGVAMHTSTSSSVTKSYISSQTNDTHKRDTYAATPRAHEVSEISVRTVYPPEEETGERVQLAHHFSEPEITLIIFGVMAGVIGTILLISYGIRRLIKKSPSDVKPLPSPDTDVPLSSVEIENPETSDQ   150
                            ************************************************************************************************:. : : : .*:.:     : .
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FIGURE 2 Changes in mRNA structure and protein sequence of *GYPA* in research subjects. (a) Location of reference sequence of *GYPA* gene in chromosome 4. (b) Exon compositions of *GYPA* mRNAs. The ones with NCBI reference number were cited from NCBI database. Subject-1 to subject-10 were sequences obtained from research subjects in this study. Nucleotide positions were indicated according to the *GYPA* gene Ref Sequence diagrammed in (a) and the exon numbering was determined according to the sequential order of all the exons in the table on the basis of their locations on *GYPA* gene. (c) Alignment of amino acid (AA) sequences of GPA proteins. The AA sequences were deduced from mRNA sequencing data of NCBI ref# L31860.1 as well as those obtained from research subjects in this study

Each pair of adjacent fragments shared a short overlapping sequence and the entire sequence was then assembled by matching overlapping sequences at the end of each fragment. The full-length sequences of *GYPA* and *GYPB* mRNAs were then aligned with *GYPA* gene and *GYPB* gene to determine exon composition of each transcript. Both *GYPA* and *GYPB* reference gene sequences were derived from chromosome 4 GRCh38.p13 (NCBI ref# NC_000004.12) as diagrammed in Figures 2a and 3a respectively.

GYP-EGFP fusion genes cloning and cell transfection

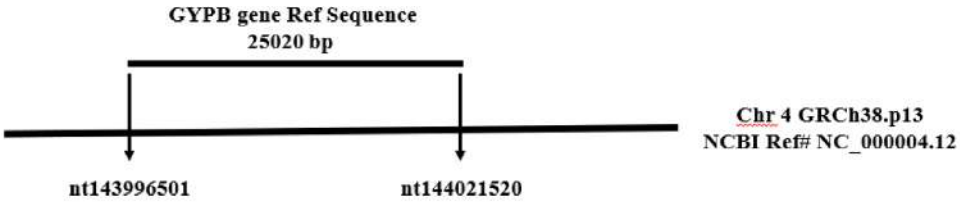
To determine the subcellular localization of the glycophorin (GP) proteins, *GYPA-EGFP* and *GYPB-EGFP* fusion genes were generated via a two-step PCR as described previously [24]. The fusion PCR products were then cloned to BamHI/XhoI site of pcDNA3.1 vector. The transfection was performed with Lipofectamine 2000 (Invitrogen/Thermo) and the transfected HEK293 cells were observed under Leica TCS SP8 confocal laser scanning microscope.

RESULTS

Fragment amplification for *GYPA* and *GYPB* mRNA sequencing

To obtain intact RNA that is good enough for long-fragment amplification, we have tried and compared several strategies to extract total RNA from fresh blood samples and found that isolation of nucleated cells was necessary for high-quality RNA purification (Figure 1a). With optimized conditions, including amplification strategy (Figure 1b) and specific primers (Figure 1c), we have successfully amplified the expected fragments that cover the whole length of *GYPA* and *GYPB* mRNAs (Figure 1d).

(a)



(b)

	Exon–1	Exon–2	Exon–3	Exon–4	Exon–5	Exon–6	Exon–7	Exon–8	Exon–9
J02982.1	1242-1288			19253-19355		21089-21128	22903-23000	24233-24263	24248-24414
NM_001304382.1	1194-1288	3656-3737		19254-19355		21089-21128	22903-23000	24233-24435	
NM_002100.6	1159-1288			19253-19355		21089-21128	22903-23000	24233-24435	
Subject-1	1242-1292		17842-17968	19254-19355		21089-21128	22903-23000	24233-24407	
Subject-2	1235-1289					21088-21128	22903-23000	24233-24407	
Subject-3	1242-1289					21088-21128	22903-23000	24233-24405	
Subject-4	1242-1289		17842-17968	19254-19355		21091-21126	22903-23000	24233-24407	
Subject-5	1242-1289					21088-21128	22903-23000	24233-24405	
Subject-6	1236-1289					21088-21128	22903-23000	24233-24405	
Subject-7	1236-1289					21088-21128	22903-23000	24233-24406	
Subject-8	1235-1289					21091-21126	22903-23000	24233-24405	
Subject-9	1235-1289	3653-3737		19254-19355		21089-21128	22903-23000	24233-24406	
Subject-10	1275-1288			19253-19299	1236-1289	21088-21128	22903-23000	24233-24405	

(c)

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J02982.1    ----MYGKIIFVLLLSEIVSISALSTTEVAMHTSTSSSVTKSYISSQTNGETGQLVHRFTVPAPVVIILIILCVMAGIIGTILLISYTIRRLIKA----------  91
Subject-1   ----------MSEEIVSISALSTTEVAMHTSTSSSVTKSYISSQTNGETGQLVHRFTVPAPVVIILIILCVMAGIIGTILLISYSIRRLIKA----------  82
Subject-2   ----MYGKIIFVLLL----------------------------------SGETGQLVHRFTVPAPVVIILIILCVMAGIIGTILLISYSIRRLIKA----------  58
Subject-3   ----MYGKIIFVLLL----------------------------------SGETGQLVHRFTVPAPVVIILIILCVMAGIIGTIPLNFLQYSPTDKGMRMRPACSPDLA  70
Subject-4   ----------MSEEIVSISALSTTEVAMHTSTSSSVTKSYISSQTNGETGQLVHRLTVPAPVVIILIILCVMAGIIGTILLISYSIRRLIKA----------  82
Subject-5   ----MYGKIIFVLLL----------------------------------SGETGQLVHRFTVPAPVVIILIILCVMAGIIGTILLISYSIRRLIKA----------  58
Subject-6   MGLRMYGKIIFVLLL----------------------------------SGEMGQLVHRFTVPAPVVIILIILCVMAGIIGTILLISYSIRRLIKA----------  62
Subject-7   ----MYGKIIFVLLL----------------------------------SGETGQLVHRFTVPAPVVIILIILCVMAGIIGTILLISYSIRRLIKA----------  58
Subject-8   ----MYGKIIFVLLL----------------------------------SGETGQLDHRFTVPAPVVIILIILCVMAGIIGAILLISYSIRRLIKA----------  58
Subject-9   ----------------------------MHTSTSSSVTKSYISSQTNGETGQLVHRFTVPAPVVIILIILCVMAGIIGTILLISYTIRRLIKA----------  65
Subject-10  ----MYGKIIFVLLL----------------------------------SGETGQLVHRFTVPAPVVIILIILCVMAGIIGTILLISYTIRRLIKA----------  58
                                                              .** *** **:**********************:* *        *.
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FIGURE 3 Changes in mRNA structure and protein sequence of *GYPB* in research subjects. (a) Location of reference sequence of *GYPB* gene in chromosome 4. (b) Exon compositions of *GYPB* mRNAs. The ones with NCBI reference number were cited from NCBI database. Subject-1 to subject-10 were sequences obtained from research subjects in this study. Nucleotide positions were indicated according to the *GYPB* gene Ref Sequence diagrammed in (a) and the exon numbering was determined according to the sequential order of all the exons in the table on the basis of their locations on *GYPB* gene. (c) Alignment of amino acid (AA) sequences of GPB proteins. The AA sequences were deduced from mRNA sequencing data of NCBI ref# J02982.1 as well as those obtained from research subjects in this study

Structures of *GYPA* and *GYPB* mRNAs in research subjects

The full sequences of *GYPA* and *GYPB* mRNAs obtained from NCBI database and research subjects (subject-1 to subject-10) were aligned with the reference sequences of *GYPA* gene (Figure 2a) and *GYPB* gene (Figure 3a) which were derived from NCBI RefSeq NC_000004.12. Totally eight candidate exons were identified in *GYPA* transcripts, including NCBI reference mRNAs and those extracted from research subjects, and they were determined E1–E8 according to their sequential order on *GYPA* reference sequence (Figure 2a,b). Meanwhile, nine candidate exons were identified in all the *GYPB* transcripts investigated in this study and they were determined E1–E9 according to their sequential order on *GYPB* reference sequence (Figure 3a,b).

Exon compositions of either *GYPA* or *GYPB* transcripts varied substantially among the research subjects. For *GYPA* mRNA, six exons in two subjects, seven exons in two subjects and eight exons in six subjects were identified respectively (Figure 2b, Table 1). For *GYPB* mRNA, four exons in six subjects and six exons in four subjects were identified respectively (Figure 3b, Table 1).

In all of the subjects, the sequence of each exon could be aligned with high identity to their corresponding genomic sequences. In almost all the subjects except *GYPB* of subject-10, the genomic locations of the exons of each mRNA were recognized in a proper order, indicating there was no gene rearrangement between homologous *GYPA* and *GYPB* or homologous *GYPB* and *GYPE* (Figures 2b and 3b).

In *GYPB* mRNA of subject-10, the fifth exon is unique as it was not found in other *GYPB* transcripts. The sequence of this exon showed high identity with that of exon1 (Figure 3b), indicating there was a gene conversion between these two regions within *GYPB* gene.

Amino acid sequences of GPA and GPB proteins that are encoded in research subjects

In all the subjects, ORFs of *GYPA* and *GYPB* mRNAs have been successfully identified on the basis of mRNA sequences; however, location of start codon varied among the research subjects. In most subjects, start codon of *GYPA* ORF was identified in first exon whereas in subject-2 the start codon located in third exon and in subject-8 the ORF started in second exon (Table 1). Similarly, start codon of *GYPB* ORF was also recognized in different exons, for example, first exon in six subjects, second exon in two subjects and third exon in two subjects (Table 1).

In most subjects, intact GPA proteins were predicted, when compared with protein encoded in reference mRNA (NCBI RefSeq L31860.1), whereas 26-AA deletion was found at N-terminal in subject-2 and subject-8 (Figure 2c). Accordingly, amino acids (AAs) representing M/N antigenic determinants were not able to be found in GPA expressed in these two subjects (Table 1).

Variation in AA sequences of GPB proteins was quite complex (Figure 3c). AA deletion in different regions of encoded GPB proteins,

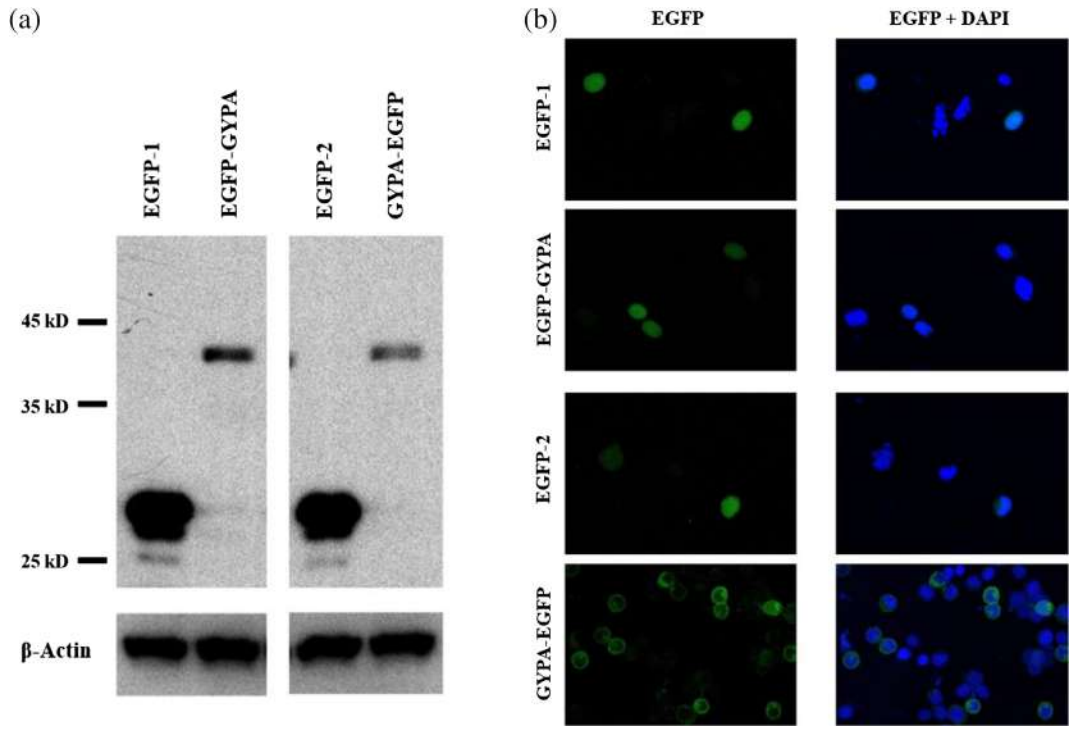

FIGURE 4 EGFP fusion at N-terminal but not C-terminal of GPA impeded its distribution on the cell surface of HEK293. (a) Western blotting to confirm overexpression of EGFP, EGFP-GPA (fusion at N-terminal of GPA) and GPA-EGFP (fusion at C-terminal of GPA) in HEK293 cells. GFP antibody (upper panel) and β-Actin antibody were used as first antibodies. (b) Detection of EGFP and DAPI staining fluorescence to show the cellular localization of target proteins and DNA in HEK293

when compared with that encoded in reference mRNA (NCBI RefSeq J02982.1), could be found in all the subjects (Figure 3c). Interestingly, presence of 's' antigenic determinant was predicted in nine subjects and 'S' antigenic determinant was recognized in only one subject (Table 1), demonstrating the 's' antigen-dominance in the Chinese population.

For each gene, only one antigen was predicted on the basis of RNA sequencing result, for example, M or N for *GYPA* and S or s for *GYPB* (Table 1). Therefore, discrepant results between serological test and mRNA-based prediction were observed in those research subjects of MN and Ss phenotypes. In addition, MN antigenic epitopes disappeared in two subjects according to the molecular prediction whereas MM or NN phenotypes were determined in serological tests.

Subcellular distribution of GPA and GPB proteins that are encoded in research subjects

To examine whether the structural changes of GPA protein (Figure 2c) and GPB protein (Figure 3c) affected their distribution on cell surface, we generated *EGFP* fusion ORFs whereby the *GYPA* or *GYPB* gene was fused with the *EGFP* gene.

First, we constructed two plasmids to compare different fusion strategies. In one plasmid, *EGFP* gene was fused at 5′-end of *GYPA* so that EGFP-GPA protein was encoded. In the other plasmid, *EGFP* gene was fused at 3′-end of *GYPA* so that GPA-EGFP protein was encoded. Plasmid encoding EGFP only was used as vector control in HEK293 cell transfection. Although the overexpression of fusion proteins in

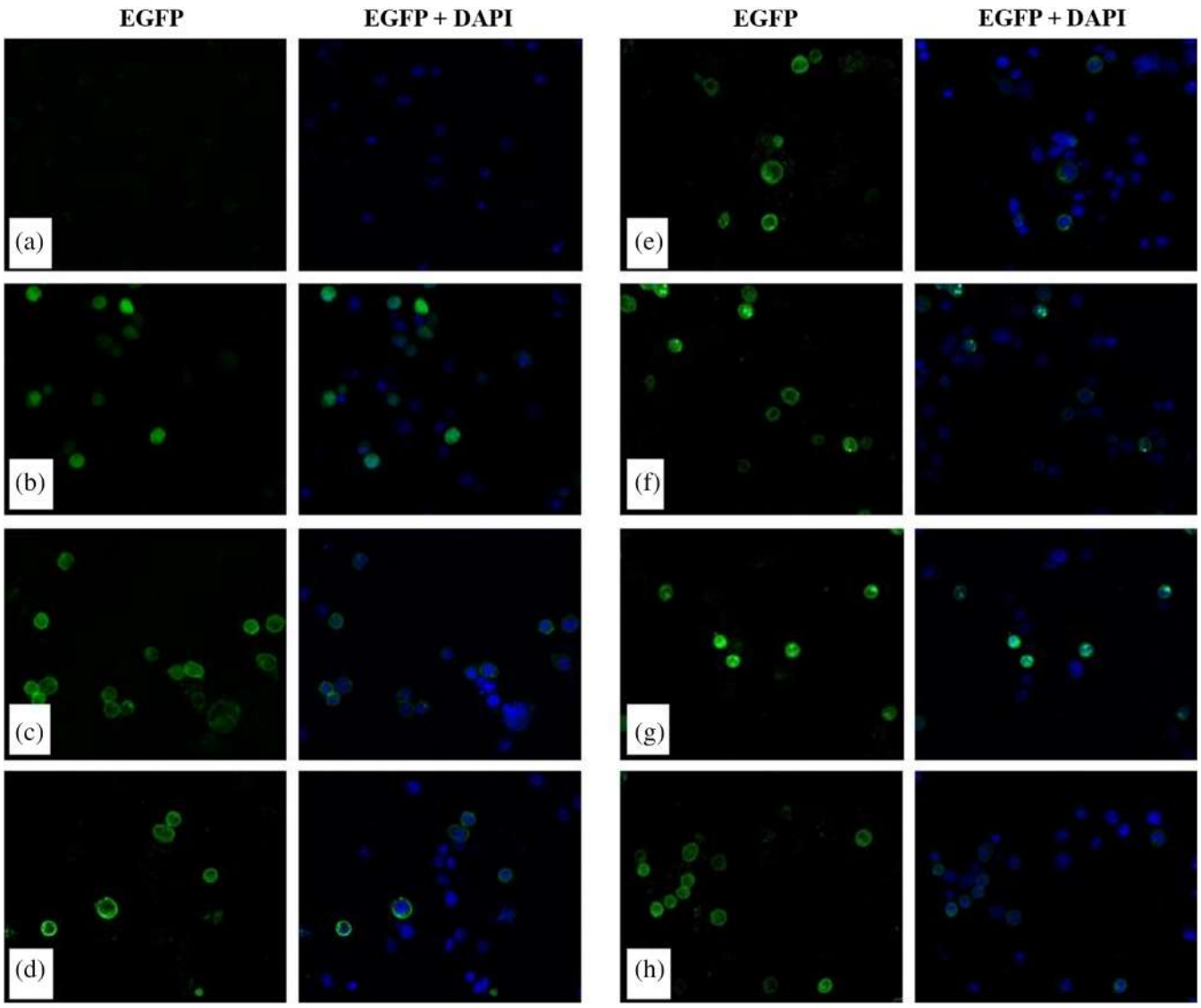

FIGURE 5 Cellular distribution of GPA and GPB proteins and their protein variants in HEK293 cells. HEK293 cells were either untransfected or transfected with plasmid encoding EGFP, GPA-EGFP or GPB-EGFP proteins. Then the cells were fixed and stained with DAPI and subsequently subjected to confocal microscopy observation. (a) Untransfected HEK293 cells. (b) EGFP only. (c, d) GPA-EGFP. Amino acid sequence was deduced according to mRNA of NCBI ref# L31860.1 (c) and subject-2. (e–h) GPB-EGFP. Amino acid sequence was deduced according to mRNA of NCBI ref# J02982.1 (e), research subject-2 (f), research subject-1 (g) and research subject-9 (h)

HEK293 cells was well confirmed (Figure 4a), only GYPA-EGFP demonstrated cell surface localization. Like EGFP protein alone, the fluorescence signal of EGFP-GPA fusion protein was diffused throughout the entire cell, indicating N-terminal structure was important for subcellular distribution of GP proteins (Figure 4b).

Secondly, wild type proteins of GPA (L31860.1 in Figure 2c) and GPB (J02982.1 in Figure 3c) as well as their representative variants discovered in the research subjects, for example, GPA of subject-2 and GPB of subject-1, subject-2, subject-9, were fused with EGFP in the GP-EGFP manner (Figure 4b) and the distribution of the fusion proteins in HEK293 cells was determined via transfection-microscopy analysis. As expected, wild type forms of both GPA (Figure 5c) and GPB (Figure 5e) showed cell surface localization very clearly. 26AA-deletion at N-terminal did not affect the cell surface distribution of GPA (Figure 5d). Similarly, AA-deletion at N-terminal also could not influence cell surface distribution of GPB, no matter whether it is a 9AA-deletion (Figure 5g) or a 26AA-deletion (Figure 5h). Amazingly, even the 33AA-deletion, which was corresponding to AA position 13–45 of GYPB encoded by NCBI RefSeq J02982.1, still could not change the cell surface distribution of GPB (Figure 5f).

DISCUSSION

Currently, 49 MNS antigens have been recognized by the International Society of Blood Transfusion [11] and it seems new antigens will be continuously discovered [7] due to the changeableness of the genomic regions encoding GPA and GPB [6]. Therefore, molecular typing will play more and more important roles in blood grouping due to its advantages in specificity, accuracy and quickness of method establishment [10].

In this work, we have adopted the method of Sanger Sequencing to characterize alternatively spliced transcript variants of *GYPA* and *GYPB* genes in the Chinese population in an effort to investigate the underneath mechanisms of the complexity of MNS antigenic features. Sequencing results of both *GYPA* and *GYPB* mRNAs have revealed complex alternative splicing of these two genes. Notably, the splicing variations often occurred in the area near 5′-end of those mRNAs whereas exons at 3′-end were relatively stable (Figures 2b and 3b). It was probably due to the fact that introns at 5′-ends of *GYPA* and *GYPB* pre-mRNAs were usually very large. For example, a 20-kb intron could be found in genomic sequence between first exon and second exon of an NCBI *GYPA* mRNA (RefSeq L31860.1, Figure 2b) and an 18-kb intron was recognized in genomic sequence between first exon and second exon of an NCBI *GYPB* mRNA (RefSeq J02982.1, Figure 3b).

Alternative splicing resulted in changes of AA sequences of GPA and GPB proteins (Figures 2c and 3c). Particularly, the splicing variation usually occurred at 5′-end which meant the N-terminal of GP proteins were affected most. Since N-terminal segments of GPA and GPB were said to be able to protrude to the extracellular compartment [25] and determine the MNS antigenic features with the help of N-linked and O-linked glycosylations [26], structural changes in this region will cause variations of MNS antigens. Interestingly, it seemed none of the structural changes of GPA and GPB proteins could affect cell surface distribution of these two proteins (Figure 5), indicating all the protein variants identified in the research subjects in this study could present certain MNS antigenic features. In addition, although Mia antigen was tested positive in some research subjects (Table 1), no hybrid GPs of phenotypes of the Miltenberger series were observed. Actually, the molecular basis of Mia antigen is not fully clear yet. Although Chen et al. proved the existence of Mia antigen as an independent entity by reporting the first monoclonal anti-Mia antibody, the epitope was not clearly determined [27]. At present, seven hybrid GPs have been reported to be able to present MIA antigen [28], raising the possibility that GP variants generated by alternative splicing of *GYP* mRNAs may also play roles for Mia antigen presentation.

We found that in some subjects, MNS antigen prediction on the basis of mRNA sequencing results was not consistent with the results of serological test (Table 1). In fact, discrepancies between phenotyping and genotyping have always been observed in blood typing [29, 30] including MNS determination [31] due to respective limitations of the typing methods. Therefore, it is essential to explore as many methods as possible to make supplement to the traditional serological test. In this study, we performed Sanger sequencing to characterize the RNA sample. It was possible that several sequence reads existed simultaneously in same subject; however, only the major one could be obtained as the final reading. That is why only one antigen was predicted on the basis of RNA sequencing result (Table 1), representing one of the limitations of this method. Actually, there were minor peaks demonstrated in some sequencing chromatograms, indicating the existence of other RNA forms at low level. We may perform TA cloning to further identify them. In addition, NGS-based RNAseq will greatly improve the sensitivity and resolution in identifying transcript variants of *GYPA* and *GYPB* genes.

To efficiently detect alloantibodies, especially those irregular antibodies, is still a challenging task to face in blood transfusion [32, 33]. In this study, we have shown that RNA characterization may help to directly predict the exact structures of antigenic proteins. Once we have identified an alternative splicing variant, we may apply the information to establish a cell model which uniquely overexpresses the particular antigenic protein and use it to detect corresponding alloantibodies. The ‘imagined’ cell model tools will significantly contribute to the accuracy and specificity in alloantibody detection that is mainly conducted with RBC-derived products at present [34].

In conclusion, our results have demonstrated complex variations of *GYPA* and *GYPB* transcripts in the Chinese population and strongly implicated the complicated molecular basis of MNS antigenic features. *GYP* transcript characterization might be an invaluable supplement to traditional serological phenotyping and DNA-based genotyping in MNS blood grouping.

ACKNOWLEDGEMENTS

Y.L. and J.R. conceptualized and supervised the study; J.R., F.Z., Y.S., J.L., S.F., Y.L., X.F. and J.L. performed the research; J.R., Y.L., Y.L., B.F.,

W.H. and Y.X. performed data analysis and summarization; J.R. wrote the first draft of the manuscript; Y.L., F.Z., W.H., F.W., S.L., Y.L. and Y.X. reviewed and edited the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SHORT REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

A case for a national registry of red blood cell antibodies

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Abstract

Background and Objectives: Red blood cell (RBC) antibody levels diminish over time and negative antibody screen are commonly seen in patients with a history of antibodies. Most hospitals do not have access to a shared registry of antibodies previously detected at other hospitals.

Materials and Methods: We describe a case where the patient was found to be at high risk of bleeding during liver transplantation. Antibody screen on admission was negative but a history of anti-Jk^a was identified on reviewing patient's history in local registry of RBC antibodies. The surgery was pushed back to arrange for antigen-negative units. The patient received a total of 16 Jk(a−) RBC units during the admission.

Results: No acute or delayed transfusion adverse reactions were seen. However, if the history of anti-Jk^a identified at another local hospital was not known, approximately three-quarters of the units transfused would have been Jk(a+). Transfusing Jk(a+) units could have potentially exposed the patient to risk of developing an acute and/or delayed haemolytic transfusion reaction which could have led to significant morbidity and perhaps mortality.

Conclusion: With this case report, we build a case for developing a national registry of RBC antibodies to help improve patient safety and outcomes.

KEYWORDS
blood safety, RBC antigens and antibodies, serological testing, transfusion reactions

Highlights

- Most hospitals do not have access to a shared registry of RBC antibodies previously detected at other hospitals.
- In a patient with negative antibody screen but history of RBC antibodies, transfusion of antigen-positive RBC unit can potentially lead to acute and/or delayed haemolytic transfusion reaction which can cause significant morbidity and perhaps mortality.
- A national registry of RBC antibodies can help improve patient safety and outcomes.

BACKGROUND

Red blood cell (RBC) alloantibody levels diminish over time and negative antibody screen are commonly seen on admission in patients with previous history of antibodies detected at other institutions. Most hospitals do not have access to a shared registry of RBC antibodies previously detected at other hospitals. Also, even if they have access to such registry, it is usually local or regional only.

CASE PRESENTATION

A 58-year-old Caucasian male patient with a history of liver cirrhosis secondary to nonalcoholic steatohepatitis (NASH) was summoned to the hospital for a liver transplant after being on the waiting list for several months. Upon admission, routine pre-transplant laboratory tests including coagulation profile, blood group typing and antibody screen were performed. Patient was found to be group O, RhD positive and antibody screen was negative by gel methodology (Ortho-Clinical Diagnostics, Raritan, NJ). Per hospital transfusion service policy, the city-wide registry of RBC antibodies maintained by local community blood centre was checked. Patient was found to have a history of anti-Jk^a. Anti-Jk^a was discovered at another local hospital about 7 months prior to the transplant admission and added to the local antibody registry by the blood centre.

The patient was found to be at high risk of bleeding with a high model for end-stage liver disease and sodium score of 24 and abnormal coagulation studies including prolonged activated partial thromboplastin time of 42 s and low platelet counts of 43,000/μl (Table 1). Per agreement between hospital transfusion service and liver transplant team, transfusion service started arranging for blood products for the liver transplantation. But with the discovery of history of anti-Jk^a, it would require extra time to procure antigen-negative units.

Transplant organ procurement and placement processes entail complex scheduling, logistics and other administrative challenges. The liver allocated to this patient was already procured and waiting for antigen negative blood would delay the transplantation. Any delays in transplantation can potentially increase the risk of graft failure. Fortunately, a second liver that had not yet been procured became available for the patient. Already procured first liver was transplanted to another patient at the same centre and patient's surgery was scheduled for later in the evening after the second liver was procured. The hospital was able to obtain a total of 14 Jk(a−) RBC units from local blood supplier and locate one Jk(a−) unit in hospital inventory by typing units for Jka antigen with antisera.

Two units were immediately transfused to the patient because of low preoperative haemoglobin of 6.2 g/dl. Liver transplant surgery was complicated with excessive blood loss (could not be quantified) and took about 10 h to complete. Thirteen RBC units were transfused during the surgery. Other blood components transfused during the surgery included 10 apheresis platelets, 31 units of thawed plasma and 8 pooled units (5-unit pools) of cryoprecipitate. Cell saver was utilized and 20 L of 5% albumin was also given to the patient during the transplant surgery. An additional unit of Jk(a−) RBC was obtained from local blood supplier which was transfused on post-operative day 2.

DISCUSSION

This case is an example of how a registry of RBC antibodies is critical to providing good patient care. The patient never received any Jk(a+) RBC units during the transplant admission. No acute or delayed transfusion adverse reactions were seen. The transplant was successful, and patient is doing well post-transplant. If our hospital transfusion service had not utilized the local registry of RBC antibodies, we would have missed the patient's history of anti-Jk^a. Kidd antibodies (anti-Jka and anti-Jkb) are well known to be capable of dropping to low or even undetectable levels after several months following exposure. And a next transfusion exposure leads to a subsequent robust antibody response in these patients (anamnestic response). Without knowing the history of anti-Jk^a, approximately three-quarters of the units transfused to this patient would have been Jk(a+) (prevalence of Jka antigen in the Caucasian population is 77%). Transfusing Jk(a+) units could have potentially exposed the patient to risk of developing an acute and/or delayed haemolytic transfusion reactions (DHTRs) [1].

Acute haemolysis during transplantation could have led to significant morbidity and perhaps mortality since anti-Jk^a can produce intravascular haemolysis. In the frenzy of a complicated transplant surgery, the haemolysis would have been devastating. The signs and symptoms of a severe acute haemolytic transfusion reaction would have certainly detracted the attention of the surgeon and anesthesiologist from the primary job at hand, replacing a cirrhotic liver. Patient could alternatively have developed DHTR which occurs 1 in 2500 transfusions in patients with negative antibody screen [2]. Most DHTR produce extravascular haemolysis that reduces haemoglobin over several days to weeks. Occasionally, anti-Jk^a induced DHTR can lead to rapid intravascular haemolytic events. Since approximately three-quarters of the red blood cells units would have been Jk(a+), the likelihood of a significant haemolytic reaction was high.

Studies have found that 4%–23% of liver transplant patients can have red cell alloantibodies pre-transplant [3]. Unfortunately, most hospitals do not have access to even a local or regional registry of RBC antibodies to look up patients' previous history. In 2005, at the

TABLE 1 Peri-transplant coagulation studies

Laboratory tests	Reference ranges	Pre-transplant	During-transplant		Post-transplant	
PT	11.4–15 s	19.1	21.2	23	**19.4**	**19.2**
INR	0.8–1.2	1.6	1.8	2	1.6	**1.6**
aPTT	22–34 s	42	49	108	–	–
Fibrinogen level	146–390 mg/dl	158	151	171	–	–
Platelet count	140–400 K/μl	43	25	51	72	87

Abbreviations: aPTT, activated partial thromboplastin time; INR, international normalized ratio; PT, prothrombin time.

concurrence of two local pathologists, Community Blood Center of Kansas City, Missouri, USA, embarked on a project to develop an electronic local registry of RBC antibodies reported by the 68 local hospitals it served [4]. As exemplified in this case report, this local antibody registry has improved patient safety and outcome on multiple occasions since its establishment. We would like to argue for a national registry of RBC antibodies, similar to the Kansas City registry and Dutch National Database [5, 6] that can be checked before any RBC unit is cross-matched (serological or electronic) and released to a patient. This will significantly reduce the risk of acute and/or DHTRs in patients with a history of RBC antibodies that are not detected in the current specimen. Another important lesson learned from this case relates to routine registry querying for all prospective liver transplant patients.

In conclusion with this case report, we build a case for developing a national registry of RBC antibodies in every country to help improve patient safety and outcomes. The case presented above presents a strong argument for the National Institutes of Health in the United States, Association for the Advancement of Blood & Biotherapies and other similar organizations in other countries to implement such a programme.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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SHORT REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

Rapid genotyping of 508G>A (rs3745635) and 1067T>A (rs3894326) of *FUT3* by a duplex Eprobe-mediated melting curve analysis

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Abstract

Background and Objectives: Lewis histo-blood group phenotypes are regulated by the action of *FUT3*-encoded α(1,3/1,4)fucosyltransferase and *FUT2*-encoded α(1,2) fucosyltransferase. Since Lewis phenotypes are suggested to be associated with various clinical conditions, a method for large-scale *FUT3* genotyping is desirable. In worldwide populations, 508G>A and 1067T>A of *FUT3* are two of three common causal single nucleotide polymorphisms for Lewis-negative alleles.

Materials and Methods: We developed a duplex Eprobe-mediated melting curve analysis for genotyping 508G>A and 1067T>A simultaneously and applied this method to 106 Ghanaian and 140 Japanese subjects.

Results: The results of both 508G>A and 1067T>A genotyping by duplex Eprobe-mediated melting curve analysis were completely in agreement with the results of a DNA sequence analysis in 106 Ghanaians and polymerase chain reaction-restriction fragment length polymorphism analysis in 140 Japanese subjects.

Conclusion: The present duplex Eprobe-mediated melting curve analysis is valid and credible for large-scale estimation of Lewis-negative alleles.

KEYWORDS
Eprobe, *FUT3*, Lewis-negative allele, melting curve analysis, rs3745635, rs3894326

Highlights

- Lewis phenotypes determined by *FUT2* and *FUT3* are suggested to be associated with various clinical conditions.
- We developed a duplex Eprobe-mediated melting curve analysis for detection of rs3745635 and rs3894326 of *FUT3*.
- The present melting curve analysis is useful for large scale association studies of *FUT3*.

INTRODUCTION

Lewis antigens, Le^a and Le^b, are the ABO(H) histo-blood group-related antigens. Expression of Lewis antigens is controlled by the action of two enzymes, *FUT3*-encoded α(1,3/1,4)fucosyltransferase (Lewis enzyme) and *FUT2*-encoded secretor-type α(1,2)fucosyltransferase (Se enzyme) [1]. The functional alleles of *FUT2* and *FUT3* (*Se* and *Le*) are dominant over the nonfunctional alleles (*se* and *le*). Accordingly, Lewis positive (*Le/Le* or *Le/le*) ABH secretors (*Se/Se* or *Se/se*) have Le(a−b+) red blood cells (RBC) and Lewis-positive non-secretors (*se/se*) have Le(a+b−) RBC while Lewis negative individuals (*le/le*) have Le(a−b−) RBC regardless of secretor status [1]. However, since Lewis antigens are not intrinsic to RBC but adsorbed onto the RBC from the plasma, it is often difficult to determine the accurate Lewis

phenotypes by traditional serological methods [2]. Therefore, a credible method of genotyping of *FUT2* and *FUT3* is desirable to deduce Lewis phenotypes.

Previous studies [3–5] and databases such as Erythrogene v0.8 (27 November 2017) (http://www.erythrogene.com/ [6]) suggested that three nonsynonymous single nucleotide polymorphisms (SNPs), 202T>C (p.W68R, rs812936), 508G>A (p.G170S, rs3745635) and 1067T>A (p.I356K, rs3894326) are common SNPs responsible for Lewis enzyme inactivation in worldwide populations. In addition, 484G>A (p.D162N, rs28362463) is prevalent in Africans [7]. The *FUT3* alleles containing 508A or 1067A always accompany 59G (59T>G, L20R, rs28362459), which was reported to be responsible for the Lewis-negative RBC and Lewis-positive saliva phenotype [6, 8]. Accordingly, 59T>G seems to be useful for estimation of *le* caused by 508G>A or 1067T>A in many populations [6], and, in fact, we recently developed three high-resolution melting (HRM) analyses for estimation of *le* using 59T>G as a proxy SNP for 508G>A or 1067T>A, 13G>A (p.G5S, rs28362458) as a proxy SNP for 484G>A and 202T>C [9].

However, the amplicon for detection of 59T>G contains another polymorphic site, 61C>T (synonymous SNP, rs28362460), that has a certain frequency in Africans (4.24%) and Americans (1.30%) but not in other populations [6]. Because it is difficult to discriminate homozygotes of 59T-61C (wild-type) and homozygotes of 59G-61T or 59T-61C/59G-61T and 59G-61C/59G-61T using this HRM assay, we may misjudge the *le* allele containing 59G-61T as the wild-type allele [9]. Therefore, it is preferable to analyse 508G>A or 1067T>A directly for a more accurate estimation of *le*, particularly in Africans and Americans. The hydrolysis probe (TaqMan) assay is one of the relatively high-throughput screening methods for genotyping SNPs. At present, a predesigned hydrolysis probe assay for determination of 508G>A is not available, probably due to the presence of *FUT5* and *FUT6*, two paralogous genes of *FUT3*, having high sequence similarity with *FUT3* [10].

Eprobe is a fluorescence probe enabling real-time polymerase chain reaction (PCR) monitoring for quantification analysis of DNA and RNA and melting curve analysis for detection of SNPs using real-time PCR machines [11, 12]. In addition, because three dyes are available, multiplex assays are capable of performing multiple tests on a single sample. In this study, we developed a duplex Eprobe-mediated melting curve analysis (Eprobe-MCA) for detection of 508G>A and 1067T>A of *FUT3* simultaneously.

MATERIALS AND METHODS

DNA samples

The ethical committee of Kurume University reviewed and approved the study protocol (Bioethics approval No. 342).

FIGURE 1 Alignment of DNA sequences of *FUT3* around 508G>A (a) and 1067T>A (b), and corresponding regions of *FUT5* and *FUT6*. 508G: wild-type allele, 508A: A allele at 508G>A of *FUT3*, 1067T: wild-type allele, 1067A: A allele at 1067T>A of *FUT3*. The Eprobe sequence for detection of 508G>A is indicated by a yellow box and that for 1067T>A is indicated by an orange box, and a mismatched nucleotide is indicated by removing the colour. Primer sequences are underlined. Boldface shows a nucleotide that is different from wild-type sequence

Genomic DNAs from 106 Ghanaians whose complete *FUT3* coding sequence had been already determined by Sanger sequencing [3] and 140 Japanese whose genotypes of three *FUT3* SNPs (59T>G, 508G>A and 1067T>A) had been determined previously by PCR-restriction fragment length polymorphism (PCR-RFLP) were used [3, 4].

Eprobe-MCA for simultaneous genotyping of 508G>A and 1067T>A of *FUT3*

Primers for amplification of *FUT3* were selected by Primer3 (https://bioinfo.ut.ee/primer3-0.4.0/), and Eprobes were selected by Edesign (https://www.dnaform.com/edesign2/). Primer and Eprobe sequences are as follows (and also indicated in Figure 1): those for detection of 508G>A (508A-Eprobe: 5′-ACATCUTCACGCCCTACAGCT-3′-AmC3; U indicates the modified T by oxazole yellow, 491–511 bp of the forward sequence of *FUT3* of 508A allele, FUT3-508-F: 5′-AGCCACCCCCTAACTGCCA-3′ and FUT3-508-R: 5′-GGTCTTGGCCGAGAGGTTGA-3′), those for detection of 1067T>A (1067T-Eprobe: 5′-TGCGCAGCATAGCGGCTTGGUTCA-3′-AmC3; U indicates the modified T by thiazole orange, 1058–1081 bp of the forward sequence of *FUT3* of 1067T allele, FUT3-1067-F: 5′-TCAGCTGGGCACTGGATTTC-3′ and FUT3-1067-R: 5′-GAGGGTTGGCCACAAAGGAC-3′). All Eprobes were synthesized by K.K. DNAFORM (Yokohama, Japan), and all primers were synthesized by Eurofins Genomics (Tokyo, Japan).

Asymmetric PCR was conducted in a reaction mixture: containing 2–20 ng of genomic DNA, 5 μl of E-Taq 2× PCR Mix (K.K.

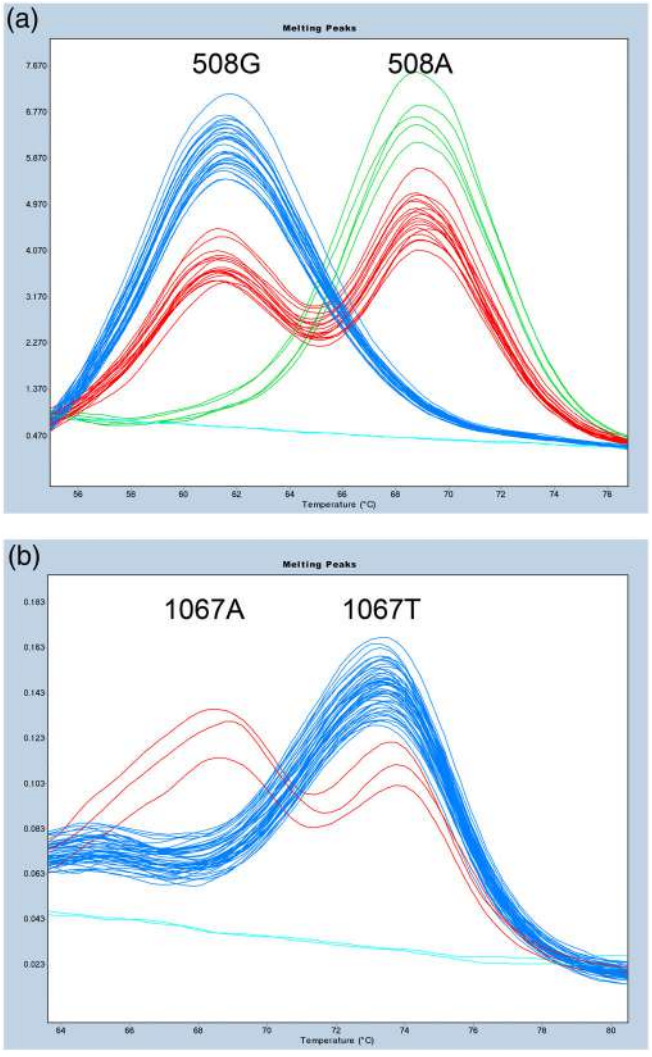

FIGURE 2 Melting peak profiles of Eprobe-MCA for detection of 508G>A (a) and 1067T>A (b) of *FUT3* obtained for 53 of 106 Ghanaians. The individuals having genotypes of G/G (blue), G/A (red) and A/A (green) at 508G>A and those of T/T (blue) and A/T (red) at 1067T>A were completely separated. There was no A/A at 1067T>A in these subjects. Light blue is a negative control

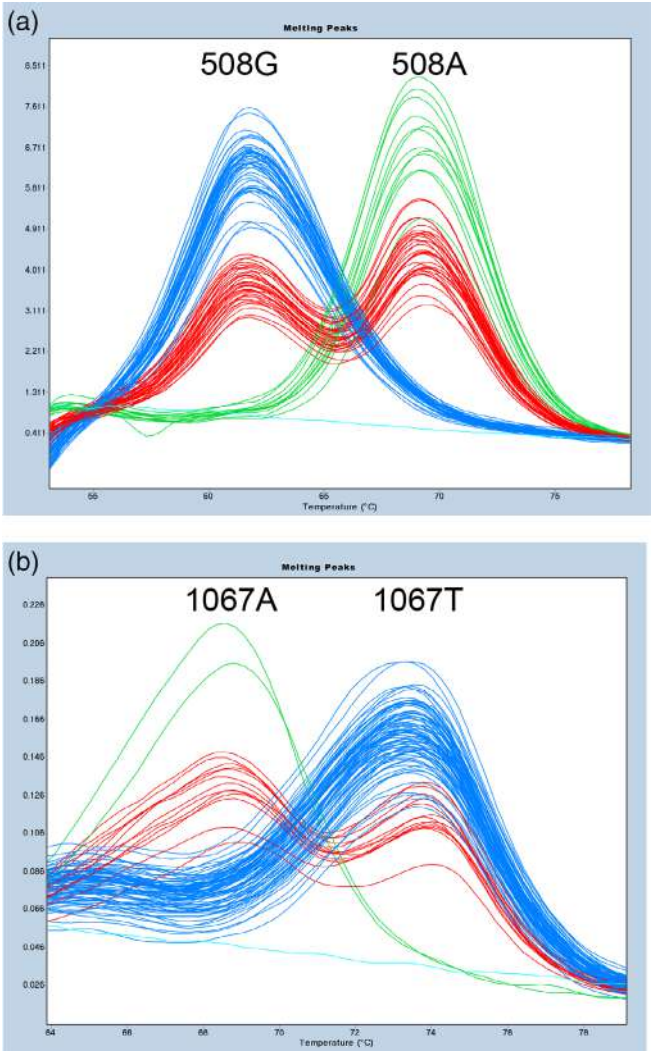

FIGURE 3 Melting peak profiles of Eprobe-MCA for detection of 508G>A (a) and 1067T>A (b) of *FUT3* obtained for 95 of 140 Japanese. The individuals having genotypes of G/G (blue), G/A (red) and A/A (green) at 508G>A and those of T/T (blue), A/T (red) and A/A (green) at 1067T>A were completely separated. Light blue is a negative control

DNAFORM), FUT3-508-F primer (50 nM), FUT3-508-R primer (250 nM), FUT3-1067-F primer (50 nM), FUT3-1067-R primer (250 nM) and the Eprobes (250 nM, for both 508A and 1067T) with PCR-grade water adjusted to a final volume of 10 μl. The temperature program was as follows: initial denaturation and enzyme activation at 95°C for 30 s, followed by 50 cycles of denaturation at 95°C for 5 s, annealing for 15 s at 58°C and extension for 15 s at 72°C. The fluorescence data for real-time PCR amplification were monitored at the end of each annealing step using the FAM (465–510 nm) filter for oxazole yellow and VIC/HEX/Yellow 555 (533–580 nm) filter for thiazole orange using a LightCycler 480 instrument II (Roche Diagnostics, Tokyo, Japan).

After PCR amplification, melting curve analysis was initiated with denaturation for 1 min at 95°C, hybridization for 1 min at 40°C, and the fluorescence data were recorded in the temperature range of 52–82°C at a ramp rate of 0.06°C/s and five acquisitions per degree Celsius using the corresponding detection filters. Melting curve genotyping was performed using LightCycler 480 gene scanning software (Roche Diagnostics) with sensitivity at default and the temperature range of 58–73°C and 66–76°C for 508G>A and 1067T>A, respectively. Samples having the same melting curve pattern were grouped automatically.

HRM analysis for genotyping of 59T>G of *FUT3*

HRM analysis for genotyping of 59T>G of *FUT3* was performed as described previously [9].

RESULTS AND DISCUSSION

As described previously [9], because HRM analysis for 59T>G of *FUT3* cannot separate one homozygote of 59G-61T from homozygotes of 59T-61C (wild-type) or two heterozygotes of 59G-61C/59G-61T from heterozygotes of 59T-61C/59G-61T in 11 selected Ghanaian subjects (Figure S1a), we may misjudge the *le* containing 59G-61T as the wild-type allele and deduce Le phenotypes.

On the other hand, Eprobe-MCA using a FAM filter can accurately discriminate homozygotes of the wild-type (G/G, melting temperature [Tm]: about 62–63°C), heterozygotes (G/A, Tm: 62 and 70°C) and A/A (Tm: about 70°C) of 508G>A including 59G-61T because 59G-61T always accompanies 508A (Figure S1b). In addition, homozygotes of the wild-type (T/T, Tm: about 73°C), heterozygotes (A/T, Tm: about 69°C and 73°C) and A/A (Tm: about 69°C) of 1067T>A were also accurately discriminated using a VIC/HEX/Yellow 555 filter (Figure S1c).

We then analysed 106 Ghanaians and Figure 2a,b show results of Eprobe-MCA obtained for 53 of 106 Ghanaian subjects. Three genotypes of 508G>A and two genotypes (T/T; wild-type homozygotes and T/A; heterozygote) of 1067T>A were separated clearly using a FAM filter and a VIC/HEX/Yellow 555 filter, respectively. The present results were completely in agreement with previous Sanger sequencing results [3]. In addition, we confirmed the repeatability of the method by two independent analyses.

Because homozygotes of the 1067A were not present in our Ghanaian subjects, we tested the duplex Eprobe-MCA on 140 Japanese subjects, and Figure 3a,b shows the results of 95 of 140 subjects. Three genotypes of 1067T>A, in addition to those of 508G>A, were separated clearly for 140 subjects, and the results were in accordance with previous PCR-RFLP ones [4].

Several studies suggested that *FUT3* polymorphisms are associated with *Helicobacter pylori* infection, enteric infections, COVID-19 susceptibility, ischemic heart diseases, inflammatory bowel diseases and ankylosing spondylitis [2, 13–17]. Large-scale replication studies in various populations are necessary to verify these associations. In addition, serum carbohydrate antigen 19-9 (CA19-9, also known as sialyl Le^a antigen) is a well-known tumour marker for the detection of pancreatic cancer. However, Lewis-negative individuals almost completely lack the secretion of CA19-9 and its clinical application is limited in these individuals [18]. Thus, a reliable method of genotyping *FUT3* for association studies or prior to CA19-9 measurement is desirable.

Previously, genotyping of *FUT3* SNPs was performed by several methods such as PCR-RFLP, PCR using sequence-specific primers, an allele-specific oligonucleotide hybridization method, TaqMan assay, HRM assay, nucleotide sequencing of PCR products and a multiplex SNaPshot assay [3–5, 9, 14, 15, 19]. As well as TaqMan and HRM assays, the advantage of Eprobe-MCA requires no post-PCR processing, is simple, cost-effective, accurate, high-throughput and faster than the conventional PCR.

We need to determine 202T>C in Asian and European populations and 202T>C and 484G>A in African populations, in addition to 508G>A and 1067T>A for more accurate estimation of Le-positive and Le-negative phenotypes. As described earlier, genotyping of 508G>A and 1067T>A instead of 59T>G may be desirable particularly for African and American populations. Using a combination of these HRM assays with the present method, a more accurate estimation of Le phenotypes can be carried out.

In conclusion, the Eprobe-MCA for simultaneous determination of two causal Le enzyme-inactivating SNPs, 508G>A and 1067T>A, is valid and feasible for association studies of *FUT3*.

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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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INTERNATIONAL FORUM

Vox Sanguinis ISBT International Society of Blood Transfusion

International Forum on the Management of Major Haemorrhage: Summary

Laura Green | Simon Stanworth | Zoe McQuilten | Victor Lin | Harriet Tucker | Bryon Jackson | Maha Badawi | Salwa Hindawi | Rahul Chaurasia | Gopal Patidar | Hem Chandra Pandey | Foluke Fasola | Shigeki Miyata | Masanori Matsumoto | Tadashi Matsushita | Naomi Rahimi-Levene | Victoria Peer | Katerina Pavenski | Jeannie Callum | Troy Thompson | Michael Murphy | Julie Staves | Marc Maegele | Chathuri Abeyakoon | Kylie Rushford | Erica Wood | Maria Antonieta Nuñez | Sandra Mellado | Edgardo Saa | Teguh Triyono | Bhirowo Pratomo | Torunn Oveland Apelseth | Nancy Dunbar

INTRODUCTION

Acute bleeding that requires large volume transfusions can occur in any clinical setting [1–3]. The optimal management of this type of bleeding is complex and has a major cost burden for health care services [4]. In the last two decades, we have seen an explosion in the number of studies evaluating the role of blood transfusion or alternatives (e.g., haemostatic agents) on clinical outcomes. Studies in trauma have demonstrated that the early and balanced transfusion resuscitation in bleeding patients improve clinical outcomes [5–8]. Delivery of these regimens for transfusion has been supported and enabled by the widespread use of major haemorrhage protocols (MHPs) in hospitals. However, it is unclear how far the results from studies in trauma, where much of the evidence on transfusion management is found, should be extrapolated to other settings of major bleeding. The different findings between results of the large randomized control trials of tranexamic acid use in acquired bleeding (i.e., trauma [9, 10], obstetric [11] and gastrointestinal bleeding) [12] clearly illustrate how a '*one-size-fits-all*' approach may not be suitable across all settings of major bleeding.

The evidence underpinning the role of MHP on the clinical outcome is primarily based on observational studies, rather than interventional trials. However, these protocols have now become a standard of care for most hospitals. For example, the UK national audit of management of major haemorrhage (MH) in 2018 where 166 hospitals–trusts were enrolled and 826 cases were analysed, showed that 99% of hospitals had MHPs in place for managing major bleeding and that the main causes for MH (defined as bleeding that triggers MHP) were surgery, followed by obstetrics, gastrointestinal bleeding and trauma [13]. Considering that these protocols consume a lot of healthcare resources, it is important that there are national and local guidelines to guide clinicians on how to implement these protocols in real life, as well as audit their use on regular basis.

Currently, there is no internationally agreed definition for massive transfusion (MT). The most common definitions used in clinical practice include transfusion of ≥10 red blood cell (RBC) units in 24 h, or ≥6 RBC units in 6 h; or ≥5 RBC units in 4 h [3, 14–16]. Other definitions such as 'ultramassive transfusion', have also been used for patients who are transfused ≥20 RBC over the course of any 2 consecutive calendar days [17]. However, these definitions have limited use in clinical management of patients, as they do not identify (massive) bleeding patients early, and more importantly they are applied retrospectively and often fail to capture patients who die in the first few hours of bleeding, known as 'survivorship bias' [18, 19]. Further, these definitions do not include other blood components (like plasma or platelets), blood products (i.e., haemostatic agents) or fluid volume, all of which are used during the initial resuscitation stage of bleeding.

Management of a massively transfused patient also poses logistical challenges for blood collection centres and hospital transfusion laboratories who need to manage blood stocks to ensure that enough blood is available for all patients who needed it and that the right blood is delivered to the right patients. This is more challenging when special requirements are needed (e.g., RhD negative RBC for women of childbearing potential or unknown patients), or during major incidents when demand for blood may exceed supply. In such

circumstance, the delivery of blood components may require a modified approach, necessitating advanced planning and knowledge of the transfusion service [20].

Recognizing these uncertainties, we designed an International Forum to explore a range of issues related to the processes and management of MH. Questions were asked to seek information on definitions of MH and/or MT, current strategies for transfusion management of bleeding (such as blood component ratios and fibrinogen target) and availability of national guidelines–policies on management of MH. An additional question explored the use of RhD negative RBC for women of childbearing potential or unknown patients, given the ongoing constraints on supply of this type of red cell component by all transfusion services. Respondent demographic data were also captured. A total of 22 sites were invited to participate in the forum and of these 13 responded, representing 13 countries.

SUMMARY OF RESPONSES

Question 1 Respondents' demographics

Of the 13 respondents, 5 were from Asia (India, Indonesia, Israel, Japan and Saudi Arabia), 3 from Europe (Germany, Norway and the United Kingdom), 2 from North and Central America (Canada and United States), 1 from Africa (Nigeria), 1 from Oceania (Australia) and 1 from South America (Chile). All respondents presented their demographic data at the local level (i.e., hospital), except Canada, which presented national-level data (Table 1).

Question 2 Definition of major haemorrhage and/or massive transfusion

Question 2 was divided into four categories, which are reported here as 2a, 2b, 2c and 2d.

Question 2a How do you define major haemorrhage and/or massive transfusion in adults?

Do you have separate definitions for MAJOR TRANSFUSION, MASSIVE TRANSFUSION and SUPRA-MASSIVE TRANSFUSION in adults?

Do these definitions apply to all clinical settings or are there different definitions used for different clinical settings in adults?

Do you think we should use one definition for MAJOR TRANSFUSION, MASSIVE TRANSFUSION and SUPRA-MASSIVE TRANSFUSION for different clinical settings in adults?

The definition of MH and/or MT was answered differently between respondents, with some providing separate definitions for MH and MT, while others either presented one answer for both or provided information only on what triggers the MHP (Table 2). By and large, these definitions applied to all clinical settings, apart from India, Saudi Arabia and Israel who had a separate definition for trauma MH. Germany only presented the definition of MH for the trauma setting.

The most common MT definition used was transfusion of 10 or more RBC units in 24 h. No country had a separate definition for *MAJOR*, *MASSIVE* and *SUPRA-MASSIVE transfusion*. Regarding the need for having one definition for *MAJOR*, *MASSIVE* and *SUPRA-MASSIVE* transfusion for different clinical settings, respondents stated that having a separate definition could be of benefit, especially in the analysis and comparison of data between studies (Australia), helping policymakers to manage blood component inventories (India) or evaluating compliance with the MHP (Saudi Arabia). However, all respondents indicated that in clinical practice having several definitions for large volume transfusions is of limited use, because they are retrospective and do not impact the decision-making process of the acute management of bleeding, and they could introduce complexities and cause confusion among clinical and laboratory teams.

Question 2b In your setting, have you adapted national or other formal guidelines for management of MH and/or MT in adults?

Do you have one main guideline that applies to all clinical settings, or are there different guidelines for different clinical settings (i.e., trauma, cardiothoracic surgery, obstetrics, gastrointestinal bleeding, surgical or medical bleeding, etc.)?

Seven respondents (Australia, Chile, Japan, Nigeria, Norway, Saudi Arabia and the United Kingdom) stated that they have adapted their own national guidelines for management of MH; however, for Norway and Saudi Arabia, this was only for trauma and obstetric settings, respectively. For non-obstetric bleedings, Saudi Arabia used the Australian guidelines, while Germany and Indonesia stated that they have adapted the European guidelines for major trauma haemorrhage [26] and for severe perioperative bleeding [27], respectively. Canada did not have a national guideline, but in 2011, they hosted an MT Consensus Conference to provide detailed guidance for clinicians on this topic [28]. All other countries adapted their own local guidelines.

Australia, Canada, Norway, United Kingdom and United States stated that their guidelines apply to all adult clinical settings, with minor modifications for different specialties, particularly for trauma, obstetric and cardiac surgery. Germany and India stated that they have specific guidelines for trauma, while Israel stated that their guideline is specific to trauma and gastrointestinal bleeding.

Question 2c Do you have clear policies or mechanisms in place for updating and/or renewing guidelines used in clinical practice for management of MH and/or MT in adults? If yes, please describe.

Guidelines are updated every 2 years (Saudi Arabia, United States), or every 3 years (Canada, Chile, Germany, United Kingdom), or sooner if evidence becomes available (Australia, Norway, United Kingdom, United States). Other countries had no policies in updating guidelines.

TABLE 1 Respondent demographics

Country	Type of hospital(s)	Geographic location (city, remote area, regional)	Inpatient bed numbers	RBC units transfused per year
Australia	Academic hospital	Serves the state of Victoria's largest metropolitan public health service	103 beds in private hospital 1850 inpatient beds	22,700
Canada	158 hospitals—of these 150 stock and issue blood components	Large cities, smaller cities and remote rural areas	23,000 inpatient beds	336,282
Chile	Tertiary and teaching hospital	City of Santiago	395 beds	5000 haemocomponents[a]
Germany	Community hospital that serves as a teaching hospital for two universities	Serving a metropolitan area in the Western part of Germany close to Dutch–Belgian border	850–950 beds	5500–7000 blood products[a]
India	Tertiary care academic hospitals	North India: serves smaller hospitals in the capital region, and receives patients from across the nation	2800 beds	75,000–78,000 units
Indonesia	Tertiary and teaching hospital	City of Yogyakarta	820 beds	26,000 units
Israel	University hospital	Urban and rural population	890 inpatient beds 110 outpatient beds	7500–8000 units
Japan	University hospitals—two hospitals (H)	H1—city of Kashihara H2—city of Nagoya	H1—992 inpatient beds H2—2200 outpatients on weekdays and 1085 inpatient beds	H1—12,000 units H2—16,611 units
Nigeria	University hospital	Receives patients from within and outside city of Ibadan	850 inpatient beds 150 outpatient beds	7000–12,000 units of blood[a]
Norway	University hospital	City of Bergen	790 beds	15,000 units
Saudi Arabia	Tertiary academic hospital	City of Jeddah	600 inpatient beds 30 outpatient beds	12,000
United Kingdom	Academic trust that included four hospitals	Three are located in the city of Oxford and one in a town (~40 miles from the city)	1100 beds	17,000 units
United States	Tertiary teaching hospital	City	767	33,000 units

Abbreviations: H, hospital; RBC, red blood cell.
[a]Reported as total blood products and not as red cell units.

Question 2d Has a national, multi-site or hospital-based audit of practice against the guidelines used in clinical practice for management of MH and/or MT in adults been undertaken?
If yes, please provide (1) the clinical setting, (2) the frequency of audit cycle and (3) the name of the organizing body that mandates or co-ordinates the audit.

Of the 13 respondents, 5 stated that they have undertaken audits of MH at a local level for different settings (Australia, Canada, Japan, Norway and the United Kingdom). Only United Kingdom had undertaken a nationwide audit in 2018 and this included all clinical settings in adults [13]. In 2018, a provincial survey of MHP of all hospitals was conducted in Canada across all clinical settings [29]: this showed marked variability, and failed to include the eight key quality metrics deemed necessary in the Ontario MHP, which was made public in 2021 [24]. Canada is planning to conduct an audit in 2022. Australia also stated that they participate in state-wide transfusion audits and other practice improvement activities, however, it was not specified if this included management of MH audits. Other countries answered as 'No' to this question.

Question 3 Blood transfusion management and tranexamic use for MH
Question 3 was also divided into five subquestions. Four subquestions focusing on blood transfusion management are reported below as 3a, 3b, 3c and 3d. Responses to subquestion 3e, focusing on tranexamic use for MH, are summarized separately.

Question 3a What is the RED BLOOD CELLS to PLASMA ratio that your guidelines recommend for management of MH?

Question 3b What is the RED BLOOD CELLS to PLATELETS ratio that your guidelines recommend for management of MH?

Question 3c What is the FIBRINOGEN trigger that your guidelines recommend for administering fibrinogen replacement therapy for management of MH?

TABLE 2 Definition of major haemorrhage and/or massive transfusion in adults

Country	Definitions of major haemorrhage (MH) and/or massive transfusion (MT)
Australia	• MT: - ≥4 RBC units in 4 h, or - Loss or transfusion of one BV over 24 h • More 'real time' definitions include - Replacement of half a BV within 4 h, or - Blood loss of >150 ml/min, or - Receipt of 10 RBC units
Canada	• ABC score (trauma only) • Shock index or resuscitation intensity[a]
Chile	• Haemorrhagic shock as: 'Type IV ATLS haemorrhage, loss ≥2000 cc'
Germany	• MH and/or MT is defined as: - ≥10 RBCs units with 24 h, which corresponds to one complete BV exchange within 24 h • Clinically MH is anticipated in the presence of bleeding with laboratory signs of shock reflected by: - Base excess and lactate, or - Failure to achieve hemodynamic stability despite volume loading in terms of non-responsiveness, or - Need for vasopressor support • In most dynamic scenarios, we use: - 50% volume exchange with 3 h, or - 4 RBC units within 1 h, or - Ongoing blood loss ≥150 ml/min
India	• MH and/or MT for trauma: - ≥4 of RBC units within 1 h • MH and/or MT for other: - ≥10 RBC units within 24 h
Indonesia	• MT: >10 units of RBC in 24 h
Israel	• MH: - Loss of >1 BV within 24 h, or - 50% of the patient's total BV lost in <3 h, or - Bleeding in excess of 150 ml/min • MH in trauma: - Systolic BP <90 mmHg and/or PR >110/min
Japan	• No uniform definition for MH and/or MT[b] • For activating MHP: - We use patient vital signs and hemodynamic instability assessed by shock index, the severity and complexity of bleeding
Nigeria	• MH: - Loss of ≥1 BV within 24 h, or - 50% of total BV lost in <3 h • For PPH, MH: - Blood loss of 1500 ml [21] • MT: - Replacement of the patient's BV with RBCs in 24 h, or - 10 RBC units within 24 h • For early identification of MT: - >5 RBC units within 4 h
Norway [22]	• MT: - ≥5 RBC units within 3 h, or - ≥10 RBC units within 24 h
Saudi Arabia	• MT: - 4 RBC units within 1 h and anticipated need for more, or - Replacement of 50% BV in 4 h, or - Rate of blood loss ≥150 ml/min • For activating MHP in trauma: - ABC score of >2 points[c]
United Kingdom	• MH [23]: - Bleeding, which leads to an HR >110 beats/min and/or systolic BP <90 mmHg - Hospitals must have locally agreed triggers

(Continues)

TABLE 2 (Continued)

Country	Definitions of major haemorrhage (MH) and/or massive transfusion (MT)
United States	• A situation in which a large number of blood products are expected to be transfused in a short amount of time • This may include adult patients who have 8–10 units replaced in 6 h or when the transfused volume equals the patient's total blood volume

Abbreviations: ABC, assessment of blood consumption; ATLS, advanced trauma life support; BP, blood pressure; BV, blood volume; HR, heart rate; MHP, major haemorrhage protocol; PPH, postpartum hemorrhage; PR, pulse rate; RBC, red blood cell.
[a]>4 units of fluid in first 30 min with '1 unit' defined as any of 1 unit RBC, 1 unit plasma, 500 ml colloid or 1 L crystalloid [24].
[b]No uniform definitions because the pathogenesis and mechanisms of coagulopathy vary depending on the clinical settings.
[c]ABC scoring 1 point for each of the following: penetrating mechanism, positive focused assessment sonography for trauma, arrival systolic blood pressure of 90 mmHg or less and arrival heart rate equal to 120 bpm or more [25].

Question 3d Do these ratios and fibrinogen trigger apply to all clinical settings or are there different recommendations for different clinical settings (i.e., trauma, cardiothoracic surgery, obstetrics, gastrointestinal bleeding, surgical or medical bleeding, etc.)?

Results are summarized in Table 3. The most common RBC:FFP ratio used was 1:1 for trauma and 2:1 for non-trauma bleeding. The RBC:platelet ratio varied more, with six responders stating that they had no specific ratio (Australia, Canada, Germany, India, Nigeria and the United Kingdom) and relied on the platelet count at the time of ongoing bleeding (Australia, Canada, Germany and the United Kingdom), four used a 1:1 ratio (Chile, Indonesia, Norway and Saudi Arabia) and others had different ratios for different clinical settings (Japan) or used the point of care testing to determine the need for platelet transfusion (Israel).

As far as the fibrinogen replacement therapy is concerned (Table 3), this was initiated if fibrinogen level was <2.0 g/L for postpartum haemorrhage or <1.5 g/L for other bleeding (Australia, Canada, Japan, Saudi Arabia and the United Kingdom), two respondents stated that they had no fibrinogen trigger (Nigeria and Indonesia), two had a trigger for trauma, but not for other settings (India and Norway) and two used one fibrinogen trigger for all settings (Chile and United States).

Question 3e Do your guidelines recommend the use of tranexamic acid for management of MH? Do these recommendations apply to all clinical settings or are there different recommendations for different clinical settings (i.e., trauma, cardiothoracic surgery, obstetrics, gastrointestinal bleeding, surgical or medical bleeding, etc.)?

Respondents from Chile, Indonesia, Nigeria and the United States stated that tranexamic acid is not in their guidelines–policies, Germany and Norway only recommended it for trauma, while other countries recommended it for all settings (Australia, Canada, India, Israel, Japan, Saudi Arabia and the United Kingdom). The respondent from Japan stated that the recommendation for administering tranexamic acid to trauma and obstetric patients who exhibit massive bleeding or are at risk of significant haemorrhage, is weak, while United Kingdom and Canada contraindicated the use of tranexamic acid in gastrointestinal bleeding following the recent publication of the HALT-IT trial [12].

Question 4 Resource utilization

Question 4a In the setting of MH, do you use group O RhD positive RBCs or Group O RhD negative RBCs for all adult patients who have unknown blood group or does your product selection differ based on patient age and/or gender?

The answers to this question are divided into five categories: (1) group O RhD negative is transfused to females of childbearing potential only (Canada, India, Israel, United Kingdom and United States); (2) group O RhD negative is provided to all patients regardless of age and sex (Australia, Chile and Saudi Arabia); (3) group O RhD negative is transfused to females of childbearing potential and children (Germany, Nigeria); (4) group O RhD positive is transfused to all patients as the prevalence of group O RhD negative in the population is very low (Japan and Indonesia) and (5) group O RhD positive whole blood or RBC is transfused to males and females older than 50 years (Norway).

Question 4b In the setting of MH, do you have a policy in your institution for when to switch RhD negative women of childbearing potential or children to RhD positive RBCs when RhD negative inventory is limited and the patient is requiring significant transfusion support such that the supply for the rest of the hospital becomes threatened?

Apart from Chile, and excluding responses from Japan and Indonesia where group O RhD positive RBC are used for all patients, others stated that they do not have a policy in place to cover this eventuality; however, all respondents stated that they will switch to group O RhD positive or group-specific RBCs, if supply for group O RhD negative is lower than demand.

Question 4c In the setting of MH, do you have a 'shortage' policy in your institution for when to discontinue RBC transfusion support when inventory is limited and the patient is requiring significant transfusion support such that the supply for the rest of the hospital might become threatened or limited? For example, in the early stages of the COVID pandemic when there were concerns about donations and blood supply, did your hospital develop such a policy in the event of a severe blood shortage?

TABLE 3 Blood component ratios

Country	RBC:FFP ratio	RBC:PLT ratio	Fibrinogen trigger
Australia	2:1 for all[a]	No ratio[b] Maintain count >50 × 10^9/L, maintain count >100 × 10^9/L for head injury	<2.0 g/L for PPH <1.5 g/L for others
Canada	2:1 for all	No ratio Maintain count >50 × 10^9/L, maintain count >100 × 10^9/L for intracranial or spinal injury	<2.0 g/L for PPH <1.5 g/L for others
Chile	1:1 for all	1:1 for all[c]	≤80 mg/dl for all
Germany	1:1 for trauma 2:1 for others	No ratio 4:1 for trauma Maintain count >50 × 10^9/L or guided by ROTEM	<1.5 g/L, or if FIBTEM is abnormal
India	1:1 for trauma No recommendation for others	1:1 for trauma No pre-defined ratio for others	<1.5 g/L for trauma No trigger for others
Indonesia	1:1 for all[d]	1:1 for all[d]	No trigger
Israel	*1:1 for all*	Guided by TEG[e]	Guided by TEG[e]
Japan	1:1, maintaining the ratio <2:1 for trauma <1:1 for cardiac, obstetric No recommendation for others	1:1, maintaining the ratio <2:1 for trauma 1:1 for cardiac Others no recommendation	150–200 mg/dl for PPH <150 mg/dl for trauma <150 mg/dl for cardiac
Nigeria[f]	5:1 for PPH No recommendation for others	No recommendation	No recommendation
Norway	1:1 if whole blood is not available	1:1 if whole blood is not available	<2 g/L for trauma No trigger for others
Saudi Arabia	2:1 for all	1:1 for all	<2.0 g/L for PPH <1.5 g/L for others
United Kingdom	1:1 for trauma 2:1 for other setting[a]	No ratio Maintain platelet count >50 × 10^9/L: to achieve platelet are requested if count <100 × 10^9/L	<2.0 g/L for PPH <1.5 g/L for others
United States	1:1 for all	6 to 1 apheresis	<100 mg/dl for all

Abbreviations: aPTT, activated partial thromboplastin time; FFP, fresh frozen plasma; FIBTEM, fibrin-based extrinsically activated test with tissue factor and the platelet inhibitor cytochalasin D; INR, international normalized ratio; PLT, platelet; PPH, postpartum hemorrhage; RBC, red blood cell; ROTEM, rotational thromboelastometry; TEG, thromboelastography.

[a]Adjust further FFP to maintain INR and aPTT to <1.5× normal.

[b]First round of blood products includes a 4:1 ratio and second round does not include platelets.

[c]1 unit of RBC per 1 apheresis platelet concentrate or platelet pool with the number of units corresponding to 1 unit per 10 kilos.

[d]This setting is not yet being a hospital policy but is particularly applied to cardiothoracic surgery when an order of 5 red blood cells and 5 platelets is usually made.

[e]A pool of 5 platelet units and fibrinogen replacement is given as part of the second pack of components.

[f]Use whole blood.

[Correction added on 28 January 2022 after first online publication: The "RBC:FFP" and "RBC:PLT" ratios for Japan were corrected in this version.]

Of the 13 respondents, 5 had a 'shortage' policy in place (Australia, Canada, Norway, Saudi Arabia, United Kingdom), while others did not (Chile, India, Germany, Israel, Nigeria). The respondent from the United States stated that they have a policy to review cases and consult with the clinical provider when RBC transfusion exceeds 100 units per patient, which could jeopardize the overall inventory. Most responders stated that if there is a blood shortage, they will postpone elective surgeries and review all emergency transfusion requests by a transfusion specialist. In the early stage of the COVID-19 pandemic, Norway implemented cold-stored apheresis platelet concentrates to mitigate the risk of insufficient platelet inventory for bleeding patients.

CONCLUSION

Treatment of MH that results in large volumes of allogeneic blood transfusion is associated with significant morbidity and mortality and costs burden for health care services [4]. This international survey showed a huge variation in the definition of MH and/or MT between countries, with most countries using the same definitions for all clinical settings. There was no country that categorized large volume transfusions into MAJOR, MASSIVE or SUPRA-MASSIVE transfusion. Respondents indicated that different large volume definitions may be important to compare data between studies–services and help policymakers to manage blood stocks, but they all specified that these definitions are not useful in clinical practice, as they do not impact on the decision-making process of the acute management of bleeding patients, and they could potentially introduce complexities and confusion for clinical and laboratory staff.

Only seven countries had a national guideline–policy in place on how to manage major bleeding with other countries using other nation's guidelines or developing their own policy. For trauma and obstetrics, the guidance on the RBC:FFP ratio and fibrinogen trigger, respectively, were clearly defined in most protocols, while for other major bleeding settings the 2:1 ratio of RBC:FFP and fibrinogen of <1.5 g/L were the most common

approaches for non-trauma bleeding for most countries. These results are not at all surprising, considering the lack of evidence in this field.

The evidence on the use of tranexamic acid for treatment of major bleeding relating to trauma, surgery, obstetrics and gastrointestinal causes, is stronger [9, 10, 12, 30] than blood transfusion ratios or fibrinogen triggers. Despite this, our results showed a diverse response on the use of tranexamic acid even for settings where there is some evidence. We believe that integrating tranexamic acid into the MHP will ensure that it is administered to all patients who need it, but this may also lead to some patients receiving it when it may be contraindicated, such as gastrointestinal bleeding.

The answer around resource utilization for the use of universal RBC group and the availability of '*blood shortage plan*' for red cells for management of bleeding also varied significantly between countries. For countries that have a low prevalence of group O RhD negative donors resource utilization of RhD negative RBC units is not an issue, however, for others this is a problem, as the demand for this resource rises to treat trauma patients who are bleeding outside the hospital setting [31–34]. Therefore, an individual risk assessment from each country is required to better understand the wider implications (for both patients and healthcare providers) of continuing to support all unknown patients with RhD negative red cell containing components and more research is needed to look at innovative technologies to develop universal blood components [35], so that we can improve overall blood component inventory.

In conclusion, among the respondents to this forum, there was wide variation on the definition of MH and MT as well as transfusion management of acquired MH. This suggests a need for the international blood transfusion community to provide further guidance in this field, so that we can harmonize treatment of bleeding disorders and research, with improved outcomes for patients and healthcare systems.

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INTERNATIONAL FORUM

Vox Sanguinis ISBT International Society of Blood Transfusion

International Forum on the Management of Major Haemorrhage: Responses

Laura Green | Simon Stanworth | Zoe McQuilten | Victor Lin | Harriet Tucker | Bryon Jackson | Maha Badawi | Salwa Hindawi | Rahul Chaurasia | Gopal Patidar | Hem Chandra Pandey | Foluke Fasola | Shigeki Miyata | Masanori Matsumoto | Tadashi Matsushita | Naomi Rahimi-Levene | Victoria Peer | Katerina Pavenski | Jeannie Callum | Troy Thompson | Michael Murphy | Julie Staves | Marc Maegele | Chathuri Abeyakoon | Kylie Rushford | Erica Wood | Maria Antonieta Nuñez | Sandra Mellado | Edgardo Saa | Teguh Triyono | Bhirowo Pratomo | Torunn Oveland Apelseth | Nancy Dunbar

UNITED STATES

Bryon Jackson

Question 1

Our hospital is a tertiary/teaching hospital located in a city with 767 beds that transfuses approximately 33,000 units of red blood cells (RBCs) each year.

Question 2

a. The transfusion service policy defines a massive transfusion event (MTE) as 'a situation in which a large number of blood products are expected to be transfused in a short amount of time'. This may include adult patients who have 8–10 units replaced in 6 h or when the transfused volume equals the patient's total blood volume. This policy definition is designed to help providers identify situations in which it may be appropriate to active an MTE. This policy applies to all clinical settings in our hospital but does not exclude clinical areas from having their own definition. There are no separate definitions for MAJOR TRANSFUSION, MASSIVE TRANSFUSION and SUPRA-MASSIVE TRANSFUSION in adults. There may be some benefit to distinguishing extremely high-volume transfusions if that resulted in differentiation of treatment (i.e., use of whole blood [WB], tranexamic acid [TXA] and prothrombin complex concentrates [PCCs]).
b. We have adopted a policy designed to implement early inclusion of plasma in a 1:1 ratio with RBCs. The policy applies across all settings.
c. The MTE guidelines exist in a formal policy that is reviewed and updated at least every 2 years but is often updated more frequently to reflect current best practices. Policy changes are proposed and reviewed by transfusion medicine physicians and a multidisciplinary transfusion practice committee.
d. No.

Question 3

a. Our guidelines recommend a 1:1 ratio of red cells to plasma across settings.
b. Our guidelines recommend a ratio of 1 apheresis platelet for every 6 red cells.
c. Guidelines recommend fibrinogen replacement below 100 mg/dl across all settings.
d. TXA is suggested as an adjunct treatment for massively transfused patients but is not part of the formal policy.

Question 4

a. Selection is based on age and gender. Females under the age of 50 are given Rh-negative red cells. All other patients are given RhD-positive RBCs.

b. Yes. RhD-negative women of childbearing potential or children are switched to Rh positive at the discretion of the transfusion physician based on evaluation of the available inventory.
c. There is a policy to review cases and consult with the clinical provider when RBC transfusion exceeds 100 units or jeopardizes the overall inventory. In the early stages of the COVID pandemic, the policy was implemented to preserve RBC inventory. Specifically, these policies increased prospective review of non-emergent transfusion requests and delayed elective procedures. There were no additional restrictions placed on emergent transfusion beyond those previously existing to prevent inventory shortages.

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SAUDI ARABIA

Maha Badawi & Salwa Hindawi

Question 1

Our hospital is a tertiary care academic hospital located in the city of Jeddah, Saudi Arabia. The hospital currently operates at a 600-bed capacity for inpatients and a 30-bed capacity for outpatients. The blood bank includes a collection centre and a transfusion laboratory and does not service other smaller hospitals.

The hospital is a referral centre for complex obstetric and cardiothoracic surgeries. It also serves patients with haemoglobinopathies. It is not a designated trauma centre, but receives trauma patients occasionally. The hospital does not include a transplant programme (for solid organs or stem cells).

Around 12,000 units of RBCs are transfused in this hospital per year.

Question 2

a. In our institution, massive transfusion is defined as the transfusion of patients with rapid blood loss, including:
   - Transfusion of 4 units of RBCs within 1 h and anticipated need for more, or
   - Replacement of 50% blood volume in 4 h, or
   - Rate of blood loss ≥150 ml/min, or

   In trauma patients, the assessment of blood consumption (ABC) score of more than 2 points may be used as an indication for activation of the massive transfusion protocol (MTP; 1 point for each of the following: penetrating mechanism, positive focused assessment sonography for trauma, arrival systolic blood pressure of 90 mmHg or less and arrival heart rate equal to 120 bpm or more) [1].

   We list multiple definitions to cover different categories of patients including paediatrics. We do not have separate definitions for major/massive/supra-massive transfusions.

   We think that simplifying and unifying the definitions may allow for better compliance with the protocol [2].
b. National guidelines are available for obstetric haemorrhage and are being developed for other clinical settings. In our institution, the MTP is adapted from the available national guidelines in addition to international published guidelines such as the Patient Blood Management Guidelines: Module 1 (Critical Bleeding/Massive Transfusion), published by the Australian National Blood Authority (NBA) [3].

   Our institutional guidelines related to massive haemorrhage are included in a single document covering all clinical settings except for obstetric haemorrhage which has different guidelines.
c. Yes. The institutional policies mandate revision of all transfusion policies every 2 years and the MTP is incorporated within hospital transfusion policies.
d. No national consensus is available regarding massive transfusion practice audits. On an institutional level, massive transfusion audits are performed by the hospital transfusion service every year. A different clinical setting is audited each year.

Question 3

a. The recommended ratio of RBCs to Plasma in the institutional guidelines is 2:1 for all clinical settings.
b. The recommended ratio of RBCs to Platelets is 1:1 for all clinical settings, where 1 platelet unit is a single whole blood-derived unit. This indicates that the patient receives an adult dose of platelets (6 whole-blood derived units or an apheresis unit) for 6 units of RBCs.
c. The recommended fibrinogen trigger for fibrinogen replacement is 1.5 g/L in all clinical settings except in obstetric haemorrhage where the trigger is 2 g/L.
d. The institutional guidelines follow national guidelines recommending the use of TXA in obstetric haemorrhage based on strong evidence [4]. TXA is included as a recommendation in other clinical settings based on available evidence [5].

Question 4

a. In the setting of major haemorrhage, institutional policies allow release of O RhD-negative RBCs only to all patients with unknown blood group, regardless of their age or gender. In cases of shortage of O RhD-negative RBC units, O RhD-positive red cells may be released to adult males and females beyond child-bearing age with authorization from the transfusion medicine physician.
b. In cases of major haemorrhage affecting RhD-negative women of childbearing potential, when transfusion laboratory staff are concerned about significant shortage, transfusion laboratory staff is informed. Additional RhD-negative units will be requested from all

neighbouring institutions. If the inventory cannot be improved on urgent basis, the release of RhD-positive red cells will be authorized by the transfusion medicine physician. After cessation of massive haemorrhage, patients are monitored and managed for any potential risks.

c. In cases of severe blood shortage, institutional policies include a number of interventions aimed to hasten recovery of the inventory and allow the best possible allocation of available resources. All non-emergency blood requests are screened by a transfusion medicine physician, elective surgeries are postponed, and intensive efforts are made for communication with blood donors. In these circumstances, decisions related to patients in massive haemorrhage are taken through close communication between the transfusion laboratory, transfusion physician and the treating physicians in order to optimize patient outcomes.

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INDIA

Rahul Chaurasia, Gopal Patidar & Hem Chandra Pandey

Question 1

Our institution, All India Institute of Medical Sciences (AIIMS), New Delhi, is located in the country's national capital region. It is one of North India's major tertiary care academic hospitals. It serves as a referral centre for smaller hospitals in the capital region, as well as receives patients from all across the nation. Our hospital offers about 2800 in-patient hospital beds and serves patients of all ages, including newborns, paediatrics, adults and geriatrics, in all medical and surgical specialties. In addition to the main hospital, there are distinct speciality centres for Advance Trauma, Cardio-thoracic and Neuroscience, Oncology, Ophthalmology and Dental Sciences. AIIMS handles around 4.4 million patients in the outpatient department every year. Our institute has three blood centres under the purview of the Department of Transfusion Medicine, which jointly collect about 80,000 whole blood units each year and completely processes them into blood components such as RBCs, platelet concentrates (PCs) and plasma. We receive around 150,000 blood component transfusion requests from various departments each year, with approximately 75,000–78,000 RBC units issued for transfusion.

Question 2

a. Major haemorrhage and/or massive transfusion for trauma patients are defined at our institution as transfusion of 4 or more units of RBCs within 1 h. For other specialties, major haemorrhage is defined as the transfusion of 10 or more RBC units within 24 h.
We do not have distinct definitions for MAJOR TRANSFUSION, MASSIVE TRANSFUSION and SUPRA-MASSIVE TRANSFUSION at our institution.
Major haemorrhage and/or massive transfusion are defined separately for trauma patients, although the rest of the specialties, such as cardiothoracic surgery, obstetrics, gastrointestinal bleeding, surgical or medical bleeding and so on do not have such standard definitions. Categorizing large-volume haemorrhage into major transfusion, massive transfusion and supra-massive transfusion can help policymakers to manage blood component inventories. However, we believe that categorizing large volume transfusions in clinical setting may be of limited use since it may not impact decision-making in the acute management of bleeding patients.

b. Since there is no standard national guideline for managing major haemorrhage and/or massive transfusion, we developed our own institutional guideline in collaboration with all stakeholders, including clinicians and nursing coordinators from trauma emergency, anaesthesia, surgical department, laboratory and transfusion medicine specialists. Our institutional policy is primarily designed for trauma patients. There are no similar guidelines for other clinical settings, and transfusion management is managed on a case-by-case basis in them.

c. Currently, we do not have such explicit policies or processes in place for updating or renewing clinical practice for management of major haemorrhage and/or massive transfusion in adults; nevertheless, any changes in clinical practice are made based on frequent clinical meetings.

d. Our institutional guideline currently does not include a mandatory provision requiring regular audit of clinical practice for the management of major haemorrhage and/or massive transfusion in adults.

Question 3

a. According to our institutional guidelines, transfusion of RED BLOOD CELLS and PLASMA in a 1:1 ratio is recommended for the

treatment of major haemorrhage in trauma settings. Other than the trauma setting, there is no pre-defined guideline for transfusion of RED BLOOD CELLS to PLASMA in the management of major haemorrhage in other clinical settings.

b. According to our institutional guidelines, transfusion of RED BLOOD CELLS and PLATELETS in a 1:1 ratio is recommended for the treatment of major haemorrhage in trauma settings. Other than the trauma setting, there is no pre-defined guideline for transfusion of RED BLOOD CELLS to PLATELETS in the management of major haemorrhage in other clinical settings.
c. According to our institutional guideline, fibrinogen replacement therapy for management of major haemorrhage in trauma situations is recommended when fibrinogen levels are <1.5 g/L. Aside from the trauma setting, there is no pre-defined guideline for fibrinogen replacement in other clinical settings for major haemorrhage management.
d. As per our institutional guideline, TXA is widely recommended in the trauma settings in accordance with recommendations from the CRASH-2 trial [1]. TXA is also widely utilized in various clinical settings such as cardiothoracic surgery, obstetrics, gastrointestinal bleeding, surgical or medicinal bleeding and so on although it is administered on a case-by-case basis with no pre-defined protocols for major haemorrhage management.

Question 4

a. In the setting of major haemorrhage, the institutional policy is to use group O RhD-negative RBCs for all adult patients who have unknown blood group. However, due to the limited inventory usually O RhD-negative red cells are preferred for female patients in reproductive age group (i.e., under 45 years of age) and O RhD-positive red cells are used for all other patients.
b. We currently do not have a standard protocol in place at our institution for switching the blood group from RhD negative to RhD positive in the setting of a major haemorrhage. It is determined on a case-by-case basis or at the inventory level.
c. We do not have a shortage policy at our institution owing to the following reasons:

i. Trauma patients are catered for by a designated specialist centre (Advance Trauma centre) with a dedicated blood collection centre that collects 10%–15% of the total blood collected at our institute. In the event of a blood shortage, blood units from the institute's other two blood centres are requested to help tide over such emergencies.
ii. Other two blood centres handling patients' rest of the specialities (other than trauma) also maintain a buffer stock of red cells on standby to handle disasters.
iii. In cases where blood donations are limited, such as the early stages of a COVID pandemic, the buffer supply is generally adequate to manage the acute shortage. However, hospital policy allows for deferment of routine surgeries and new admissions so that blood inventory could be utilized for emergent situations.

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NIGERIA

Foluke Fasola

Question 1

University College Hospital is located in the city of Ibadan one of the largest cities in Nigeria with a total population of 3,649,000 as of 2021. The hospital is a Federal teaching hospital attached to the University of Ibadan. The hospital receives patients from within and outside Ibadan. Being a tertiary hospital, patients are referred from the primary and secondary health centres to the hospital. The hospital has 850 inpatient and 150 outpatient beds. A range of 7000–12,000 units of blood is transfused per year depending on activities of the hospital which has been interrupted by frequent industrial strike actions and COVID-19 in the recent time.

Question 2

a. Major haemorrhage is defined by the loss of more than one blood volume within 24 h or 50% of total blood volume lost in less than 3 h while massive transfusion is the replacement of the patient's blood volume with packed RBCs in 24 h or transfusion of 10 units of packed red blood cells (PRBCs) within a 24-h period. For early identification of massive transfusion, transfusion of greater than 5 units within 4 h is a useful definition. In obstetrics haemorrhage particularly in post-partum haemorrhage, loss of 1500 ml of blood should be considered as major haemorrhage [1]. Irrespective of how much blood units are transfused, there is no different

definition for major transfusion, massive transfusion and supra-massive transfusion; however, I think major transfusion should have a different definition. Major transfusion should be defined to include transfusion of significant units of blood that does not cause dilution of the coagulation factors to the extent that a need for fresh frozen plasma (FFP), cryoprecipitate and PC is required. Oftentimes, transfusion of 4–6 units of blood within 24 h is considered a major transfusion. Since the clinical outcome of transfusion within the context of massive transfusion is poor and clinical outcome super-transfusion is likely to be similar, there might not be any need to give different definitions for the two terms.

b. Even though protocol-based management of these patients using major haemorrhage and/or MTP have shown improved outcomes, currently the national guideline in place was produced in 1991 [2]. The Obstetrics and Gynaecology Department of the hospital have a formal guideline in place for management of post-partum haemorrhage which could be adapted to manage major haemorrhage in obstetrics [3]. General guideline for managing major haemorrhage and/or massive transfusion is applied to all clinical settings irrespective of whether it is trauma, cardiothoracic surgery, obstetrics, gastrointestinal bleeding, surgical or medical bleeding.
c. There are no clear policies or mechanisms in place for updating and/or renewing guidelines.
d. Presently, there is no national, multi-site or hospital-based audit of practice against the guidelines on management of major haemorrhage and/or massive transfusion.

Question 3

a. Use of whole blood is recommended in the National guideline, therefore it does not specify the RBCs to plasma ratio for management of major haemorrhage. The protocol used by the Obstetrics and Gynaecology Department of the hospital for post-partum haemorrhage recommends 1 unit of FFP for every 5 units of red cell concentrate. Other clinical settings do not have formal guidelines. In our hospital, cardiothoracic surgery routinely gives one unit of FFP for every 3 units of red cell concentrate use. The haematologist is consulted to co-manage in most cases where there is no guideline.
b. RBCs to platelets ratio is not stated in the guideline since whole blood administration is advised. This applies to all clinical settings. The recommendation for whole blood might be because while FFP is may be available stored in blood bank, PC is strictly prepared on demand.
c. There is no recommended fibrinogen trigger for administering fibrinogen replacement therapy for management of major haemorrhage. This might be because whole blood is recommended and coagulation profile during emergency management of major haemorrhage is checked by whole blood clotting time, prothrombin time and activated partial thromboplastin time. In the event of a deranged coagulation profile despite the use of FFP, the haematologist is consulted and cryoprecipitate is recommended.
d. The guideline does not recommend or discourage the use of tranaxemic acid.

Question 4

a. In major haemorrhage, group O RhD-positive RBCs are administered to all male patients and females above childbearing age with unknown blood group while group O RhD-negative RBCs are administered to female patients within childbearing age and children.
b. There is no policy in my institution for when to switch RhD-negative women of childbearing potential or children to RhD-positive RBCs.
c. There is no 'shortage' policy in my institution.

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JAPAN

Shigeki Miyata, Masanori Matsumoto & Tadashi Matsushita

Question 1

Nara Medical University Hospital (NMUH) is located in the city of Kashihara, which has a population of 130,000 people. Our hospital is a teaching institute and serves as a tertiary hospital for a target population of 1 million people. NMUH has 992 inpatient beds. Approximately 12,000 units of RBCs were transfused in 2020.

Nagoya University Hospital (NUH) is located in the centre of the city of Nagoya, which is the third largest city in Japan, with a population of approximately 2.5 million. We are responsible for tertiary emergency services and one of four teaching hospitals near Nagoya. Currently, our regional collaborative medical network is limited to non-emergency cases, but we have an active ambulance service. We have 2200 outpatients on weekdays and 1085 inpatient beds. We transfused 16,611 units in 2020.

Question 2

a. Fortunately, the number of acts of terrorism, injuries resulting from gunshot accidents and criminal homicides is low in Japan. In addition, we do not see patients from battlefields with injuries including massive bleeding. Therefore, the major causes of massive

haemorrhage in our patients result from cardiovascular surgery, trauma caused by traffic accidents or falls, post-partum haemorrhage and gastrointestinal bleeding.

As described in detail below for Question 2b, the first official 'Japanese transfusion guidelines for patients with massive bleeding' was established in 2019 [1] by our expert physicians who belong to the Japanese Societies of Transfusion Medicine and Cell Therapy, the Japanese Society for Cardiovascular Surgery, the Japanese Association for Thoracic Surgery, the Japan Society of Obstetrics and Gynecology, the Japan Association of Obstetricians and Gynecologists, the Japanese Association for the Surgery of Trauma, the Japanese Association for Acute Medicine, the Japanese Society of Anesthesiologists or three other related medical societies. During the meetings in which we established the Guidelines, we discussed how to define 'massive transfusion' and 'target patient population', although 'massive transfusion' is commonly defined worldwide as transfusion therapy that includes administration of 10 or more units of red cell concentrates within 24 h of the event onset. As a result, we concluded that it is very difficult to determine a uniform definition regarding major haemorrhage and/or massive transfusion because the pathogenesis and mechanisms of coagulopathy and impaired haemostasis vary depending on the clinical settings (i.e., trauma, cardiothoracic surgery, obstetrics, gastrointestinal bleeding etc.). Rather, we concluded that patient vital signs and haemodynamic instability of patients with bleeding, which can be assessed by indexes such as shock index, the severity and complexity of trauma, pregnancy status and surgery, should be considered when determining whether to activate an MTP or transfusion therapy with concentrated blood products such as PCs, fibrinogen concentrates (FCs) and cryoprecipitates.

Therefore, we do NOT separate the definitions for MAJOR TRANSFUSION, MASSIVE TRANSFUSION and SUPRA-MASSIVE TRANSFUSION for adults in these guidelines.

b. Massive bleeding occasionally involves severe coagulopathy (acute dilutional and consumptive coagulopathies and hyperfibrinolysis) that is induced by tissue injuries and early administration of a large volume of crystalloids, colloids and RBCs. Therefore, earlier and more appropriate transfusion of blood products such as FFP, PC and FC can lead to early improvements for patients with coagulopathy with a high potential to reduce the observed mortality rate and overall use of blood products.

When we established the first official 'Japanese transfusion guidelines for patients with massive bleeding', we systematically reviewed the scientific literature for evidence related to the following clinical questions (CQs):

In patients with massive bleeding,

1. How does FC affect patient outcomes? If it is effective, at what fibrinogen level should FC be transfused?
2. How does the MTP, involving earlier and more balanced transfusion of blood products, affect patient outcomes? If it is effective, how do the dose, timing and ratio of RBC to FFP or PC influence patient outcomes?
3. How does the administration of recombinant activated factor VII or prothrombin complex concentrate affect patient outcomes?
4. How does anti-fibrinolytic therapy affect patient outcomes?

We identified 5322 articles related to these CQs. After assessing the quality of each article, 81, 228, 115 and 72 articles were finally selected for the following patient categories: cardiovascular surgery, trauma, obstetrics and other settings, respectively. Therefore, we have different guidelines for different clinical settings (i.e., cardiovascular surgery, trauma, obstetrics and other settings). The selected articles were then systematically reviewed to formulate a complete and exhaustive summary of the literature, constituting the current body of evidence, to provide grading recommendations. The Grading of Recommendations Assessment, Development, and Evaluation approach was used to assess evidence and make recommendations with some modifications [2]. To adequately implement the recommendations from these transfusion guidelines in actual clinical practice in Japan, we asked 11 related medical societies (listed in Question 2a) to assess the appropriateness of the guidelines before finalizing the recommendations. Finally, we published these guidelines [2] and posted them on the website of the Japanese Society of Transfusion Medicine and Cell Therapy [3]. Some parts of these guidelines were added to the National Transfusion Guidelines provided by Japanese Government (the Ministry of Health, Labour and Welfare) [4].

c. We have no defined policy for updating or renewing guidelines in Japan. We are planning to revise it as needed.

d. To date, we do not have a national or multi-site audit of practice against the guidelines used in Japan. A small number of hospitals performed a hospital-based audit of practice against the guidelines.

Currently, our research group, supported by the Ministry of Health, Labour and Welfare of Japan, is investigating how the guidelines that were developed are used in real transfusion practices in Japan. Based on the data from 4953 hospitals in Japan (response rate 52%), only in about 20% of the facilities, the transfusion services routinely investigated whether or not physicians complied with the guidelines for each blood product. The compliance rate for adhering to the guidelines was generally high in large hospitals, but the rate was lower in smaller hospitals. Approximately 20% of the institutions routinely inquired about inappropriate blood transfusion orders that were requested by physicians.

Question 3

a. As described in detail in Question 2b, we published the first official 'Japanese transfusion guidelines for patients with massive bleeding' in 2019 [2, 3]. Several related medical societies and associations, including the Japanese Society of Transfusion Medicine and Cell Therapy, recommend that these guidelines are used to manage treatment protocols for patients with major haemorrhage and/or massive transfusion in all hospitals, and some parts of the guidelines were added to the National Transfusion Guidelines provided by Japanese Government (the Ministry of Health, Labour and

Welfare) [4]. Hence, we describe the recommendations from the guidelines to answer Questions 3a through 3d as follows.

Trauma

We strongly recommend that an MTP be implemented for trauma patients who exhibit massive bleeding or are at risk of significant haemorrhage (1C). As an initial transfusion therapy for these patients, we strongly recommend early administration of FFP and PC in addition to RBCs, maintaining a ratio of FFP:PC:RBC as close as possible to 1:1:1 and minimally at >1:1:2 (1C).

Cardiovascular surgery

We weakly recommend that an MTP be implemented for cardiovascular surgery patients who exhibit massive bleeding or are at risk of significant haemorrhage (2C). When an MTP is used for this patient population, we strongly recommend that FFP and PC be administered, maintaining a ratio of FFP:PC:RBC as close as possible to 1:1:1 and a ratio of FFP:RBC of >1:1 (1C).

Obstetrics

The MTP is effective for massive post-partum haemorrhage. We suggest that FFP be administered, maintaining a ratio of FFP:RBC of >1:1 (2C).

Other settings

We recommend an MTP for the patients who exhibit massive bleeding or are at risk of significant haemorrhage in other clinical settings (2C). However, we cannot determine the adequate FFP:PC:RBC ratio because we lack sufficient evidence for this recommendation.

b. The recommended RED BLOOD CELLS to PLATELETS ratio is given above in the response to Question 3a.

c. *Cardiovascular surgery*

Acquired hypofibrinogenemia (<150 mg/dl) caused by massive bleeding leads to increased bleeding volume and increased usage of allogeneic blood components (B). We weakly recommend that fibrinogen replacement therapy, using FC or cryoprecipitate, be implemented for the complex cardiovascular surgery patients who exhibit massive bleeding or are at risk of significant haemorrhage (2C). Initial administration doses of 50 mg/kg for FC and 3–4 ml/kg for cryoprecipitate are recommended (2C). We strongly recommend monitoring fibrinogen concentration in plasma or whole blood and specifically recommend using point-of-care testing such as a viscoelastic device (1B).

Obstetrics

We propose administering FC or cryoprecipitate to patients with massive post-partum haemorrhage (2C). We propose using a plasma fibrinogen concentration of 150–200 mg/dl as the fibrinogen trigger (2C).

Trauma

We weakly recommend administering cryoprecipitate or FC to trauma patients, with a plasma fibrinogen concentration <150 mg/dl, who exhibit massive bleeding or are at risk of significant haemorrhage (2C). Because FC is not yet approved by the Japanese government for acquired hypofibrinogenemia in bleeding trauma patients and cryoprecipitate should be produced in-house in individual hospitals, careful discussion is required in each hospital regarding the off-label use of FC for fibrinogen replacement therapy.

Other settings

We recommend fibrinogen replacement therapy using FC or cryoprecipitate in other clinical settings (2C). However, we cannot determine the appropriate fibrinogen tripper or the clear efficacy of fibrinogen replacement therapy because we lack sufficient evidence for this recommendation.

d. *Trauma*

We weakly recommend administering TXA to trauma patients who exhibit massive bleeding or are at risk of significant haemorrhage as soon as possible (ideally within 3 h after injury), independent of trauma severity (2B).

Cardiovascular surgery

We weakly recommend early administration of TXA to the cardiovascular surgery patients who are at risk of significant haemorrhage to reduce allogeneic blood component transfusion (2C). However, it should be noted that administering high doses of TXA increases the incidence of seizures but not mortality.

Obstetrics

We propose that TXA be administered to patients with massive post-partum haemorrhage as soon as possible and within 3 h after delivery (2B).

Other settings

We recommend administering TXA to patients who exhibit massive bleeding, are at risk of significant haemorrhage, and/or exhibit signs of hyperfibrinolysis in orthopaedic surgery, gynecologic surgery or abdominal surgery in order to reduce bleeding volume and allogeneic blood component transfusion (2B).

Question 4

a. For major haemorrhage, we use group O RhD-positive RBCs for all patients who have an unknown blood group because only 0.5% of the Japanese population is RhD negative. This selection does not differ based upon patient age or gender. The probability of RhD incompatibility is quite low.

b. For NMUH: If it is a life-threatening case, we would use RhD-positive RBCs. We would avoid transfusing RhD-positive RBCs to a young woman or child who is RhD negative.

For NUH: In such cases, RhD-positive red cells would be used, although we have not experienced such a case in the last decade. Of course, we avoid transfusing RhD-positive cells to RhD-negative young women.

c. The number of blood donations was maintained at pre-pandemic levels even during the early stages of the COVID-19 pandemic. Blood supply and demand dropped by approximately 10% in the early stages of the COVID-19 pandemic (April and May of 2020) in Japan because of the postponement of elective surgeries and non-emergency treatments. Afterwards, blood supply and demand were maintained at levels similar to those recorded pre-pandemic. Therefore, we have not encountered obvious shortages of blood components in Japan.

For NMUH: When there is a shortage of RBCs that matches the ABO blood group of the patient, we use blood group O RBCs.
For NUH: Never at all so far.

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ISRAEL

Naomi Rahimi-Levene & Victoria Peer

Question 1

Our hospital is a government and university hospital, with a trauma centre, which treats an urban and rural population of above 1.5 million people in Israel. We are not part of a network. There are 890 in-patient beds and 110 out-patient beds in our hospital. We transfuse 7500–8000 units of RBCs per year.

Question 2

a. The massive haemorrhage bleeding definition we use is: 'loss of more than one blood volume within 24 h, 50% of the patient's total blood volume lost in less than 3 h or bleeding in excess of 150 ml/min' [1]. An unstable trauma patient bleeding with systolic blood pressure below 90 mm^3 and/or pulse above 110/min is treated as massive bleeding until proven otherwise. We do not have separate definitions for major transfusion, massive transfusion and supra-massive transfusion in adults or for different clinical settings. We use one definition for all massive bleeding episodes in the hospital, occurring mainly in the emergency room, operating theatre and obstetric department.
b. There is a national requirement in Israel that every hospital has an MTP. We have an MTP applicable to the emergency room including mainly trauma and gastrointestinal bleeding.
c. We do not have a policy for updating our guidelines.
d. There is no national or local audit at this stage in Israel.

Question 3

a. The RBC:plasma ratio in our massive bleeding protocol is 1:1. This applies to massive bleeding in the emergency room setting. The first pack of components includes 4 RBC units and 4 plasma units.
b. We administer random donor pooled platelets. A pool of 5 platelet units is given as part of the second pack of components which includes 4 RBCs, 4 units of plasma and 10 units of cryoprecipitate. Further management is continued after clinical assessment and viscoelastic study, in our case thromboelastography (TEG).
c. Our second pack of components also includes 10 units of cryoprecipitate. Again, further management is according to clinical assessment and viscoelastic study, in our case TEG.
d. Yes, 1 g of TXA is administered to the unstable, massively bleeding patient within 1 h after admission to the emergency room. If necessary, the dose is repeated after 8 h.

Question 4

a. In principle, we use group O RhD-positive RBCs for adults with unknown blood group. There are 2 group O RhD-positive units in the emergency room which are administered as part of the first pack of components. If possible, for women under the age of 50 years, 2 units of O RhD negative are ordered from the blood bank.
b. There is no specific policy, however, when the expected need for RBCs exceeds our capability, we switch to RhD-positive units for the episode of bleeding and try to continue with RhD-negative RBCs when the patient is stabilized and needs less components.
c. We do not have a shortage policy and have not experienced a scenario when the supply for the rest of the hospital was threatened or limited. Our hospital is close enough to our supplier in order to receive an urgent transport of components. During the COVID epidemic, we have so far not experienced a blood shortage; therefore, we have not developed such a policy.

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CANADA

Katerina Pavenski, Jeannie Callum & Troy Thompson

Question 1

We are responding on behalf of the province of Ontario. Ontario includes large cities, such as the greater Toronto area with a population of 6 million, as well as smaller cities and remote rural areas; the total population of Ontario is over 13.5 million and its territory is 1,076,395 km^2. Ontario has 158 hospitals, of which 150 stock and issue blood components. There are estimated 23,000 inpatient acute care beds and 6.4 million emergency visits per year. Between 1 April 2020 and 31 March 2021, Ontario hospitals transfused 336,282 RBC units, 52,735 platelet doses and 43,053 units of plasma.

Question 2

a. The Ontario Massive Haemorrhage Protocol (MHP) does not define major, massive or supra-massive haemorrhage [1]. The Protocol recommends using one of the following validated published activation criteria: ABC score (trauma only), shock index or resuscitation intensity (>4 units of fluid in first 30 min with '1 unit' defined as any of 1 U RBC, 1 U plasma, 500 ml colloid or 1 L crystalloid). The same activation criteria are to be used in all settings, except paediatrics, where a modified paediatric shock index is recommended. Our Protocol also recommends using a single protocol for all settings to avoid confusion and facilitate implementation. There are recommendations in the protocol for specific populations of patients where the MHP is modified based on evidence (e.g., a fibrinogen target in post-partum haemorrhage of 2.0 g/L). This recommendation was supported by a modified Delphi exercise involving a multidisciplinary panel conducted for the development of our MHP.

b. Currently, there are no such national guidelines in Canada. In 2011, Canada hosted a Massive Transfusion Consensus Conference which provided detailed guidance for clinicians on this topic [2]. Following publication of the Ontario MHP in March 2021 and development of implementation tools (simulation material, checklists, posters, videos and a podcast; www.transfusionontario.org), implementation and harmonization of policies across our province are ongoing. The Ontario MHP detailed 36 recommendations and 8 quality metrics to ensure all massively haemorrhaging patients receive state-of-the-art care in our region [1]. Other regions of Canadian have elected to also implement the Ontario MHP. We have a single protocol, with additional specific guidance for select patient populations/clinical situations (e.g., obstetrical bleeding, cardiac surgery).

c. Written into our Ontario MHP is the requirement for a review at a minimum of every 3 years. The responsibility for initiating a review rests with the project leads and Ontario Regional Blood Coordinating Network (ORBCON). The Protocol is to be reviewed by a multidisciplinary panel, including physicians, surgeons, nurses, medical laboratory professionals, blood supplier representatives and others. This frequency of revision was chosen through the modified Delphi exercise.

d. In 2018, we have conducted a provincial audit of all hospitals with a transfusion service and across all clinical settings [3]. The audit was coordinated by ORBCON. This audit determined that the content of the hospitals were diverse and failed to include key components deemed necessary in the Ontario MHP. The provincial MHP was made public in 2021 and it includes a section on tracking eight key quality metrics. We are planning to conduct our next audit in 2022, once most hospitals have had the chance to implement their first MHP or revise their current MHP to align with provincial requirements. This repeat audit will be sponsored by ORBCON. Our future goal is to have continuous reporting of quality metrics by the hospitals through a web-based platform. In 2022, the quality metric platform will be validated at 3 adult and 1 paediatric centre before rollout across the region.

Question 3

a. Our protocol dictates a minimum 2:1 RBC to plasma ratio during massive haemorrhage. This ratio applies across all clinical settings. This ratio was first adopted after the 2011 Massive Transfusion Consensus Conference [2] and then selected through the modified Delphi exercise [1]. This ratio-based resuscitation terminates as soon as laboratory test results can be utilized to personalize the haemostatic resuscitation.

b. There is no recommended ratio for platelets. Platelets are to be transfused as per platelet count or for suspected/known platelet dysfunction. Minimum laboratory resuscitation target for platelets is 50 $\times$ 10^9/L (with a higher target of 100 $\times$ 10^9/L for patients with intracranial or spinal injury).

c. In our protocol, the trigger for fibrinogen replacement is 1.5 g/L for all settings except obstetrics; recommended target is 2.0 g/L in obstetrical haemorrhage. Moreover, our MHP calls for empiric fibrinogen replacement (4 g FC) in Box 3 (Box 1:

4RBC; Box 2: 4RBC and 4FP; Box 3: 4RBC, 2FP and 4gFC). In cardiac surgery, the MHP recommends the use of viscoelastic testing to guide the use of blood components and haemostatic adjuncts.

d. Our MHP recommends that all patients receive TXA within 3 h of MHP activation (unless already given prior to MHP activation). This recommendation was developed prior to publication of the HALT-IT trial. Therefore, the more recently developed toolkit includes the following statement: '… universal administration of TXA in patients with gastrointestinal bleed cannot be recommended. Decision to use TXA in this clinical scenario should be made by the clinical team on a case by case basis'. One of the eight quality metrics that will be tracked across the province is the proportion of patients administered TXA within 60 min of protocol activation.

Question 4

a. As per our MHP, if the patient's blood group is unknown, O RhD-negative RBCs should only be used for female patients of childbearing potential (45 years of age or less).

b. The Protocol dictates to switch patients to their own blood group as soon as the group has been determined. One of the eight quality metrics being tracked across the province is the proportion of patients transitioned to group-specific blood within 90 min of protocol activation.

   The answer to the latter question above is no, this is not currently covered by our MHP. However, this issue is addressed in the national position paper *Utilization and Inventory Management of O Rh D Negative Red Cells* (www.nacblood.ca).

c. Blood shortage contingency plans are mandated by our national transfusion standards. We have the national shortage plan, the provincial Ontario shortage plan and each hospital has its own blood shortage plan. Guidance on when not to initiate RBC transfusion support (Emergency Framework) is included in the national plan (www.nacblood.ca). Guidance on when to discontinue RBC transfusions is not included in our MHP, but is a requirement for hospitals to include in the hospital's blood shortage plans. This includes triage teams in the unlikely event that blood stocks are insufficient to support all haemorrhaging patients.

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UNITED KINGDOM

Michael Murphy & Julie Staves

Question 1

Our local health service comprises four hospitals, three of which are located in Oxford and one in a town about 40 miles north of the city. It delivers care from many other locations in our region including in patients' homes. It is an academic medical centre. It is the regional centre for many acute specialties including trauma, haematology and vascular and cardiothoracic surgery. It has 1100 beds.

Approximately 17,000 units of RBCs are transfused each year.

Question 2

a. While there are arbitrary definitions of massive blood loss, for example, loss of one blood volume within a 24-h period, 50% blood volume loss within 3 h, loss of 150 ml/min, these may be difficult to apply in the acute situation [1]. The national arbitrary definition of major haemorrhage is bleeding which leads to a heart rate of more than 110 beats/min and/or systolic blood pressure of less than 90 mmHg [1].

   We do not have separate definitions for MAJOR TRANSFUSION, MASSIVE TRANSFUSION and SUPRA-MASSIVE TRANSFUSION in adults. There is only one definition of major haemorrhage as described above.

   One definition is sufficient for immediate patient management as it is intended to trigger immediate actions as outlined in the local major haemorrhage protocol. Further definitions are not particularly helpful because they can only be made retrospectively, for example after a given number of transfusions have been administered.

b. Yes. Please see reference [1].
We have one main guideline for our local health service. It is based on the national guideline [1]. It does include specific recommendations for different types of major haemorrhage, for example, trauma, post-partum, intracranial and gastrointestinal.
c. Yes, our policy is to update the local guideline every 3 years or sooner than that if new evidence suggests that it needs to be updated earlier. We are not certain when the national guideline will be updated.
d. A national audit led by NHS Blood & Transplant and supported by the National Blood Transfusion Committee was conducted in 2018 [2]. One hundred and sixty-six hospitals enrolled in the audit and 826 cases were analysed where a major haemorrhage episode was reported. A further national audit of major haemorrhage has not yet been scheduled.

Question 3

a. The initial resuscitation in major haemorrhage is recommended to be a 2:1 unit ratio of red cells to FFP until the results for coagulopathy tests are received. Both the local and national guidelines recommend aiming at a ratio of 1:1 in traumatic bleeding.
b. Both the local and national guidelines recommend aiming to keep the platelet count >50 $\times$ 10^9/L, and suggest that platelets should be requested if there is ongoing bleeding and the platelet count has fallen below 100 $\times$ 10^9/L. The local guideline recommends a higher target level of 100 $\times$ 10^9/L for patients with multiple high-energy trauma or central nervous system injury, and that empirical platelet transfusion may be required when platelet function is abnormal such as is found after cardiopulmonary by-pass or in patients taking anti-platelet therapy.
c. Both the local and national guidelines recommend fibrinogen supplementation should be given if the fibrinogen level falls below 1.5 g/L; the target level for fibrinogen should be at least 1.5 g/L in trauma and surgery and 12 g/L in obstetric haemorrhage.
d. Yes, both the local and national guidelines recommend the use of TXA as soon as possible after adult trauma at a dose of 1 g intravenously over 10 min followed by a maintenance infusion of 1 g over 8 h. It should not be given if more than 3 h have elapsed from the time of injury. The local guideline points out that ambulance crews frequently administer intravenous TXA at the scene of the injury, and so confirmation of the date and time of its administration must be obtained at the time of handover of the patient from the ambulance crew.
The local guideline also includes evidence from recent trials indicating that:
    1. All women with post-partum haemorrhage should receive intravenous TXA as soon as possible after bleeding onset [3];
    2. Adults admitted to hospital with traumatic brain injury should be administered intravenous TXA within 3 h of the injury [4];
    3. TXA did not reduce mortality in patients with acute gastrointestinal haemorrhage but was associated with an increased risk of venous thromboembolic events and seizures [5].

Question 4

a. The local guideline and national recommendations for the use of group O blood indicate it is acceptable to give O RhD-positive cells to males and postmenopausal females of unknown blood group in an emergency. Females of childbearing age whose blood group is unknown should be given O RhD-negative red cells in order to avoid sensitization and the risk of haemolytic disease of the newborn in subsequent pregnancy.
b. There is no local policy indicating how to manage this scenario as there are many variables that are important in deciding when to switch RhD-negative women of childbearing potential or children to RhD-positive RBCs including the likelihood of continuing major haemorrhage and the local and national inventory for RhD RBC units. Each situation is managed after consideration of these factors.
c. We manage blood shortage situations in accordance with the recommendations in national guidelines (see https://www.transfusionguidelines.org/uk-transfusion-committees/national-blood-transfusion-committee/responses-and-recommendations).

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GERMANY

Marc Maegele

Question 1

Our Hospital/Medical Centre is an academic Level 1 trauma centre located in the western part of Germany close to the Dutch/Belgian border serving a metropolitan area of approximately 1.5 million inhabitants. The Hospital is a community hospital affiliated to a private University and serves as a teaching hospital for two universities. The Hospital serves as one of two supra-regional trauma centres within the local trauma network. The hospital has 850–950 beds and an in-house blood bank (Institute for Transfusion Medicine [ITM]) that also serves as a reference centre for blood transfusion for EuroTransplant and is accredited by the European Federation of Immunogenetics. According to information provided by our ITM, approximately 5500–7000 blood products are being delivered and transfused in-hospital on an annual basis but with overall numbers consistently declining.

Question 2

a. The definition of major haemorrhage and/or massive transfusion usually follows the standard definition of ≥10 PRBCs with 24 h which corresponds to one complete volume exchange within 24 h. Clinically major haemorrhage is anticipated in the presence of major haemorrhage with laboratory signs of shock reflected by base excess and lactate, failure to achieve haemodynamic stability despite volume loading in terms of non-responsiveness, need for vasopressor support. In most dynamic scenarios also the definition of 50% volume exchange with 3 h, transfusion of 4 PRBCs within 1 h or ongoing blood loss ≥150 ml/min may be used. This is the distinction between major transfusion, massive transfusion and supra-massive transfusion. The above definition is mainly used for trauma cases, however, there may be use of distinction between bleedings arising from different entities as dynamics and pathophysiology may differ between the settings!
b. In our setting, we follow the European Guideline for Major Trauma Haemorrhage which is currently updated with our support [1]. In our setting, this guideline is mainly applied to trauma patients as we do not have any cardiothoracic surgery or obstetrics units in our house.
c. We have mainly adopted the above-referenced guideline which is updated every 3 years; our local guideline corresponds to this guideline and is synchronically being updated [1].
d. There is no such policy in place in our Hospital/Medical Centre.

Question 3

a. According to the above-referenced guideline the FFP:PRBC ratio is at least 1:2; this is for trauma as outlined above [1].
b. The administration of platelets is not according to the ratio in our setting; in case of massive transfusion on average 1 PC may be given after 4 PRBCs. Alternatively, platelets are given in case of ongoing haemorrhage and when platelet counts drop below 50 or according to rotational thromboelastometry (ROTEM) amplitude differences in extrinsically activated assay with tissue factor (EXTEM) and extrinsically activated assay with tissue factor and the platelet inhibitor cytochalasin D (FIBTEM). Again, this is all for trauma.
c. In acute settings, there is a rather liberal policy in place for the supplementation with FC. In extreme cases, arriving patients in haemorrhagic shock receive 4–6 g of FC blindly. In less severe cases, fibrinogen is kept >1.5 g/L or given when FIBTEM A5 is ≤9 mm to target to a FIBTEM A5 ≥12 mm. Again, this is all for trauma.
d. TXA is given either pre-hospital or early in-hospital blindly 1 g iv to bleeding patients in shock where the reason for shock is most likely haemorrhage. Attention is given that TXA administration occurs before supplementation of fibrinogen. In less severe cases, TXA is given according to ROTEM signals of lysis.

Question 4

a. In general, males are being transfused with O positive products, females of childbearing potential and children with O negative products. If possible, blood group testing is being performed which is usually fast and then matched products are being delivered by our in-house blood bank.
b. This is usually done case-based after consulting with a transfusion specialist from our ITM or with the treating physician-in-charge. If possible, there is no switch as usually sufficient quantities of blood products are available and provided by our in-house blood bank.
c. As outlined, usually there is no shortage. If there is a shortage, feedback will be given to our in-hospital crisis management team and elective surgeries with the potential need of blood products will be postponed as was the case during peak COVID-19 times.

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AUSTRALIA

Chathuri Abeyakoon, Kylie Rushford & Erica Wood

Question 1

Located in Melbourne, Australia, Monash Health is the state of Victoria's largest metropolitan public health service, providing an academic and referral service across six major campuses, including emergency services and all major paediatric and adult speciality care; it is

not a designated trauma centre. A 103-bed private hospital is co-located. Monash has approximately 1850 inpatient beds, many outpatient facilities and beds and delivers over 3 million episodes of care per year, including over 230,000 emergency department visits and over 10,000 obstetric deliveries. Between July 2020 and June 2021, the network transfused 22,700 RBCs.

Question 2

a. We define massive transfusion in adults as receiving 4 or more units of RBCs in 4 hours, or loss or transfusion of one blood volume over 24 hours. This definition applies to all clinical settings, including trauma, surgical, medical and obstetric bleeding contexts. Other, more 'real time' definitions include replacement of half a blood volume within 4 hours, blood loss of more than 150 mL per minute, or receipt of 10 units of RBCs. These definitions are clearly documented in our hospital massive transfusion protocol (MTP) which is one of the suite of documents easily accessible to all Monash healthcare providers. Blood loss or transfusion meeting these definitions is not required for activation of the MTP, which can be triggered as below.

   Triggers used to activate our MTP include, 1) ongoing bleeding despite 4 units of RBC transfusion, 2) loss of approximately 50% of blood volume and (3) anticipated massive transfusion due to clinical scenarios, such as severe thoracic, abdominal, pelvic or multiple long bone trauma, or major obstetric, gastrointestinal or surgical bleeding. The MTP may be activated through any of the following mechanisms, which include: direct activation by a senior clinician or member of the treating team, indirectly by the blood bank following the 5th unit of RBCs ordered within a 4-h period, or by the haematology transfusion registrar/on-call haematologist depending on the clinical scenario.

   We do not use separate definitions for major transfusion and supra-massive transfusion.

   Separate definitions for major transfusion, massive transfusion and supra-massive transfusion could be of benefit, especially in the analysis and comparison of data between studies. However, a potential drawback could be that complexities of having multiple definitions may cause confusion amongst clinical and laboratory teams, especially amongst those who are not very familiar with managing these emergency situations with significant blood loss, with uncertain potential impact on patient management. Therefore, on balance, we feel that using one definition for clinical purposes minimizes confusion and simplifies communication. Further classification into supra-massive and so on could be applied for research purposes.

b. Yes. Patient blood management guidelines for critical bleeding and massive transfusion published by Australia's NBA and by the Australian and New Zealand Society of Blood Transfusion are followed at our health service, where recommendations are adapted into our institutional MTP [1, 2]. Optimal ratios of blood product transfusion remain unclear, with no specific recommendations made by our national guidelines (noting that these were published in 2011 and are currently being updated). We have adopted a protocol where we alternate between two rounds of blood products: the first round includes 4 units of RBCs, 2 units of FFP and 1 dose of platelets; the second round includes 4 units of RBCs, 2 units of FFP and 10 whole-blood-derived units of cryoprecipitate (or equivalent). Our MTP applies generally to all adult clinical settings, with minor modifications of the fibrinogen targets in obstetric haemorrhage (as detailed below) and modifications in the context of cardiothoracic surgery in patients undergoing cell salvage and coming off cardiopulmonary bypass, where special coagulation packs are utilized to replace coagulation factors and platelets but where additional RBCs may not be routinely required.

c. All hospital policies are regularly reviewed and updated by the Monash Blood Management Committee, which has input from Transfusion Medicine, Haematology, Surgery, Obstetrics, Nursing, Emergency and Intensive Care. Our MTP is updated periodically as per hospital requirements, with further modifications made as the national guidelines are updated (these are currently under review) and/or new research becomes available.

d. Yes. We conduct regular institutional audits of the activations and outcomes of the MTP, with the most recent audit conducted in 2021. These ensure our performance complies with national standards set out by the Australian Commission on Safety and Quality in Health Care (ACSQHC), and allow us to review progress including in blood product utilization, and to identify areas for further improvement [3]. These audits are performed under the governance of the Monash Blood Management Committee and coordinated by our institutional transfusion working group (transfusion nurse consultants, laboratory scientists, haematologist registrars and haematologists). We also participate in state-wide transfusion audits and other practice improvement activities through the Victorian 'Blood Matters' collaborative.

Question 3

a. We issue two alternating rounds of blood products in all clinical scenarios. Each round has an RBC to FFP ratio of 2:1. We adjust the amount of FFP transfusion to aim for an international normalized ratio (INR) and activated partial thromboplastin time (aPTT) of less than 1.5× normal, noting that these laboratory-based test results may correlate poorly with clinical bleeding.

b. We do not specify or recommend a target ratio of RBCs to platelets in any clinical setting. Our first round of blood products includes an RBC to platelet ratio of 4:1; our second round does not include platelets. Additional platelets are given as required, aiming to maintain a platelet count of >50 × 10^9/L (or >100 for head injury).

c. We aim to maintain a fibrinogen concentration of >1.5 g/L for patients with major haemorrhage in most settings, with the specific exception of obstetric haemorrhage, where we replace fibrinogen to target a level of 2 g/L instead of 1.5 g/L, given early

disseminated intravascular coagulation is often a feature in such situations, and low fibrinogen levels have been reported as an early independent risk factor for severe PPH. We do not specify a fibrinogen trigger for the administration of fibrinogen replacement. Our second round of blood products issued as part of our MTP includes 10 units of cryoprecipitate. We recommend performing ongoing coagulation tests every 30–60 min, and in consultation with the haematology transfusion registrar/on-call haematologist, tailoring transfusion of cryoprecipitate to aim a fibrinogen level of >1.5 g/L.

d. Yes. We strongly recommend an initial TXA dose of 1 g intravenously over 10 min to be administered as soon as possible, followed by an infusion of 1 g over 8 h in all clinical situations in which the MTP has been activated, given the favourable outcomes observed with early use in the CRASH-2 and WOMAN trials [4, 5].

Question 4

a. In emergency situations of major haemorrhage, whilst laboratory tests are underway to confirm a patient's blood group and antibody status, group O RhD-negative RBCs are provided in the first instance. For male patients and females over the age of 50 years, we may change to O RhD-positive RBCs if there are delays in pre-transfusion testing and high RBC requirements. We do not specify a specific trigger for this switch of products, but would suggest that this be considered after 6–10 units of RBCs. The Monash Health Blood Bank scientist would make this decision following consultation with the transfusion registrar or haematologist. We also recommend early activation of cell salvage wherever possible in major bleeding scenarios, which can further minimize the requirement for allogeneic RBCs to be transfused.

b. We do not have a policy specifying when to switch RhD-negative women of childbearing potential or children to RhD-positive RBCs when our RhD inventory is limited during times of major haemorrhage. If appropriate RhD-negative products could not be sourced in a timely manner, RhD-positive RBCs would be supplied following approval from a haematologist. This would be a protocol deviation, managed in consultation with the treating clinical team. We would consider administration of RhD immunoglobulin ('anti-D') in such situations, depending on the individual patient's clinical situation. We also recommend early activation of cell salvage wherever possible in major bleeding scenarios to minimize the use of allogeneic RBCs.

c. We have national and institutional policies to guide practice in the event of a severe blood shortage. Australia has a national blood service (Australian Red Cross Lifeblood) which manages supply nationally and locally to hospitals. The blood supplier issues an alert to the hospitals and laboratories if their supply falls below predefined stock levels. According to the alert level, Monash Health Blood Bank scientific staff liaise with the transfusion registrar and the clinical teams, and blood product requests are reviewed and approved, as needed, on a patient-by-patient basis by either a haematologist or transfusion registrar.

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CHILE

Maria Antonieta Nuñez, Sandra Mellado & Edgardo Saa

Question 1

My hospital is located in Santiago of Chile; this city is the capital of the country.

It is a highly complex hospital with almost all the specialties; haematopoietic progenitor and solid organ transplants are performed.

It does not provide services to other hospitals.

It has 395 beds and we transfuse approximately 5000 haemocomponents per year.

Question 2

a. It is defined as haemorrhagic shock as: 'Type IV Advanced Trauma Life Support (ATLS) haemorrhage, loss ≥2000 cc'. We do not have

separate definitions for MAJOR TRANSFUSION, MASSIVE TRANSFUSION and SUPRA-MASSIVE TRANSFUSION in adults. These definitions apply to all clinical settings.

b. No national guidelines exist; we adapt guidelines from other countries' guides.
Yes, there is a blood transfusion indication guide as an official document in our Institution
c. The indication guide is reviewed every 3 years or earlier if required.
d. No, no audit of any of the aforementioned audits has been carried out.

Question 3

a. A 1:1 ratio is recommended.

Applies to Emergency Services.

b. One unit of RBCs per 1 apheresis PC or platelet pool with the number of units corresponding to 1 unit per 10 kilos.

Applies to Emergency Services.

c. In my country, lyophilized FC is rarely used; cryoprecipitate is used in haemorrhage when the fibrinogen concentration is ≤80 mg/dl.

Applies to all clinical settings.

d. This recommendation is not found in the blood transfusion indication guidelines.

Question 4

a. The first unit is O RhD negative, after classification if RhD positive it continues with RhD positive in all patients.
b. Yes, the recommendation is found in the transfusion indication guide, which indicates that RhD-positive transfusion in RhD-negative patients can only be performed in situations of extreme urgency with associated vital risk, when RhD-negative components are definitely not available and will not be available in a short period of time. It can be used in non-sensitized male patients and in non-sensitized postmenopausal women. This criterion cannot be applied in newborns or in transfusion-dependent patients.
c. During the pandemic, elective surgeries were suspended and each unit of blood indicated was previously validated by the physician of the Transfusion Medicine Unit. So far, we have been able to meet the transfusion requirements.

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INDONESIA

Teguh Triyono & Bhirowo Pratomo

Question 1

Dr Sardjito Hospital located in the city of Yogyakarta, Indonesia, is a hospital in the provincial capital, a tertiary hospital and teaching hospital and as a referral hospital. As a referral hospital, this hospital has specialist and sub-specialist services which are also the referral destination for lower-class surrounding hospitals. Dr Sardjito Hospital Yogyakarta has outpatient services equipped with 39 polyclinics. The number of outpatient visits is approximately 500,000 a year. It has 820 beds with the number of inpatient visits being approximately 30,000 in a year. Approximately 26,000 RBC units are transfused out of a total of 48,000 transfusions per year.

Question 2

a. Our criterion of massive transfusion is defined as the transfusion of >10 units of PRBCs in 24 h [1], usually in cases of major bleeding or elective surgery that could potentially require massive bleeding. Most massive transfusions performed in our hospital were found in cardiothoracic surgery. The terms major transfusion and supra-massive transfusion are relatively unfamiliar to clinicians here. Factually, the definition of massive transfusion is used in different clinical settings in the service, usually activated in cases of surgical bleeding or in the emergency room. Supra-massive transfusion probably refers to a larger number of transfusions than massive transfusion but is not recognized in our transfusion standard procedure. More certain definition for *MAJOR TRANSFUSION*, *MASSIVE TRANSFUSION* and *SUPRA-MASSIVE TRANSFUSION* should be considered.
b. As far as I know, there is no national guideline regarding major haemorrhage/massive transfusion in adults yet in Indonesia. Likewise, there is no agreement for the guidelines used in hospital. Each department uses different references depending on the patient's situation setting and area of specialties. It is possible that

centres or hospitals in different cities use different references. 'Management of severe perioperative bleeding: guidelines from the European Association of Anesthesiology' is widely referred [2].

c. No guidelines used in clinical practice for management of major haemorrhage and/or massive transfusion in adults in our hospital.
d. Not applicable

Question 3

a. In general, the implementation of RBCs and plasma in massive transfusion uses the 1:1 ratio in all clinical settings. This setting is not yet being a hospital policy but is particularly applied to cardiothoracic surgery when an order of 5 RBCs and 5 FFP is usually made.
b. Similar with RBCs to plasma ratio, the use of RBCs and platelets with 1:1 ratio was performed in all clinical settings. This setting is not yet being a hospital policy but is particularly applied to cardiothoracic surgery when an order of 5 RBCs and 5 platelets is usually made.
c. Fibrinogen replacement therapy is not yet familiar for management of major haemorrhage. Most of the clinicians will request a cryoprecipitate component to replace the reduced fibrinogen level.
d. The use of TXA for management of major haemorrhage has been practiced in our hospital for a long time but has not become a hospital guideline. Almost all clinical settings have used TXA in the treatment of massive haemorrhage.

Question 4

a. In Indonesia, the prevalence of RhD negative is very small, approximately 1%–3%, so that in cases requiring massive transfusion, no RhD negative available and the patient blood group is unknown, then group O RhD-positive donor blood is used as an emergency setting. Age or gender remains a consideration.
b. This setting was not built in our hospital, due to the low prevalence of RhD negative in Indonesia. In case the patient is an RhD negative, if possible it is sought from donors in the RhD-negative donor group in the city to come and make donations.
c. In shortage conditions, for example, at the beginning of the COVID-19 pandemic, we endorsed family donors or substitute donors. We realize that this is not the best option, but it must be applied to meet the needs of donor blood, especially in the event of massive transfusion.

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NORWAY

Torunn Oveland Apelseth

Question 1

Haukeland University Hospital is a regional level-one trauma centre and a national burn centre located in the western region of Norway. The hospital has 790 beds and serves as a local hospital for the city of Bergen, with a population of 285,000. It is a teaching hospital, affiliated with the University of Bergen. Our hospital-based blood bank provides the following services: blood collection, production of blood components, an immunohematology laboratory, stem cell collection, hospital transfusion services and a research facility. Approximately 15,000 red cell units are transfused in our hospital per year. Our blood bank also serves several smaller hospitals, both in the city and a smaller hospital 90 min drive from Bergen.

Question 2

a. The overall transfusion guidance document for Haukeland University Hospital defines a massive transfusion as 5 or more red cell units transfused within 3 h or 10 red cell units or more transfused within 24 h. This definition is in accordance with the overall Norwegian Trauma Guideline [1]. These definitions apply to all bleeding patients regardless of the clinical setting in which the bleeding occurs. We do not have separate definitions for major, massive or supra-massive transfusions and have not yet observed a need to separate between these for the practical purpose of ensuring the adequate supply of blood components for these patients.
b. The Norwegian trauma guideline provides recommendation on management of patients with life-threatening bleeding in the trauma setting [1]. In our hospital, we have a local guideline for treatment of patients with life-threatening bleeding. We have one main guideline that is used for all clinical settings and referred to by the different wards/services.

c. The guideline is revised regularly based on revisions of clinical practice (including our local quality registry for massive transfusions), discussions with clinicians, updates of national regulations and guidelines and relevant literature.
d. There has not been performed a national audit of practice against the guidelines used in clinical practice for management of major haemorrhage and/or massive transfusion, but we regularly perform local audits and evaluate practice by use of our quality registry of massive transfusions.

Question 3

a. Our primary choice for resuscitation of patients with life-threatening bleeding is whole blood for all patients, male, female, adult and children. Whole blood provides a 1:1 ratio of RBCs to plasma, and in addition, it includes platelets. If whole blood is not available, we provide RBCs, plasma and platelets in a 1:1:1 ratio as part of a massive transfusion package.
b. As described for the question above, our primary choice for resuscitation of patients with life-threatening bleeding is whole blood for all patients, male, female, adult and children. Whole blood provides a 1:1 ratio of RBCs to platelets.
c. In Norway, we do not use cryoprecipitate for fibrinogen substitution, only FC. We have not included information on fibrinogen trigger in our overall guidelines for massive transfusion, but this is described in procedures for the different wards/services. The overall Norwegian Trauma guidelines recommend infusion of FC if fibrinogen is <2 g/L.
d. We have not included information on TXA in our overall guidelines for massive transfusion, but this is described in procedures for the different wards/services. The overall Norwegian Trauma guidelines recommend the use of TXA.

Question 4

a. In the setting of a major haemorrhage, we use RhD-positive whole blood or RBCs for males and females older than 50 years. We perform early type and screening for erythrocyte antibodies of all patients.
b. We do not have a predefined algorithm for when to switch RhD-negative women of childbearing potential or girls from RhD-negative to RhD-positive RBCs. We have a predefined whole blood-based blood preparedness plan, which includes systems for emergency collection of blood in case of risk of emptying of our blood inventory. This plan is based on the walking blood bank principle and we have a large number of donors readily available for emergency blood collection if needed. As part of this plan, evaluation of the need for RhD-negative whole blood units is performed by the blood bank physician on a call together with the physician in charge of the clinical treatment of the actual patient(s).
c. We have a predefined whole blood-based blood preparedness plan, which includes systems for emergency collection of blood in case of risk of emptying of our blood inventory. This plan is based on the walking blood bank principle and we have a large number of donors readily available for emergency blood collection if needed. We have also included a plan for emergency collection of platelets by apheresis. In the early stages of the pandemic, we implemented cold-stored apheresis PCs to mitigate the risk of insufficient platelet inventory for bleeding patients.

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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

Does ABO blood group influence antibody response to SARS-CoV-2 vaccination?

In two recent studies published in *Vox Sanguinis*, de Freitas Dutra et al. and Bloch et al. investigated whether ABO blood group was associated with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) antibody response following natural infection in convalescent plasma donors [1, 2]. de Freitas Dutra et al. found lower infection prevalence, and neutralizing and anti-nucleocapsid antibody titres among blood types O and B compared to A and AB [1]. Bloch et al. also found significant differences in neutralizing antibody titres according to ABO blood groups [2].

The impact of ABO blood group on SARS-CoV-2 infection risk and disease severity has been investigated by several studies. Results of a recent systematic review and meta-analysis suggest that non-O blood groups are associated with a significantly increased probability of SARS-CoV-2 infection compared to individuals with blood group O [3]. It has been proposed that anti-A antibodies in blood group O individuals could interfere in the interaction between SARS-CoV-2 and its receptor, decreasing the susceptibility to infection of blood group O individuals [3].

The antibody titre before SARS-CoV-2 vaccination has been identified as a significant predictor of postvaccination response [4]; we conducted a cross-sectional study to investigate whether ABO blood group influences antibody response to SARS-CoV-2 vaccination. Participants were enrolled among medical students of the University of Turin, in Northern Italy, undergoing vaccination with Pfizer/BNT162b2 SARS-CoV-2 mRNA vaccine. The study was approved by the University's bioethical committee (protocol number 280344). Serum samples were obtained from participants 2 weeks after

TABLE 1 Anti-spike IgG antibody titres following full vaccination cycle with Pfizer/BNT162b2 SARS-CoV-2 mRNA vaccine, stratified by ABO blood groups, in a sample of medical students of the University of Turin, Italy, June 2021 ($N = 85$)

Blood group	*N* (%)	Median (IQR), RU/ml
O	35 (41.18)	1240.80 (788.56–2009.28)
A	28 (32.94)	1254.80 (817.68–1553.36)
B	14 (16.47)	1212.84 (676.56–1789.44)
AB	8 (9.41)	1299.16 (1203.52–2129.4)

Abbreviations: IQR, interquartile range; RU, relative units.

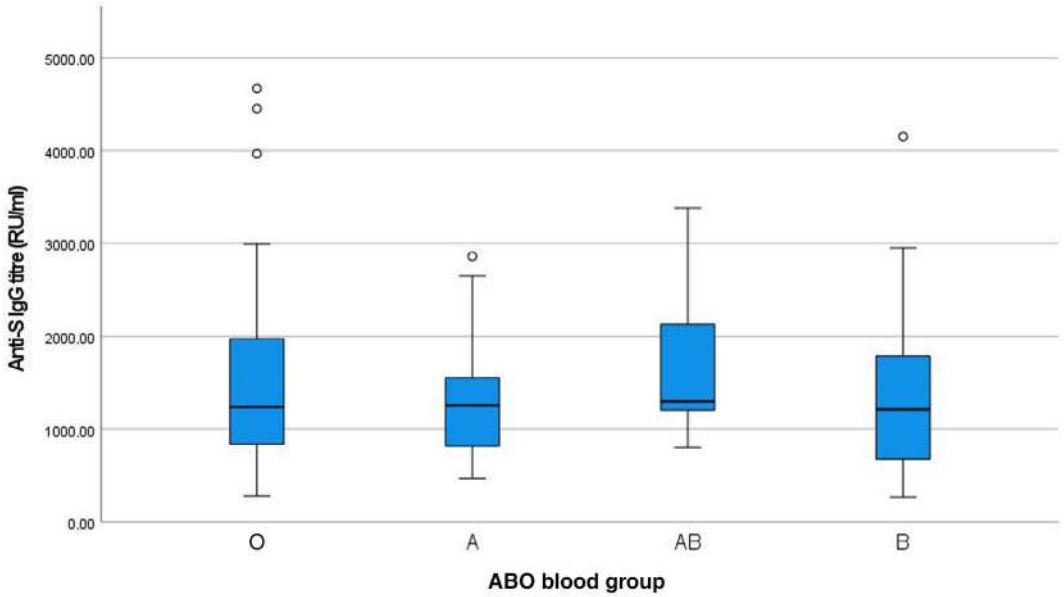

FIGURE 1 Box plots representing anti-spike IgG antibody titres following full vaccination cycle with Pfizer/BNT162b2 SARS-CoV-2 mRNA vaccine, stratified by ABO blood groups, in a sample of medical students of the University of Turin, Italy, June 2021 ($N = 85$). RU, relative units

completing a full vaccination cycle (two doses, 21 days apart), in June 2021. Informed consent was obtained prior to the collection of data and specimens. SARS-CoV-2 anti-S antibodies were assayed using the EUROIMMUN QuantiVac ELISA kit (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany). This method detects IgG antibodies against the S1 domain of the spike protein including the receptor-binding domain (RBD). Results were expressed in relative units, RU/ml. Due to non-normal distribution (Shapiro–Wilk test), the Kruskal–Wallis test was conducted to evaluate possible differences between ABO blood groups. All analyses were conducted using SPSS version 27.0 (IBM, Armonk, NY).

In total, 85 medical students were enrolled, of which 57.6% ($n = 49$) were female and 42.4% ($n = 36$) were male. Only 5.88% ($n = 5$) had a previous COVID-19 infection confirmed by reverse-transcription polymerase chain reaction (RT-PCR) testing. The overall mean anti-S IgG titre was 1497.64 RU/ml (SD 937.28 RU/ml). Table 1 reports anti-S IgG titres stratified by blood groups. As shown in Figure 1, no significant differences in antibody levels among ABO blood groups were identified in our sample (p 0.771 at Kruskal-Wallis test).

Despite limitations due to our small sample, which only included medical students from a single University, our results do not support an influence of ABO blood group on vaccine response, and could be useful to inform vaccine strategy.

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See also http://www.isbtweb.org/congresses/	
10.2.2022	The European Hematology Association (EHA) and the European Society for Blood and Marrow Transplantation (EBMT) - 4th edition of the jointly organized European CAR T-cell Meeting.
15-16.3.2022	The IPFA/EBA Symposium on Plasma Collection and Supply will take place fully physical in Amsterdam, the Netherlands on March 15 - 16, 2022.
23.3.2022	Eye Drops from Human Origin - First EDHO Workshop on Current Standards and Future Developments organized by the ISBT Working Party Cellular Therapies.

