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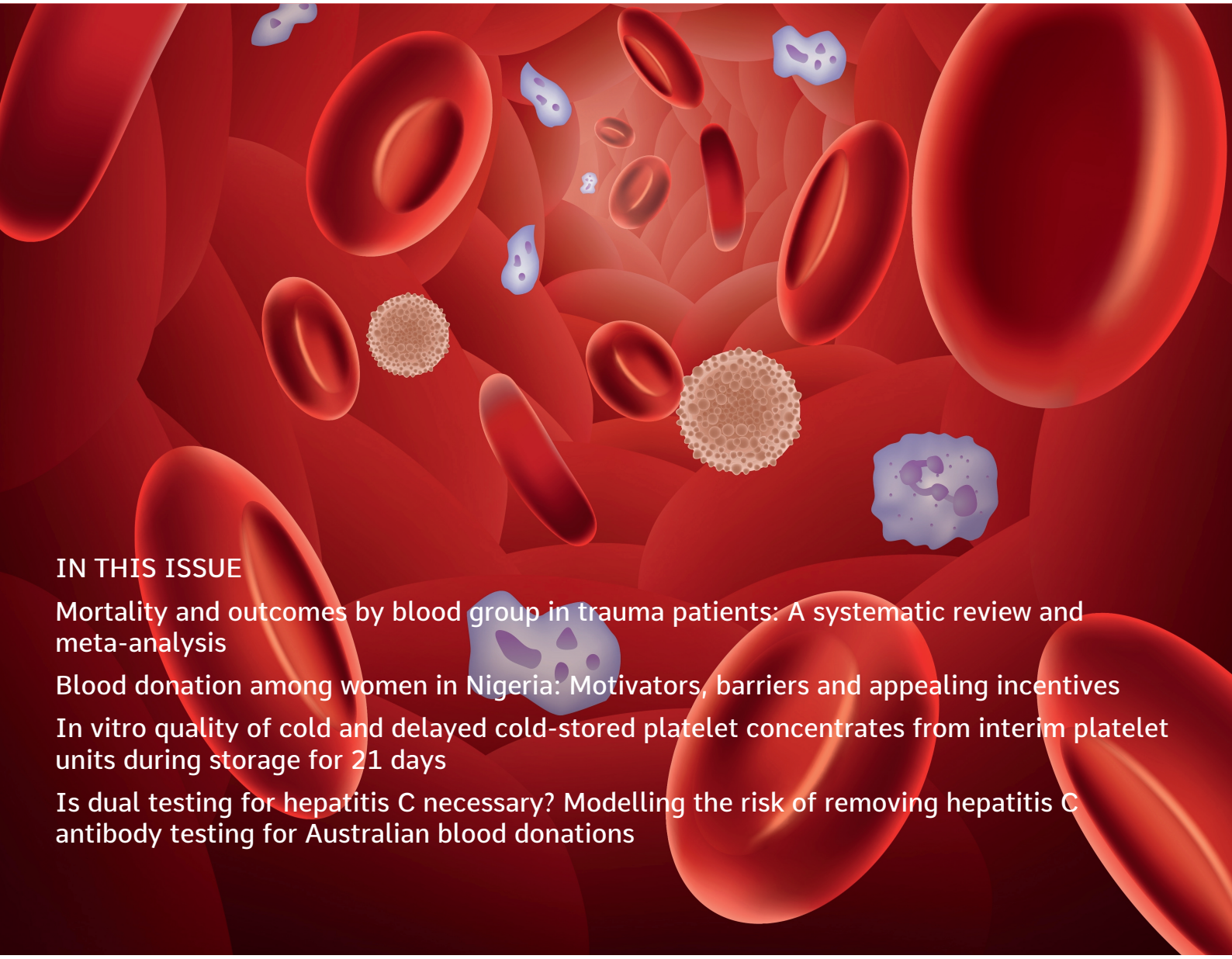

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International Journal of Blood Transfusion

Official Journal of the International Society of Blood Transfusion

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Vox Sanguinis

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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;
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3. Donors and Donations: Donor recruitment and retention; donor selection; donor health
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6. International Forum: Section in which topics related to any aspects of the transfusion chain (from technical to scientific, including socioeconomic and ethical facets) are discussed by invited participants and summarized by guest editors
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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;

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REVIEW

Vox Sanguinis ISBT International Society of Blood Transfusion

Mortality and outcomes by blood group in trauma patients: A systematic review and meta-analysis

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Abstract

Background and Objectives: Blood group O contains lower levels of factor VIII and von Willebrand factor. Higher incidence of bleeding among group O is reported in multiple contexts. Results of studies vary regarding outcomes stratified by blood group in trauma. We systematically reviewed the literature for outcomes related to blood group in trauma patients. Meta-analysis of studies evaluating mortality related to blood group was performed.

Materials and Methods: The PubMed and Embase databases were searched for studies analysing relationships between blood group and outcomes in trauma patients. Preferred Reporting Items in Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed. We synthesized outcomes data related to blood group. Meta-analysis compared mortality rates between group O and non-O patients.

Results: Inclusion criteria were met by 13 studies. Statistically significant differences by blood group were reported in 3 of 10 (30%) studies evaluating mortality, 2 of 3 (66.7%) evaluating mortality from haemorrhage and 2 of 9 (22.2%) evaluating transfusion requirement. Meta-analysis was performed on seven studies evaluating mortality (total $n = 11{,}835$). There was significant heterogeneity among studies ($I^2 = 86\%$, $p < 0.00001$). No difference was found in mortality between group O and non-O patients (relative risk = 1.21, 95% confidence interval = 0.89–1.64, $p = 0.23$).

Conclusion: Existing literature does not consistently demonstrate a mortality difference between trauma patients with O and non-O blood groups. High variability in the methods and results among studies limits this conclusion, and further research is needed to delineate under what circumstances blood group may influence outcomes.

Keywords

blood group, blood type, meta-analysis, mortality, systematic review, trauma

Highlights

- The existing literature does not consistently demonstrate a mortality difference between trauma patients with O and those with non-O blood groups.
- There is high heterogeneity in results and methods among studies evaluating outcomes related to blood group in trauma patients.
- Further research is needed to delineate under what circumstances blood group may influence trauma patient outcomes.

INTRODUCTION

Blood group, or type, has been associated with patient outcomes and risk of developing complications in a wide variety of disease processes and clinical scenarios [1–3]. Particular emphasis has been placed on the relationship between blood group and haemorrhage, as well as the risk of thromboembolic events [4]. This association has been most thoroughly explored when dichotomizing O versus non-O blood. Some authors suggest that decreased circulating levels of factor VIII and von Willebrand factor (vWF) in patients with group O blood may lead to higher bleeding risk and lower thromboembolic risk in these patients [5, 6]. Given that O is the most common blood group, comprising 45% of the US population, these differences may play an important role in patient risk stratification.

While only around 18% of trauma patients require blood product transfusion and only 2% require massive transfusion (>10 blood product transfusions in 24 h), haemorrhage remains the most common cause of preventable death in traumatically injured patients [7, 8]. Haemorrhaging trauma patients are among the most likely populations to be affected by subtle differences in coagulation due to trauma-induced coagulopathy and the need for large-volume resuscitation [9–12]. While these differences may not appear clinically relevant in healthy individuals, they could profoundly alter patient outcomes after the massive insult of a major trauma. Even after survival of an initial injury, distinctions associated with different blood groups may also contribute to the high incidence of thromboembolic events, delayed bleeding complications and organ injury secondary to systemic inflammation seen among trauma patients [13, 14].

To date, despite numerous individual studies investigating blood groups and trauma patient outcomes, none has reviewed or pooled associations among blood groups and outcomes. In this study, we present a systematic review of the existing literature on this topic and perform a meta-analysis of studies evaluating mortality related to blood group in trauma patients.

MATERIALS AND METHODS

Search methodology

Search methods were conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (checklist available as Supporting information) [15]. The search was not preregistered. Electronic searches were performed in the PubMed and Embase databases for studies that assessed for a relationship between clinical outcomes and patient blood type or group in trauma patients. The PubMed search terms used were: (trauma[Title/Abstract] OR trauma[MeSH Terms]) AND ((blood group[Title/Abstract]) OR (blood type[Title/Abstract]) OR (abo blood group system[MeSH Terms])). A similar search strategy was used for Embase. The literature search was repeated most recently on 5 July 2022. References of the included articles and other related articles were investigated for further relevant studies not found in our initial database search, but no additional studies meeting inclusion criteria were identified in this manner.

Selection criteria

Inclusion and exclusion criteria were defined a priori. Only full articles written in English evaluating a relationship between clinical outcomes and patient blood group in human trauma patients were included in this systematic review. Two independent reviewers (D.T.L. and J.-M. V.G.) performed the initial search, and no automation tools were employed. After duplicate publications were deleted, each abstract was screened its entirety. If exclusion criteria were not met based on the abstract, the full article was retrieved to determine proper categorization. Full-text reports were then assessed for eligibility by each independent reviewer and studies were excluded if no original data were reported. Any cases of incongruent assessments were arbitrated by a third reviewer (J.B.B.).

Data collection and statistical analysis

Data collected for all articles when available included: first author, publication year, type of trauma patients included, number of patients total and with each blood group, mean injury severity score (ISS), mortality rate by blood group, evaluated relationships between blood group and clinical outcomes and key study findings. If mean or median ISS was reported only within blood groups and not for the overall study population, then a weighted mean was calculated to represent the entire study population. If mortality was reported for blood groups only as a percentage, then the absolute number of patients in each blood group suffering mortality was calculated from the total number of patients in each blood group.

Meta-analysis was performed to evaluate for a difference in mortality rate between trauma patients with group O blood compared to non-O blood. Statistical analysis was performed using RevMan software (Version 5.4, The Cochrane Collaboration, London, UK) using the inverse variance method and random effects analysis model given the expected heterogeneity between study methods and populations. I^2 was calculated to evaluate heterogeneity between studies with $I^2 > 50\%$ interpreted as representing substantial heterogeneity [16]. Sensitivity analyses were performed by sequentially removing every single study and repeating our meta-analysis to determine which studies contributed most to heterogeneity [17]. Risk of bias in reported mortality was assessed in the studies included for meta-analysis using the Risk of Bias in Non-randomized Studies of Exposure (ROBINS-E) tool as recommended by the Cochrane Handbook [16, 18]. Evaluation of the certainty of our results was performed according to the Grading of Recommendations, Assessment, Development and Evaluations tool (GRADE tool) using the GRADEpro Guideline Development Tool Software (McMaster University and Evidence Prime, 2022. Available from gradepro.org). Publication bias was evaluated by the creation of a funnel plot to assess for symmetry in the reported effect size. Per

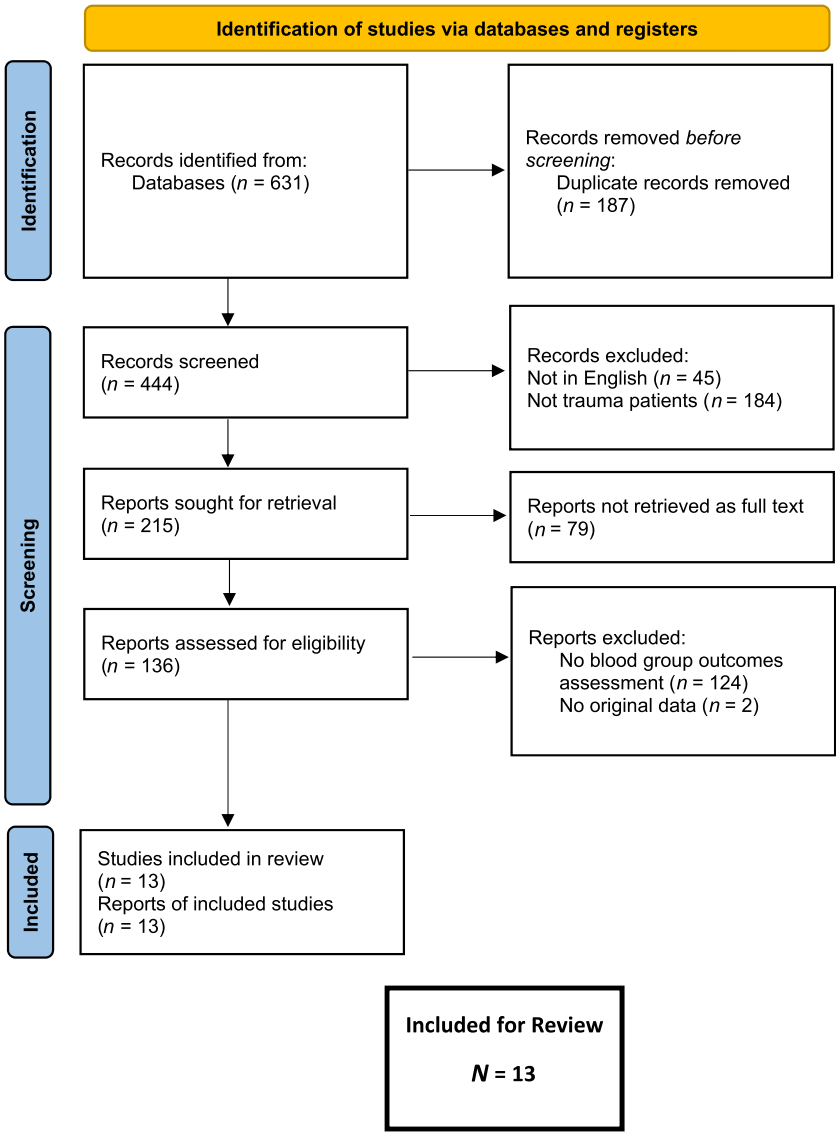

FIGURE 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) search diagram.

recommendations published in the Cochrane Handbook, no statistical tests were performed to assess for asymmetry given the small number of included studies [16]. The full study protocol, data extraction templates, and analytic coding are available upon request to the corresponding author.

RESULTS

Study characteristics and relationships

A total of 631 studies were identified, of which 13 met the inclusion criteria for review [19–31] (Figure 1). Study details and a summary of their key findings are included in Table 1. The studies were published between 2002 and 2022 and the number of included patients ranged from 497 to 3913. Mean ISS when reported ranged from 9.87 to 24.85, with only one study with a mean ISS < 15 [28]. There was variability in the types of trauma patients included, with 3/13 (23.1%) studies including only trauma intensive care unit (ICU) patients with no isolated traumatic brain injury (iTBI), 1/13 (7.7%) including all trauma ICU patients, 5/13 (38.5%) including all trauma patients, 1/13 (7.7%) including only iTBI patients, 1/13 (7.7%) including only isolated abdominal trauma patients, 1/13 (7.7%) including only trauma patients receiving a transfusion within 1 h of presentation and 1/13 (7.7%) including only hip fracture patients. The outcomes most commonly assessed for a relationship with blood group are included in Table 2. Studies were only counted as demonstrating a significant relationship if it was present in the entire patient population and not only in some sub-populations. A significant relationship was found between blood group and mortality in 3/10 (30%) studies, mortality from haemorrhage in 2/3 (66.7%) studies, transfusion requirement in 2/9 (22.2%) studies, ICU days in 0/4 studies, ventilator days in 2/6 (33.3%) studies, length of stay in 0/4 studies, acute kidney injury (AKI) in 0/4 studies, deep vein thrombosis (DVT) in 0/4 studies, pulmonary embolism

TABLE 1 Reviewed studies and key findings.

Author	Year	Trauma group	*n*	ISS	Key finding
Carter et al.	2002	All	686	17.05	No relationship between blood group and DVT
Reilly et al.	2014	ICU only, no iTBI	732	23.57	Group A White patients are more likely to develop ARDS, but no relationship is seen in Black patients
Uzoigwe et al.	2014	Hip fracture	2896		Group A associated with higher mortality but this may be due to higher comorbidities among group A patients in this study population
Reilly et al.	2015	ICU only, no iTBI	497	22	Group A White patients are more likely to develop AKI, but no relationship is seen in Black patients
Takayama et al.	2018	All	901	18.5	Group O is associated with increased mortality, mortality due to haemorrhage and mortality from TBI
Hamsen et al.	2019	ICU only	1281	21.25	No relationship between blood group and mortality or thromboembolic events
Griffin et al.	2020	All	3913	24.85	No relationship between blood group and mortality or other measured outcomes
Reilly et al.	2021	ICU only, no iTBI	1212	22.96	A1 genotype associated with a higher risk of ARDS
Sauder et al.	2021	All	3779	9.87	No overall association between blood group and mortality or outcomes. In penetrating trauma, group O had increased mortality and transfusion requirements. In blunt trauma, blood group AB had higher mortality
Takayama et al.	2021	Abdominal trauma	920	18.11	Group O had higher mortality, transfusion requirements and rates of intervention. Group AB had lower mortality
Tutunjian et al.	2021	iTBI only	949	16.95	No difference between group O versus non-O in the progression of ICH on repeat CT, surgical intervention after repeat CT, mortality or other outcomes
Kander et al.	2022	All	1583		No difference between group O versus other blood groups in mortality or other outcomes
Miller et al.	2022	Transfusion <1 h	544	26	No difference between group O versus non-O in transfusion requirement, time to haemostasis, DVT, PE, AKI, MOF, stroke or 24-h or 30-day mortality

Note: ISS reported as the mean value of the entire trauma patient population.
Abbreviations: AKI, acute kidney injury; ARDS, acute respiratory distress syndrome; CT, computed tomography; DVT, deep venous thrombosis; ICH, intracranial haemorrhage; ICU, intensive care unit; ISS, injury severity score; iTBI, isolated traumatic brain injury; MOF, multiple organ failure; PE, pulmonary embolism; TBI, traumatic brain injury.

in 0/3 studies and acute respiratory distress syndrome (ARDS) in 1/3 (33.3%) studies.

Meta-analysis

Meta-analysis was performed on 7 studies evaluating mortality (Table 3) out of a total of 10 studies evaluating this outcome. Tutunjian et al. [24] was not included as this study included only iTBI patients. The other analysed studies included primarily polytrauma patients with one even excluding iTBI patients [20], and it, therefore, was felt to represent a patient population with a primary mechanism of death too different from the other studies (devastating neurologic injury vs. haemorrhagic shock) to combine data for meta-analysis. Uzoigwe et al. [31] was not included for similar reasons as this study included only hip fracture patients in a primarily elderly population. Kander et al. [19] was not included as this study included all types of ICU patients (not exclusively trauma) and did not report the necessary information for their trauma subset to include in the meta-analysis. A combined total of 11,835 patients were represented in the meta-analysis. The weighted combined mortality rate was 9.4% for group A patient, 10.2% for group B, 7.4% for group AB, 11.0% for group O and 9.6% for all non-O groups combined. Mortality rate for any single blood group ranged from 2.8% to 28.2%.

On meta-analysis, there was no significant difference found in mortality between group O and non-O patients (relative risk [RR] = 1.21, 95% confidence interval [CI] = 0.89–1.64, $p = 0.23$) (Figure 2). Risk of bias in reported mortality in studies included for meta-analysis was, overall, determined to be low, with four studies [21–23, 26] found to be at low risk of bias and three studies [20, 28, 30] found to have some concern for bias (Table 4). The certainty of our meta-analysis result as evaluated by the GRADE tool was found to be low (Table 5). This was primarily due to the observational nature of these studies and the high variability in results between studies.

Significant heterogeneity was found in the designs and outcomes of the studies ($I^2 = 86\%$, $p < 0.00001$). Sensitivity analysis

TABLE 2 Blood group and relationship with commonly assessed outcomes.

Outcome assessed	Significant relationship	No significant relationship
Mortality	3	7
Mortality from haemorrhage	2	1
Transfusion requirement	2	7
ICU days	0	4
Ventilator days	2	4
Length of stay	0	4
AKI	0	4
DVT	0	4
PE	0	3
ARDS	1	3

Note: Statistical significance is defined as $p < 0.05$. Relationship only counted as significant if present in the entire tested population.
Abbreviations: AKI, acute kidney injury; ARDS, acute respiratory distress syndrome; DVT, deep venous thrombosis; ICU, intensive care unit; PE, pulmonary embolism.

was performed to address the high observed heterogeneity. The two Takayama et al. studies [23, 26] were found to most significantly contribute to heterogeneity. Exclusion of both was required to achieve an I^2 value below 50%. Removal of both reduced I^2 to 47% with $p = 0.11$ and confirmed no significant difference in mortality between group O and non-O patients (RR = 1.00, 95% CI = 0.82–1.23, $p = 0.97$). A funnel plot was created (Figure 3) illustrating the risk ratio of mortality for group O patients versus study population size to evaluate for publication bias. This demonstrated some asymmetry with more smaller studies finding a lower risk ratio than the estimated true effect size.

DISCUSSION

Blood group in the existing literature

While blood group is best known for its critical role in crossmatching patients for blood product transfusions, it has also been found to play a potential role in numerous other clinical scenarios. For example, Liu et al. [32] demonstrated in a recent systematic review and meta-analysis that groups A and B exhibit a higher susceptibility to COVID-19 infection, and group O appears to be protective. This is thought to be due to a protective effect of the anti-A and anti-B antibodies that are produced by group O patients but are not produced in groups A and B, respectively, to avoid autoimmune haemolysis. ABO grouping is also an important determinant of circulating factor VIII and vWF levels, with group O patients exhibiting 25%–35% lower circulating concentrations [33–35]. These molecules play vital roles in coagulation and haemostasis with the absence or dysfunction of either resulting in severe coagulation disorders [4, 36]. In a 2013 meta-analysis, Dentali et al. [6] demonstrated a higher risk of haemorrhage of any aetiology among group O patients compared with non-O. Our group has previously reviewed the contribution of blood group to VTE risk, with group O possibly playing a protective role potentially also due to decreased circulating vWF and factor VIII levels [5, 37].

Synthesis of the meta-analysis

Overall, we found a high degree of variability among the reviewed studies both in their methods and outcomes. As detailed above, 5/13 (38.5%) [19, 22, 26–28] of the reviewed studies included all types of trauma patients and 4/13 (30.8%) [20, 21, 25, 27, 29] further narrowed this population to only ICU patients. Per Hamsen et al. [21], including only ICU patients ensured that 'patients were severely enough injured with a high risk of bleeding'. Additionally, the three Reilly et al. studies [20, 25, 29] excluded patients with iTBI, as many believe the determinants of outcomes for these patients are substantially different from those of polytrauma patients. On the other hand, Tutunjian et al. [24] included only iTBI patients. Miller et al. [30] included only trauma patients who received a transfusion within the first hospital hour, and Uzoigwe et al. [31] included only hip fracture patients. All studies other than Sauder et al. [28] that reported ISS had a mean ISS of >15, which is generally considered to be the cutoff for major trauma. Given the variation in study populations, it is unsurprising that there was also significant heterogeneity in the results of the studies.

Only 2 out of 10 studies evaluating mortality found a significantly lower survival in group O patients. Of note, both studies were conducted by Takayama et al. [23, 26], and these were also the only two studies to report higher mortality from haemorrhage and more ventilator days in group O patients. Across all studies included for meta-analysis, there was a weak trend towards higher average mortality rate among group O patients compared to non-O (11.0% vs. 9.6%); however, our meta-analysis did not find a statistically significant difference ($p = 0.23$). One potential explanation for this lack of significant difference among trauma patients is that factor VIII and vWF are acute phase reactants. The intense physiologic stress of a severe trauma may boost their circulating levels more effectively than other contexts in which a difference in outcomes related to blood group has been previously observed, such as cardiac surgery [38–40]. This may negate the effect of baseline differences in factor VIII and vWF among blood groups.

The slightly higher mortality in group O patients appears to be largely due to the very high group O mortality rates reported in the Takayama et al. studies, 28.2% among 284 group O patients in 2018 and 22.2% among 288 group O patients in 2021. These rates are much higher than even the next highest reported mortality rate among all other blood groups in all other included studies, 14.4%. On sensitivity analysis, the Takayama studies were found to contribute most significantly to heterogeneity, and exclusion of both was required to achieve an I^2 value <50%. The median ISS in the Takayama et al. studies were 18.5

TABLE 3 Mortality by blood group.

Author	Year	Mortality A	Mortality B	Mortality AB	Mortality O	Mortality non-O	*p* value
Reilly et al.	2015	21 (13%)	5 (6%)	1 (4%)	15 (7%)	27 (10.0%)	0.11
Takayama et al.	2018	30 (10.5%)	30 (14.4%)	11 (8.9%)	80 (28.2%)	71 (11.5%)	<0.001
Hamsen et al.	2019	55 (9.7%)	17 (10.4%)	5 (6.8%)	61 (12.7%)	77 (9.6%)	>0.05
Griffin et al.	2020	180 (13.0%)	70 (13.8%)	13 (13.8%)	262 (13.8%)	263 (13.0%)	>0.05
Sauder et al.	2021	57 (4.0%)	12 (2.8%)	8 (6.2%)	72 (4.0%)	77 (3.9%)	0.34
Takayama et al.	2021	48 (14.0%)	27 (14.5)	5 (5.0)	64 (22.2%)	80 (12.7%)	<0.001
Miller et al.	2022				24 (8.9%)	36 (13.3%)	0.11
Total		391 (9.4%)	161 (10.2%)	43 (7.4%)	578 (11.0%)	631 (9.6%)	

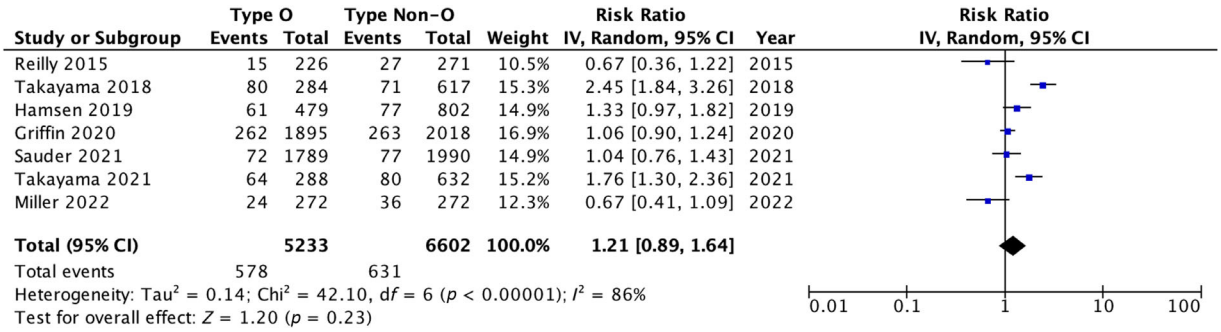

FIGURE 2 Mortality meta-analysis and forest plot.

TABLE 4 Evaluation of risk of bias in mortality results.

Study	Confounding	Measurement of exposure	Participant selection	Post-exposure interventions	Missing data	Measurement of outcomes	Selection of reported result	Overall bias
Reilly et al. (2015)	+/−							+/−
Takayama et al. (2018)	+/−							
Hamsen et al. (2019)	+/−							
Griffin et al. (2020)	+/−							
Sauder et al. (2021)	+/−							+/−
Takayama et al. (2021)	+/−							
Miller et al. (2022)	+/−							+/−

Note: Shaded areas illustrate risk of bias: green, low risk of bias; yellow, low risk of bias except uncontrolled confounding; orange, some concerns for bias; +/−, unclear if biased towards a positive or negative result.

(2018) and 18.11 (2021), which are on the lower end for mean ISS of the included studies. There was also no significant difference in ISS between blood groups, suggesting that higher injury severity alone does not account for the high mortality rates seen among group O patients in these two studies. Additionally, their 2021 study included patients from 12 different hospitals, making it unlikely the observed difference is due to the patient population or practices of a single hospital. As acknowledged by the authors, the patient population was primarily of Japanese descent, and the observed differences could be due to additional genetic variation that may not be present in other populations. Reilly et al. provide further evidence for different effects of blood group on different populations in their studies, which demonstrate that White trauma patients with group A blood are more likely to develop ARDS and AKI but did not find this to be true in Black patients [20, 25].

Limitations

Our study has limitations that are important to consider for its interpretation. This is a relatively smaller review with only 13 studies included and only 7 studies felt to be sufficiently comparable to include in meta-analysis. The small number of included studies increases the chance that anomalous findings in a single study will affect the analysis of the combined data, as was at least partially

TABLE 5 Summary of findings

Certainty assessment						Number of patients		Effect		
Number of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Group O	Group non-O	Relative (95% CI)	Absolute (95% CI)	Certainty
7	Observational studies	Not serious[a]	Very serious[b]	Not serious	Serious[c]	578/5233 (11.0%)	631/6602 (9.6%)	RR 1.21 (0.89–1.64)	20 more per 1000 (from 11 fewer to 61 more)	⨁⨁◯◯ Low

Abbreviations: CI, confidence interval; RR, risk ratio.
[a]Risk of bias present due to non-randomized studies.
[b]High inconsistency due to high degree of variability in results.
[c]High imprecision due to large 95% CI.

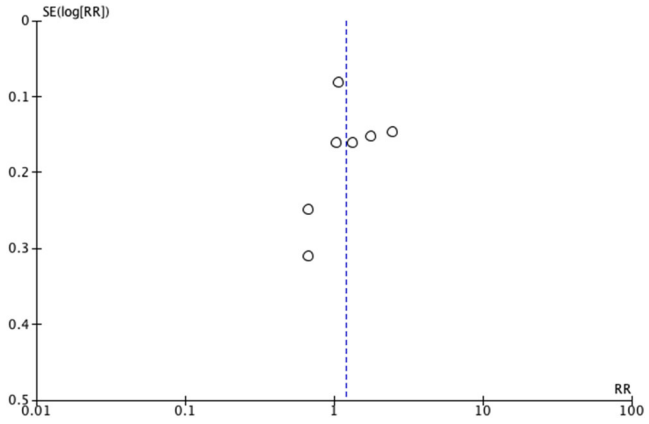

FIGURE 3 Funnel plot of risk ratio of mortality for group O patients versus study population size.

demonstrated in the sensitivity analysis. Furthermore, due primarily to the high variability of results between studies, the certainty of the results of our meta-analysis was assessed to be low as evaluated using the GRADE tool. There was also not a sufficient number of existing studies to perform meta-analysis evaluating for a relationship between blood group and patient outcomes other than mortality. Trauma patients encompass a broad population of injury mechanisms and patient demographics, and as previously discussed, there was high variability between studies in the populations included. This variability makes it difficult to draw conclusions about trauma sub-populations as well as to determine under what specific circumstances blood group may contribute to patient outcomes. We also did not investigate Rhesus antigens, nor were genotypes considered, though some evidence exists for clotting factor variation among the $A_1A/A_1B/BB$ versus A_1O/BO versus OO genotypes [41]. Finally, as in all reviews, our study was also subject to publication bias and could not integrate results from studies that may not have been published. The funnel plot for our study demonstrated potential bias towards negative findings with more smaller studies finding a lower risk ratio than the estimated true effect size. However, this tool is limited in its ability to accurately detect bias given the small number of studies.

Areas for further research

In this study, we find that the existing literature demonstrates uncertainty regarding whether there is a mortality difference between trauma patients with O and non-O blood groups. There is high variability in results and methods among studies, and further research is needed to delineate under what circumstances the blood group may influence patient outcomes. Here we provide suggestions to guide future research on this topic. As it is done in many trauma cohorts, patients with severe iTBI can be analysed separately as this subgroup experiences an inherently different hospital course and its outcomes may be uniquely affected by differences among blood groups [37]. Future analysis of mortality can be stratified by cause of death with a particular focus on exsanguination, as blood group may play a significant role in this mechanism but may not in others. The contribution of blood group to patient outcomes among different patient populations may warrant further investigation given multiple studies finding variation in the role of blood group based on demographic factors. Lastly, further studies are needed examining the relationship between blood group and trauma patient complications other than mortality such as AKI, ARDS and DVT, as there are currently few such studies published.

CONCLUSION

In this systematic review and meta-analysis, we find no consistent difference in trauma patient outcomes based on blood group. Due to high variability between studies in methods and results, further research is needed to determine if blood group contributes to trauma patient outcomes under specific clinical conditions and in particular patient populations.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

An international comparison of haemoglobin deferral prediction models for blood banking

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Abstract

Background and Objectives: Blood banks use a haemoglobin (Hb) threshold before blood donation to minimize donors' risk of anaemia. Hb prediction models may guide decisions on which donors to invite, and should ideally also be generally applicable, thus in different countries and settings. In this paper, we compare the outcome of various prediction models in different settings and highlight differences and similarities.

Materials and Methods: Donation data of repeat donors from the past 5 years of Australia, Belgium, Finland, the Netherlands and South Africa were used to fit five identical prediction models: logistic regression, random forest, support vector machine, linear mixed model and dynamic linear mixed model. Only donors with five or more donation attempts were included to ensure having informative data from all donors. Analyses were performed for men and women separately and outcomes compared.

Results: Within countries and overall, different models perform similarly well. However, there are substantial differences in model performance between countries, and there is a positive association between the deferral rate in a country and the ability to predict donor deferral. Nonetheless, the importance of predictor variables across countries is similar and is highest for the previous Hb level.

Conclusion: The limited impact of model architecture and country indicates that all models show similar relationships between the predictor variables and donor deferral. Donor deferral is found to be better predictable in countries with high deferral rates. Therefore, such countries may benefit more from deferral prediction models than those with low deferral rates.

Marieke Vinkenoog and Jarkko Toivonen shared first authorship.

Mikko Arvas and Mart Janssen shared last authorship.

Keywords
donor health, haemoglobin deferral, haemoglobin measurement, prediction

Highlights

- Within countries, different haemoglobin deferral prediction models perform similarly well.
- The relative importance of predictor variables is very similar across countries.
- Performance of models in different settings is dependent on the deferral rate. As a result, prediction models may be of higher value in countries with higher deferral rates.

INTRODUCTION

To avoid blood donations by donors at risk of becoming anaemic, blood banks test the donors' haemoglobin (Hb) levels. In case of pre-donation testing, a low Hb level leads to on-site deferral, which is demotivating for donors and makes them less likely to return to the blood bank than non-deferred donors [1, 2]. Additionally, it is in the interest of blood banks to keep deferral rates low to save time and costs. The ability to accurately predict low Hb deferral and adjust donation intervals based on these predictions likely decreases deferral rates. In the last 15 years, various Hb deferral prediction models, such as multiple logistic regression models [3], Bayesian linear mixed models (LMM) [4, 5] and ensemble models [6], have been evaluated by blood banks. Most prediction models use donors' previous Hb measurements in combination with donor characteristics such as age and sex, but the prediction accuracy has been modest. Nonetheless, even models with modest accuracies could be beneficial in practice [5]. Accurate prediction of Hb levels and/or deferral remains a difficult task, as many factors affect Hb, and both intra- and inter-individual variation is large. Therefore, it stands to reason that machine learning models might improve the prediction accuracy over the traditional regression models, as they are capable of learning more complex associations between predictors and outcome variables. Support vector machines (SVMs) have been shown to predict Hb deferral in Dutch donors reasonably well [7], as do random forests (RFs) in Finnish donors [5].

Most prediction models are developed and validated on donation data of a single country [3, 6]. Between countries, sets of available predictor variables differ widely. Ferritin levels, genotyping data, smoking status and iron supplementation are examples of variables that are associated with Hb levels but are not systematically measured or recorded by most blood banks [8]. Therefore, prediction models using such variables cannot be applied to data from other blood banks. Additionally, differences in blood bank policies regarding donor deferral require models to be calibrated for each country separately.

The SanguinStats group is a collaboration of statisticians and epidemiologists from several countries carrying out research in the area of donor health. It currently consists of researchers from blood banks in Australia, Belgium, Denmark, Finland, the Netherlands, South Africa and the United Kingdom, as well as researchers with statistical expertise who are associated with research institutes other than blood banks. The aim of the SanguinStats group is to combine the available expertise and data sources to develop and evaluate the outcome of state-of-the-art models in various settings.

In this first joint paper, we present a comparison of various Hb deferral prediction models on data from five blood banks. The goal of this research is not to create the best performing predictor, but rather to use exactly the same models for all datasets and to compare the performance and importance of variables between countries. Therefore, only basic predictor variables that are available in all individual countries are included in the models. Comparing the importance of variables between countries will show whether models show the same relationships between the variables and Hb deferral.

This is the first study to compare multiple Hb deferral prediction models on datasets from multiple countries. The results can be used by other blood banks to anticipate benefits from collecting additional measurement data and the use of various predictors for the prediction of donor deferral.

MATERIALS AND METHODS

Data sources and variables

Within each country, data were extracted from the blood banks' database, selecting data from whole blood donors from the past 5 years. The exact years differ per country because of the availability of up-to-date datasets. For each country, the timeframe of data collection was carefully selected to minimize iron-related blood bank policy changes in the dataset. In Australia, Finland and the Netherlands, there is one national blood bank (Australian Red Cross Lifeblood, Finnish Red Cross Blood Service and Sanquin Blood Bank, respectively), and data from these blood banks were used. In Belgium, data from Red Cross Flanders were used, which covers the whole of Flanders. In South Africa, data from South Africa National Blood Service were used, which is the major blood bank in the country.

For this study, only donors with five or more donation attempts were included to balance the trade-off between prediction accuracy (which has been shown to decrease with shorter time series at least in LMM) and data availability, as data becomes scarcer with higher thresholds of minimum donation numbers [5].

The following donation-level variables are used in the prediction models:

- Donor age ('Age')
- Days to previous donation ('Days to previous whole blood donation')
- Time of day at the start of the donation ('Time')
- Hb level at first donation ('First Hb') (not used by dynamic linear mixed model [DLMM])
- Hb level at previous donation ('Previous Hb') (not used by linear mixed model [LMM])
- Low Hb at previous donation ('Previous visit low Hb')
- Warm season (April–September for Northern hemisphere and October–March for Southern hemisphere) ('Warm season')
- Number of consecutive deferrals since previous successful donation ('Consecutive deferrals')
- Number of successful donations in last 5 years ('Recent donations')
- Number of low Hb measurements in the last 2 years ('Recent low Hb')

Models were fitted separately for male and female donors. Unless otherwise specified, the analyses presented in this study were performed on a random subset of 10,000 donors per sex, to prevent differences in model performance between countries due to different dataset sizes. The outcome is a dichotomous variable: deferral or non-deferral.

Statistical methods

Five prediction models were compared in this study: a baseline model, RF, SVM, LMM and DLMM. Note that these models are fundamentally very different. Each of the models is briefly described below.

The baseline model is a simple logistic regression model that estimates the likelihood of deferral as a function of only the Hb level at the previous donation.

RF is a classification algorithm that consists of several decision trees, fitted on sub-samples of the data. It uses averaging to improve predictive accuracy and prevent overfitting. The prediction output of an RF is the class selected by the majority of the decision trees. The RF takes as input all predictor variables listed in the previous section.

SVM is a classification algorithm that aims to find the best hyperplane to separate both outcome classes in a multi-dimensional space. The SVM again takes all predictor variables listed in the previous section as input. Note that none of the three models mentioned above explicitly models the subsequent donations, but rather uses aggregated information on donation history (see list above). This is where these differ from LMM and DLMM, which include a donor-specific intercept as the only random effect.

LMM does not include previous Hb as a predictor, but instead uses the first Hb level. DLMM, however, does include the previous Hb as a predictor. Both LMM and DLMM are regression models that predict not Hb deferral but the actual Hb level. If this predicted Hb level is lower than the country-specific donation threshold, deferral is predicted. These LMMs were trained in a Bayesian setting with weakly informative conjugate priors. They are described in more detail in a previous article [5], and they are essentially simplified versions of the models proposed by Nasserinejad et al. [4], excluding the modelling of the temporary reduction in Hb after blood donation.

Model performance is assessed using the area under the precision–recall (AUPR) curve. As no perfect model exists, each model provides an estimate of the probability of deferring a donor. Depending on the probability that is applied as a classification threshold (so anyone with a higher probability of deferral is labelled 'deferral' and the others 'non-deferral'), a different number of correct and incorrect predictions will be found. The precision–recall curve is a graph in which the recall versus the precision of a prediction model at varying classification thresholds is shown, where precision is the proportion of correctly predicted deferrals of all predicted deferrals and recall is the proportion of all deferred donors that were correctly labelled as such. The higher the AUPR curve, the better the prediction model's performance. To fairly compare AUPR across countries, we adjusted the AUPR values by subtracting the countries' deferral rate. The adjusted value now indicates the improvement by the model over always predicting non-deferral.

SHapley Additive exPlanations (SHAP) values were used to quantify the contribution of each predictor variable to the prediction for each individual observation [9]. Because SHAP values are model-agnostic, they can be calculated and compared for each model. This results in variable importance measures even for models that do not have interpretable coefficients, such as RF and SVM.

Docker container

To ensure that all collaborators perform exactly the same analyses, but without having to export data outside of their organization or between jurisdictions, we implemented all models for Hb-deferral prediction in a Docker container whose development was started earlier [5]. The Docker platform is easy to install on all major operating systems. After installation, the Docker container image can be downloaded and the user can run all models presented in this paper in a secure environment (without requiring an internet connection). For this study, we added an implementation of the SVM to the container, in addition to some specific improvements to facilitate the comparison of outputs. Both the ready-to-use container image [10] and its source code [11] are freely available through Dockerhub and Github, respectively. All analyses presented in this paper were obtained using version 0.32 of the container. Analyses of the results were performed using the R language and environment for statistical computing (version 4.2.0) [12], using packages dplyr (version 1.0.9) [13] and tidyr (version 1.2.0) [14] to handle data, and ggplot2 (version 3.3.6) [15] to create graphs.

RESULTS

Table 1 shows the distribution of the predictor variables in all countries.

TABLE 1 Distributions of predictor variables in all four datasets.

Visits by male donors					
Variable	**Australia**	**Belgium**	**Finland**	**Netherlands**	**South Africa**
Number of donors	10,000	8552	10,000	10,000	10,000
Age in years	41 (29–54)	39 (25–52)	53 (41–60)	52 (39–60)	44 (33–54)
Mean consecutive deferrals	0.003	0.025	0.018	0.029	0.213
Days to previous donation	98 (84–167)	99 (90–182)	106 (77–168)	92 (70–147)	73 (59–118)
Hb in g/L	149 (142–157)	153 (147–159)	154 (147–162)	148 (142–156)	153 (142–163)
Proportion of Hb deferrals	0.004	0.022	0.018	0.029	0.129
First Hb level in g/L	150 (143–158)	154 (148–160)	155 (147–162)	150 (143–158)	153 (140–163)
Time of day as hour between 0 and 24	13.1 (10.8–15.6)	18.9 (17.8–19.7)	14.8 (13.1–16.4)	16.3 (13.1–18.7)	12.8 (11.2–14.6)
Hb level at previous visit in g/L	148 (139–156)	151 (143–158)	153 (144–161)	148 (140–155)	151 (137–162)
Proportion of low Hb at previous visit	0.003	0.020	0.018	0.030	0.124
Mean recent low Hb	0.008	0.066	0.074	0.127	0.553
Recent donations	4 (2–6)	4 (2–6)	5 (2–9)	5 (2–9)	4 (2–7)
Warm season proportion	0.500	0.477	0.491	0.494	0.524
Visits by female donors					
Variable	**Australia**	**Belgium**	**Finland**	**Netherlands**	**South Africa**
Number of donors	10,000	9028	10,000	10,000	10,000
Age in years	37 (25–50)	34 (21–47)	50 (35–58)	47 (31–57)	41 (31–52)
Mean consecutive deferrals	0.016	0.131	0.040	0.057	0.146
Days to previous donation	104 (87–183)	111 (91–196)	140 (106–224)	154 (132–222)	84 (62–156)
Hb in g/L	133 (127–140)	135 (130–141)	140 (133–147)	135 (129–142)	136 (128–144)
Proportion of Hb deferrals	0.021	0.106	0.038	0.054	0.141
First Hb level in g/L	134 (128–141)	137 (132–143)	141 (134–147)	135 (129–142)	135 (128–144)
Time of day as hour between 0 and 24	13.1 (11.0–15.4)	18.7 (17.7–19.6)	15.3 (13.5–16.6)	15.5 (13.1–18.3)	13.0 (11.4–14.7)
Hb level at previous visit in g/L	131 (124–138)	134 (126–140)	138 (130–146)	134 (127–142)	134 (125–143)
Proportion of low Hb at previous visit	0.017	0.096	0.040	0.057	0.127
Mean recent low Hb	0.039	0.287	0.110	0.151	0.390
Recent donations	3 (1–5)	3 (1–5)	4 (2–6)	3 (1–6)	3 (1–6)
Warm season proportion	0.506	0.472	0.504	0.491	0.523

Note: Numerical variables are described by their median and (interquartile range) unless otherwise stated. Dichotomous variables are described by the proportion of visits where the value was true.
Abbreviation: Hb, haemoglobin.

TABLE 2 Haemoglobin (Hb) measurement and donor deferral policies per country.

Country	When and how is Hb measured?	When is the donor deferred?
Australia	Capillary skin-prick Hb measurement by haemoglobinometer before each donation. If the Hb is below the threshold, a venous sample is taken from the non-donation arm and Hb is measured using the haemoglobinometer at the donation site to confirm.	Hb levels below 120 g/L (women) or below 130 g/L (men) as well as donors with a 20 g/L drop in Hb level relative to their previous donation.
Belgium	Haematology analyser Hb measurement from venous sample after every successful donation. Capillary skin-prick Hb measurement before donation for new donors and for donors with a venous Hb below the eligibility threshold at the previous donation.	Hb level below 125 g/L (women) or below 135 g/L (men) at previous and current donation.
Finland	Capillary skin-prick Hb measurement point of care (POC) before each donation. If the Hb is below threshold, venous sample is taken and Hb measured by POC device at donation site [19].	Hb level below 125 g/L (women) or below 135 g/L (men) as well as donors with a 20 g/L drop in Hb level relative to their previous donation.
The Netherlands	Capillary skin-prick Hb measurement before each donation. If a Hb level is below the threshold, the measurement is repeated (up to three times in total). The highest value is used for the deferral decision. Since late 2017, donors are also deferred for low ferritin levels.	Hb level below 125 g/L (women) or below 135 g/L (men).
South Africa	Capillary skin-prick Hb measurement before each donation.	Hb level below 120 g/L (women) or below 130 g/L (men). Before 2020, cut-off levels of 125 and 135 g/L were used.

Hb measurement and deferral policies

All participating countries use Hb measurements to defer donors, but there are differences in how Hb is measured and when donors are deferred. Table 2 shows a summary of Hb-deferral-related policies per country.

Comparison of model performance

Figure 1 shows the AUPR values (adjusted for deferral rate) and their confidence intervals for all models for all countries. All models outperform the baseline model in all countries. Performance of different models does not differ greatly within one country, except for Australian female donors, for which RF and SVM clearly outperform the LMM and DLMM. The same pattern is visible in South African male donors, although less obvious, and slightly in Belgium. In general, variation in within-country model performance is much smaller than variation between countries. Belgium and South Africa obtain significantly higher AUPR values than the other three countries in all models, except for the high-performing RF and SVM on Australian female donors.

Tables 3 and 4 show the predicted versus observed outcomes of the model with the lowest AUPR (baseline model, female donors, Finland; unadjusted AUPR = 0.07) and the model with the highest AUPR (RF, male donors, South Africa; unadjusted AUPR = 0.69) to illustrate the AUPRs with actual case counts to make the results more tangible.

Figure 2 shows the deferral rate and AUPR for all countries and models. Even though the AUPR values are adjusted for the deferral rate, there is still a positive correlation between deferral rate and (adjusted) AUPR. All models show the same pattern for this association. Again, we see that for Australian female donors the RF and SVM obtain a much higher AUPR than expected based on the deferral rate.

To further investigate whether the low deferral rates indeed affect the ability of the models to predict deferral, we intentionally modified the deferral rate of the Belgian datasets by removing a varying proportion of the deferred donors from the dataset and refitting the models on these adapted datasets. The results are shown in Figure 3. This figure clearly shows the positive association between deferral rate and AUPR. There is no monotonically increasing association even though the datasets with lower deferral rates are subsets of the datasets with larger deferral rates. The fact that classification tasks are more difficult when there is a large imbalance between outcome classes is a well-known phenomenon in statistics [16].

Importance of individual variables

Figure 4 shows the variable importances derived from SHAP values calculated on a random subset of 1000 donors from the validation data. Variable importances are presented as mean absolute attribution (MAA) values. Variables are sorted by MAA over all countries and models (represented by the horizontal bars). For each individual country, the MAA values are provided and connected by a line.

RF and SVM

Comparing variable importances between countries within the same model allows identification of differences in predictive power of individual model parameters. In the RF and SVM models, previous Hb is the most important predictor for all countries and sexes and has almost twice the MAA of the second-most important predictor. The MAA for most variables is similar across countries. There are some

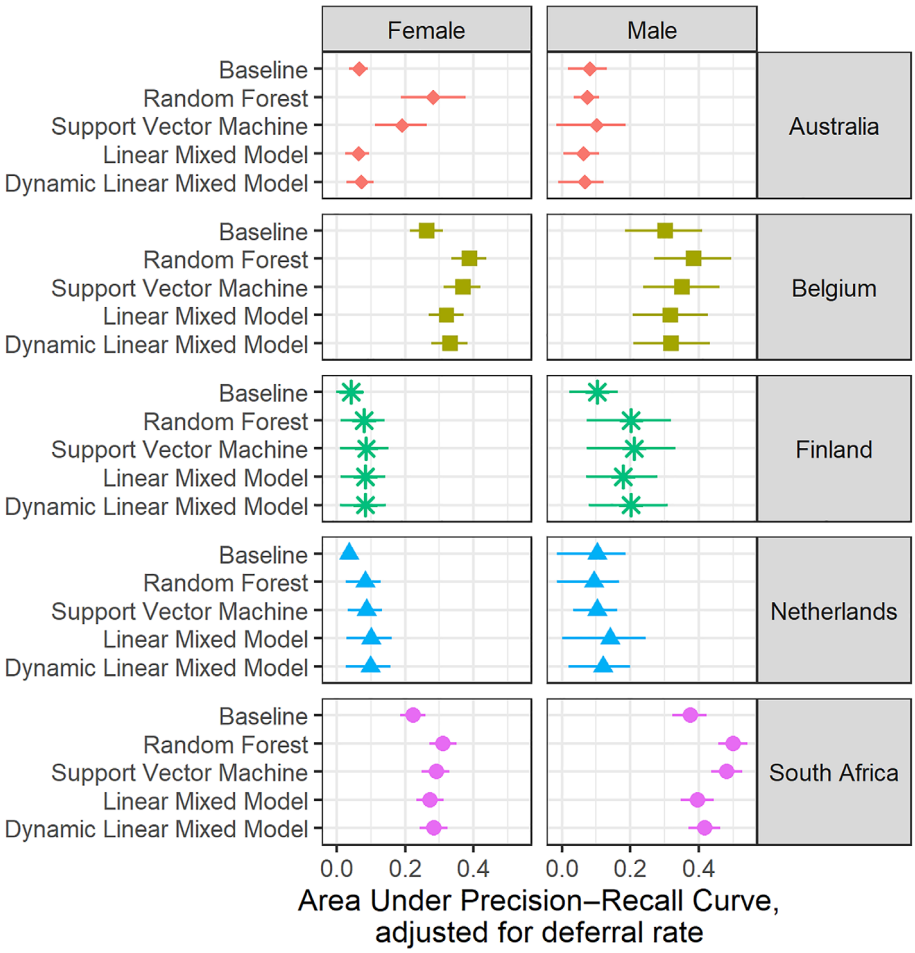

FIGURE 1 Area under the precision–recall (AUPR) curve for all countries and all models. Note that each AUPR curve is adjusted by subtraction of the country's deferral rate.

TABLE 3 Observed versus predicted outcomes of the baseline model applied to female Finnish donors.

	Observed outcome	
Predicted outcome	**Accepted**	**Deferred**
Accepted	1146	10
Deferred	807	37

Note: This is the model with the lowest area under the precision–recall (0.07). The precision of class deferral is 0.04 and the recall is 0.79.

TABLE 4 Observed versus predicted outcomes of the random forest model applied to male South African donors.

	Observed outcome	
Predicted outcome	**Accepted**	**Deferred**
Accepted	1433	108
Deferred	195	264

Note: This is the model with the highest area under the precision–recall (0.69). The precision of class deferral is 0.58 and the recall is 0.71.

exceptions, however: for South Africa, the number of recent low Hb measurements is much more important than in other countries, as well as the deferral status of the previous blood bank visit. For Belgium, whether the donation visit took place during the warm season is more important than in the other countries.

Linear and dynamic linear mixed models

For the LMMs, the MAA of variables show the highest similarity between countries. A donor's first Hb measurement is the most important predictor, and all other predictor variables have a relatively low MAA in comparison. Conversely, for DLMMs, there is much more variation in MAA values between countries and between sexes. For female donors, the most important predictor is age, and previous Hb is only the third-most important predictor, which deviates considerably from what was found for all other models. In both LMM and DLLM, the difference in MAA for age between sexes is much larger than in RF and SVM models.

Unlike the RF and SVM models, the LMM and DLMM estimate regression coefficients that may be compared across countries. For

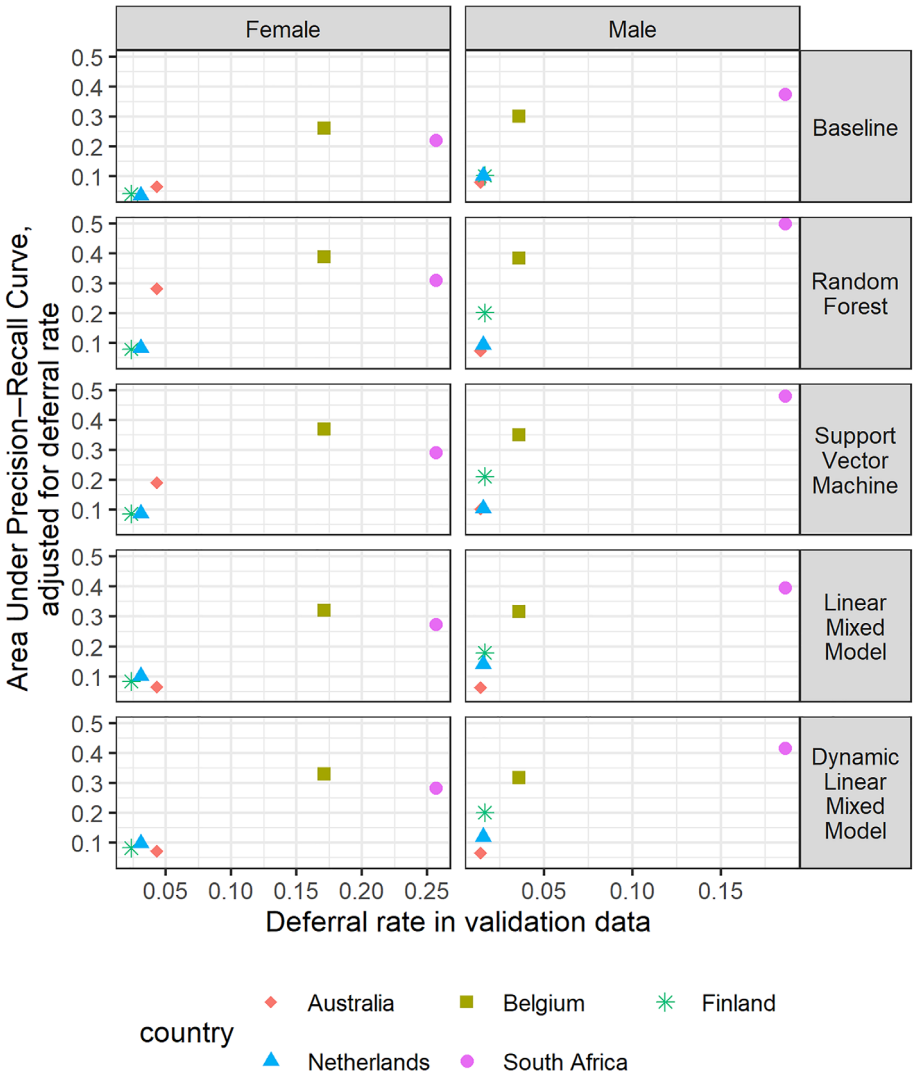

FIGURE 2 Adjusted area under the precision–recall value versus deferral rate in various settings for various models.

consistency with other model results, we compared the MAA output rather than regression coefficients. A comparison of regression coefficients can be found in Supplementary Material. For all variables except for 'Low Hb at previous visit' (which is the second to last most important predictor), coefficients are very similar between countries and 95% highest posterior density intervals mostly overlap.

Absolute value of MAA per model

It should be noted that the MAA values for different models are on different scales. In the baseline and SVM, SHAP values are on the log-odds scale, while for the RF and (dynamic) LMM, these are expressed on the probability scale. Since only the relative size of MAA values within models are compared, the difference in scales has no effect on the interpretation of the results.

The effect of sample size

We fitted the same models as above on the full datasets from Finland, the Netherlands and Australia to see whether this improves performance. This experiment showed that using the full dataset increases performance only by a very small amount and within the size of the confidence interval for the subsample of 10,000 donors.

DISCUSSION

In this paper, various prediction models for Hb deferral were applied to blood bank visit data from five countries to investigate the performance of prediction models in different settings. In all countries, the baseline was outperformed by all other models, although the overall performance was quite low for all models in all countries. Model performance, however, varies considerably between countries, and a high deferral rate is associated with better model performance. The relative

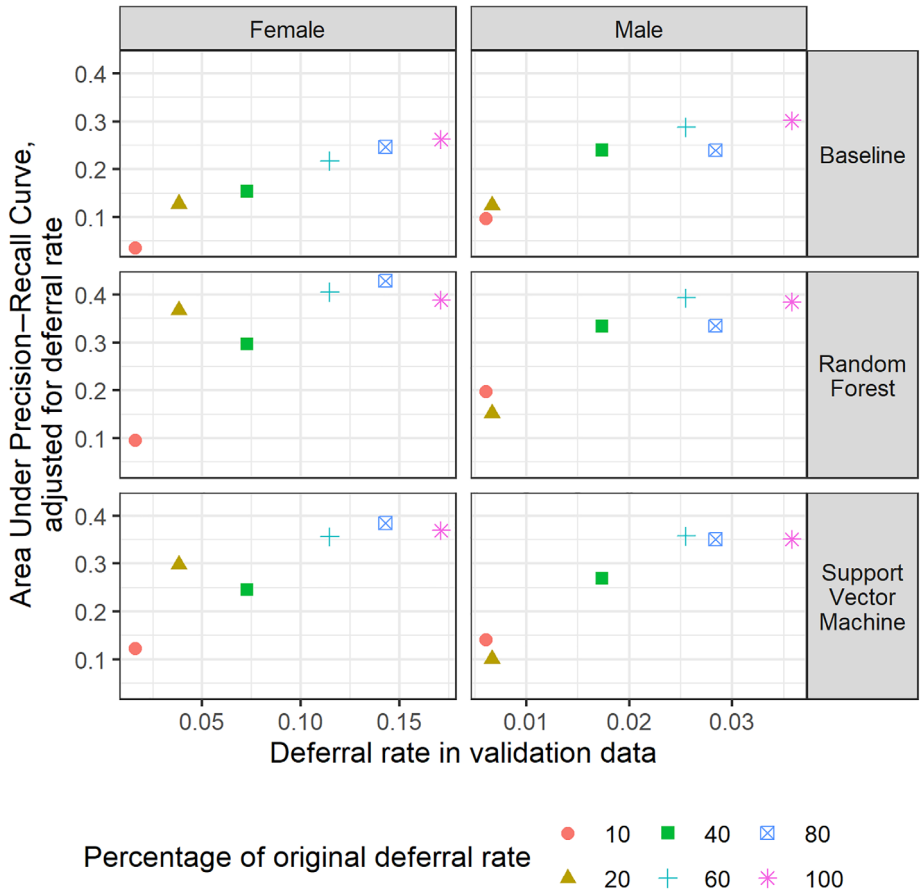

FIGURE 3 Adjusted area under the precision–recall as a function of the deferral rate for various deferral levels in the Belgian dataset. The reduction in deferral rate was obtained by sequentially removing an increasing number of deferred donations from the data.

importance of individual predictors is very similar in different countries. In particular, the Hb level at previous donation is an important predictor for donor deferral in almost all models. This indicates that models learn the same associations in different settings, which supports the idea that these associations are the result of similar biological processes underlying donor deferral.

The similarity of the relative importance of predictors also indicates that the differences in performance are not caused by different associations between predictors and Hb deferral. Rather, deferrals are more difficult to predict in countries with low deferral rates as there are fewer deferrals. The experiment with the Belgian data, which shows that the predictability collapses with a decrease in deferral rate, supports this finding. However, there appears to be an exception with the Australian data on female donors, where a relatively high AUPR is obtained for two models despite the very low deferral rate. Another possible explanation for the difference in performance could be that data collected in some countries is more informative than in others, for instance due to differences in the accuracy of Hb measurements and/or differences in deferral policies. However, we were unable to confirm this as a plausible hypothesis: Hb deferral is based on the same capillary measurement in South Africa and the Netherlands, and yet model performance on South African data is much higher than on Dutch data.

This study is the first to compare prediction models for Hb deferral across different settings. By focusing on the comparison of models between countries rather than optimizing model performance based on variables available within a single country, the effect of the setting on model performance becomes visible. We show that low deferral rates substantially limit model performance, although they do not hinder the model in learning the same associations as with higher deferral rates. Comparing results for male donors from Australia and South Africa illustrates this perfectly: the deferral rate in South Africa is more than 10-fold than in Australia (18.6% vs. 1.4%), resulting in a much higher AUPR (0.50 vs. 0.08 for RF), yet the variable importance is very similar.

Our findings are also in line with previously published work on Hb deferral prediction, which consistently shows that previous Hb measurements are by far the most important predictor [3, 5, 8]. Another interesting finding is that LMM, which is the only model to use a donor's first Hb instead of the previous Hb, performs just as well as the other models. This may indicate that most donors' Hb levels are quite stable over time, and that predictions of personalized donation intervals can already be made after a first Hb measurement at donor intake. To account for sudden drops in Hb level, inclusion of the previous Hb seems to be more relevant. The importance of first Hb levels is also shown by others [17], which indicates that iron dynamics

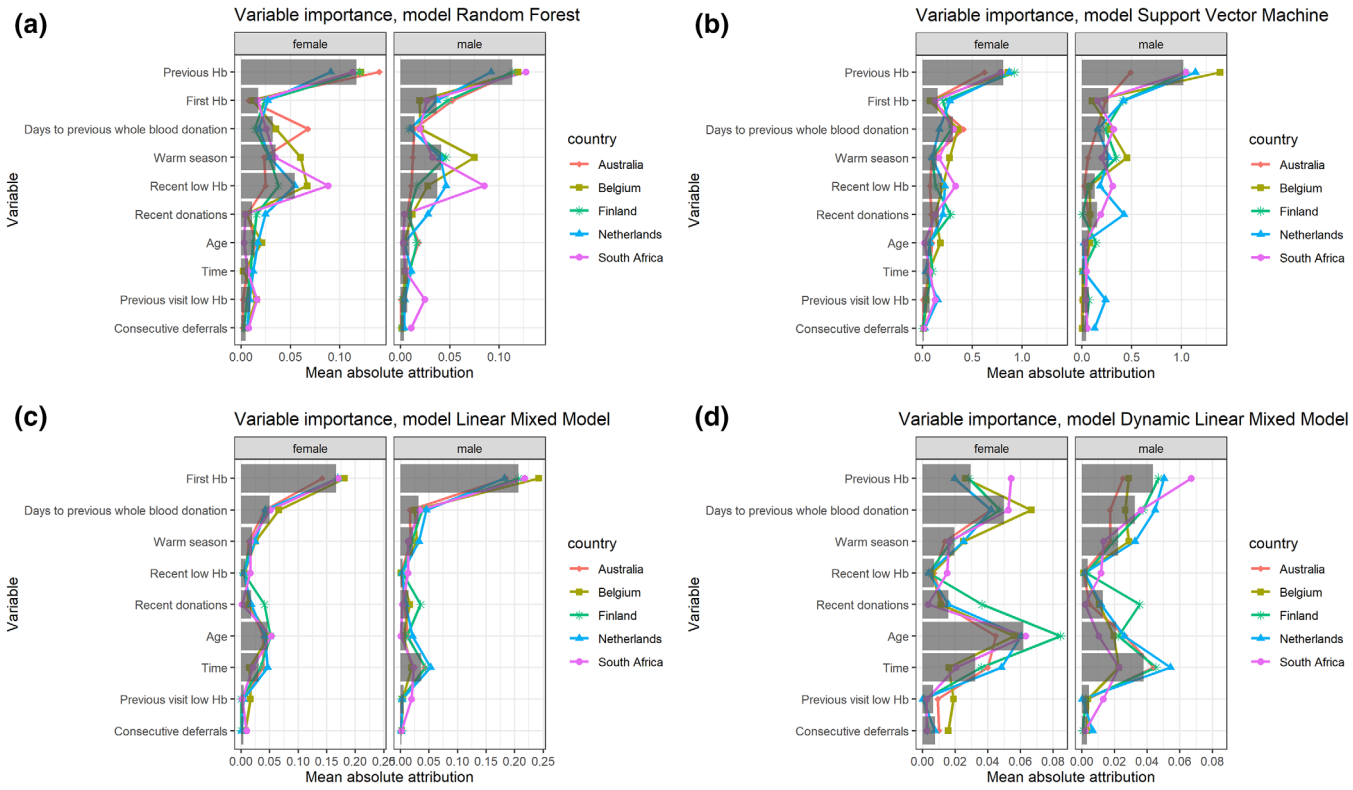

FIGURE 4 (a–d) Variable importance (mean value and per individual country) determined by the mean absolute attribution according to SHapley Additive exPlanations values for various models and sexes. The bars indicate the mean over all countries. Variables are ordered by the mean mean absolute attribution over both sexes and all models. Hb, haemoglobin.

(Hb and ferritin levels) in blood donors can be predicted over a longer period from the Hb and ferritin levels at donor intake.

Although this study offers new insights into the predictability of donor deferral in different settings, the actual predictive value of the models is low, which may be explained by the substantial variability in Hb measurement outcomes [18]. Note also that all analyses were done on donors with at least five donation attempts, which limits the generalizability of the models to the full donor population. Many blood banks collect more variables than were used in the predictions in this study and including those may improve model performance. Improved performance is paramount, as a model will create added value for the blood bank only when the benefits of the correctly predicted deferrals will outweigh the loss due to incorrectly predicted deferrals. The prediction of a potential reduction of donation intervals by some donors by the model may again add to the value of applying such prediction models.

Currently, the development of prediction models requires extensive expertise and data to enable prediction of donor deferral. Ideally, the work and insights developed by this collaboration would result in strategies that could also be of use to countries with limited resources.

In conclusion, this study shows that model architecture in most cases has a limited impact on the performance of prediction models for donor deferral, but in some cases, exemplified by Australia, certain model architectures can capture the data better than others. It would be recommended for any new country starting with Hb deferral prediction to try several architectures if possible. Adding better predictor variables to the different model could considerably improve predictive performance. Performance is strongly affected by the donor deferral rate. For most countries with low deferral rates, prediction models are unlikely to contribute to an effective reduction of donor deferral rates. Conversely, deferral prediction models may be applied in countries with high deferral rates to reduce on-site deferral of donors. Hb deferral remains a relevant topic, as it negatively affects both donors and blood services. By joining efforts, we can enhance our understanding of which generic factors affect donor deferral and to what extent. Also, only by studying the performance in different settings, organization-specific and operational characteristics may be identified that enhance or deteriorate prediction models' performance, which may indicate directions for further research and meaningful policy changes.

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M.V., T.B., D.d.C., S.K. and M.W. analysed the data; M.V. aggregated the results; M.V. wrote the paper; M.V., J.T., T.B., D.d.C., V.C., S.K., M.W., A.M., K.v.d.H., J.v.R., E.L., M.A. and M.J. reviewed and edited the paper.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Evolving deferral criteria for blood donation in France: Plasma donation by men who have sex with men

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Abstract

Background and Objectives: Since the advent of AIDS, men who have sex with men (MSM) have often been deferred from blood donation. In France, quarantine plasma donation by MSM donors with the same deferral rules as for other donors was introduced in July 2016 and continued up to March 2022. At this time, MSM-specific deferral criteria were lifted for all blood or plasma donation. The donor deferral, as well as rate of infectious markers in plasma donors who would have been otherwise deferred for MSM activity, was evaluated and compared with those of the other donors during the same time period from June 2016 to March 2022.

Results: A total of 8843 MSM donors made 12,250 plasma donation applications. The overall deferral rate was very high (75.2%), mainly due to the absence of apheresis capacity at the donation site. The deferral criteria for sexual risk were present in 12.1% of MSM donors compared with 1.0% in other plasma and blood donors ($p < 0.001$). Overall, 994 MSM donors made 2880 plasma donations. Of these, one donation was HIV positive (34.7 vs. $0.6/10^5$ donations by other donors, relative risk [RR]: 61.0 [95% confidence interval [CI]: 8.5–437.7]), one was HBV positive (34.7 vs. $4.5/10^5$, RR: 7.7 [95% CI: 1.1–54.6]) and none were HCV positive (0 vs. $2.4/10^5$). Additionally, 21 donations were syphilis positive (729.2 vs. $10.7/10^5$, RR: 67.9 [95% CI: 44.2–104.4]). A post hoc analysis of eligible MSM donors who were unable to donate plasma due to logistic constraints yielded similar findings.

Conclusion: Plasma donation by donors who would have been otherwise deferred for MSM activity was associated with both an increased deferral rate for sexual risk and an increased rate of infectious markers, notably syphilis.

Keywords

blood donation deferral criteria, blood donor, infectious risk, men who have sex with men, quarantine plasma donation

Highlights

- Plasma donation by donors who would have otherwise been deferred for being men who have sex with men (MSM) was associated with an increased deferral rate for sexual risk and an increased rate of infectious markers.

- This increased rate of infectious markers was primarily due to syphilis as assessed by treponemal serology.
- These initial results regarding infectious markers in MSM donors who are subject to the same deferral rules as other blood donors require further studies.

INTRODUCTION

In addition to ensuring the quality of blood components and appropriateness of transfusion, the prevention of transfusion-transmitted infections is of paramount importance [1]. The prevention of known and emerging transfusion risks primarily relies on blood donor deferral policies for risk exposure, pathogen screening of blood donation and/or pathogen reduction of blood component. Since the advent of AIDS, and up until recently, male blood donors reporting histories of sexual contact with other men (MSM) were often permanently deferred from donating blood [2].

Increasingly, highly sensitive screening tests have limited the HIV and hepatitis virus transfusion-transmission risks to donations during a very short infectious window period. Therefore, temporary deferral may be applied without incurring any significant risk to recipients [3]. Furthermore, MSM-specific blood donation deferral may be viewed as disproportionate compared with deferrals for other risks exposures, and therefore be perceived as discriminatory and overall be misunderstood [4]. Lastly, such a perception could reduce the willingness to donate blood, particularly among young people, and possibly increase overall non-compliance with deferral criteria. A better understood individualized behaviour-based deferral policy may therefore favour blood donation while increasing donor adherence to deferral criteria and ultimately increasing recipient safety.

In France, the deferral period for MSM for blood donation was reduced from permanent to one year since last sexual activity in July 2016 [5]. A large proportion of plasma intended for transfusion is quarantined until repeat testing at the time of a new donation at least 2 months later, enabling the detection of blood-borne virus window period donation [6]. It was reasoned that such a blood donation could be made by MSM under the same conditions as other donors, that is, no more than one sexual partner over the last 4 months. By means of repeat testing at a gap after donation, quarantined apheresis plasma donation also offered the unique opportunity to safely assess the epidemiology of sexually transmissible pathogens such as HIV among MSM blood donors subjected to the same deferral rules as other donors. It was therefore decided to allow one notable exception to the MSM one-year deferral criteria: quarantined apheresis plasma donation with deferral rules identical to those applied to all other plasma and blood donors. This measure was implemented in July 2016 [7].

Time-dependent deferral for MSM was further reduced to 4 months in April 2020 in France following reassuring risk analyses [8]. Plasma for fractionation apheresis donation by MSM with deferral rules identical to those applied to other plasma and blood donors was authorized in April 2020 as well. In March 2022, all MSM-specific deferral criteria were lifted for blood or plasma donation in France [9]. The only new deferral criteria introduced in March 2022 is a 4-month deferral for those who have used pre- or post-exposure HIV prophylaxis. Importantly the deferral criteria for more than one sexual partner over the last 4 months for the donor as well as their partner has been in place in France for over 30 years.

These changes in MSM deferral periods relied on risk analysis demonstrating no increase in the residual risk of HIV transmission when reducing the deferral period from 12 to 4 months, and a slight increase when removing MSM-specific deferral criteria (HIV residual risk: 0.23 per 1 million donations [95% credibility interval (CrI), 0.05–0.56] vs. 0.16 per 1 million donations [95% CrI, 0.04–0.35]) [8].

The termination of all MSM-specific deferral criteria in March 2022, associated with the cessation of information collection regarding sexual orientation of blood donors, resulted in the termination of the MSM-specific plasma donation program.

Here, we evaluate donor accrual, donor deferral and rate of infectious markers in MSM plasma donors who would have otherwise been deferred, in comparison with those of other blood donors, from July 2016 to March 2022.

MATERIALS AND METHODS

Blood, plasma and platelet collection in France

In France, whole blood, plasma and platelet collection as well as blood component manufacturing and testing are undertaken mainly within the remit of EFS, the French transfusion public service. Furthermore, EFS issues over 80% of the blood components transfused to patients. Between 2016 and 2022, approximately 1.6 million donors made 2.8 million donations every year, comprising of approximately 2.4 million whole blood donations, 300,000 plasma apheresis donations and 100,000 platelet/plasma apheresis donations. Plasma obtained from whole blood donations (known as recovered plasma) or from apheresis (known as source plasma) undergoes fractionation for plasma-derived medicinal product manufacturing or is transfused as a blood component. Most transfused plasma is quarantined until repeat testing upon renewed donation 2–5 months later, while approximately 10% undergoes pathogen reduction (Intercept, Cerus Corporation). In the absence of a timely repeat donation, quarantine plasma is reoriented towards fractionation. All donations are screened for HIV (individual donation - nucleic acid testing (ID-NAT) and Ab), HBV (ID-NAT, HBsAg and anti-HBcAb), HCV (ID-NAT) and Ab), HTLV-1 (Ab, restricted to first-time donors in mainland France since April 2019), syphilis (anti-treponemal Ab), HAV and Parvovirus (96 pool NAT, for

the purpose of plasma for fractionation). Additionally, a fraction of plasma intended for transfusion (quarantine or pathogen-reduced) to high-risk patients is tested for HEV (6 pool NAT).

Donor selection and deferral criteria

In France, donor selection and deferral criteria are determined by health authorities. These criteria included a permanent deferral for MSM until July 2016. As mentioned earlier, deferral for MSM was reduced to 12 months in July 2016 with the exception of quarantine plasma donation, where no time-specific deferral criteria were applied for MSM donors. The deferral for MSM for all other blood donations was further reduced to 4 months in March 2020 before being abolished in April 2022 (Figure 1). In France, other sexual risk deferral criteria for all donors were unchanged during the study period and included: (1) having more than one sexual partner over the last 4 months, (2) knowledge that one's sexual partner has had one or more other sexual partners over the last 4 months, (3) engaging in sex in exchange for money or drugs over the last 12 months (donor or their partner), (4) having a sexually transmissible disease over the last 4 months (donor or their partner), (5) HIV, HBV, HCV or HTLV infection (permanent deferral for the donor or 12-month deferral for their partner, except for HCV in the absence of HCV RNA for more than one year and for HBV [HBsAg-positive partner] if the donor is vaccinated with confirmed post-vaccination immunization) and (6) having syphilis infection/seropositivity (12 months deferral for the donor as per regulation, permanent in practice due to the frequent difficulty to distinguish an ongoing infection from a resolved past infection).

Donation frequency

Plasma apheresis in France is allowed up to 24 times/year (with a minimum interval of 14 days between two donations). Up until July 2016, at least one successful whole blood donation was required before undergoing a plasma or platelet/plasma apheresis (as per EFS internal procedures).

Data collection and analysis

Since 1992, all blood centres in France report quarterly the total numbers of donors and donations according to donor status (first-time or repeat, type of donation) and the epidemiological characteristics of donors confirmed positive (serology and/or NAT) for blood-born pathogen to the national surveillance system for blood donors [10]. More than three-quarters of donors found to be HIV, HBV, HCV or syphilis positive return for a post-donation interview, during which repeat testing is performed, and further information regarding the date and probable mode of transmission of the pathogen is investigated.

All plasma donations made by donors who self-identified as MSM during on-site donor interviews, and who would have otherwise been deferred due to MSM activity (since less than one year up to March 2020, and less than 4 months subsequently), were evaluated and compared with the other blood, plasma and plasma/platelet donations and donors. Furthermore, the rate of infectious markers in the involved MSM plasma donors was compared with the frequency of such markers in first-time male blood donors, a group of donors associated with the highest rate of infectious markers within the general donor population [11]. In an additional ad hoc evaluation, the rate of infectious markers in involved MSM donors who gave a non-therapeutic donation because of unavailability of apheresis capacity at the time of donation was also evaluated. As one could not be completely sure that these donors would have undergone plasma donations if logistically feasible, the results of this additional evaluation were kept separate from the main analysis.

For the sake of clarity and brevity, the term 'MSM donors' in the rest of the manuscript refers to the plasma donors evaluated in this study, that is, the donors who would have otherwise been deferred for MSM activity since less than one year up to March 2020, and since less than 4 months subsequently.

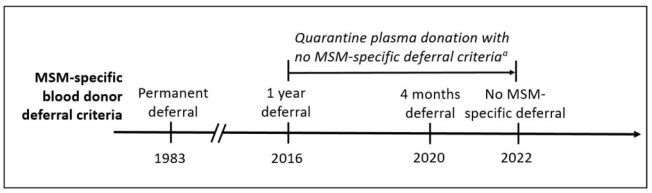

FIGURE 1 Timeline for changes regarding men who have sex with men (MSM) deferral criteria's in France. [a]Plasma for fractionation donation with no MSM-specific deferral since 2020.

RESULTS

MSM donors and donations

From July 2016 to March 2022, all donors who self-identified as MSM with sexual activity since less than one year (up to March 2020), and since less than 4 months thereafter, were registered as MSM donors potentially eligible for plasma donation. A total of 12,250 plasma donation applications were made by 8843 MSM donors between July 2016 and March 2022. The overall deferral rate was 75.2% (9220/12,250), and 57.7% when considering non-therapeutic blood donations (for laboratory, teaching or research purposes) in addition to standard plasma donations. The main reason for this very high deferral rate was the absence of apheresis capacity or availability at the donation site, particularly in mobile donation sites.

Among these candidate donors, a sexual risk deferral criterion (such as more than one partner over the last 4 months, a high-risk partner or prior sexually transmissible infection) was present in 12.1% (1480/12,250) versus 1.0% in the other blood and plasma donors ($p < 0.001$). The most frequent sexual risk deferral criterion encountered was reporting more than one sexual partner over the last

4 months (donor: 70%; donor partner: 24%). Similar frequencies were observed in the other donors. A non-sexual deferral criterion was present in 8.0% of the MSM candidate donors versus 9.7% in the other donors. The main non-sexual deferral criteria were recent piercing or tattooing, nosocomial risk (such as endoscopy) and intercurrent (non-sexual) infection.

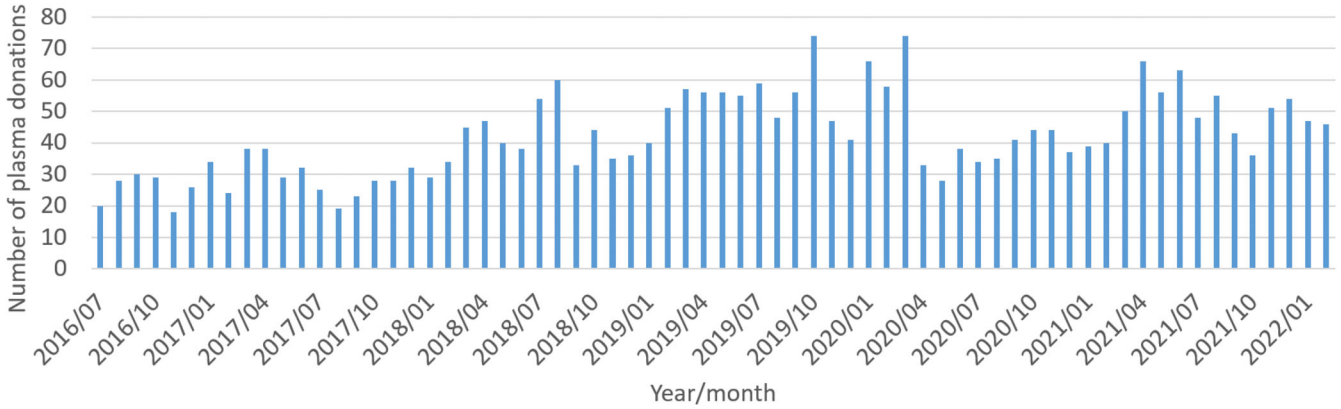

FIGURE 2 Number of plasma donations by men who have sex with men (MSM) donors who would have been otherwise deferred for MSM activity.

TABLE 1 Rate of infectious markers in plasma donations by men who have sex with men (MSM) donors who would have been otherwise deferred for MSM activity in comparison to all other blood, plasma, plasma/platelet donations and donors (A), and in comparison to first-time male blood donors (B).

(A)

	Donations by MSM ($n = 2880$)		All other donations ($n = 16,164,468$)				
Donations	*n*	Rate (/100,000 donations)	*n*	Rate (/100,000 donations)	Relative risk (MSM donations vs. other donations)	Relative risk 95% CI	*p**
HIV	1	34.7	92	0.6	61.0	8.5–437.7	0.016
HCV	0	0.0	380	2.4	–	–	1.00
HBV	1	34.7	731	4.5	7.7	1.1–54.6	0.12
Syphilis	21	729.2	1736	10.7	67.9	44.2–104.4	<0.0001
	MSM donors ($n = 994$)		**All other donors ($n = 3,769,294$)**				
Donors	*n*	Rate (/100,000 donors)	*n*	Rate (/100,000 donors)	Relative risk (MSM vs. other donors)	Relative risk 95% CI	*p**
HIV	1	100.6	92	2.4	41.2	5.7–295.7	0.024
HCV	0	0.0	380	10.1	–	–	1.00
HBV	1	100.6	731	19.4	5.2	0.7–36.9	0.17
Syphilis	21	2112.7	1736	46.1	45.9	29.8–70.5	<0.0001

(B)

	MSM donors ($n = 994$)		First-time male donors[a] ($n = 672,865$)				
Donors	*n*	Rate (/100,000 donors)	*n*	Rate (/100,000 donors)	Relative risk (MSM donors vs. first-time male donors[a])	Relative risk 95% CI	*p**
HIV	1	100.6	31	4.6	21.8	3.0–160.0	0.046
HCV	0	0.0	204	30.3	–	–	1.00
HBV	1	100.6	607	90.2	1.1	0.2–7.9	0.59
Syphilis	21	2112.7	741	110.1	19.2	12.4–29.6	<0.0001

Abbreviation: CI, confidence interval.
[a]Excluding MSM plasma donors.
*Fisher's exact test.

TABLE 2 Rate of infectious markers in donations by men who have sex with men (MSM) donors who would have been otherwise deferred for MSM activity and who gave whole blood for laboratory or research purposes because of unavailability of apheresis capacity at time of donation, in comparison with blood, plasma, plasma/platelet donations and donors.

	Donations for laboratory or research purposes by MSM donors ($n = 1682$)		Blood, plasma, plasma/platelet donations[a] ($n = 16,164,468$)		Relative risk (MSM donations vs. other donations)	Relative risk 95% CI	p*
Donations	***n***	**Rate (/100,000 donations)**	***n***	**Rate (/100,000 donations)**			
HIV	2	118.9	92	0.6	208.9	51.5–847.3	<0.0001
HCV	0	0.0	380	2.4	–	–	1.00
HBV	0	0.0	731	4.5	–	–	1.00
Syphilis	24	1426.9	1736	10.7	132.9	89.1–198.2	<0.0001
	MSM donors who gave blood for laboratory or research purposes ($n = 1350$)		**Blood, plasma, plasma/platelet donors[b] ($n = 3,769,294$)**		**Relative risk (MSM donors vs. other donors)**	**Relative risk 95% CI**	p*
Donors	***n***	**Rate (/100,000 donors)**	***n***	**Rate (/100,000 donors)**			
HIV	2	148.1	92	2.4	60.7	15.0–246.1	<0.001
HCV	0	0.0	380	10.1	–	–	1.00
HBV	0	0.0	731	19.4	–	–	1.00
Syphilis	24	1777.8	1736	46.1	38.6	25.9–57.5	<0.0001

Abbreviation: CI, confidence interval.
[a]Other than the plasma donations by MSM reported in Table 1.
[b]Other than the MSM donors reported in Table 1.
*Fisher's exact test.

Over the study period, 994 MSM donors made 2880 plasma apheresis donations (i.e., 0.13% of all plasma donations over the same time period in France). The number of plasma donations by MSM donors over time is depicted in Figure 2. The COVID-19 crisis impacted donor recruitment, as evidenced by the 50% drop in donation numbers in early 2020.

An interim analysis pertaining to the first 3 years of the program (July 2016–June 2019) and involving 456 MSM donors and 1035 plasma donations revealed that among these donors, only 124 were new donors (27%), while a majority (73%, $n = 332$) had given blood before July 2016, that is at a time when most likely a large majority should have been deferred.

Infectious markers

Out of the 2880 plasma donations, one donation was found to be HIV positive (Ab and RNA) with a negative recency (detuned) serology test (infection >6 months) [12], one donation was HBV positive (HBsAg, anti-HBcAb and DNA), and none were HCV positive. Additionally, 21 donations were found syphilis positive (with a testing algorithm including Treponema Pallidum hemagglutination assay, chemiluminescent microparticule immunoassay and immunoblotting). Of 15 evaluable cases and considering the date of a previous negative donation and/or disease history reported by the donor, syphilis infection was deemed very recent (<3 months) in two donors (13.3%) and recent (<3 years) in six donors (40%). This is in line with the almost 50% rate of recent (<3 years) infection observed in repeat blood donors found positive for syphilis in the general blood donor population over the same time period (Sauvage et al., unpublished data). Lastly, one donation was found to be HAV-RNA positive (in July 2017, at the time of an HAV outbreak in France [13, 14]).

The rates of infectious markers among MSM plasma donors and donations were compared with the rates in all other donors and donations over the same time period (Table 1A). Relative risks (RRs) were found to be significantly increased for HIV and syphilis. With only one occurrence of HIV and HBV among MSM plasma donations, the resulting RR for these two viral infections should be viewed with caution. On the contrary, the results regarding syphilis appear more robust with strikingly high RRs (donations RR: 67.9 (95% confidence interval [CI]: 44.2–104.4), donors RR: 45.9 [95% CI: 29.8–70.5]). A similar trend was observed when the MSM donors were compared with male first-time donors (other than MSM plasma donors) with RRs at 21.8 (95% CI: 3.0–160.0) and 19.2 (95% CI: 12.4–29.6) for HIV and syphilis, respectively (Table 1B).

In an additional post hoc evaluation, 2 out of 1350 fully eligible MSM donors who gave 1682 non-therapeutic blood donations because of plasma apheresis unavailability at the time of donation were found to be HIV positive (Ab and RNA) with negative recency tests. The RRs for HIV and syphilis in these donations and donors were found to be significantly increased as well (Table 2), similarly to the findings in the MSM donors who undertook plasma donation.

Lastly, no returning MSM donor after a quarantine plasma donation was found to have seroconverted for HIV, HBV and HCV while two donors had seroconverted for syphilis (the two cases with very recent infection reported earlier). Quarantined plasma for these two donors was destroyed according to standard procedure.

DISCUSSION

Overall assessment of the close to 6-year period in France during which donors otherwise deferred for MSM activity could give plasma while other type of blood donations were subject to a deferral period for MSM activity (12 months, then 4 months) reveals a low donor accrual, a high deferral rate for sexual risk, as well as a differing infectious epidemiology when compared with that of the general donor population. However, the low donor accrual with <3000 plasma donations by involved MSM donors, while at the same time over 16 million blood or plasma and plasma/platelets donations were made in France, limits the conclusions that could be made regarding the infectious epidemiology in donors who would have been otherwise deferred for MSM activity.

A number of reasons may have contributed to the low donor accrual, including insufficient knowledge among potential candidates, limited access to apheresis donation which was, and remains, often unavailable, notably in mobile collections sites, the impact of the COVID-19 crisis as well as low attractiveness of this type of donation, especially for new donors who have not previously donated whole blood. Furthermore, while plasma donation by otherwise deferred MSM could be viewed as a way forward regarding deferral criteria that are viewed as disproportionate and discriminatory, such a MSM-specific donation pathway could still be perceived as stigmatizing [15]. Lastly, the observation, early on, that a substantial proportion of MSM donors who gave plasma had given blood earlier, at a time when permanent deferral for MSM was enforced, further increases the uncertainties as to how representative these donors are of MSM likely to give blood in the absence of MSM-specific deferral criteria. Indeed, such a specific donation program because of its novel nature and limited access, might have attracted more 'militant' donors possibly less compliant with donor deferral criteria.

These limitations acknowledged, some striking differences regarding infectious epidemiology were observed, notably regarding syphilis with a RR of 67.9 (95% CI: 44.2–104.4) and 45.9 (95% CI: 29.8–70.5) when comparing MSM donations and donors with all other donations and donors, respectively. This increased RR persisted when comparing the MSM plasma donors with the group of donors with the highest rate of infectious markers, that is, male first-time blood donors [11].

These findings, associated with the high deferral rate for sexual risk, suggest that at least a fraction of the MSM plasma donors were potentially at higher risk of sexually transmitted infections compared with other blood donors. The detection of a donor with an HIV infection, a donor with an HBV infection, as well as another donor with an HAV infection within this small cohort of MSM donors (plus two HIV infections in eligible MSM donors who made non-therapeutic blood donations) further strengthens this assertion. Importantly, none of the three HIV cases were related to recent HIV infections and so would not have posed a transfusion safety risk per se.

Syphilis testing has been in place since the 1940s, at a time of frequent transfusion-transmitted syphilis [16]. Introduction of effective syphilis treatment as well as pre-donation screening and evolving, processing and storage conditions of blood components have significantly reduced the risk of transfusion-transmitted infection [17]. No cases of transfusion-transmitted syphilis have been reported in France since more than 40 years. Similarly, no cases have been reported in the United States since 1966 [18]. Persisting routine serological syphilis testing has also been justified as a surrogate marker for other blood-born sexually transmissible diseases such as HIV. However, the usefulness of such a marker with increasingly sensitive detection technologies is questioned, at least for well-identified and detectable pathogens [19]. Furthermore, current syphilis screening strategies rely mainly on detection of treponemal antibody that may persist at low levels in donors who have been infected and cured years to decades earlier and who are no longer infectious [20]. Nevertheless, syphilis screening appeared useful in the context of this study, by showing that MSM donors with no other deferral criteria may be at higher risk for prior infection by this pathogen.

Syphilis incidence and prevalence are higher in the MSM population compared with the general population and have been found to be increasing in France as well as in other high-income countries over the last 10 years [21, 22]. Increased syphilis seropositivity in MSM blood donors is therefore not unexpected and may only be a witness of an infection earlier in their life and not indicative of a current lifestyle associated with an increased risk of sexually transmissible infections. Nevertheless, two very recent (<3 months) and six recent (<3 years) syphilis infections were evidenced among the 15 evaluable donors found seropositive.

Among the deferral criteria for sexual risk, the most frequent criteria encountered by far was having more than one sexual partner over the last 4 months. This deferral criteria, applied to all blood donors in France since more than 30 years, is viewed as particularly relevant when moving from MSM time-based deferral criteria to more individualized behaviour-based deferral criteria [23]. Accordingly, several other jurisdictions have such a similar or approaching criteria (multiple partner or a new sexual partner) in place for a long time (e.g., Spain and Italy) [24] while others have recently introduced it, such as in the UK for all blood donors (not as a deferral criterion per se, but as a criterion, if positive, leading to a second question pertaining to anal sex) [25] or in the Netherlands for MSM donors only [26]. Such a deferral criterion applied to all blood donors has some drawbacks. In addition to leading to the deferral of large number of blood donors, notably young donors, not necessarily engaged in a high-risk sexual activity, this deferral criterion regarding more than one sexual partner over the last 4 months may be associated with lower compliance compared with other deferral criteria [27].

In conclusion, the infectious epidemiology among MSM plasma donors who would have been otherwise deferred for MSM activity within the last 12 months, then last 4 months, appeared different from that of the general donor population, especially for syphilis. However, syphilis seropositivity may be indicative of an enhanced risk of sexually transmitted infections in the past and not at the time of donation. Furthermore, the small number of MSM donors evaluated in this study, as well as uncertainty as to their representativeness, limits the ability to rely on these findings to robustly assess infectious epidemiology among MSM donors subjected to the same deferral rules than other blood donors. Nevertheless, as France and several other countries have recently moved away from time-dependent deferral

for sexually active MSM, a close monitoring of transfusion-transmitted disease markers in blood donors following these changes is warranted.

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P.T. coordinated the research and wrote the first draft of the manuscript. P.T., S.Le., J.H., L.M., T.T., P.R. and S.La. acquired and analysed the data. All authors reviewed and edited the manuscript.

CONFLICT OF INTEREST STATEMENT

P.T., S.Le., J.H., L.M., T.T., T.B., C.B., P.M., P.R. and S.La. are employed by Etablissement Français du Sang, the French transfusion public service in charge of collecting, manufacturing, testing and issuing blood components in France.

DATA AVAILABILITY STATEMENT

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

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ORIGINAL ARTICLE

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Blood donation among women in Nigeria: Motivators, barriers and appealing incentives

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Abstract

Background and Objectives: Women are grossly under-represented among blood donors in Nigeria. We, therefore, determined the barriers, motivators and appealing incentives to blood donation among women in Nigeria.

Materials and Methods: This was an internet-based cross-sectional study among women aged 18–65 years. A well-structured questionnaire was used to determine sociodemographic characteristics, motivation, barriers and appealing incentives. Motivational and barrier differences in some sociodemographic characteristics were determined using the chi-squared test. A *p*-value of 0.05 was considered statistically significant.

Results: The most common motivators among blood donors were 'when family or friend is in need of blood', 'health benefits' and 'reminders to donate'. One-time donors who were willing to become regular donors were more motivated by reminders to donate than those not willing ($p = 0.000$). The most common barriers among non-donors were 'poor attitude of hospital staff' and 'fear of contracting infections'. Younger women and those of the Hausa tribe were more debarred by 'lack of privacy during blood donation exercise' than older women and those of the other tribes (*p*-values of 0.008 and 0.006, respectively). The most appealing incentives for blood donation were medical consultation and a blood donation certificate.

Conclusion: Women's participation in blood donation in Nigeria can be improved by sending regular reminders to donors, especially one-time donors and by infrastructural adjustments to improve privacy at the blood donation sites. Specific and targeted capacity-building initiatives should also be put in place to drive a paradigm shift in the attitude of hospital staff to work in Nigeria.

Keywords

barrier, blood donation, incentives, motivation, Nigeria, women

Highlights

- Women are grossly under-represented among blood donors in Nigeria.
- One-time donors who are willing to donate are motivated by reminders during shortages.
- Lack of privacy during blood donation is a barrier among women of the Hausa ethnic group, who are predominantly Muslims.

INTRODUCTION

Nigeria with a population of about 200 million requires about 1.8 million units of blood annually to meet demand but only about 500,000 units are collected annually from mainly family replacement donors with voluntary non-remunerated blood donation accounting for only 8% of total blood collection [1, 2]. This is because the practice of donating to family members and friends is more popular than voluntary donation in Nigeria [3]. Blood and blood products are therefore in short supply to meet demand. Women and children constitute a significant proportion of blood transfusion recipients [4]. The first target of sustainable development goal 3 is to reduce the global maternal mortality ratio to less than 70 per 100,000 live births by 2030 and the second is to end preventable deaths of newborns and children under 5 years of age [5]. The current maternal mortality rate in Nigeria is 814 per 100,000 live births with postpartum haemorrhage being the leading cause, accounting for about 27% of all maternal deaths in Nigeria [6]. The maternal mortality rate reduction target cannot be possibly met, going by the current shortfalls in the blood required to manage haemorrhage as the leading cause of maternal death.

Women constitute more than half of the population and require more blood transfusion than their male counterparts because obstetrics and gynaecological complications predispose them to haemorrhage. However, they are grossly under-represented among blood donors in Nigeria [7]. The proportion of females in the donor population range from 0.3% to 0.64% in the Northwest [4, 8] to 1.0% in the Northcentral [9], 7.8% in the Southwest [10] and 10.8% in Southeast Nigeria [11]. In some developed countries like the United Kingdom, the Netherlands, Denmark and Finland, where the blood supply is optimal, the contribution of women to the donation pool is either at par or even slightly more than that of men [12]. With the increasing demand for blood and blood products, women need to participate in blood donation as much as their male counterparts as is the case in these developed countries. This will go a long way in bridging the gap between the demand and supply of blood in Nigeria.

Studies conducted in sub-Saharan Africa have identified altruism, donating blood for family, and incentive as motivators while fear due to lack of knowledge and discouraging spiritual, religious, and cultural perceptions of blood donation were identified as barriers [13]. Nigerian studies have identified the desire to help and save the life of relatives and acquaintances as motivational factors, while poor attitudes of blood bank staff and concerns that donated blood may be sold have been identified as some of the barriers to blood donation [14–16]. However, these studies were predominantly among blood donors who are dominated by men. Some studies conducted among female participants outside Nigeria have identified altruism, the feeling of responsibility, positive effect on health and moral or religious obligation as motivators [17, 18], while fear of anaemia or weakness and dizziness were some barriers identified [17]. Very little is known about female blood donors and the inherent factors that could promote or deter them from blood donation in Nigeria. In addition, national data are unavailable on female factors in blood donation and female-directed strategies to improve the current abysmal availability of blood and blood products in the country. Whereas, knowing the inherent female factors that determine the behaviour of the female population towards blood donation will inform policymakers and stakeholders at both local and national levels. The aim of this study, therefore, was to determine the potential barriers, motivators and appealing incentives to blood donation among women in Nigeria.

MATERIALS AND METHODS

This was an internet-based cross-sectional study among women in Nigeria. The survey link was distributed via snowball sampling from each of the six geopolitical zones. Participation was voluntary. Women, aged 18–65 years, who consented and fully completed the online survey were included. The study was approved by the Ethics Review Committee of the University of Ilorin Teaching Hospital, Ilorin (approval number: ERC PAN/2021/09/0198).

The questionnaire was pre-tested with 20 volunteers including 10 each of female donors and non-donors. The questionnaire was also thoroughly scrutinized by some trained donor organizers and consequently, some adjustments to the questions were made to ensure questions were understood by participants with no ambiguity and adequate to elicit the intended information. The survey questions were in four parts:

Part 1: Sociodemographic and blood donation-related information

These items included sociodemographic characteristics and blood donation experiences.

Part 2: Motivation for blood donation

This section was for blood donors and was used to assess the factors that motivated them to donate. The questions were designed based on literature review results of common motivational factors for blood donation, the responses from the pre-test and other blood donation practices in Nigeria that serve as motivations such as the opportunity to know haemoglobin level, blood phenotype and HIV status. A 5-point Likert scale ranging from 'strongly disagree' to 'strongly agree' was used.

Part 3: Barriers to blood donation

This section was intended for non-donors and used to assess barriers to blood donation. The questions were also designed based on literature review results of common barriers to blood donation and responses from the pre-test. A 5-point Likert scale was also used.

Part 4: Appealing incentives

This was a multi-select multiple choice question in which participants were provided with a list of common incentives given to voluntary donors in Nigeria (e.g., souvenirs like T-shirts and face caps, snacks, etc.) and others like free consultation and treatment gotten from the result of the pre-test.

Data analysis

Results of the online survey were extracted from Google Sheets using Excel and then imported and analysed using IBM Statistical Package for Social Sciences (SPSS) version 23. Frequency tables and cross-tabulations were generated for sociodemographic characteristics, motivators, barriers and appealing incentives. The Likert scale for barrier and motivation was recoded into three (strongly disagree/disagree, neutral and agree/strongly agree) for ease of analysis. A chi-squared test was used to determine the association between some sociodemographic factors with the motivators and barriers. A p-value of 0.05 was considered statistically significant.

RESULTS

A total of 1023 women responded to the survey but 8 were excluded due to incomplete responses. So, analysis was conducted with 1015 women aged 18–65 years. Majority were married (57.7%), employed (71.1%) and of the Yoruba ethnic group (47%). The majority (81%) of participants from the Hausa ethnic group were Muslims, while the majority of participants from Yoruba (79.2%), Igbo (100%) and other ethnic groups (64.6%) were Christians. Other sociodemographic characteristics of the participants are shown in Table S1. Overall, 225 (22.2%) had donated blood previously and were classified as blood donors, and 790 (77.8%) had no previous history of blood donation and were classified as non-donors. Among the non-donors, only 326 (41.3%) were willing to donate regularly.

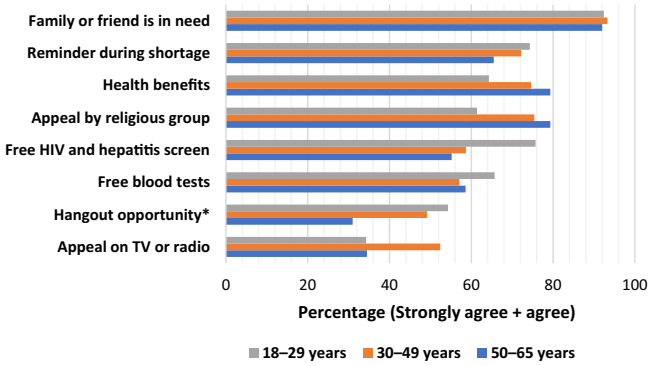

FIGURE 1 Motivation by age group among women blood donors in Nigeria. *$p < 0.05$.

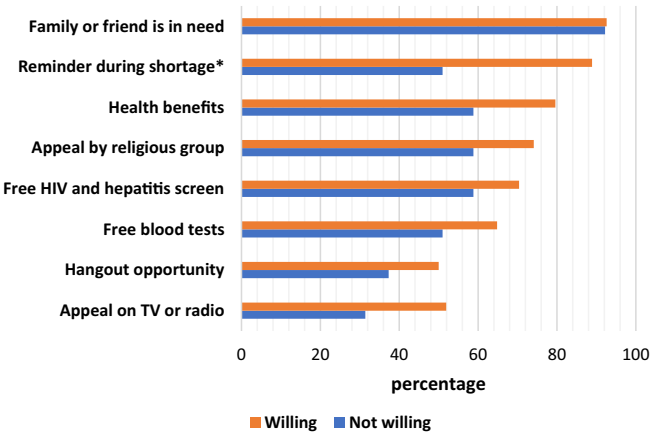

FIGURE 2 Motivation by willingness to donate regularly among one-time women blood donors in Nigeria *$p = 0.000$.

Motivation for blood donation among the blood donors

The most common motivation to which over 50% of the women donors strongly agreed or agreed were 'when family or friend is in need of blood' (94.7%), 'a reminder to donate during shortage' (72%), 'health benefits derived from blood donation' like cardiovascular

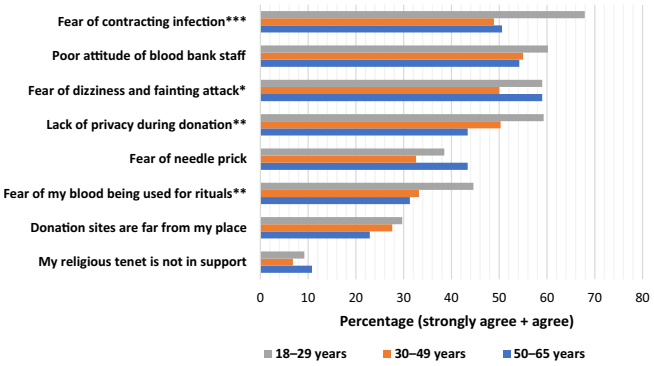

FIGURE 3 Barriers to blood donation by age group among women non-donors in Nigeria. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

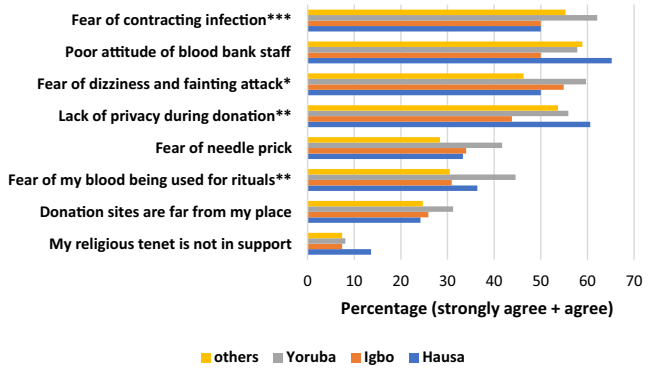

FIGURE 4 Barriers by ethnicity among women non-donors in Nigeria. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

protection (72%), 'blood donation appeal by religious group' (71.6%), 'opportunity to screen for HIV and hepatitis' (63.6%) and 'opportunity to do free blood tests' like haemoglobin electrophoresis and blood typing (60%). Others are found in Table S2. There was no significant difference between the three age groups regarding these motivational factors except for opportunity to hang out with friends to which 54.3% of donors aged 18–29 strongly agreed or agreed and this was significantly higher than in the other two age groups (49.2% for 30–49 years and 31% for 50–65 years; $p = 0.047$; Figure 1). A significantly higher proportion of one-time donors who were willing than those not willing to donate regularly were motivated by a reminder to donate during shortage (88.9% vs. 51%; $p = 0.000$; Figure 2).

Barriers to blood donation among the non-donors

The most common barriers to blood donation for which over 50% of the non-donors strongly agreed or agreed were 'poor attitude of blood bank staff' (57.1%), 'fear of contracting an infection during donation process' (57%), 'fear of dizziness and fainting attacks' (54.7%) and 'lack of privacy during blood donation' (53.3%). About one-third are afraid of their blood being used for rituals (37.7%) and of needle pricks (36.2%; Table S3). A significantly higher proportion of women aged 18–29 than those aged 30–49 years and 50–65 years, strongly agreed or agreed to 'fear of contracting an infection during donation process' (67.9% vs. 48.9% and 50.6%, respectively; $p = 0.000$) and 'lack of privacy during blood donation' (59.3 vs. 50.3 and 43.4, respectively; $p = 0.008$) as barriers to blood donation while a significantly higher proportion of those aged 18–29 and 50–65 years than those aged 30–49 years strongly agreed or agreed to fear of dizziness and fainting attacks (59% vs. 50%; $p = 0.019$). Fear of donated blood being used for rituals was significantly higher among young non-donors aged 18–29 years than those in the 30–49 and 50–65 years age group (44.6% vs. 33.2 and 31.3, respectively; $p = 0.001$; Figure 3).

There was a significant ethnic difference ($p = 0.006$) in 'lack of privacy at blood donation sites' as a barrier to blood donation, with the Hausa ethnic group most debarred (60.6%) and the Igbo tribe least debarred (43.8%; Figure 4). In terms of willingness to donate, a significantly higher proportion of those not willing to donate than those willing to donate were debarred by fear of dizziness and fainting attacks (61% vs. 45.7%; $p = 0.000$) and fear of needle prick (40.5% vs. 30.1%; $p = 0.001$; Figure 5).

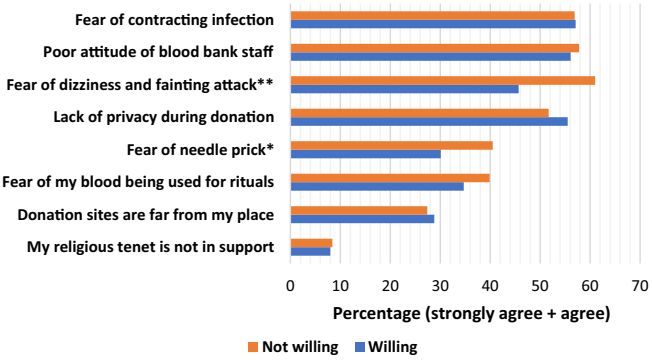

FIGURE 5 Barriers by the willingness to donate among women non-donors in Nigeria *$p < 0.01$; **$p < 0.001$.

Appealing incentives to blood donation among women in Nigeria

The incentives for blood donation that were appealing to over 50% of the participants were medical consultation (55%) and donation certificate (51%). Others that were appealing to over 40% of the participants include blood credits (48%), medical treatment (46%) and souvenirs (42%). Cash gift was appealing to 23% while 6% were not interested in any incentives but were ready to practice altruism (Figure 6). Among the non-donors, a significantly higher proportion of those willing than those not willing to donate endorsed donation

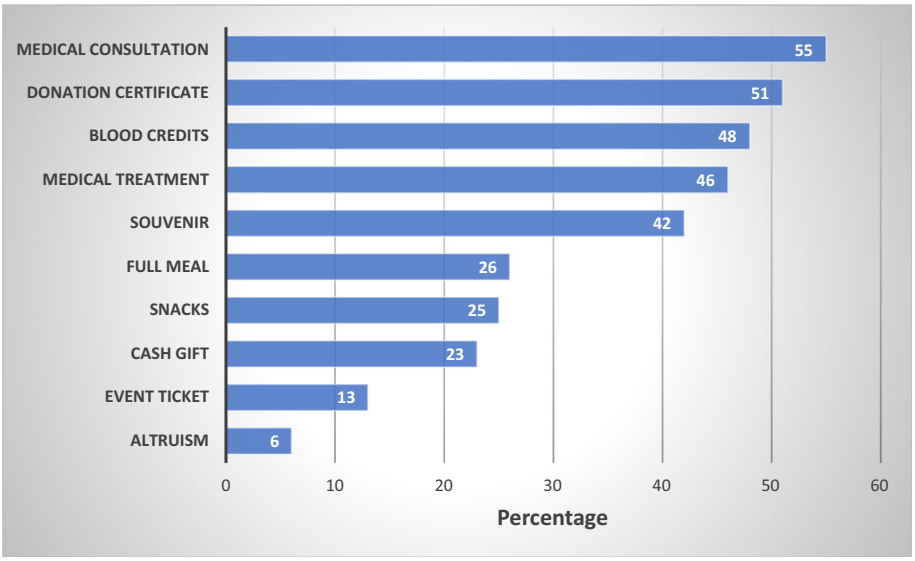

FIGURE 6 Appealing incentives to blood donation among women in Nigeria (*note*: multiple options were possible).

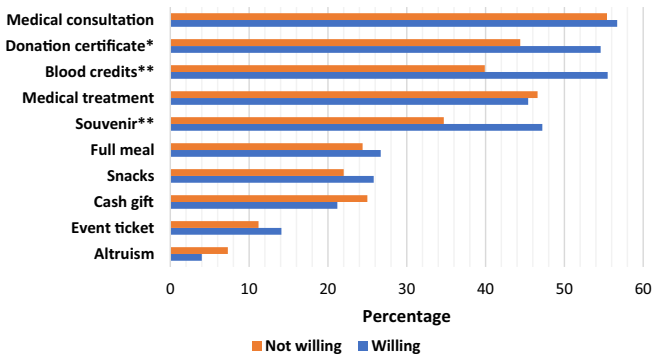

FIGURE 7 Appealing incentive by the willingness to donate among women non-donors in Nigeria. *$p < 0.01$; **$p < 0.001$.

certificate (54.6% vs. 44.4%; $p = 0.005$), blood credits (55.5% vs. 39.9%; $p = 0.000$) and souvenir (47.2% vs. 34.7%; $p = 0.000$; Figure 7).

DISCUSSION

The most common motivator to donate blood endorsed by nearly all (94.7%) of the participants in this study is 'to help family or friends in need'. This finding is similar to previous studies in Nigeria [3, 15], Ghana [19], Bulgaria [20] and Pakistan [21]. In contrast, in a study by Kasraiana et al. among Iranian women and by Weidmann et al. in Germany, nearly all the participants (94.4% and 98.1%, respectively) endorsed altruism [17, 22]. Just like in other sub-Saharan African countries, community-oriented culture favours family donors in Nigeria [3, 23]. These family donors can be converted to regular voluntary donors through donor education and advocacy.

Reminder to donate when there is a blood shortage was endorsed by 72% of the participants in this study. This finding is in keeping with previous studies [17, 19]. A significantly higher proportion of one-time donors who were willing than those not willing to donate regularly were motivated by a reminder to donate during shortage. The national blood transfusion service needs to leverage this by designing an online application programmed to send regular reminder messages to both voluntary and family replacement donors, especially one-time donors.

Health benefit like cardiovascular protection derived from blood donation was also endorsed by 72% of participants in this study. This is much higher than results from previous studies in Iran and the United States where between 11% and 38% were motivated by health benefits [24–26]. Several studies conducted to determine the effect of regular blood donation on the heart have been inconsistent and whether or not blood donation protects against cardiovascular diseases remains unclear [27]. This health benefit should therefore be downplayed during blood donation campaigns until definite conclusions are drawn. Appeal by religious groups was endorsed by 71.6% of the participants in this study, this is in keeping with the study among female students in Saudi Arabia [18]. Religious organizations usually make these appeals when one of their members is in need of blood with a good response, especially from men. In order to increase the participation of women, the national blood transfusion service and hospital blood banks could partner with religious organizations in organizing regular women-targeted blood donation drives, especially during women's conferences and in commemoration of women's and mothers' days.

Free HIV screening was endorsed by 63% while free blood tests like haemoglobin electrophoresis and blood typing were endorsed by 60% of the participants in this study. This is in keeping with studies in India [28] and Qatar [29] but contrary to the study done in Southwest Nigeria [14]. Test-seeking behaviour should be discouraged because

of the possibility of non-disclosure of personal high-risk behaviour which can jeopardize blood safety. In a study by Marantidou et al. in Greece, 5% of those who were motivated by free blood tests gave some false information in the pre-donation questionnaire [30].

Opportunity to hang out with friends was endorsed by over 50% of women aged 18–29 years and this was significantly higher than endorsement by older women. This is to be expected because most blood donation drives in Nigeria are conducted in higher institutions like Polytechnics and Universities where music with soothing vibes for young people is played to attract donors. Blood donation appeals on radio and television were endorsed by less than 45% of the participants. This is contrary to the finding in the Ghana study in which about 70% of the donors were motivated by appeals on radio and television [19]. This is not surprising because in Nigeria most people, especially young people, do not listen to the radio nor watch local television, they would rather browse the internet or watch cable television [31]. Online blood donation campaigns and appeals are therefore likely to be more effective than the appeal on local radio and television.

The most common barrier to blood donation endorsed by approximately 60% of the women was the 'poor attitude of blood bank staff'. This is similar to the finding of the study in Ghana (63.7%) [19] but contrary to a study by Finck et al. in Los Angelis, where only 7% of the female participants were debarred by poor attitude of the staff [24]. The poor attitude to work of the Nigerian worker has been found to be independent of the geopolitical zone, religious affiliation, sex or age [32]. It has been attributed to poor work conditions and remuneration, inadequate training and development, and job insecurity [33, 34]. Fear of contracting an infection during the donation process was also endorsed by approximately 60% of the participants in this study, this is higher than the results of the study in Ghana (39.7%) [19], Malaysia (24%) [35] and Qatar (10.3%) [29]. Fear of contracting HIV as a barrier to blood donation can be attributed to ignorance and lack of trust in the system of ensuring an aseptic blood donation process. In a similar study done in Southwest Nigeria, only 19% of participants were not willing to donate because of fear of contracting HIV infection, this may be due to the fact that the latter study was conducted among health workers who are more knowledgeable about the mode of transmission of HIV infection [14].

'Fear of dizziness and fainting attacks' was endorsed by about 55% of women in this study, this is not in keeping with the Ghana study where only about 40% had this factor as a barrier [19]. Lack of privacy during blood donation was also endorsed by about 55% of women and this is in keeping with the Ghana study [19]. About one-third of the participants in this study were afraid of needle pricks. This is consistent with the studies in Ghana [19], Qatar [29], Malaysia [35] and Pakistan [21] but contrary to the study in Los Angeles [24] where less than 15% were afraid of needle pricks. Fear of donated blood being used for rituals was also endorsed by about one-third of participants in this study and this is in keeping with the Ghana study [19]. The ritual mentality is common in African settings especially because of the sacrality attached to blood. Fear of contracting HIV infection, fear of dizziness and fainting attacks, lack of privacy, and fear of donated blood being used for rituals were endorsed more by women aged 18–29 years. These need to be addressed, through donor education and improvement of infrastructure to ensure adequate privacy during blood donation exercises, especially among young people that are the main target of blood donation drives. Fear of dizziness and fainting attacks and fear of needle pricks was also significantly endorsed more by those not willing to donate. This is in keeping with the previous studies in Nigeria [14] and Brazil [36]. There needs to be a lot of advocacy and enlightenment efforts to allay these fears in order to increase women participating in blood donations.

Participants of the Hausa ethnic group were most significantly debarred from blood donation because of lack of privacy during the blood donation exercise while participants from the Igbo tribe were least affected. This correlates with the predominant religion in these ethnic groups, the Hausa participants were mostly Muslims (81%) and all the Igbo participants (100%) were Christians. This finding is consistent with the tenets of the Islamic religion which prescribes privacy and covering for females which is why the use of the Hijab (a traditional covering for the head and neck) is part of their dressing. Measures that address the issue of privacy should therefore be put in place in the donor bays and during blood donation drives, especially among Muslim-dominated communities. Women of the Yoruba tribe were significantly more debarred from blood donation because of fear of contracting HIV infection, dizziness, fainting attacks and fear of donated blood being used for rituals. This may be related to some mythical beliefs among the Yoruba tribe. Advocacy for blood donation should therefore include strategies to dispel these mythical beliefs.

Incentives that help to save the cost of medical bills including free medical consultation, blood donation certificate, blood credits and free medical treatment were the most appealing incentives endorsed by over 45% of the participants. Blood donation certificates and blood credits are meant to be used to secure free blood transfusion in the future for self and sometimes for relatives. Endorsement of these incentives may be attributed to the low coverage of health insurance in Nigeria in which about 97% are uninsured [37]. In the study conducted by Weidmann in Germany [22], where there is almost 100% health insurance coverage, only 10% of donors were motivated by medical consultation. In another study in Iran [25], only 4.5% were motivated by donation certificates.

Souvenirs like T-shirts and face caps are popular incentives given to blood donors in Nigeria and these were appealing to 42% of the participants in this study. In a similar study in Iran, only 4.5% endorsed souvenirs as incentives [25]. Provision of snacks for donors as incentives is a general practice during blood donation drives in Nigeria; however, only about one-quarter of participants endorsed free snacks and full meals. Then even though altruism is being encouraged and financial benefits discouraged, 23% of participants subscribed to cash gifts while only 6% of participants would rather practice altruism. In the Iranian study [25], 5.8% supported financial benefits. The incentives that were more appealing to non-donors who were willing than those not willing to donate include souvenirs, blood credits, and donation certificates. Souvenirs are already popular; blood transfusion

services should therefore consider blood credits and donation certificates as incentives to encourage non-donors to donate and become regular donors.

This study has some limitations. The survey was online so only women who had access to the internet could participate. A 5-point Likert scale was used to assess motivation and barriers to blood donation, participants did not have the opportunity to add their own free text comments which may have provided additional suggestions for improving the participation of women in blood donation. Further research among women who do not have access to the internet and focus group discussions may therefore supplement the findings in this study.

In conclusion, the most common motivator for blood donation among women in Nigeria is 'to help family or friends in need'. One-time donors who are willing to become regular donors are more motivated by reminders than those not willing. Younger women are more motivated by the opportunity to hang out with friends during blood donation drives than older women. The most common barriers to blood donation among women in Nigeria include; 'poor attitude of hospital staff' and 'fear of contracting infections'. Younger women and those of the Hausa ethnic group are significantly more debarred by 'lack of privacy during blood donation exercise' than older women and other ethnic groups. The most appealing incentives for blood donation are medical consultation and a blood donation certificate. Women's participation in blood donation in Nigeria can therefore be improved by sending regular reminders to donors, especially one-time donors, and by infrastructural adjustment to improve privacy at the blood donation sites. The National Orientation Agency, which is an organ of the Nigerian government that has the spread and capacity for public enlightenment and sensitization campaigns, should be engaged in donor education and advocacy to allay fears and dispel mythical beliefs about blood donation among women. Specific and targeted capacity-building initiatives should also be put in place to drive a paradigm shift in the attitude of hospital staff to work in Nigeria.

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H.O.O. designed the research, supervised the conduct of the research, the data analysis and interpretation, and wrote the first draft of the manuscript. F.A.F. supervised the research in the Southwest zone. T.I.O, I.A.D., K.O.O. and M.K.O. supervised the research in the Northcentral zone. N.I.U. supervised the research in the Southeast zone and was responsible for continuity. I.P.I. supervised the research in the Northwest zone. A.M.A. supervised the research in the Northeast zone. U.I.M. supervised the research in the Southsouth zone. J.O.O. supervised the research in the Southwest zone and analysed the data. All authors participated in the design of the questionnaire, interpretation of data analysis and in the review and editing of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Long-term poor sleep quality is associated with adverse donor reactions in college students in Central China: A population-based cross-sectional study

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Abstract

Background and Objectives: Adverse donor reaction (ADR) could adversely impact the recruitment and retention of blood donors, but the evidence of effect of sleep quality on ADR is limited and controversial. The goal of this study was to explore the association between the sleep quality and ADR among college students in Wuhan.

Materials and Methods: The college student blood donors in Wuhan from March to May 2022 were recruited. Self-compiled general information questionnaire and Pittsburgh sleep quality index (PSQI) were investigated by convenience sampling. Univariable and multivariable logistic regression analyses were used to estimate the association.

Results: Among 1014 participants included in this study, 63 were in the ADR group and 951 were in the non-ADR group. Compared with the non-ADR group, the PSQI scores of ADR group were higher (3.44 ± 1.81 vs. 2.78 ± 1.82, $p < 0.01$). The results of multivariable logistic regression analysis showed that after adjusting gender, body mass index, blood donation history and other potential confounding factors, higher PSQI scores were related to the occurrence of ADRs (odds ratio = 1.231, 95% confidence interval 1.075–1.405), that is, the worse the sleep quality, the more likely the ADRs will occur.

Conclusion: The long-term poor sleep quality of college students is a risk factor for the occurrence of ADRs. It should be identified early before blood donation to reduce the incidence of ADRs and improve the safety and satisfaction of donors.

Keywords

adverse donor reaction, college students, sleep quality

Highlights

- Poorer sleep quality is associated with adverse sleep reactions in young blood donors.
- With regard to gender (male), body mass index (18.5–24.0 kg/m^2), degree subject (non-medical), donation history (first-time) and donation volume (400 mL), subgroup analyses showed that poorer sleep quality could increase the risk of adverse sleep reactions.
- Sleep quality should be considered when interviewing college student blood donors.

Songqing Ke, Pu Xu and Jing Xiong contributed equally to this work and share first authorship.

INTRODUCTION

The adequacy and safe of blood supply play a vital role in public health [1]. Most blood products come from voluntary, uncompensated, eligible blood donors. In the face of the unremitting need and seasonal shortage for blood components, blood centres undertake an important task to maintain the balance between supply and demand of blood products and to ensure the safety of blood donors [2]. Blood donation is recognized as an extremely safe and well-tolerated procedure; however, a small percentage of donors occasionally do experience adverse donor reaction (ADR) during or after the process, especially in blood donors who lack experience and knowledge of blood donation [3, 4]. Most of ADRs are usually vasovagal reactions and haematomas, counting for over 98% of all ADR [5].

Don or characteristics that have been observed to predispose to ADR include younger age, female gender, predonation fear, low weight, first-time donation status, low estimated blood volume and low haemoglobin levels [2, 4, 6–9]. Previous studies have demonstrated that ADR could leave a negative influence on recruiting and retaining blood donors [10–12]. Besides, young donors are more likely to have ADR [13], and they are the essential part of blood donation population. Therefore, blood centre should make efforts to identify the potential risk factors of ADR earlier and minimize the incidence of ADR ultimately in order to promote donor safety and satisfaction.

Sleep is an essential part of our growth and survival. Sleep disorder is common in general population and has shown significant association with hypertension, anxiety and depression [14, 15]. However, very few studies have explored the relationship between the sleep quality and ADR, and the findings of previous studies were controversial. Takanashi et al. found that short sleep time may increase the risk of ADR [16]. Shivhare et al. also found that having disturbed sleep the night before blood donation could lead to vasovagal reaction [17]. However, another study conducted in America reported that hours of sleep were not significantly related to ADR risk in young donors [18]. Given this, our study intended to use Pittsburgh sleep quality index rather than sleep time to assess the sleep quality comprehensively.

Based on a population-based cross-sectional study in Wuhan, this study was aimed to systematically identify the association of sleep quality with ADR among college students and provide theoretical support of donor health management.

MATERIALS AND METHODS

Participants and study design

A cross-sectional, online, questionnaire-based survey was conducted among college students of whole blood donation in Wuhan through the Wenjuanxing application (a survey platform) using a convenience sampling method between March and May 2022. In our study, eligible whole blood donors were selected to investigate after they donated blood. Wuhan Blood Center is a public health institution that is responsible for undertaking voluntary blood donation recruitment, blood collection and testing and clinical blood supply for nearly 160 clinical medical institutions in Wuhan. According to the China national standard for the eligible donor selection criteria (GB 18467-2011), all donors filled the health consultation questionnaire; thereafter, they underwent primary screening, including the blood pressure, body temperature, determination of ABO group, rapid test for hepatitis B surface antigen (HBsAg), alanine aminotransferase (ALT) and haemoglobin. The donors who failed the inspection were

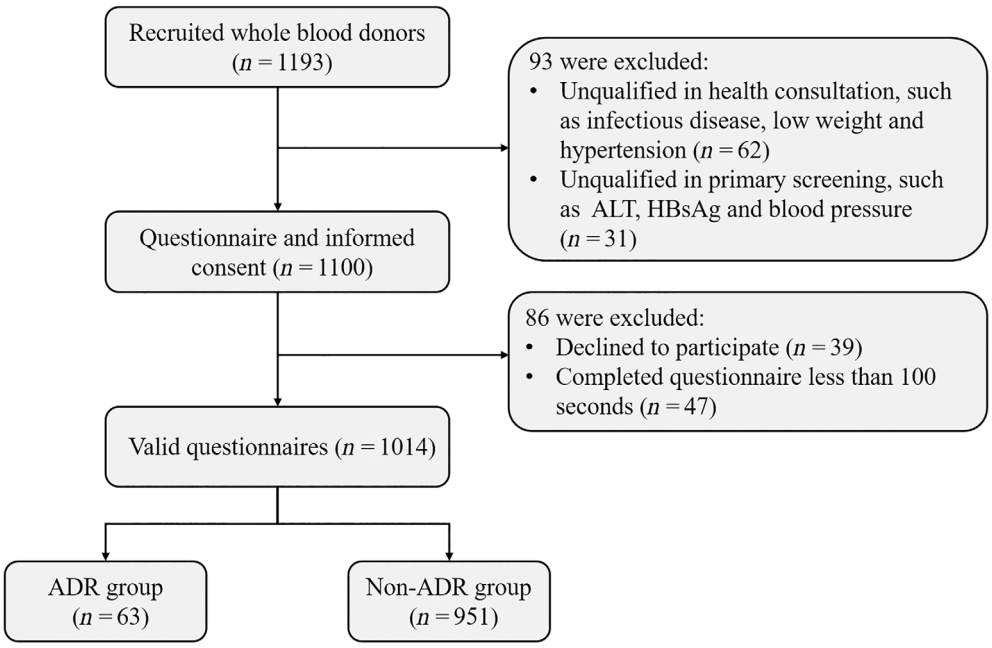

FIGURE 1 Flow diagram of participants recruitment ($n = 1014$, Wuhan, China). ADR, adverse donor reaction; ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen.

temporarily deferred. During the rest period after donation, they scanned QR codes on their mobile phones to fill out questionnaires.

To prevent repetitive filling, we restricted the same IP address to fill in the questionnaire only once. In order to ensure quality, quality control questions were set in the questionnaire, and questionnaires completing less than 100 s were considered invalid. The detailed flow diagram of the participants' selection process is shown in Figure 1. This study follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

Ethics statement

The entire survey was carried out using voluntary, anonymous and confidential principles. Before filling out the questionnaire, participants provided informed consent. The study was approved by the ethics committee of the Wuhan Blood Center.

Outcome assessment

The National Health Commission of the People's Republic of China issued their guidelines on the Classification of Blood Donation Adverse Reaction (WS/T551-2017) in 2017, which specified the classification, severity assessment and relevance of adverse reactions during blood donation. According to this guideline, ADRs, judged and recorded by professional blood sampling nurses, refer to local bleeding, pain, allergy or systemic vasovagal response at the puncture site in some blood donors during or after blood donation.

TABLE 1 Characteristics of study population.

Characteristics	Total ($n = 1014$)	ADR group ($n = 63$)	Non-ADR group ($n = 951$)	t/χ^2	p
Gender (*n*, %)				0.013	0.909
Male	508 (50.10)	32 (50.79)	476 (50.05)		
Female	506 (49.90)	31 (49.21)	475 (49.95)		
Age (mean ± SD, years)	19.83 ± 1.83	20.06 ± 2.29	19.81 ± 1.80	0.864	0.288
BMI group (*n*, %)				6.226	0.044
<18.5	102 (10.06)	12 (19.05)	90 (9.46)		
18.5–24.0	726 (71.60)	42 (66.67[a])	684 (71.92)		
>24.0	186 (18.34)	9 (14.29)	177 (18.61)		
Ethnicity (*n*, %)				-	1[b]
Han	943 (93.00)	59 (93.65)	884 (92.95)		
Minority	71 (7.00)	4 (6.35)	67 (7.05)		
Grade (*n*, %)				2.079	0.556
Freshman	506 (49.90)	30 (47.62)	476 (50.05)		
Sophomore	222 (21.89)	11 (17.46)	211 (22.19)		
Junior	171 (16.86)	12 (19.05)	159 (16.72)		
Senior and above	115 (11.34)	10 (15.87)	105 (11.04)		
Degree subject, *n* (%)				5.131	0.024
Medical	124 (12.23)	2 (3.17)	122 (12.83)		
Non-medical	890 (87.77)	61 (96.83)	829 (87.17)		
Blood type, *n* (%)				3.198	0.362
A	290 (28.60)	23 (36.51)	267 (28.08)		
B	268 (26.43)	18 (28.57)	250 (26.29)		
AB	97 (9.57)	4 (6.35)	93 (9.78)		
O	359 (35.40)	18 (28.57)	341 (35.86)		
Donation history, *n* (%)				7.204	0.007
First-time	645 (63.61)	50 (79.37)	595 (62.57)		
Repeat	369 (36.39)	13 (20.63)	356 (37.43)		
Donation volume, *n* (%)				9.551	0.002
<400 mL	485 (47.83)	42 (66.67)	443 (46.58)		
400 mL	529 (52.17)	21 (33.33)	508 (53.42)		

Abbreviation: ADR, adverse donor reaction.
[a]Due to rounding, the total is not equal to 100%.
[b]Using the Fisher's exact test.

Survey materials

The survey questionnaires consisted of two sections: sociodemographic information and Pittsburgh sleep quality index. Sociodemographic information included age, gender, ethnicity, height, weight, grade, degree subject (medical or non-medical), blood type, donation history and donation volume. Body mass index (BMI) was calculated as body weight divided by the squared height (in kilograms per square metre). The Pittsburgh sleep quality index (PSQI), complied by Buysse et al. [19], has been frequently utilized to assess the sleep quality of participants within the last month [20, 21]. It contains 19 items in 7 dimensions including subjective sleep quality, sleep latency, sleep persistence, habitual sleep efficiency, sleep disorder, use of hypnotic drugs and daytime dysfunction. Each dimension is scored 0–3, and the cumulative scores range from 0 to 21. The lower PSQI scores indicate better sleep quality.

Statistical analysis

The continuous variables were expressed as mean and standard deviation, and *t*-test was performed to evaluate the difference of continuous variables. The categorical variables were expressed as number and percentage, and the chi-square test or Fisher's exact test was used for comparison where appropriate. The restricted cubic spline function was conducted to observe the relationship between the global scores of PSQI and ADR. Then, we carried out univariable and multivariable logistic regression models to evaluate the association between the PSQI scores and ADR, and we calculated the odds ratio (OR) and 95% confidence interval (95% CI). Furthermore, stratified analyses were performed according to gender, BMI, degree subject, donation history and donation volume. Statistical analysis was conducted with R 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism 8.3.0. The R statistical packages 'rms', 'ggplot2' and 'forestplot' were used to draw restricted cubic spline curves and forest plot. All statistical tests were two-sided, with statistical significance set at 0.05.

RESULTS

Basic characteristics of participants

A total of 1014 college students were included in our study, including 508 males and 506 females. The response rates of questionnaire were 92%. The characteristics of participants stratified by whether or not ADR occurred are shown in Table 1. Between ADR and non-ADR groups, there were significant differences in the BMI group, degree subject, donation history and donation volume ($p < 0.05$). Gender, age, ethnicity, grade and blood type did not exhibit any significant differences in these two groups ($p > 0.05$).

As Figure 2 shows, compared with the non-ADR group, the ADR group was more likely to have higher scores of subjective sleep quality ($p < 0.001$), sleep persistence ($p < 0.05$), habitual sleep efficiency

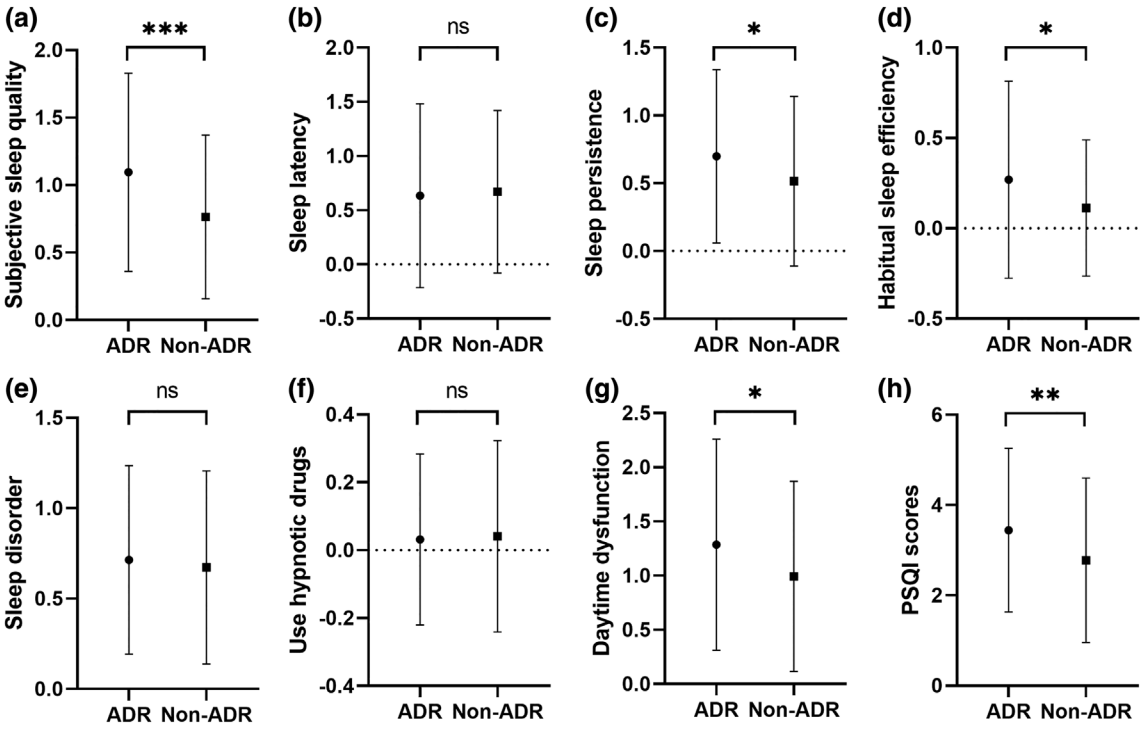

FIGURE 2 Analysis of different dimensions of Pittsburgh sleep quality index (PSQI) scores between adverse donor reaction (ADR) and non-ADR groups. Mean ± standard deviation, $n = 1014$. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not statistically significant.

($p < 0.05$) and daytime dysfunction ($p < 0.05$). The global PSQI score of ADR group was higher than that of the non-ADR group (3.44 ± 1.81 vs. 2.78 ± 1.82, $p < 0.01$).

Association of sleep quality with ADRs

The restricted cubic spline curve of global scores of PSQI with the ADRs is displayed in Figure 3. Positive correlation and linear association were found between PSQI scores and ADR with the increase in PSQI scores.

Logistic regression analyses were performed to assess the relationship between ADR and PSQI scores (Table 2). In the unadjusted model, the higher PSQI scores could increase the risk of ADR (OR = 1.195, 95% CI 1.051–1.351, $p = 0.005$). After adjustment of a series of covariates, the PSQI scores remained showing positive significant associations with ADR ($p < 0.05$). In the full adjusted model, PSQI scores showed positive significant association with ADR (OR = 1.231, 95% CI 1.075–1.405, $p = 0.002$).

Subgroup analyses

The results of subgroup analyses are shown in Figure 4. A significant association of increased PSQI scores with ADR was observed in subgroup analyses. The OR was 1.335 (95% CI 1.113–1.597) for male, 1.232 (95% CI 1.058–1.426) for BMI between 18.5 and 24.0 kg/m^2, 1.208 (95% CI 1.060–1.370) for non-medical students, 1.192

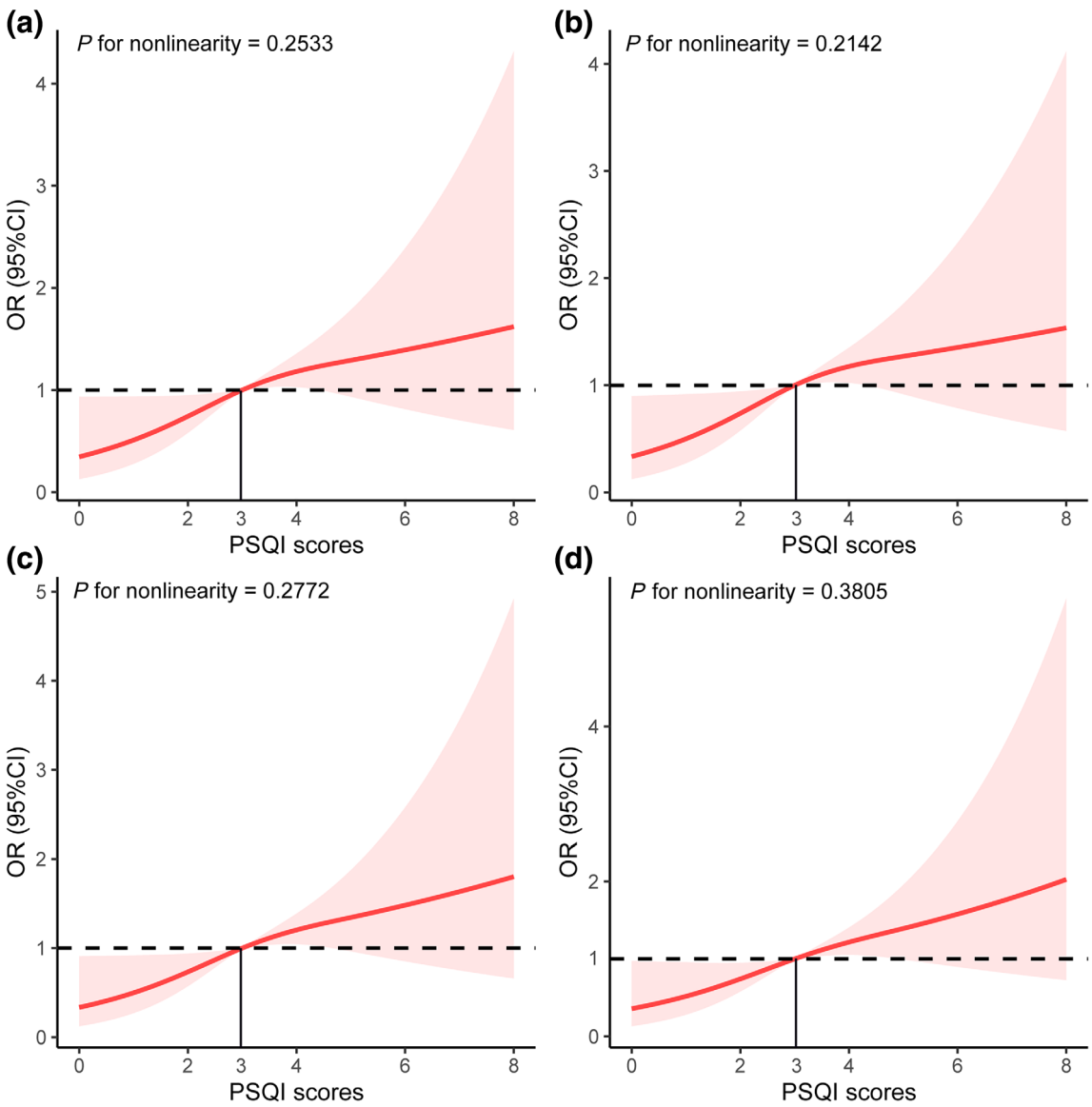

FIGURE 3 Dose–response relationship of the Pittsburgh sleep quality index (PSQI) scores with adverse donor reaction among college students. A knot is located at the 25th, 50th and 75th percentiles for the PSQI scores. (a) Unadjusted model; (b) model was adjusted for age, gender and BMI; (c) model was additionally adjusted for degree subject, donation history and donation volume; and (d) model was additionally adjusted for ethnicity, grade and blood type. OR, odds ratio.

TABLE 2 Logistic regression analyses of adverse donor reaction and the Pittsburgh sleep quality index scores.

	OR	95% CI	*p*
Unadjusted model	1.195	1.051–1.351	0.005
Model 1	1.191	1.049–1.347	0.006
Model 2	1.219	1.068–1.385	0.003
Model 3	1.231	1.075–1.405	0.002

Note: Model 1 was adjusted for age, gender and BMI; Model 2 was additionally adjusted for degree subject, donation history and donation volume; Model 3 was additionally adjusted for ethnicity, grade and blood type.
Abbreviations: CI confidence interval; OR: odds ratio.

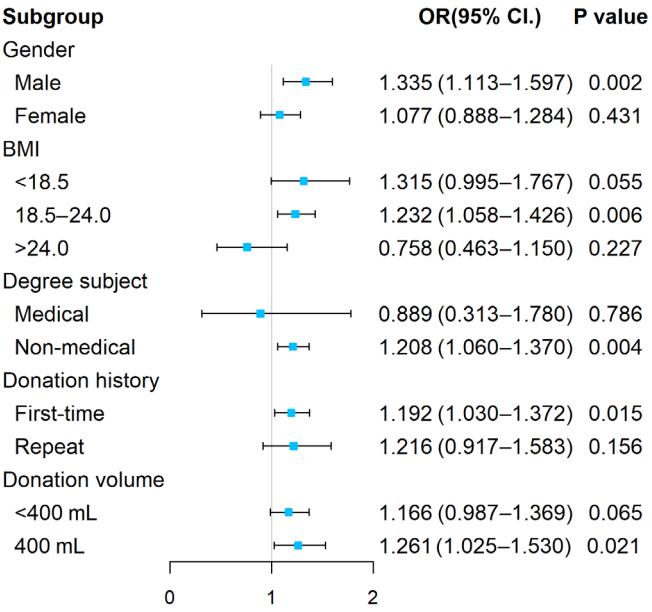

FIGURE 4 Adjusted stratification analysis of the association between adverse donor reaction and the Pittsburgh sleep quality index (PSQI) scores by logistic regression among college students.

(95% CI 1.030–1.372) for the first-time donation and 1.261 (95% CI 1.025–1.530) for 400 mL of donation volume.

DISCUSSION

In this study, we have demonstrated the association between long-term sleep quality and ADR in college students and found that poor sleep quality was an independent risk factor of ADRs. In each dimension of PSQI scores, the scores of subjective sleep quality, sleep persistence, habitual sleep efficiency and daytime dysfunction in the ADR group were higher. As sleep quality is a modifiable risk factor, in the development of recruitment strategy in college donors, blood centre's staffs could advise them to have good sleep in advance, and the condition of sleep quality could become a criterion of selecting blood donors to reduce the risk of ADR.

A number of studies have demonstrated that sleep disorder could increase the risk of depression, obesity, hypertension, cardiovascular diseases and other chronic diseases [14, 15, 22, 23]. It is usually recommended that blood donors get a good night's sleep before blood donation to prevent vasovagal reaction [24], but the evidence supporting this advice is sparse; very few studies in the medical literature have evaluated the association between sleep quality and ADR, and the results are discordant. Takanashi et al. investigated 48,872 donors of which 4924 with vasovagal reaction, and they observed that sleep of less than 6 h increased the risk of vasovagal reaction, with a dose–response relationship, both in whole blood and apheresis donors [16]. And Shivhare et al. also found that a significant correlation existed between having disturbed sleep the night before blood donation and vasovagal reaction (OR: 4.4; 95% CI 2.8–6.9) [17]. These studies were similar to the findings in our study. Furthermore, in Jeddah, inadequate sleep has been one of the commonest causes of blood donor deferral (about 6%) [25, 26]. In contrast, a survey among high school blood donors conducted by France et al. [18] found that reported sleep duration of donors was not significantly related to the risk of vasovagal reactions (OR: 1.05; 95% CI, 0.96–1.15). When considered as a whole, the inconsistent results may be due to regional and ethnic differences, and sleep duration may not reflect sleep quality comprehensively. PSQI has been widely used to measure sleep quality in many researche studies, which have been proved as an effective tool [27, 28]. Assessment of sleep quality may enhance the prediction of ADR among college student blood donors. Nurses and phlebotomists working in donation centres should be aware of the necessity of consulting sleep quality in the process of donor health assessment, so that ADR could be anticipated before donation.

The mechanisms of poorer sleep quality with a higher risk of developing the ADR remain unclear. However, some studies may provide potential insights. The vasovagal reactions were the most common type of ADR, accounting for 67%–95% of all ADR [29]. Vasovagal reactions result from a neurophysiological reflex during or after venipuncture, including nausea, dizziness, sweating, pallor changes or even fainting [17]. The autonomic nervous system plays an important role in the coordination of several physiological functions including the sleep process. And there was a bidirectional relationship between the impaired autonomic nervous system function and sleep disorder [30, 31]. The lack of sleep duration could affect the sympathovagal balance and generate higher sympathetic activity [32]. And the relation between sleep and cardiovascular system is bidirectional, and sleep deprivation could sympathetic activity and neuroendocrine response to stressor stimuli [33]. Then, the stimulation of venous pooling induces vasovagal reactions. Besides, needle fear and blood fear are psychological risk factors for ADR [6, 18]. There may be a synergistic effect between sleep quality and psychological factors, which may cause ADR together, and further research is needed.

In subgroup analysis, it revealed that the association between sleep quality and ADR was modified by gender, BMI, degree subject, donation history and donation volume. Significant effect of poorer sleep quality on ADR was observed in participants among males, those BMI 18.5–24.0 kg/m^2, non-medical students, first-time

donation, 400 mL of donation volume. Females generally are more prone to ADR than males [7], but evidence showed that males with short sleep duration had higher risk of vasovagal reactions than female [16], which was consistent with our findings. Overweight participants (BMI > 24 kg/m^2) showed that poorer sleep quality did not increase the risk of ADR. Obesity is a protective factor of ADR [34, 35], which may buffer poor sleep quality for ADR. In addition, students who are studying medicine may have more knowledge about donation and tend to feel less fear and anxiety while donation, thus weakening the effect of sleep quality on ADR. Besides, the experience of blood donation could also reduce the fear of blood donation. The effect of sleep quality on the ADR risk was not significant for individuals with <400 mL of donation volume, while it was significant for group with 400 mL of donation volume, probably because of the small sample size of group with <400 mL of donation volume.

This study has some strengths. First, we conducted a prospective study. Second, our study had high response rates of >90%, so the selection bias was alleviated. There are some limitations in our study. First, evaluation of sleep quality was based on self-report rather than clinical diagnosis, which induced recall bias and detection bias. Therefore, the measurement of sleep quality needs to be improved in the further study. Second, the ADR includes vasovagal reaction and other pathophysiological reactions; whether sleep quality affects the subtype of ADR require further investigation. Last, the current sample was restricted to young blood donors in college, and the results cannot be generalized to the overall blood donation population.

In conclusion, our study demonstrates the association between sleep quality and ADR among college students in central China. And we found that long-term poorer sleep quality before blood donation could increase the risk of ADR with a dose-dependent relationship. Long-term sleep quality of blood donors should be considered at the reception of donors to promote the safety and satisfaction of donors.

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S.K. and L.X. conceived the study. J.X. collected the data. S.K. performed the statistical analyses. M.M. and X.D. provided statistical consultion. The first draft of the manuscript was written by S.K. and P.X., reviewed by R.Y. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

The authors have disclosed no conflicts of interest.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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ORIGINAL ARTICLE

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In vitro quality of cold and delayed cold-stored platelet concentrates from interim platelet units during storage for 21 days

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Abstract

Background and Objectives: Based on previous success using apheresis platelets, we wanted to investigate the in vitro quality and platelet function in continuously cold-stored and delayed cold-stored platelet concentrates (PCs) from interim platelet units (IPUs) produced by the Reveos system.

Materials and Methods: We used a pool-and-split design to prepare 18 identical pairs of PCs. One unit was stored unagitated and refrigerated after production on day 1 (cold-stored). The other unit was stored agitated at room temperature until day 5 and then refrigerated (delayed cold-stored). Samples were taken after pool-and-split on day 1 and on days 5, 7, 14 and 21. Swirling was observed and haematology parameters, metabolism, blood gas, platelet activation and platelet aggregation were analysed for each sample point.

Results: All PCs complied with European recommendations (EDQM 20th edition). Both groups had mean platelet content >200 $\times$ 10^9/unit on day 21. The pH remained above 6.4 for all sample points. Glucose concentration was detectable in every cold-stored unit on day 21 and in every delayed cold-stored unit on day 14. The cold-stored group showed a higher activation level before stimulation as measured by flow cytometry. The activation levels were similar in the two groups after stimulation. Both groups had the ability to form aggregates after cold storage and until day 21.

Conclusion: Our findings suggest that PCs from IPUs are suitable for cold storage from day 1 until day 21 and delayed cold storage from day 5 until day 14.

Keywords

blood component storage, cold-stored platelets, delayed cold-stored platelets, interim platelet units, platelet concentrate

Highlights

- High glucose concentrations in platelet concentrates (PCs) from interim platelet units support prolonged cold storage.

- Cold-stored PCs had the ability to form aggregates throughout storage until day 21, measured by light transmission aggregometry.
- Delayed cold-stored PCs had a higher ability to be activated by agonists, measured by flow cytometry.

INTRODUCTION

Platelet concentrates (PCs) have been transfused to patients to reduce bleeding or as prophylaxis to prevent bleeding since the 1950s [1]. Platelets were initially stored cold to prevent bacterial growth. The discovery that room temperature-stored platelets had longer circulation time than cold-stored platelets led to a shift in practice regarding platelet storage temperature [2]. While some hospitals kept a dual platelet inventory, the majority switched to room-temperature storage [2–4]. Whole blood was the primary choice for massively bleeding patients and the need for platelets was primarily for prophylactic transfusions.

PCs are now routinely stored agitated at room temperature for 4–7 days [5, 6]. Transport of PCs from blood service providers to hospitals or from bigger hospitals to smaller hospitals further narrows the window for transfusion. In some cases, blood components are transported across borders to provide military hospitals with lifesaving components. This makes PCs a vulnerable resource, as blood banks and hospitals experience high wastage and a constant threat of bacterial growth from product contamination.

Several recent publications have shown that cold-stored or delayed cold-stored PCs comply with transfusion guidelines and have better in vitro function than room temperature-stored PCs [7–9]. The platelet storage lesion, which occurs during storage as biochemical, structural and functional changes, is mitigated by cold storage [10]. Cold storage reduces the risk of bacterial growth and preserves mitochondrial integrity, which potentially extends shelf life [11–15]. Cold storage shortens platelet circulation time in vivo to 1–3 days post-transfusion; however, the improved in vitro platelet function indicates a more optimal blood product for actively bleeding patients [9, 15].

The majority of PCs are used for prophylactic transfusions for non-bleeding patients, for which room temperature storage is preferred, but high wastage rates challenge platelet inventory management [16]. Wastage could be cut significantly if hospitals initially reserved PCs for prophylactic use and thereafter transferred unused units to cold storage for actively bleeding patients. Implementation of delayed cold-stored PCs may reduce wastage and provide an alternative for actively bleeding patients [17].

Quality control data from our hospital show that as much as 80% of PCs are used for prophylactic transfusions. Following a clinical study of cold-stored platelet transfusion in cardiac surgery patients in 2020, we introduced a dual inventory of PCs where room temperature-stored PCs were supplemented with a small inventory of continuously cold-stored apheresis PCs for actively bleeding patients [18, 19]. This aimed to ensure a sufficient inventory of PCs during the COVID-19 pandemic. In 2021, the dual inventory consisted of 96% room temperature-stored and 4% continuously cold-stored PCs. While the wastage of room temperature-stored PCs was 17%, the figure for cold-stored PCs was 36%.

Our research group has performed two laboratory studies, one in preparation for submission, involving delayed cold-stored apheresis PCs on day 7 [8]. We chose day 7, corresponding to the end of shelf life for our routinely stored PCs. This was found to be too late for delayed cold storage [8]. Previous publications indicate that platelets may be delayed cold-stored after 4 days of storage [7, 20]. At our hospital, the main PC production method is from single donation interim platelet units (IPUs). We wanted to explore the effect of relocating IPU PCs from room temperature storage to cold storage near the end of their shelf life aiming to reduce platelet wastage and the workload of our production unit. Additionally, IPU PCs have a slightly higher plasma content than apheresis PCs, which may influence platelet storage lesion [21].

In this study, we investigated the in vitro quality and platelet function from day 1 to day 21 in cold-stored IPU PCs and compared this to delayed cold-stored IPU PCs when transferred from room temperature to cold storage on day 5.

MATERIALS AND METHODS

Study design

This prospective single-centre experimental study was performed at the Department of Immunology and Transfusion Medicine at Haukeland University Hospital, Bergen, Norway from August 2021 to April 2022. The Regional Committee for Medical and Health Research Ethics approved the study (REC id: 2017/157). Blood from healthy regular blood donors, both female and male with blood type A or O were used in the study. The participants consented to their blood samples being used for research purposes.

Production of IPU concentrates

IPU PCs were prepared from five IPU units from whole blood donations. In brief, 450 mL whole blood with 63 mL citrate phosphate dextrose (Reveos, Terumo BCT) was separated using the Reveos system with the 3C program within 3–14 h of donation. Each IPU unit consisted of approximately 30 mL of platelets in autologous plasma. The IPUs rested for 1 h before being moved to a room temperature horizontal agitator for overnight incubation. The next morning five IPUs with an estimated total yield of >300 $\times$ 10^9/unit were sterile connected (TSCDII, Terumo BCT) to a Reveos platelet pooling set (Terumo BCT), and 220 mL platelet additive solution (PAS) (T-PAS+,

Terumo BCT) was then added before the IPU units were passed through a leukocyte filter. The leukocyte-depleted IPU PCs were placed in an agitator for a minimum of 10 min before further manipulation.

Pool-and-split, storage and sampling

The trial included 36 IPU PCs pooled and split into 18 identical pairs. Two ABO-identical IPU PCs were sterile connected and combined in one bag. The bag was mixed gently for a minimum of 5 min before storage bags (Platelet Storage Bag, Terumo BCT) were sterile connected to the pooling bag and the content split into two identical IPU PCs. Each pair was stored for 21 days, where one IPU PC was stored agitated at room temperature (20–24°C) for 5 days before transfer to cold storage without agitation, while the other IPU PC was stored continuously cold without agitation.

Sampling was performed after pool-and-split on day 1 and on storage days 5, 7, 14 and 21. After gentle mixing in an agitator for a minimum of 10 min, we sterile connected sampling bags (BB*T015CM, Terumo BCT) to the storage bags and transferred 25 mL (days 1 and 21) or 15 mL (days 5, 7, and 14) for testing.

Analyses

We tested for residual white blood cells (rWBC) using the BD Leucocount kit and a flow cytometer (BD FACSCanto II with FACSDiva version 8.0.1, BD Biosciences), following established procedures. Swirling was documented at each sample point by gently mixing the storage bags beneath a light source and looking for swirling patterns.

Tubes for haematology analyses contained K2EDTA anticoagulants (BD Bioscience) to obtain single platelets for analysis. Platelet count (PLT) and mean platelet volume (MPV) were analysed on the Sysmex XN-9100 (Sysmex Europe GmbH).

Glucose, lactate, pH, base excess (BE), pO_2 and pCO_2 were analysed at 22°C on a blood gas analyser (ABL825 FLEX, Radiometer Medical ApS).

To measure platelet function, we investigated platelet aggregation responses to 0.5 mM arachidonic acid (AA) and 30 μM thrombin receptor-activating peptide 6 (TRAP-6) (Roche Diagnostics GmbH) with a light transmission aggregometer (Chrono-log Model 700, Chrono-log Corporation). For light transmission analyses (LTA), we centrifuged samples of IPU PCs at 1500*g* for 15 min to obtain platelet-poor plasma (PPP). Platelet-rich plasma (PRP) was prepared by diluting a sample of IPU PC in PPP to a concentration of $\sim 200 \times 10^9$/L platelets. After 3 min of incubation at 37°C, LTA was performed with 1000 rpm stirring at 37°C with either 0.5 mM AA or 30 μM TRAP-6 as agonists until maximum aggregation was achieved. Maximum amplitude (MaxA) indicated distance from PRP (0%) and PPP (100%). The slope indicated maximum speed of aggregation formation (%/min) during analysis. Aggregation response was investigated in duplicate, and results were reported as the mean of the two parallels. We also ran one sample without an agonist to ensure no spontaneous aggregation occurred.

We also recorded the activation response to the same agonists using a flow cytometer (BD FACSCanto II with FACSDiva version 8.0.1, BD Biosciences). For flow cytometry analyses, we diluted IPU PCs with Isoton™ II Diluent (Beckman Coulter Life Sciences) to a concentration of $\sim 200 \times 10^9$/L. Three aliquots of 50 μL from each diluted sample were either added without agonist, 0.5 mM AA or 30 μM TRAP-6. All samples were added 12.5 μL anti-CD61 PerCP and 2.5 μL anti-CD62P PE (BD Biosciences). After mixing, we incubated the samples in the dark at room temperature for 30 min. Before flow cytometer analysis, we adjusted the volume with 2 mL FACSFlow (BD Biosciences) to allow optimal dilution. Samples were run immediately after dilution. The platelets were identified using anti-CD61 (BD Biosciences) and platelet activation response using anti-CD62P (BD Biosciences). A fixed gating setup based on CD61 positivity was used for all samples, irrespective of storage time, and 15,000 events were recorded per sample at a medium flow rate.

Statistics

Our data were analysed using R version 3.5.0 [22] with the NLME package version 3.1-137 [23] (The R Foundation for Statistical Computing). The graphics were derived using GraphPad Prism 9.4.0 for Windows (GraphPad Software). For each outcome, we fitted a linear mixed effect model using time, group and their interaction as predictors. In the time domain, we applied simple contrast (i.e., change from baseline). The interaction describes the change in difference between the groups, with respect to baseline measurements on day 1. Results are presented as mean (95% CI). We considered $p < 0.05$ as significant.

RESULTS

General requirements

The mean ratio of plasma to PAS was 42:58, with plasma concentration ranging from 40% to 43%. All IPU PC units complied with the requirements in the Guide to the Preparation, Use and Quality Assurance of Blood Components (EDQM, 20th edition) after pool-and-split [6]. Mean (95% CI) volume per 60×10^9 platelets was 62 mL (59, 64) in the cold and 62 mL (59, 65) in the delayed cold group ($p = 0.827$). After pool-and-split on day 1, rWBC was 0.04×10^6/unit (0.02, 0.05) in the cold and 0.05×10^6/unit (0.02, 0.07) in the delayed cold group ($p = 0.443$). We graded swirling to 3+ on day 1 in all units. We only downgraded one room temperature-stored unit to 2+ on day 5. No cold-stored or delayed cold-stored units had detectable swirling.

There were no statistically significant differences in any of the analyses on day 1, which indicates that our pool-and-split model resulted in equal pairs. The complete results are presented in Tables S1 and S2.

Haematology and blood gas analyses

There was an overall decline in PLT per unit during storage in both groups (Table 1), however; both groups had PLT > 200 × 10^9/unit on day 21. Cold storage led to shape change and increased MPV (Table 1).

Figure 1a shows that there was a steeper decline in glucose concentration in the delayed cold-stored group, which continued after cold storage ($p < 0.001$). Correspondingly, we saw an increase in lactate concentration (Figure 1b). There was measurable glucose in every unit in the cold-stored group on day 21, while there was one unit without detectable glucose on day 21 in the delayed cold-stored group. This unit had the lowest percentage of plasma from production (40%), that is, a low glucose concentration (6.6 mmol/L) and, due to a high platelet concentration (376 × 10^9/unit), the lowest volume per 60 × 10^9 platelets (52 mL).

During storage, pH declined in both groups with the biggest decline in the continuously cold-stored group on day 21 ($p < 0.001$). However, the lowest value of 6.9 was well above the recommended limit of 6.4 for PCs [6]. pCO_2 and BE declined similarly in both groups, while pO_2 increased with cold storage (Table S1).

Platelet function

Measurements of aggregation response by LTA showed that units not stored cold on day 1 (both groups) and until day 5 (delayed cold-stored group) were unable to produce irreversible aggregation with AA as agonist (Figure 2a). Samples from cold-stored units produced irreversible aggregation, and the cold-stored group had better aggregation by AA, measured by MaxA and slope from day 5 and throughout storage (Figure 2a and Table S2) when compared to the delayed cold-stored platelet group. Room temperature-stored units had better aggregation measured by MaxA and slope with TRAP-6 as agonist. Transfer to cold storage led to a decline in TRAP-induced aggregation in both groups, with a steady decline throughout storage. The continuously cold-stored group showed better aggregation response on days 14 and 21 compared to the delayed cold-stored group (Figure 2b and Table S2). There was no difference in spontaneous aggregation between groups and it did not affect the analysis (Table S2).

Unstimulated platelets had higher CD62P mean fluorescence intensity (MFI) in the continuously cold-stored group from day 5 ($p = 0.010$) and throughout storage (Figure 3a). CD62P MFI when stimulated by AA increased during storage, but the change was not statistically different in the two groups throughout storage (Table S2). CD62P MFI stimulated by TRAP-6 decreased during storage and was similar in both groups until day 7. The continuously cold-stored group had higher MFI from day 14, showing a change between groups ($p = 0.021$). However, the stimulation response measured as difference in MFI between stimulated and unstimulated platelets was higher in the delayed cold-stored group from day 5 to day 21 (Figure 3b).

TABLE 1 Platelet content, platelet count and mean platelet volume.

	Observed values		LME: change	
Outcome	Warm 5 days Mean (95% CI)	Cold no agitation Mean (95% CI)	In time *p*-value	Between groups *p*-value
PLT (10^9/unit)				
Day 1	329 (314, 344)	328 (312, 344)	–	0.724
Day 5	294 (280, 307)	298 (285, 311)	<0.001	0.138
Day 7	274 (261, 288)	280 (267, 293)	<0.001	0.055
Day 14	249 (236, 261)	245 (232, 258)	<0.001	0.482
Day 21	213 (203, 224)	205 (194, 215)	<0.001	0.013
PLT (10^9/L)				
Day 1	982 (936, 1027)	976 (928, 1023)	–	0.307
Day 5	945 (901, 989)	957 (916, 999)	<0.001	0.033
Day 7	938 (891, 985)	941 (896, 985)	<0.001	0.275
Day 14	892 (848, 936)	868 (823, 913)	<0.001	0.028
Day 21	812 (772, 851)	764 (723, 805)	<0.001	<0.001
MPV (fL)				
Day 1	10.0 (9.8, 10.1)	9.9 (9.8, 10.0)	–	0.255
Day 5	10.1 (9.9, 10.2)	10.9 (10.7, 11.0)	0.039	<0.001
Day 7	11.0 (10.9, 11.2)	10.8 (10.7, 10.9)	<0.001	0.024
Day 14	11.2 (11.0, 11.3)	10.7 (10.6, 10.9)	<0.001	<0.001
Day 21	11.2 (11.1, 11.4)	10.7 (10.6, 10.9)	<0.001	<0.001

Abbreviations: LME, linear mixed effect; MPV, mean platelet volume; PLT, platelet count.

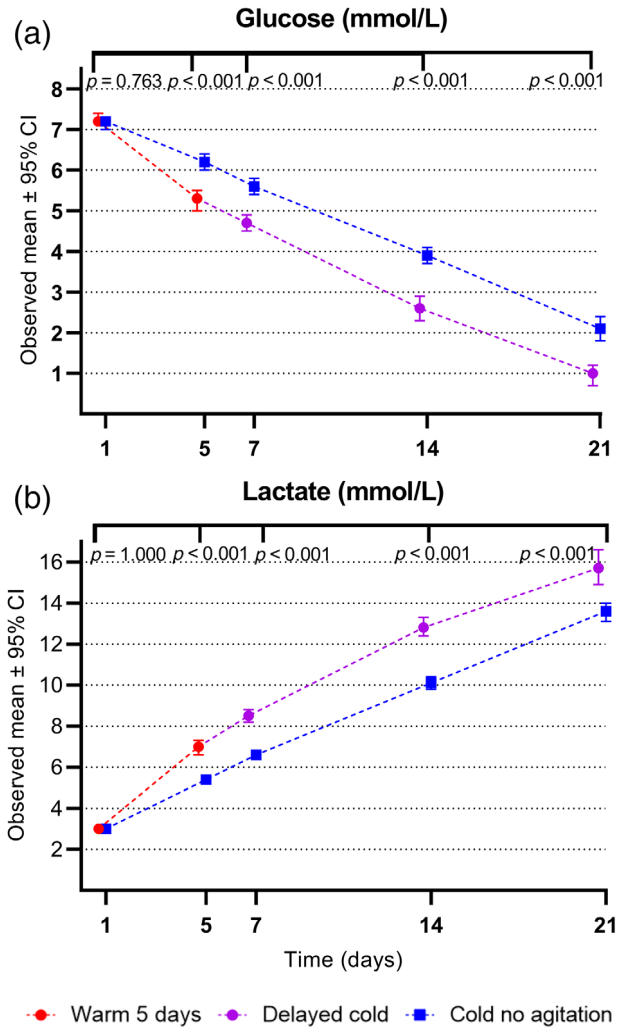

FIGURE 1 Glucose and lactate concentrations. (a) Glucose and (b) lactate in platelet concentrates from interim platelet units stored cold for 21 days (cold-stored, blue) or agitated at room temperature until day 5 and then cold until day 21 (delayed cold-stored, red). The *p* values represent change in difference between groups, with respect to baseline measurements on day 1 (linear fixed-effect model, R version 3.5.0 with the NLME package version 3.1-137). Points show observed mean with 95% CI.

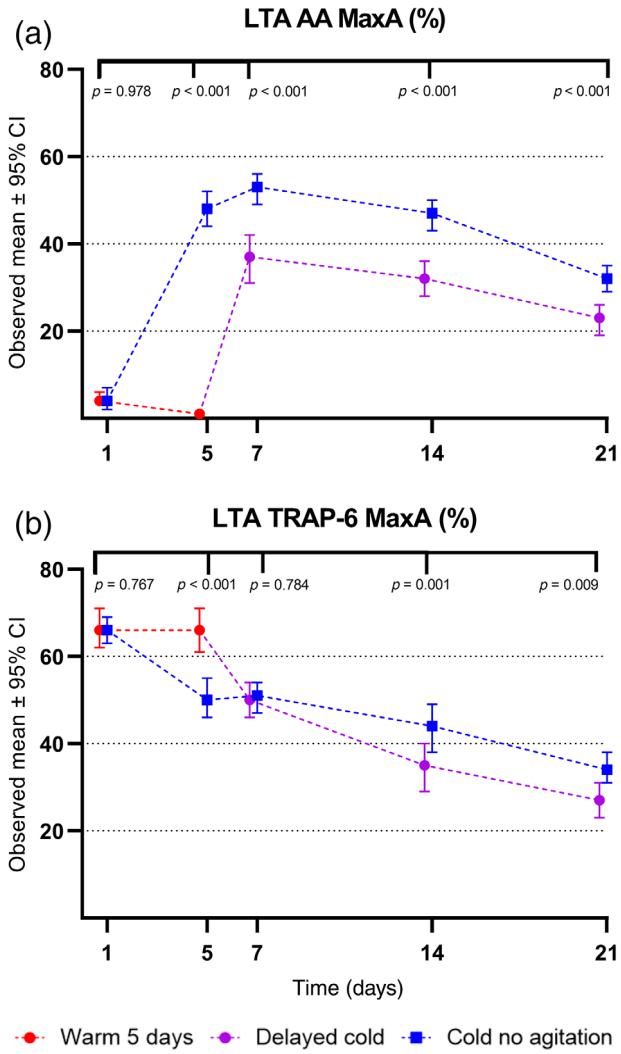

FIGURE 2 Platelet function measured by light transmission aggregometry. Platelet aggregation after stimulation with (a) arachidonic acid (AA) or (b) thrombin receptor-activating peptide 6 (TRAP-6) in platelet concentrates from interim platelet units stored cold for 21 days (cold-stored, blue) or agitated at room temperature until day 5 and then cold until day 21 (delayed cold-stored, red). Maximum amplitude (MaxA) indicates distance from platelet-rich plasma (0%) and platelet-poor plasma (100%). The *p* values represent change in difference between groups, with respect to baseline measurements on day 1 (linear fixed-effect model, R version 3.5.0 with the NLME package version 3.1-137). Points show observed mean with 95% CI. LTA, light transmission analyses.

DISCUSSION

In this experimental study, we found an in vitro quality and function of IPU PCs supporting both cold and delayed cold storage. Throughout storage, PLT per unit was above the recommended regulatory requirements [5, 6].

The study design resulted in 10 different donors in each IPU PC pooled unit. This minimized donor variability between the pooled units. To achieve a high platelet yield per unit, our department uses five IPUs, which provides additional plasma compared to four IPUs in the same amount of PAS. The additional plasma is beneficial for prolonged storage because it entails higher levels of glucose in the units. All units, except one delayed cold-stored, still contained glucose on day 21. Previous findings from studies on apheresis PCs, with delayed cold storage on day 7, showed glucose deprivation as early as on day 14 [8]. Johnson et al. found that glucose exhaustion was associated with apoptotic changes, which indicates that PCs should not be stored beyond glucose deprivation [24]. The lactate concentration found on day 14 in delayed cold-stored PCs and cold-stored PCs on day 21, respectively, is comparable to lactate concentration on day 7 in room temperature-stored PCs in plasma [25]. While pH is a poor predictor of platelet quality due to the presence of acetate and phosphate in the PAS [26], the detection of glucose seems to coincide with platelet function. The one unit without detectable glucose on

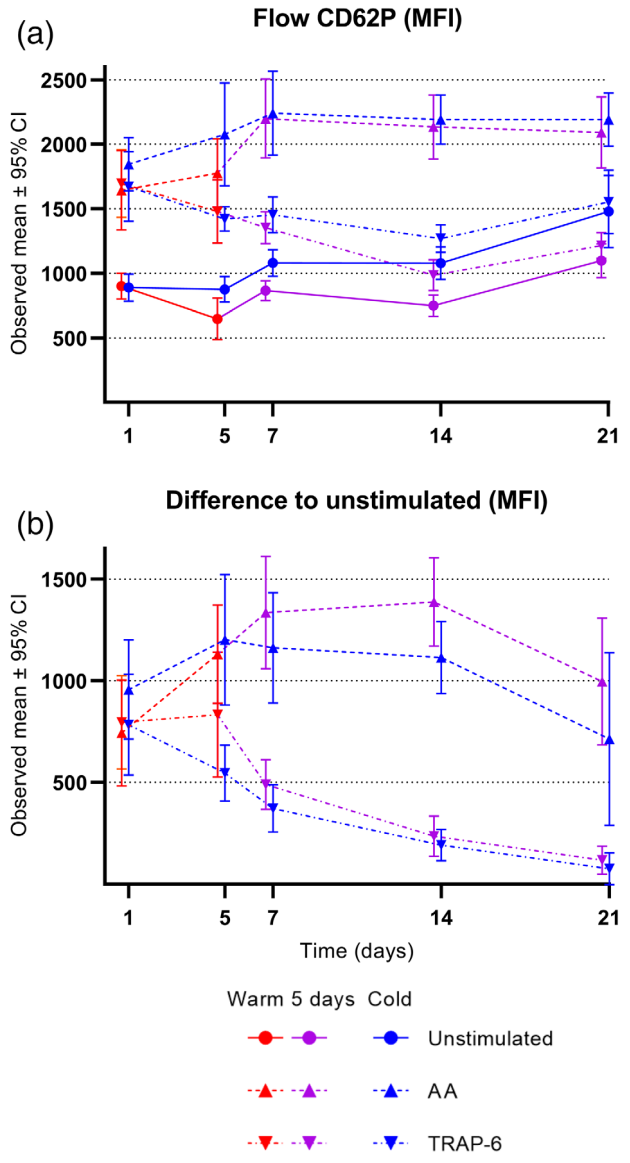

FIGURE 3 Platelet function measured by flow cytometry. (a) Activation response by use of anti-CD62P measured as mean fluorescent intensity (MFI) with flow cytometry in platelet concentrates from interim platelet units stored cold until day 21 (cold-stored, blue) or agitated at room temperature until day 5 and then cold until day 21 (delayed cold-stored, red). Points show observed mean with 95% CI. Circles indicate unstimulated platelets, triangles pointing upward indicate platelets stimulated with arachidonic acid (AA) and triangles pointing downward indicate platelets stimulated with thrombin receptor-activating peptide 6 (TRAP-6). The platelets were identified by use of anti-CD61 and platelet activation response by use of anti-CD62P. (b) MFI difference to unstimulated (baseline) after stimulation with AA (triangles pointing upwards) or TRAP-6 (triangles pointing downwards).

day 21 lacked the ability to aggregate with AA or TRAP-6 measured with LTA and also the ability to be stimulated with AA in flow analysis compared to the other units in the same group. We have found corresponding results in our previously published study of apheresis PCs [8].

Our flow cytometry analyses indicate that the cold and delayed cold-stored platelets have continuous high activation potential, especially with AA as agonist. We also found that the cold and delayed-cold platelets had the ability to form aggregates throughout storage with both AA and TRAP-6 as agonists. Room temperature-stored platelets did not form aggregates with AA as agonist (Figure 2a). Increasing the concentration of agonist in the study might enhance aggregate formation. The shape change that occurs after cold storage leads to clustering of GPIbα [27], which may enhance the effect of AA on the activation of platelet aggregation [28]. Tohidi-Esfahani et al. report that cold-stored PCs are more activated than room temperature-stored PCs [29]. The increased CD62P MFI of unstimulated platelets in the continuously cold-stored units indicates an in vitro activated state [30]. Due to lower activation levels in the unstimulated samples, there was a higher ratio between unstimulated and stimulated CD62P MFI in the delayed cold-stored platelets. However, CD62P MFI levels were similar in the two groups after stimulation.

The MPV of delayed cold-stored units where the volume continued to increase during storage may indicate a storage lesion not seen in the continuously cold-stored units. The cold-stored units decreased in size from day 5, which may be due to microparticle formation. The presence of microparticles in the units may contribute to enhanced in vitro function [31].

Logistically, IPU PCs are feasible for cold and delayed cold storage. It is easier to organize the production of cold-stored IPU PCs from the previous day's whole blood donations than to find suitable apheresis donors, and collect and produce a specific amount of apheresis PCs. In addition, IPU PCs may be stored cold immediately after production while, in our department, apheresis PCs may need to rest until the following day at room temperature to reduce aggregates from the apheresis process, which may delay cold storage. Another benefit of using platelets from whole blood is the resting period before production, where leukocytes are able to phagocytose possible bacteria.

Implementation of a dual inventory of platelets with a small cold-stored inventory with a prolonged shelf life for actively bleeding patients may reduce wastage [17]. The prolonged shelf life of cold-stored PCs may simplify logistics for blood providers and hospitals. If a new pandemic, or another severe event, occurs, a dual inventory may mitigate wastage and improve the availability of platelets as part of overall measures to establish control over blood supply and demand [32, 33]. Unagitated, cold-stored PCs may be suitable for transport to military hospitals or rural civilian hospitals and thereby ensure the availability of a platelet-containing product for the resuscitation of bleeding patients in a logistically challenging setting [34].

In conclusion, our results suggest that IPU PCs are suitable for cold storage from day 1 until day 21 and delayed cold from day 5 until day 14. The cut-offs expressed here are based on levels of glucose measured in the PCs and the results of platelet function analysis. Further studies are encouraged to further examine the optimal storage time of cold-stored platelets.

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H.B., T.O.A., T.H.F.L. and G.S. designed the research study; H.B. and T.H.F.L. collected the data; J.A. designed statistical analyses, tables and figures; H.B., T.H.F.L. and T.O.A. analysed and interpreted the data; H.B and T.O.A. wrote the manuscript, while all the other authors reviewed and approved the manuscript for publication.

CONFLICT OF INTEREST STATEMENT

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Norwegian Armed Forces Joint Medical Services.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and online supporting tables.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Removing hepatitis C antibody testing for Australian blood donations: A cost-effectiveness analysis

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Abstract

Background and Objectives: The risk of transfusion-transmitted hepatitis C virus (HCV) infections is extremely low in Australia. This study aims to conduct a cost-effectiveness analysis of different testing strategies for HCV infection in blood donations.

Materials and Methods: The four testing strategies evaluated in this study were universal testing with both HCV antibody (anti-HCV) and nucleic acid testing (NAT); anti-HCV and NAT for first-time donations and NAT only for repeat donations; anti-HCV and NAT for transfusible component donations and NAT only for plasma for further manufacture; and universal testing with NAT only. A decision-analytical model was developed to assess the cost-effectiveness of alternative HCV testing strategies. Sensitivity analysis and threshold analysis were conducted to account for data uncertainty.

Results: The number of potential transfusion-transmitted cases of acute hepatitis C and chronic hepatitis C was approximately zero in all four strategies. Universal testing with NAT only was the most cost-effective strategy due to the lowest testing cost. The threshold analysis showed that for the current practice to be cost-effective, the residual risks of other testing strategies would have to be at least 1 HCV infection in 2424 donations, which is over 60,000 times the baseline residual risk (1 in 151 million donations).

Conclusion: The screening strategy for HCV in blood donations currently implemented in Australia is not cost-effective compared with targeted testing or universal testing with NAT only. Partial or total removal of anti-HCV testing would bring significant cost savings without compromising blood recipient safety.

Keywords

blood donation testing, blood safety, cost effectiveness, hepatitis C

Highlights

- In Australia, the risk of transfusion-transmitted hepatitis C virus (HCV) infections is extremely low.

- Anti-HCV testing in addition to HCV RNA nucleic acid testing for screening blood donations does not prevent any additional morbidity in recipients but uses significant resources.
- Partial or total removal of anti-HCV testing would bring significant cost savings without compromising blood recipient safety.

INTRODUCTION

Hepatitis C virus (HCV) is a bloodborne virus of global public health concern. In Australia, the main transmission route is the sharing of needles among people who inject drugs. Globally, transmission via the reuse or inadequate sterilization of medical equipment and the transfusion of unscreened blood and blood products are important considerations. Australian Red Cross Lifeblood (Lifeblood) is responsible for the collection and distribution of blood and blood products in Australia. Collections are made from voluntary non-remunerated donors in Australia. HCV antibody (anti-HCV) testing is currently used in parallel with HCV RNA nucleic acid testing (NAT) to detect current or past HCV infections. With approximately 1.6 million blood donations collected annually [1], no transfusion-transmitted HCV infections have occurred with current testing [2].

A previous international modelling study demonstrated that the additional blood safety provided by anti-HCV testing is minimal with universal NAT [3]. However, universal donor testing with both anti-HCV testing and NAT continues not only in Australia but also in other developed countries [4, 5]. The current screening strategy is considered as a 'belt and braces approach' primarily to mitigate the remote risks of test failure. While the rate of HCV infection in first-time donors is higher than repeat donors [6], approximately 90% of Australian blood donations are contributed by repeat donors [1]. In addition, plasma for further manufacture collections for plasma-derived medicinal products has been steadily increasing and now outnumbers whole blood collections. The fractionation process includes pathogen reduction steps that substantially reduce the HCV residual risk, so anti-HCV testing in this context is unlikely to provide any clinically relevant safety benefit.

The shift in the management of chronic hepatitis C provides further justification for reconsidering HCV donation testing strategies. The use of dual testing was adopted at a time when direct-acting antiviral (DAA) therapy was not available, and the majority of those infected by HCV would progress to chronic hepatitis C. In the past, testing donors for HCV reduced the incidence of severe conditions such as liver failure, as well as avoiding significant costs associated with managing these conditions [7]. With the advent of DAA, which has demonstrated a cure rate of over 95% [8], in the vast majority of cases, diagnosed HCV infection can be cured.

Given the context of finite healthcare resources and the limited incremental risk–benefit contributed by anti-HCV testing, a targeted anti-HCV testing strategy or no anti-HCV testing may be favourable over current testing. Removing or targeting anti-HCV will lower the total costs of HCV screening for blood donations, but how this will affect the residual risk of HCV infection and the long-term costs and health outcomes requires investigation. Cost-effectiveness analysis is such a tool that can incorporate all available evidence and assist decision-making in the transfusion medicine [9, 10]. Previous studies have investigated the cost-effectiveness of adding NAT to anti-HCV testing in blood donation [11–13], but the cost-effectiveness of removing anti-HCV testing or applying anti-HCV testing dependent on donor risks, remains unknown. Therefore, this study aims to conduct a cost-effectiveness analysis of different testing strategies for HCV infection in Australian blood donations.

MATERIALS AND METHODS

Alternative HCV testing strategies

In this study, four HCV testing strategies were proposed for comparison:

1. Universal testing with both anti-HCV and NAT (status quo).
2. Anti-HCV and NAT for first-time donors and NAT for remaining donations.
3. Anti-HCV and individual donor-NAT (ID-NAT) for transfusible component donations and pools of 16 donations (MP16-NAT) for plasma for further manufacture.
4. Universal testing with NAT only.

Under strategies 1, 2 and 4, ID-NAT is used for transfusible component donations and MP16-NAT for plasma for further manufacture.

Decision-analytical modelling

The decision-analytical model for assessing the cost-effectiveness of alternative HCV testing strategies consisted of a decision tree model (Figure 1) and a Markov model (Figure 2) implemented in TreeAge Pro 2021 [14]. The decision tree started with one of the four alternative testing strategies. Following each testing strategy, there was a chance that the transfusion recipient was infected by HCV, which was determined by the residual risk estimate from a separate analysis (see Table 1, [6]). Once the recipient developed acute hepatitis C, it was assumed that the condition would either clear spontaneously or progress to chronic hepatitis C. For blood transfusion recipients who have achieved blood safety (i.e., no infection transmitted) or whose acute infection cleared spontaneously, it was assumed that they would survive or die of any causes in the following years. For those who progress to chronic infection, a Markov model was used

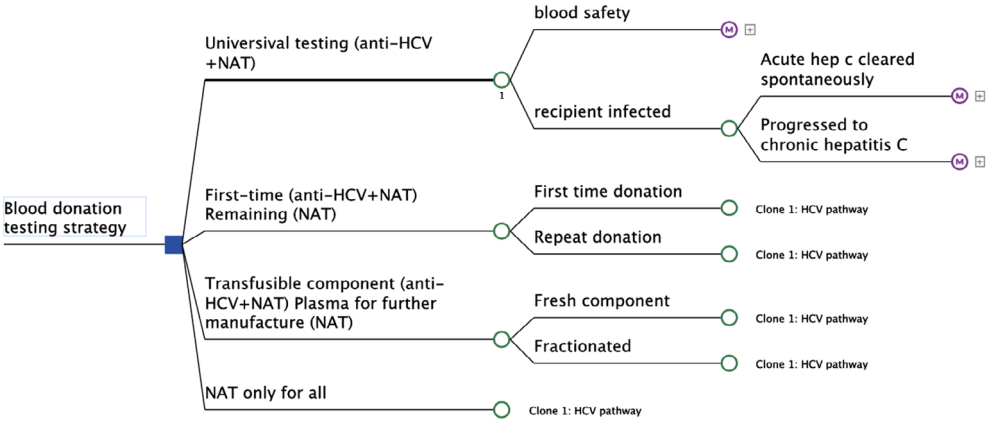

FIGURE 1 Decision tree model structure for hepatitis C virus (HCV) testing in blood donations. Anti-HCV, hepatitis C virus antibody; NAT, nucleic acid testing.

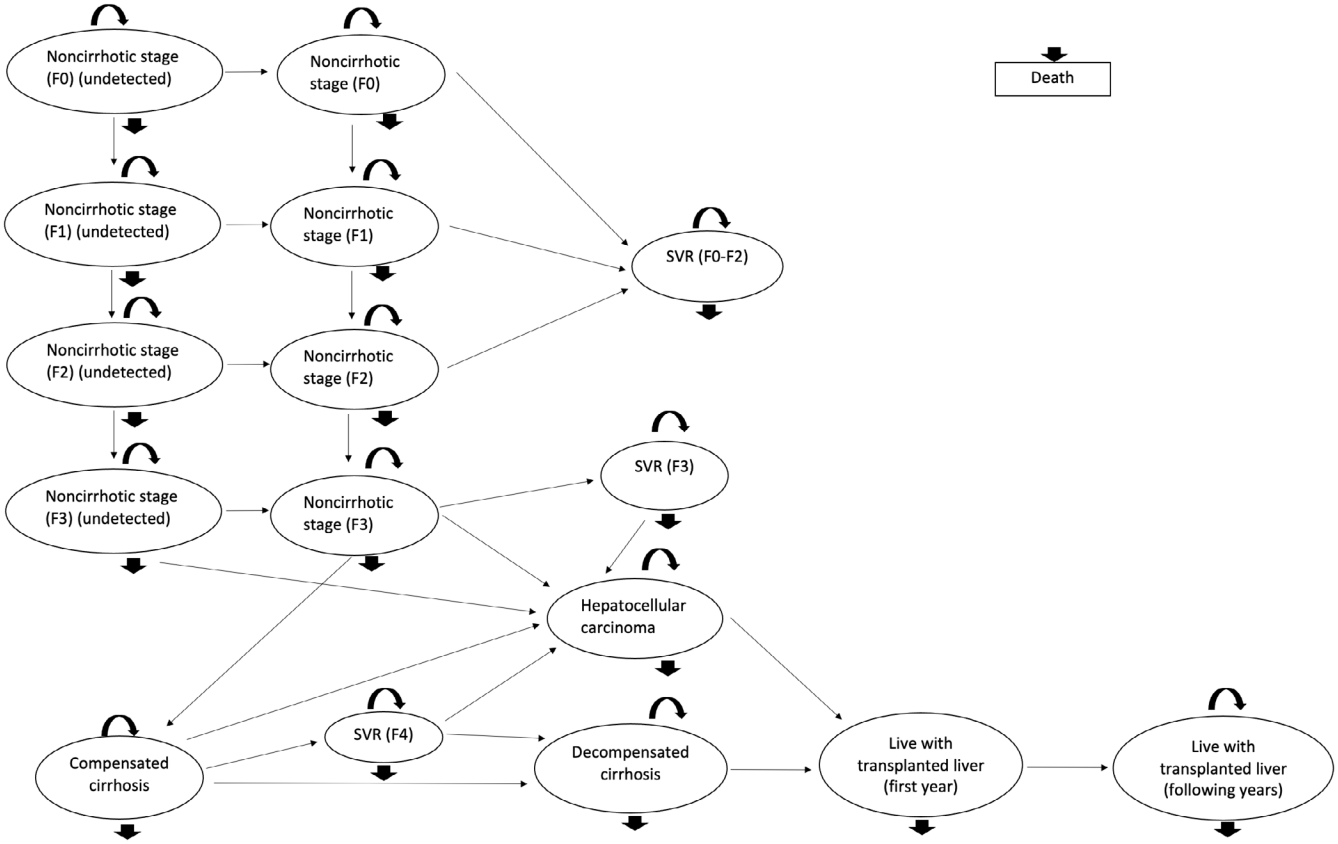

FIGURE 2 Markov model structure for chronic hepatitis C disease progression. SVR, sustained virological response.

to simulate the disease progression of chronic hepatitis C. The model consisted of a set of mutually exclusive health states where patients either stayed in their current state or transited to other health states or died. Patients would progress through non-cirrhotic stages (F0–F3) before developing compensated cirrhosis. While in non-cirrhotic stages, there was a probability that patients had their chronic hepatitis C detected. It was assumed that all diagnosed HCV infections would be treated by DAA therapy, and there was a high chance of achieving sustained virological response (SVR), whereby HCV is not detected in the blood 12 weeks or more after treatment. Once in the 'compensated cirrhosis' state, patients would no longer be able to achieve a full recovery as the liver damage done by cirrhosis could not be reversed. Other advanced disease stages of chronic hepatitis C included decompensated cirrhosis and hepatocellular carcinoma, with another proportion of patients receiving a liver transplant.

TABLE 1 Residual risk estimate of hepatitis C virus (HCV) infection following each testing strategy used in the model [6].

Testing strategy[a]	Residual risk
1. Universal testing with anti-HCV and NAT	1 in 151 million
2. First-time donor (anti-HCV and NAT), remaining donor (NAT)	1 in 111 million
3. Transfusible component (anti-HCV and ID-NAT), plasma for further manufacture (MP16-NAT)	1 in 151 million
4. Universal testing with NAT only	1 in 66 million

Abbreviations: ID, individual donor; MP16, minipool 16; NAT, nucleic acid testing.
[a]For strategies 1, 2 and 4, ID-NAT is used for transfusible component donations and MP16-NAT for plasma for further manufacture.

Model parameters

Risk of transfusion-transmitted HCV infection

In this study, the residual risk refers to the risk that a donation from an HCV-infected donor is not detected by testing, leading to a transfusion recipient becoming infected with HCV. The residual risk for different testing strategies was estimated based on an in-house purpose-built risk model [6]. The more conservative mid-estimates were used in the baseline analysis (Table 1).

Transition probabilities

The proportions of first-time donations and transfusible component donations were taken from Lifeblood 2020 donation data (Table 2, [6]). The spontaneous viral clearance rate in acute infections was derived from a systematic review where the proportion achieving clearance within 12 months following infection was 0.36 [15]. The transition probabilities associated with chronic hepatitis C disease progression were sourced from previous modelling studies [16-19]. The mortality rates for non-cirrhotic stages and compensated cirrhosis were based on the mortality rates of blood transfusion recipients, who have higher mortality than the general population (Table S1). Elevated mortality rates were assigned for 'decompensated cirrhosis' and 'hepatocellular carcinoma' health states using published estimates [23]. The mortality rates following liver transplant were taken from a project that investigated the future health and economic burden of hepatitis C in Australia [24]. As there is no universal testing for HCV among the general Australian population, we assumed that for non-cirrhotic stages, only 1% (probability 0.01) of blood transfusion recipients would get tested for HCV each year.

Resource use and costs

This cost-effectiveness analysis was conducted from a healthcare system perspective where only direct costs of providing testing and treatment of HCV infection were considered. The costs of testing HCV in blood donations were the rolled-up costs including the costs of tests and labour. As NAT is a multiplex test that also tests for human immunodeficiency virus (HIV) and hepatitis B virus in blood donations, it should be noted that the costs of NAT listed in Table 2 are not just for hepatitis C alone. In the baseline analysis, the total costs of NAT were used as the costs of NAT for HCV. An alternative assumption that the costs of NAT for HCV were a third of the total costs of NAT was tested in the sensitivity analysis. The costs of managing different stages of chronic hepatitis C were sourced from an Australian study that assessed the cost-effectiveness of treating people who inject drugs with DAA therapy [20]. All cost items were valued in 2020 Australian dollars.

Health outcome measure

The health impact of HCV infection was quantified using quality-adjusted life years (QALYs). QALYs are calculated by multiplying the utility weight associated with a health state by the number of years lived in that state. The utility weights range between 0 and 1, with 0 representing death and 1 representing full health. The utility weight for the Australian general population was used for those blood transfusion recipients who were not infected by HCV [25]. The utility weights for different stages of chronic hepatitis C were informed by a recent systematic review and meta-analysis of health utilities in patients with chronic hepatitis C, including people who are treated with DAA [26]. As the chronic hepatitis C condition deteriorates, the utility weight would decrease accordingly. Those infected but achieving SVR were assumed to experience improved quality of life, but the utility weight would still be lower than for the general population. We also applied a disutility of 0.1 to the utility weights to all health states to account for the impact of health conditions that required transfusion.

Model evaluation

Baseline analysis

Three age groups were modelled in this study to account for different survival rates following blood transfusion (0–35 years, 36–65 years and 66+). The Markov model was run with an annual cycle length and a time horizon of 50, 30 and 20 years for the three age groups, respectively. The costs and health outcomes (QALYs) were accumulated as the model ran through health states until reaching the end of the time horizon. Both costs and QALYs were discounted at a rate of 5% per year in line with Australian government guidelines [27,28]. The main outputs from the Markov model were fed into the decision tree model where each pathway was associated with cost and health outcome payoffs. The expected cost and health outcomes of implementing a testing strategy were computed by summing the costs and health outcomes weighted by the probability of each outcome

TABLE 2 Model parameters.

Model parameters	Value	Reference
Blood donation (year 2020)		Lifeblood internal data
Total number of donations	1,595,364	
First-time donations	108,544	
Transfusible component donations	774,919	
Number of transfusions (year 2020)	1,194,285	Lifeblood internal data
Transition probabilities (annual)		
Spontaneous clearance	0.3610	[15]
F0–F1	0.1310	[16–18]
F1–F2	0.0885	[16–18]
F2–F3	0.1345	[16–18]
F3 to compensated cirrhosis	0.1235	[16–18]
F3 to hepatocellular carcinoma	0.0020	[16, 17, 19]
Compensated cirrhosis to decompensated cirrhosis	0.0300	[16, 17, 19]
Compensated cirrhosis to hepatocellular carcinoma	0.0360	[16, 17, 19]
Probability of liver transplant with decompensated cirrhosis	0.0330	[20]
Probability of liver transplant with hepatocellular carcinoma	0.1000	[20]
Mortality following blood transfusion	Age-specific (Table S1)	[21, 22]
Mortality (decompensated)	0.0390	[23]
Mortality (hepatocellular carcinoma)	0.1760	[23]
Mortality (liver transplant first year)	0.1690	[24]
Mortality (liver transplant following years)	0.0340	[24]
Cost (AUD 2020)		
HCV testing (*aggregated cost per donation*)		Lifeblood internal estimate
Anti-HCV testing	$7.20[a]	
ID-NAT (transfusible component)	$9.40[a]	
MP16-NAT (plasma for further manufacture)	$5.30[a]	
Health state cost		
F0–F2	$554.85	[20]
F3	$858.11	[20]
Compensated cirrhosis	$1161.43	[20]
Decompensated cirrhosis	$18,883.02	[20]
Hepatocellular carcinoma	$13,364.74	[20]
Transition cost		
HCV diagnosis	$ 1163.39	[20]
DAA interferon-free therapy	$12,661.22	PBS code 11147Q
Post-treatment for SVR	$377.04	[20]
Transition from F3 to compensated	$702.62	[20]
Transition to HCC	$1205.18	[20]
Liver transplant	$180,807.05	[20]
Utility		
Health state utility		
Australian population norms	0.91	[25]
SVR (F0–F2)	0.85	Assumed
SVR (F3)	0.82	Assumed
SVR (F4)	0.79	[26]
Non-cirrhotic stages (F0–F3)	0.75	[26]

(Continues)

TABLE 2 (Continued)

Model parameters	Value	Reference
Compensated cirrhosis	0.67	[26]
Decompensated cirrhosis	0.60	[26]
Hepatocellular carcinoma	0.66	[26]
Live with transplanted liver	0.66	[26]

Abbreviations: anti-HCV, hepatitis C virus antibody; ID, individual donor; MP16, minipool of 16 donations; NAT, nucleic acid testing; SVR, sustained virological response.
[a]This is an aggregated estimate of test cost per donation for the purposes of the economic analysis including, but not representative of, the cost of consumables procured by Lifeblood.

TABLE 3 Expected total costs and quality-adjusted life years (QALYs) per 1000 blood donations.[a]

Testing strategy[b]	Costs (AUD)	QALYs (0–35 years)	QALYs (36–65 years)	QALYs (66+ years)
1. Universal testing with anti-HCV and NAT	$14,492	22,817.24	9432.32	4633.82
2. First-time donor (anti-HCV and NAT), remaining donor (NAT)	$7781	22,817.24	9432.32	4633.82
3. Transfusible component (anti-HCV and ID-NAT), plasma for further manufacture (MP16-NAT)	$10,789	22,817.24	9432.32	4633.82
4. Universal testing with NAT only	$7292	22,817.24	9432.32	4633.82

Abbreviations: anti-HCV, hepatitis C virus antibody; ID, individual donor; MP16, minipool 16; NAT, nucleic acid testing.
[a]Incremental cost-effectiveness ratios were not reported as the difference in QALYs was negligible.
[b]For strategies 1, 2 and 4, ID-NAT is used for transfusible component donations and MP16-NAT for plasma for further manufacture.

('rollback' analysis). The total testing costs for HCV in blood donations in Australia were also estimated using the actual number of blood donations in 2020 and 2021.

Sensitivity analysis

To account for the model parameter uncertainty, we conducted a sensitivity analysis to assess the impact of varying parameter values on model outputs. Moreover, we conducted a threshold analysis to determine the parameter values required for the testing strategies to become cost-effective.

RESULTS

Baseline analysis

The expected costs and QALYs associated with 1000 blood donations under different testing strategies are presented in Table 3. As the residual risks are so low, the number of potential cases of acute hepatitis C and chronic hepatitis C approximates to zero in each scenario. Thus, the costs of managing HCV infections had virtually no impact on the total costs (which were determined by the costs of testing), and the total QALYs contributed by uninfected blood transfusion recipients were the same for all four testing strategies. As a result, universal testing with NAT only is the preferred strategy in our analysis as it had the lowest testing cost for all three age groups.

TABLE 4 Number of blood donations and estimated total testing costs for years 2020–2021.

	2020	2021
Number of blood donations		
Total	1,595,364	1,603,507
First-time	108,544	94,916
Repeat	1,486,820	1,508,591
Transfusible component	774,919	848,637
Plasma for further manufacture	820,445	754,870
Costs (AUD)		
1. Universal testing with anti-HCV and NAT	$23,119,218	$23,523,249
2. First-time donor (anti-HCV and NAT), remaining donor (NAT)	$12,414,114	$12,661,394
3. Transfusible component (anti-HCV and ID-NAT), plasma for further manufacture (MP16-NAT)	$17,212,014	$18,088,185
4. Universal testing with NAT only	$11,632,597	$11,977,999

Abbreviations: anti-HCV, hepatitis C virus antibody; ID, individual donor; MP16, minipool 16; NAT, nucleic acid testing.

Based on the actual number of blood donations in 2020 and 2021, the costs of testing blood donations for HCV using different strategies were calculated and are presented in Table 4. The costs of dual testing were estimated to be A$23 million in 2020 and increased as the volume of blood donations increased in 2021. If universal testing with NAT only were to be implemented, the total costs of testing

would be halved, and the annual cost savings could reach A$11 million.

Sensitivity analysis

Given that the background risks of HCV transmission are extremely low, varying residual risks and changing the value of parameters related to managing HCV infection had virtually no impact on the baseline results. We greatly increased the residual risk of other testing strategies in the threshold analysis. The results show that for the current practice to be cost-effective, the residual risk of other testing strategies would need to be at least one HCV infection per 2424 donations, which is over 60,000 times the baseline estimate for residual risk (1 in 151 million, Table 1).

DISCUSSION

To our knowledge, this is the first published study to assess the cost-effectiveness of removing anti-HCV blood donation testing. We tested different scenarios where NAT was applied solely for repeat donations, fractionated donations or all blood donations. Given that the residual risks of acquiring HCV following blood transfusion are extremely low for each proposed testing strategy, our modelling predicts that almost no one will develop acute or chronic hepatitis C, incur substantial costs associated with managing hepatitis C and experience reduced quality of life. As a result, the cost-effectiveness of different testing strategies is almost completely determined by the cost of testing. Therefore, strategy 4 (the NAT-only testing) is clearly the optimal strategy due to its lower testing cost.

Although we are not aware of published studies assessing the impact of removing anti-HCV testing from blood donation screening, our finding that a single test is more cost-effective than dual testing is consistent with previous cost-effectiveness studies [11–13]. These studies assessed the cost-effectiveness of adding NAT to serological (antibody and antigen) testing for blood donations and all reported that adding the additional test would not be cost-effective. This is because using a serological test alone already reduces residual risks to a very low level. Adding NAT further lowers the residual risk but the additional reduction in viral transmission is minimal, while the additional cost of testing is significant. Similarly in our case, although removing the anti-HCV test would result in a slightly higher residual risk, the impact on total costs of managing HCV infections is negligible given an already very low background risk. The results from our threshold analysis also showed that the background risk needs to be elevated to a very high level for the dual testing strategy to be cost-effective. It should be noted that our study did not include a scenario where anti-HCV alone was used for screening. The residual risk of using anti-HCV alone is many fold higher at 1 in 800,000, as estimated by the Lifeblood internal modelling. In addition, NAT also tests for hepatitis B and HIV. A strategy with anti-HCV testing alone was, therefore, not considered a realistic option in the Australian setting. Although our study findings may not be comparable to those reported in the previous cost-effectiveness studies, this study and previous cost-effectiveness studies are concordant in determining that a single test would suffice in blood donations, and dual testing is not cost-effective.

One factor distinguishing this study from earlier cost-effectiveness analyses is that we assessed targeted testing based on donation collection types. We found that the costs of targeted testing would increase as the proportion of donations receiving dual testing increases. In our case, first-time donations represented approximately 10% of total donations, so screening first-time donations with dual testing and repeat donations with NAT only is the second least costly testing strategy. Screening transfusible component donations with dual testing and screening plasma for further manufacture with MP16-NAT only would cost much more because almost 50% of blood donations are transfusible component donations, although as Australia becomes more plasma focused this proportion will change.

A limitation is the lack of population-level HCV screening rate data. In Australia, HCV screening is not conducted routinely for blood transfusion recipients nor for the general population, so we conservatively assumed a 1% annual diagnosis of theoretically infected blood transfusion recipients. Although the HCV screening rate would directly impact on the chronic HCV disease progression and associated costs (with earlier detection and treatment preventing severe complications), it does not affect the analysis due to the very small risks of viral transmission.

Cost-effectiveness analysis is one important assessment in considering blood safety risk management but risk-based decision-making includes other assessments, including stakeholder view, reputational risk and ethics [29,30]. Historically, blood operators have tended to risk-mitigate at any cost, but with risk-based decision-making principles, blood operators are moving to risk reduction. One recent example includes a transfusion-transmitted hepatitis C case in Germany that occurred with minipool testing that would likely have been prevented by ID-NAT [31]. Despite this, the authors concluded that it remains a very rare event, and the implication is, therefore, that the risk is considered tolerable. Our cost-effectiveness analysis clearly concludes that the screening strategy for HCV in blood donations currently implemented in Australia is not cost-effective compared with targeted testing or universal testing with NAT only. Partial or total removal of anti-HCV testing would bring significant cost savings without compromising blood supply safety. The purpose of risk management is not to eliminate risk but to use resources appropriately to minimize or accept the risk, and with overwhelming evidence of ineffectual resource use in our case, this provides a strong argument to cease or change anti-HCV testing.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflicts of interest.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Is dual testing for hepatitis C necessary? Modelling the risk of removing hepatitis C antibody testing for Australian blood donations

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Abstract

Background and Objectives: Parallel testing of blood donations for hepatitis C virus (HCV) antibody and HCV RNA by nucleic acid testing (NAT) has been standard practice in Australia since 2000. Meanwhile, NAT technologies have improved, and HCV has become a curable disease. This has resulted in a significant reduction in the risk and clinical consequences of HCV transmission through transfusion. This study aimed to estimate the residual risk (RR) under various testing options to determine the optimal testing strategy.

Materials and Methods: A developed deterministic model calculated the RR of HCV transmission for four testing strategies. A low, mid and high estimate of the RR was calculated for each. The testing strategies modelled were as follows: universal dual testing, targeted dual testing for higher risk groups (first-time donors or transfusible component donations) and universal NAT only.

Results: The mid estimate of the RR was 1 in 151 million for universal dual testing, 1 in 111 million for targeted dual testing of first-time donors, 1 in 151 million for targeted dual testing for transfusible component donations and 1 in 66 million for universal NAT only. For all testing strategies, all estimates were considerably less than 1 in 1 million.

Conclusion: Antibody testing in addition to NAT does not materially change the risk profile. Even conservative estimates for the cessation of anti-HCV predict an HCV transmission risk substantially below 1 in 1 million. Therefore, given that it is not contributing to blood safety in Australia but consuming resources, anti-HCV testing can safely be discontinued.

Keywords

blood donation testing, blood safety, hepatitis C virus, transfusion-transmissible infections

Highlights

- Parallel testing of blood donations for hepatitis C virus (HCV) antibody and HCV RNA by nucleic acid testing (NAT) has been standard practice in many jurisdictions since 2000.
- Testing technology refinements as well as curative treatment for HCV has changed the transfusion-transmission risk paradigm in Australia.

- Using a deterministic model to estimate residual risk (RR) for several HCV testing strategies, we conclude that if HCV RNA ID-NAT is performed, anti-HCV testing is no longer required to maintain a tolerable RR.

INTRODUCTION

Since the discovery of the hepatitis C virus (HCV), the risk of transfusion-transmitted HCV has decreased substantially. Whilst immunoassay development [1] and subsequent improvement in testing resulted in substantial reductions in [2] the risk of anti-HCV testing, it remained limited by the 7–8-week antibody testing window period [3]. Nucleic acid testing (NAT) targeting HCV RNA developed in the late 1990s significantly reduced the residual risk (RR) [4].

The current testing strategy for HCV in blood donations for transfusible components in Australia involves performing both anti-HCV testing and individual donor-nucleic acid testing (ID)-NAT. Plasma for further manufacture donations are currently tested for anti-HCV and NAT in 16 donation pools (MP-16). The HCV context has substantially changed. The development of direct-acting anti-viral drugs (DAAs) has made chronic HCV a curable disease [5]. Anti-HCV testing does not differentiate between the decreasing small number of donors at risk of transmitting with active infection and resolved infection.

The Alliance of Blood Operators' Risk-Based Decision-Making Framework determines transmission risk and cost effectiveness as fundamentals in blood safety decision-making [6, 7]. Proposed interventions should be assessed for their likelihood of mitigating the risk and the proportional resource allocation in comparison with similar risks to the blood system or health system [8]. Australian Red Cross Lifeblood's (Lifeblood) risk tolerability framework defines the tolerable risk for HCV transmission as less than 1 in 1 million (Lifeblood document), which considers other important factors in the risk assessment process including reputational risk, stakeholder assessment and societal viewpoints.

An international NAT study assessing over 10 million donations parallelly tested with NAT and anti-HCV concluded that anti-HCV testing was adding very little additional risk reduction [9]. Lifeblood remains committed to providing both a safe and cost-justified service to the Australian public, which has prompted consideration of alternative screening testing strategies in the changing HCV context.

This study models the risk of transfusion-transmitted HCV in four alternative testing strategies, enabling a subsequent economic analysis of cost effectiveness [10] to determine the optimal HCV testing strategy.

MATERIALS AND METHODS

A simple deterministic risk model was developed under various testing strategies with a risk evaluation model considering the RR as calculated and other risks, including an evaluation of risk tolerability with any testing failure and recipient impacts.

Selection of targeted-testing strategies

Consideration of various potential testing strategies was made based on Australian donation patterns and the known HCV donor prevalence. Lifeblood is plasma collection focussed with approximately 55% of all 2020 donations being apheresis plasma. The rate of HCV-positive donations (defined as being either anti-HCV-positive and/or NAT-positive) was 51.5 and 0.67 per 100,000 donations in new and repeat donors, respectively [11]. First-time donor donations were categorized as a higher testing positive risk. While this is a cumulative prevalence and does not translate to the equivalent impact on transmission, this impacts on the prevalence of detection of anti-HCV in blood donations, considered separately in the risk assessment.

Transfusible component donations were categorized as higher risk compared to plasma for further manufacture because of dedicated viral inactivation and removal processes during fractionation [12], which are effective against enveloped viruses such as HCV [13].

Therefore, the status quo was compared to three alternative testing strategies (Table 1). For each testing strategy, the RR was calculated as either a low (likely most representative), mid (midpoint between low and high) or high (worst case) estimate based on varying assumptions expanded on below.

Testing strategy 1 (status quo: ID anti-HCV testing for all donations, ID-NAT for transfusible components, MP-NAT for plasma for further manufacture)

Testing strategy 1 is the baseline current testing strategy. The RR for this testing strategy was derived using Lifeblood HCV donation testing data for the 6-year period (2015–2020) using the model established by Weusten et al. [14], which is calculated routinely by Lifeblood (see Supporting information).

Testing strategies 2 and 4

The RR for testing strategies 2 and 4 was calculated by adjusting the baseline RR by adding the estimated RR increase if anti-HCV was removed from the selected population in the respective testing strategies. The additional risk was calculated by the product of the following parameters:

1. Prevalence of anti-HCV-positive, NAT-non-reactive donations in the population if anti-HCV testing is no longer performed in either repeat donors (strategy 2) or removed completely (strategy 4) (internal data).

TABLE 1 Proposed hepatitis C testing strategies for blood donation.

Testing strategy 1	Universal testing with both NAT and anti-HCV (current testing strategy in Australia)
Testing strategy 2	Targeted testing with NAT and anti-HCV testing for first-time donors and NAT for remaining donations
Testing strategy 3	Targeted testing with ID-NAT and anti-HCV for transfusible components and MP-16 NAT for plasma for further manufacture
Testing strategy 4	Universal donation NAT only (complete removal of anti-HCV testing)

Note: Nucleic acid testing (NAT) is for individual donor for transfusible component donations and MP-16 for plasma for further manufacture.
Abbreviation: HCV, hepatitis C virus.

Note that if anti-HCV testing is withdrawn, anti-HCV-positive donors would no longer be detected and therefore the true, but now undetected anti-HCV prevalence in the donors would increase. Importantly, this does not constitute a change in the HCV incidence, but rather non-detection of past infections. The purpose of the model is to estimate potentially infectious anti-HCV-positive ID-NAT non-reactive donations. The rate of these would not be expected to further change in our model, given that non-detectable viraemia in anti-HCV-positive donations is assumed to occur after seroconversion during clearance of viraemia (i.e., during a defined period during viral clearance), and, unlike in occult hepatitis B infection, is not expected to occur intermittently given that occult HCV is defined by the absence of RNA detection in the serum [15].

The prevalence of anti-HCV-positive, NAT-non-reactive donations was derived from the 2016 to 2020 Lifeblood donation testing data.

2. The percentage of potentially infectious anti-HCV-positive, NAT-non-reactive donations.

In brief, El Ekiaby et al. [16], in the previous highest world-wide estimated HCV prevalence country, aimed to determine the prevalence of low-level viraemia in donors with a testing pattern consistent with resolved HCV infection (i.e., antibody-positive, NAT-negative donations). This is dependent on the incidence, given that this event is postulated to occur in the period during which infectivity is resolving and there will be a period, similar to the acute window period, where the virus will be present but undetectable by NAT. In this study, 175 resolved samples were evaluated for presence of low-level vireamia by replicate HCV-RNA testing ($n = 10$) in the Grifols Ultrio NAT. In the Egyptian population, 2 of 174 (0.114%) were positive. This estimate was used as our 'high estimate', but given that the incidence of HCV in Egypt as demonstrated by the same studies' NAT yield results was 1 in 7145 donations and total first-time donor HCV prevalence was 2.86%, this is a beyond 'worst case' Australian estimate. An adjustment factor for the Australian population was derived by comparing the anti-HCV-positive prevalence in Australian first-time donors (107 positive of 501,450 donations) compared to Egyptian donors (937 antibody-positive only per 119,756 donations), which was 36.7 times lower.

TABLE 2 Strategy 2 and 4 risk adjustment methodology.

Risk adjustment methodology	Data source
Prevalence of anti-HCV-positive, ID-NAT-non-reactive donations in the donor population if anti-HCV testing is no longer performed (internal data)	See Table 3
Proportion anti-HCV infectious [16]	0.114%
Adjustment factor (proportion first-time donors anti-HCV positive: Egypt to Australia)	36.7 times lower High estimate: No adjustment factor Low estimate: Full adjustment factor Mid estimate: Half adjustment factor
Infectious donation transmission risk [16]	Red cells: 0.025 Clinical plasma: 0.215
Proportion donations clinical plasma Proportion red cell or equivalent (Lifeblood internal data)	0.237 0.763

Abbreviations: HCV, hepatitis C virus; ID-NAT, individual donor-nucleic acid testing.

3. The percentage of assumed infectious anti-HCV-positive but NAT-negative donations resulting in a recipient infection (transmission factor).

The transmission factor was from El Ekiaby et al. [16]. Using Poisson distribution formulas [17] and a minimum infectious dose of 316 virions for anti-HCV-reactive transfusions [18], the authors estimated that the two low viraemic donations (which contained 0.5 and 1.8 copies/mL HCV-RNA, respectively) had probabilities of 1.1% and 3.9% to be infectious after transfusion of a red blood cell unit containing 20 mL of plasma and 10.4% and 32.6% for transfusion of a 200-mL fresh frozen plasma (FFP) unit. Therefore, the transmission factor for infectious donations used was 0.025 for red cells and 0.215 for clinical plasma. Australian 2020 donation data were used to determine the proportion of red cells and plasma. Given that Lifeblood platelets are either suspended in a platelet additive solution or a small amount of plasma, platelets were given the same transmission factor as red cells. In 2020, 76.3% of Lifeblood's components were red cells or platelets and 23.7% clinical plasma. It is noted that Lifeblood has a significant cryoprecipitate inventory, so the mean volume of clinical plasma is less than the modelled risk.

An overview of the calculation methodology is presented in Table 2. The proportional increase for each strategy was added to the original RR.

Testing strategy 3

Plasma for further manufacture incorporates viral reduction/removal processes. The assumption was that any additional low NAT-positive

TABLE 3 Number of Lifeblood blood donations that tested positive using anti-hepatitis C virus (HCV), HCV nucleic acid testing, or both, between 2016 and 2020.

Donation categories	Anti-HCV-positive, NAT reactive	Anti-HCV-positive, NAT non-reactive	Anti-HCV-negative, NAT reactive (NAT yield)
First-time donations (n = 501,450)	101	107	0
Repeat donations (n = 6,605,800)	17	41	1
Transfusible component donations (n = 3,845,771)	105	133	1
Plasma for further manufacture (n = 3,261,479)	13	15	0
Total	118	148	1

Abbreviation: NAT, nucleic acid testing.

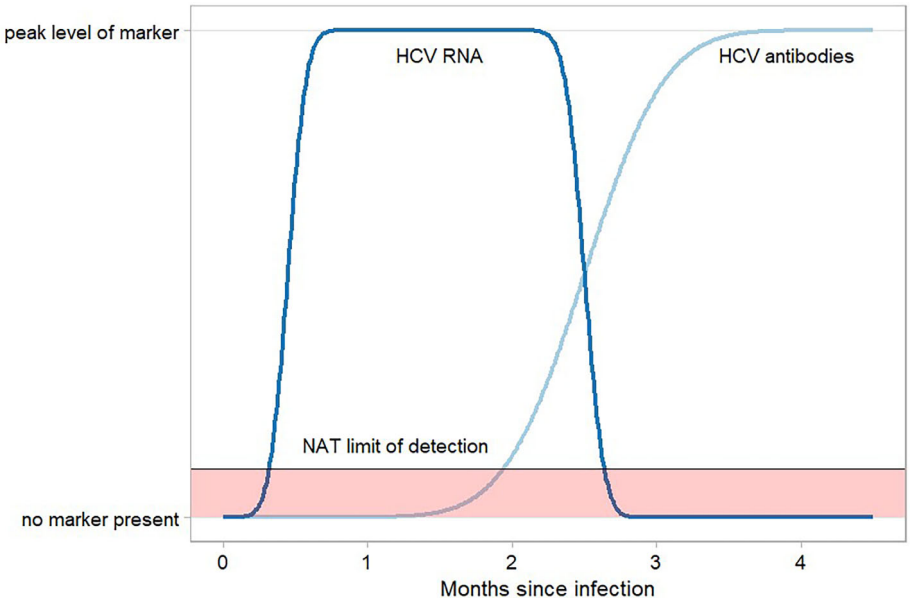

FIGURE 1 Schema of periods where nucleic acid testing can be false negative as below the NAT limit of detection following infection and ramp-up (window period, where HCV antibodies are not detectable) and during resolving infection where HCV antibodies are detectable. NAT, nucleic acid testing; HCV, hepatitis C virus (figure by Claire Styles).

donation undetected by MP-NAT would not materially alter the RR, given the low incidence. Therefore, the potential for a positive donation below the level of detection undergoing fractionation was considered when determining the RR for testing strategy 3, and how much this would increase if not testing anti-HCV donations.

A classical incidence-window period deterministic model was used to calculate the baseline RR with testing adjusted for pools of 16 (MP-16) (see Data S1). The additional risk of an anti-HCV infectious donation was added by determining the prevalence of anti-HCV untested donations based on the number of anti-HCV-positive donations of plasma (see Table 3), assuming that 0.114% were RNA positive but not detected [16], with no adjustment for the Australian population or minipool testing. These two risk figures were then added together to determine the estimated number of potentially infectious but NAT-negative donations for a total risk.

The risk of an infectious donation below the level of detection in MP-16 (48 IU/mL) was then used to calculate the maximum viral load in an 850-mL bag of plasma. A log reduction with factor of 10.6 [19] was then applied to calculate the maximum virion load in an 850-mL bag following fractionation to determine transmission risk.

Other risks to recipients

The assumption in the model is that anti-HCV-positive/NAT-negative donations are infectious only for a limited period (see Figure 1) and a single donor does not constitute an ongoing transmission risk. This assumption is based on evidence that a single test at 12 weeks after treatment is adequate to be considered cured [20] and a high concordance of 12-week results [21]. Australian donation guidelines have a 2-week donation interval, and very few donors donate at the minimum interval. However, there is a risk of passive transfer of anti-HCV to a recipient. Prior to widespread anti-HCV screening of blood donors, high concentrations in immunoglobulin preparations were

TABLE 4 Estimated residual risk of hepatitis C virus transmission for each testing strategy.

	Low estimate	Mid estimate	High estimate
Testing strategy 1	1 in 151 million	1 in 151 million	1 in 151 million
Testing strategy 2	1 in 148 million	1 in 111 million	1 in 89 million
Testing strategy 3	1 in 151 million	1 in 151 million	1 in 151 million
Testing strategy 4	1 in 146 million	1 in 66 million	1 in 43 million

reported, prompting a recommendation to implement plasma donor anti-HCV screening to prevent passive transfer from interfering in patient monitoring [22]. This could result in test misinterpretation, predominately in recipients of plasma donations, and this is mitigated by removing anti-HCV-positive donations. The risk of transfusing an anti-HCV-positive donation with the capacity to interfere with recipient test results was calculated, considering the prevalence would be the first-time donor prevalence, if testing was discontinued.

Risk of a process failure

Continuing anti-HCV testing provides redundancy in case of a process failure of NAT, where both tests have to fail for transfusion-transmission. To demonstrate that this is largely a theoretical risk, a process failure model was developed with the current anti-HCV-positive RNA-positive donations for testing strategies 2 and 4. The rate of positive RNA donations was determined from internal data for first-time and repeat donors. For *no testing*, the rate of RNA-positive donations was the total rate in first-time and repeat donors, and for *first-time donor testing*, the rate was the rate in repeat donors. For plasma for fractionation, the model accounted for MP-16 (the risk increased by 16).

Process failure rate additional risk
= Various risks of a process failure × risk of an RNA
− positive donation that does not have dual testing under the strategies.

RESULTS

Screening data

Over the 2016–2020 period, 7,107,210 donations were included. Of these, 118 donations tested positive for HCV by both NAT and antibody testing, 148 anti-HCV positive only and 1 NAT yield (see Table 3).

Residual risks

Table 4 presents the four testing strategies' HCV transmission RRs.

The RR in a plasma for further manufacture was estimated to introduce an additional 0.17 extra donations below the threshold of NAT but positive in the 5-year period, with a risk of a NAT-negative donation containing infectious virions being 1 in 16.4 million. The estimated number of virions left after fractionation with a worse case unit was 2.77×10^{-6}. This number is substantially less than the postulated 50% minimum infectious dose of 7–20 copies [18]. Therefore, we conclude that the RR in a plasma for further manufacture is no greater than the baseline risk and the RR is unchanged.

Passive transfer risk

If anti-HCV antibody testing was discontinued, the rate of donations testing positive could be expected to stabilize at the first-time donor rate of approximately 1 in 4700.

Process failure risk

The process failure risks are outlined in Table 5. For each strategy of no testing, first-time donor testing and transfusible component testing, a process failure would need to occur, on average 1 in every 16.7, 2.6 and 103 donations, respectively, to increase release of a viraemic unit to more than 1 in 1 million.

DISCUSSION

Our modelling demonstrates that anti-HCV testing is not required to maintain a tolerable transfusion-transmission HCV risk, even using our unrealistically high estimate. Therefore, changing to a testing strategy that is more cost effective should be considered, and accordingly the risks derived here have been applied to a separate cost-effectiveness analysis [10].

Cappy et al. [23] estimated that 0.5% of anti-HCV NAT-negative (including a period of pooled testing) had low-level RNA. This value is lower than our high estimate. A formal RR was not performed in this work and, importantly, our argument is about cost effectiveness [10] for a test that does not materially change the RR.

Our findings of dual testing inefficiency are not novel. A large, multi-country NAT study published in 2015 demonstrated that the efficacy of HCV NAT in removing HCV transmission risk per unit of blood was 99.98% in first-time donors and 97.94% in repeat donors [9]. The authors concluded that the efficacy increase of anti-HCV testing when ID-NAT screening is performed was minimal.

The Australian overall notification rate of HCV declined by 31% over a 10-year period, as has the proportion of potentially infectious donors (i.e., RNA-positive cases) [11]. Australia provides free

TABLE 5 Event of a process failure and impact.

Process failure rate of the NAT (1 in *x*)	Testing strategy 4: Additional risk (1 in *x*)	Testing strategy 2: Additional risk (1 in *x*)	Testing strategy 3: Additional risk of contaminated fractionated plasma unit (1 in *x*)
100,000	5,972,478,992	38,857,647,059	962,481,250
10,000	597,247,899	3,885,764,706	96,248,125
1000	59,724,790	388,576,471	9,624,812
100	5,972,479	38,857,647	962,481
10	597,248	3,885,765	96,248
2	119,450	777,153	19,249

Abbreviation: NAT, nucleic acid testing.

treatment for HCV with DAAs [24], resulting in cure in over 95% [5]. Not only has this contributed to a decreasing transfusion-transmitted RR because of declining HCV incidence, but it has also lessened morbidity/mortality. These developments favour transitioning to a more cost-effective HCV donation testing strategy.

Performing two HCV screening tests to address a potential process failure associated with a single test process is one argument to maintain anti-HCV testing. However, when we modelled the process failure rates and associated RRs by strategy, it was clear that very high failure rates in a single test (NAT) were required to result in an intolerable RR. To breach Lifeblood's 1 in 1 million intolerable threshold, a process failure for NAT would need to occur on average 1 in every 2.6–16.7 donations. While the exact Lifeblood failure rate of NAT is unknown, the demonstrated failure rates required to impact materially are clearly outside the bounds of how often a process failure would occur with good laboratory practices and is therefore a negligible risk.

Blood donation testing for traditional transfusion-transmitted infections evolved over time in Australia with the addition of more sensitive tests [25]. However, single-test serological strategies were used effectively in Australia prior to NAT implementation [26, 27]. West Nile virus single testing, which uses NAT [28], provides adequate protection against viraemia that may be as high as 1 in 1057. The exemplary safety profile of ID-NAT for HCV is supported by the absence of any ID-NAT reported cases of transfusion-transmission. In addition, in the resolving phase of infection with viraemia below the level of detection, there is decreased infectivity [18] compared to the same viral load in the ramp-up phase, which is thought to be due to viral particle immune complexes and neutralizing antibodies.

Although complete cessation of anti-HCV testing has operational advantages and is the most cost-effective [10], there are reasons why first-time donor testing may be regarded as the optimal initial change. Given that first-time donors are only 11.5% of total Lifeblood tests (and decreasing over time) and account for 73% of all anti-HCV positives, anti-HCV testing costs could be reduced by approximately 90%. First-time donor screening would also prevent the majority of true-positive anti-HCV donors with cleared infection entering the blood donor pool, which would avoid potential downstream issues, such as passive transfer. Passive transfer could theoretically cause transient false positivity in recipients receiving FFP and concentrated plasma products if tested in the months post transfusion. However, given that this is a measure of past exposure, this would not impact on a treatment plan. If first-time anti-HCV-positive donors were tested and removed from the donor pool, this risk would be sufficiently rare to be a negligible for transfusible components, given the dilution factor. In Australia, we have recently ceased serological screening for both human T-cell lymphotropic virus and syphilis in plasma donations for further manufacture, excluding first-time donor testing, because of a negligible transmission risk. This is despite the potential for passive transfer, demonstrating that this consequence alone is not a reason to continue testing in the absence of a transmission risk.

False-positive results are a significant issue in testing blood donations [29]. False positivity is a random event, and therefore eliminating the majority of testing (repeat donors) will eliminate much of the issue of false-positive results. This would reduce the operational load of complex and time-consuming counselling as well as costly discard of non-issuable false-positive donations.

We considered anti-HCV cessation and the potential risk of occult HCV. Occult HCV is defined as the presence of HCV RNA in hepatocytes or peripheral blood mononuclear cells (PBMCs) while being absent in the serum [30]. Considering NAT measures HCV RNA in the serum, it is unable to detect occult HCV infections. No Australian cases of occult HCV have been reported. Studies estimate from 0.15% to 85% of being able to detect RNA, depending on the population group. However, all testing involves PBMCs or hepatocytes [30] and this is not synonymous with a transfusion-transmission risk, as demonstrated with other viruses such as EBV, which, while detectable lifelong in lymphocytes, do not represent a chronic transmission risk [31]. We consider that occult HCV remains only a theoretical transfusion-transmission risk.

Anti-HCV testing in addition to multiplex NAT (which simultaneously mitigates HIV, HBV and HCV risk) does not contribute to blood safety in Australia, while adding substantial cost. In keeping with risk-based decision-making principles and blood operators moving away from preventing extremely rare risks at any cost, as evidenced by the recent argument in favour of continuation with MP NAT in Germany despite an extremely rare HCV minipool transmission [32], our risk tolerability threshold incorporates societal expectations and appropriate resource use. Given that the marginal risk

reduction does not materially change the RR for transfusion recipients, our modelling demonstrates that, even using conservative assumptions, this is an ineffectual use of resources. Complete cessation of anti-HCV testing is operationally the simplest option. Based on our findings, we intend to progress an application to our regulator to cease anti-HCV testing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

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ORIGINAL ARTICLE

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Application of droplet digital PCR combined with TaqMan real-time PCR in Dombrock blood group genotyping in Northwest China

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Abstract

Background and Objectives: The Dombrock blood group system is based on the *DO* gene. *DO*A* and *DO*B* antigens are the result of a single-nucleotide polymorphism (SNP) on this gene. The introduction of Do antigens through blood transfusion or other invasive factors like infection may result in the production of Do antibodies, which may cause serious haemolytic transfusion reactions. In this study, TaqMan real-time PCR and droplet digital PCR were used to detect rare *DO*A* allele, guide the search for rare *DO*A* allele donors, and calculate *DO* alleles frequencies in mixed populations in Northwest China.

Materials and Methods: In this study, the highly sensitive and accurate TaqMan real-time polymerase chain reaction (PCR) method was used to detect and screen *DO* genotype SNPs in combination with droplet digital PCR. We also searched for rare *DO*A* allele donors and calculated the frequencies of *DO* alleles in mixed populations.

Results: A total of 1202 donor DNA samples were collected from Northwest China, of which 202 were used to detect *DO* allele SNPs using TaqMan real-time PCR. The rare *DO*A* allele was detected in the other 1000 blood donors by droplet digital PCR, and gene frequencies were inferred from dual channel droplet digital PCR data. Among 1202 donors from Northwest China, the allele frequencies of *DO*A* and *DO*B* were 0.1128 and 0.8872, respectively.

Conclusion: The sequencing results confirmed that this new way of detecting *DO* alleles by droplet digital PCR with specific probes can detect rare *DO*A* allele to predict the presence of the rare antigen Do^a and infer *DO* allele frequencies. This method is highly sensitive and specific.

Keywords

Dombrock blood group system, droplet digital polymerase chain reaction, genotype, TaqMan real-time polymerase chain reaction

Zhuolin Li and Yufeng Wang contributed equally to this work.

Highlights

- A new method that combines TaqMan real-time polymerase chain reaction (PCR) with droplet digital PCR was used to detect and screen Dombrock (DO) blood group genotype single-nucleotide polymorphisms.
- The allele frequencies of the DO blood group system in Northwest China were *DO*A* = 0.1128 and *DO*B* = 0.8872.
- This method could replace traditional methods and improve the efficiency of gene frequency measurement in the population.

INTRODUCTION

Gene frequency has become an academic frontier issue in recent years within population genetics [1]. To address this need, it is important to have a fast, convenient and precise method for producing gene and genotype frequency statistics. As a population genetics marker, blood group inheritance is the theoretical basis of clinical blood detection, transfusion technology, organ matching and transplantation technology and can be used in paternity test, disease association analysis, ethnographic evolutionary research, and so on [2]. Affected by genetic drift or microbiological environment, the frequencies of blood type genes vary by region and race [3, 4]. The distribution frequencies of blood type genes have also become an important research focus in population genetics.

Most blood group antigens are monogenic final expression products. Blood group genes are closely linked to the expression of blood group antigens. Therefore, genetic level changes, like insertion, deletion, conversion, alternative splicing or single-nucleotide polymorphisms (SNPs) [5], may lead to antigen differences, even the complete loss of or creation of new antigens [6]. In 1965, a new red blood cell antigen, Do^a, was found in the blood of a White patient, Mrs. Dombrock, through serological testing [7]. The Do^b antigen was found 8 years later, thereby expanding the Dombrock blood group system [8]. According to the International Society of Blood Transfusion (ISBT) nomenclature, the allele for the Do^a antigen is *DO*A*, and the allele for the Do^b antigen is *DO*B* in the Dombrock blood group. The Dombrock blood group is based on the *ART4* gene (GenBank accession number X95826) on the short arm of *Homo sapiens* chromosome 12, and has been renamed as the *DO* gene (GenBank accession number XM_017877) [9]. Its antigens are anchored to the red blood cell membrane by glycosylphosphatidylinositol [10]. The *DO*B* allele has three altered nucleotides in exon 2, two of which are synonymous variants (c.378T>C; p.Tyr126= and c.624C>T; p.Leu208=). The other is a missense variant (c.793G>A; p.Asp265Asn). The SNP c.793G>A sequence at this position constitutes the genetic basis of alleles, *DO*A* and *DO*B* [11, 12].

Studies have shown that blood groups have different frequencies in different populations [3]. In a White population, the frequencies of the *DO* allele were found to be *DO*A* = 0.425 and *DO*B* = 0.575; in a Black population, the allele frequencies were *DO*A* = 0.33 and *DO*B* = 0.67; in a Japanese population, the allele frequencies were *DO*A* = 0.125 and *DO*B* = 0.875; in a Thai population, the allele frequencies were *DO*A* = 0.07 and *DO*B* = 0.93 [9]; in a Chinese Han population, the allele frequencies were found to be *DO*A* = 0.1027 and *DO*B* = 0.8973 [13]. It can be seen that, compared with Europeans, the prevalence of the *DO*B* allele in Asians is much higher. At present, it has not been reported that DO antibodies cause haemolytic disease in newborns, but in adults, abundant serious haemolytic transfusion reactions caused by anti-DO antibodies have been reported [11, 14, 15]. Therefore, to reduce the occurrence of antigen-mediated transfusion reaction, we need an efficient and accurate genotyping method to determine the Dombrock blood group.

Several methods have been reported to detect the frequency of *DO* alleles in the population. These include serological methods [7], polymerase chain reaction and restriction fragment length polymorphism analysis (PCR-RFLP) [16], microarray single-nucleotide polymorphic gene chips technology (Array-SNP) [17] and PCR with sequence-specific primer (PCR-SSP) [13]. Serological methods have several key weaknesses—there are frequent false positives, they have low sensitivity, take a long time to process and may determine only one blood type at a time [18]. PCR-RFLP likewise has limitations: it has low fluxes and not every SNP has suitable restriction endonuclease, which leads to the limitations of the application [19]. So far, PCR-SSP, which is inexpensive and convenient to operate, is the most frequently used technology, but it still cannot classify point mutations accurately and has a high false-positive rate [20]. For the past few years, because of the specificity and high sensitivity of its detection, TaqMan real-time PCR has been increasingly used to detect and determine allele frequencies [21], and it serves as a very practical detection tool. But even though TaqMan real-time PCR technology can detect *DO* alleles precisely, it cannot detect rare alleles in the mixed samples, which were mixed from the same amount of blood samples in batches, nor can it conduct absolute quantitative analysis. It still requires for each sample detection, which multiplies the cost of reagents, materials and manpower. Hence, it is not appropriate for studying gene distribution frequency in countries with large population bases like China. Therefore, we saw a need to develop a new technology combining simplicity, efficiency, low cost and quantitative capacity in mixed samples to study the allele frequencies of the Dombrock blood group.

Droplet digital PCR creates micro-drops from the samples before traditional PCR amplification [22]. After PCR amplification, it detects each droplet one by one. According to the Poisson

distribution principle, by using the number or proportion of positive droplets, the initial concentration or copy number of the target nucleotide molecule can be obtained [23]. As droplet digital PCR is a highly precise and absolute quantitative detection system, the use of droplet digital PCR may help to detect rare alleles in blood donors [24]. Moreover, the method can also be used for multiple samples or mixed samples simultaneously, which greatly reduces the time required, difficulty, cost and efficiency. Therefore, droplet digital PCR can be widely used in population mutation screening theoretically [25].

The purpose of this study was to design and try a new droplet digital PCR technology that can determine allele frequencies in a population to detect the Dombrock blood type, facilitating the search for more rare blood donors in the population. Determining the applicability of this method is the first step to screening *DO* alleles and preventing Do antibody-mediated transfusion reactions.

MATERIALS AND METHODS

Donor blood samples

We recruited 1202 blood sample donors from the northwest of China. The experiment used an ethylenediaminetetraacetic acid tube to collect 2 mL of fresh blood from each donor, of which 202 were used for the TaqMan real-time PCR for genotyping and for examining the droplet digital PCR accuracy of allele detection. Another 1000 samples were prepared as 20 random 50-person blood pools, each pool was mixed from 50 equal amounts of blood, to calculate the *DO* allele frequency. Proceeding according to the manufacturer's instructions, we selected and used the Ezup Column Blood Genomic DNA Purification Kit (Sangon Biotech) to extract genomic DNA from each sample to carry out follow-up experiments.

TaqMan real-time PCR genotyping

A TaqMan real-time PCR method for DNA genotyping was established in this study. The TaqMan real-time PCR amplicon was designed with a length of 107 bp. The primers (Sangon Biotech) and probes (Sangon Biotech) were designed as shown in Table 1 (primers are 1-1 and 1-2; probes are 2-1 and 2-2). Using 0.8 μL of each probe, 0.4 μL per primer, along with 10 μL of 2× Premix Ex Taq (Probe qPCR), 1 μL of 30 ng genomic DNA, 0.4 μL of 50× ROX Reference Dye II (Premix Ex Taq™ (Probe qPCR); TAKARA) and 6.2 μL of ddH_2O were added to the 20 μL of the reaction mixture. In total, 202 DNA samples were individually genotyped, and the SNPs were standardized for the evaluation using an ABI 7500 Fast (Applied Biosystems) TaqMan real-time PCR system with the following conditions: pre-amplification at 60°C for 1 min, followed by 95°C for 10 min, 40 cycles at 95°C for 15 s and 61°C for 1 min, and finally, post-amplification at 60°C for 1 min.

TABLE 1 Primers and probes used in *DO*A*/*DO*B* (Dombrock blood group system) genotyping and sequencing.

Number	Primers and probes	Sequences
1-1	DOMBROCK FP	5′-agtcttgatccctccctatga-3′
1-2	DOMBROCK RP	5′-agttatatgtgctcaggttccc-3′
2-1	DOMBROCK A	5′ VIC-tgagctaccacccaaga ggaaact-3′ MGB
2-2	DOMBROCK G	5′ 6-FAM-tgagctaccacccaaga ggagact-3′ MGB
3-1	*DO* ID FP	5′-gaggtgcattataggacgaagg-3′
3-2	*DO* ID RP	5′-ccatcattaccgaaggctagtt-3′

Nucleotide sequencing

After genotyping using TaqMan real-time PCR, each sample needed to be amplified and sequenced separately to test whether the results of TaqMan real-time PCR were accurate. The sequence of amplification and sequencing primers (Sangon Biotech) were the same, as shown in Table 1 (3-1 and 3-2); the amplified region included the *DO* SNP region. A 30 μL reaction mixture was prepared comprising 15 μL of 2× Taq PCR mix (RUNDE), 1.5 μL each of forward and reverse primers (10 μM, Sangon Biotech), 3 μL of 30 ng genomic DNA, as well as 9 μL of ddH_2O. Amplification was performed using a thermal circulator (ProFlex PCR System, Applied Biosystems by Life Technologies) at 95°C for 10 min, with 40 cycles at 94°C for 30 s, 61°C for 1 min, 72°C for 30 s, then 98°C for 10 min, followed by 4°C indefinitely. Each sample sequence was sent for Sanger sequencing after amplification.

Detecting rare *DO*A* allele by blood pool organization

To verify the sensitivity and accuracy of droplet digital PCR and detect the rare *DO*A* allele, six blood pools with 50 samples were established for droplet digital PCR based on the DNA of 202 donors with known genotypes as determined by TaqMan real-time PCR. The sample blood pools were designed as follows:

1. Pool 1.0 contained 50 randomly selected blood samples of *DO*(*A*−*B*+) donors as the control group;
2. Pool 1.1 contained blood samples from 1 *DO*(*A*+*B*+) donors and 49 *DO*(*A*−*B*+) donors;
3. Pool 1.2 contained blood samples from 2 *DO*(*A*+*B*+) donors and 48 *DO*(*A*−*B*+) donors;
4. Pool 1.3 contained blood samples from 3 *DO*(*A*+*B*+) donors and 47 *DO*(*A*−*B*+) donors;
5. Pool 1.4 contained blood samples from 4 *DO*(*A*+*B*+) donors and 46 *DO*(*A*−*B*+) donors;
6. Pool 1.5 contained blood samples from 5 *DO*(*A*+*B*+) donors and 45 *DO*(*A*−*B*+) donors.

Inferring *DO* alleles frequencies by blood pool organization

As droplet digital PCR has the capacity for absolute quantification, we randomly selected blood samples from the remaining 1000 donors with unknown Dombrock genotypes to form 20 blood pools of 50 individuals named pools 2.1–2.20. Droplet digital PCR was used to detect the rare alleles as well as to calculate the frequencies of *DO* alleles.

Droplet digital PCR

The same probes and primers were used for droplet digital PCR as those for TaqMan real-time PCR (Table 1). Before the droplet digital PCR experiment, a 20 μL pre-mixed solution was prepared with 10 μL of droplet digital PCR supermix for probes (Bio-Rad), 0.1 μL of each of the probes and primers (Bio-Rad), 9.1 μL of ddH_2O and 0.5 μL of DNA from the 50-donors blood pool. All of the reaction solution was added into a Droplet Generator DG8 Cartridge (Bio-Rad) using eight compartments to generate droplets. The entire droplet was then transferred into the 96-well PCR plate (Bio-Rad). This PCR plate was then covered with aluminium foil and sealed with high heat in the PX1 PCR Plate Sealer (Bio-Rad). The PCR plate was then placed in a GeneAmp PCR System 9700 (Applied Biosystems) with the following program: 95°C for 10 min, 40 cycles of 94°C for 30 s, 61°C for 1 min and 98°C for 10 min, followed by 4°C indefinitely. After the PCR, the PCR plate including droplets was moved into a QX200 droplet reader Digital PCR System (Bio-Rad) to analyse as well as quantify the PCR targets using QuantaSoft software version 1.7.4.0917 as the number of copies per microliter (copies/μL).

Statistical analysis

According to the Poisson distribution model, the numerical calculation was performed using Microsoft Excel software. The statistical data of this experiment were analysed by GraphPad Prism 9.3.0 software (GraphPad Software).

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of Air Force Medical University. Informed consent was obtained from all subjects involved in the study.

RESULTS

TaqMan real-time PCR analysis and sequence analysis

A total of 202 samples were genotyped by TaqMan real-time PCR. Figure 1 shows the genotyping results of TaqMan real-time PCR of the Dombrock blood group in individual samples. Individuals with three genotypes, *DO*(*A*+*B*−), *DO*(*A*−*B*+) and *DO*(*A*+*B*+), can be identified clearly in the amplification plots. To verify the accuracy of the TaqMan real-time PCR results, all of the 202 nucleic acid samples were subjected to Sanger sequencing involving SNP regions. Primers were designed to amplify a specific segment of the *DO* gene 430 bp region fragment (including c.793G>A) for sequence analysis. It was accurate and specific in the identification of the *DO* gene SNP in the 202 samples. The TaqMan real-time PCR analysis system gave the same results as the Sanger sequencing results. This demonstrated the accuracy and specificity of TaqMan real-time PCR for genotyping. Further analysis of 202 blood donors' *DO* gene from Northwest China (Table 2) revealed that only 3 donors (1.49%) were homozygous for *DO*A*, 34 donors (16.83%) were heterozygous for *DO*A* and *DO*B* and 165 donors (81.68%) were homozygous for *DO*B*. Since the Dombrock blood group system conforms to Hardy–Weinberg equilibrium [26], it has been proven that *DO*A* homozygous blood donors are rare donors. TaqMan real-time PCR can accurately guide us to find such rare blood donors, but it can only perform single sample typing each time and cannot detect large numbers of blood donors together. At this point, we need to use droplet digital PCR for detection.

Droplet digital PCR analysis

In the Asian population, the incidence of the *DO*A* allele in the population is extremely low. Traditional methods cannot detect rare *DO*A* alleles in batches; so, droplet digital PCR may be suitable to screen rare blood donors of the *DO*A* phenotype by quantifying rare *DO*A* alleles in blood pools.

It was verified that droplet digital PCR was accurate for the determination of all three Dombrock genotypes (Figure 2a–d). As shown in the results, *DO*A* allele concentrations were accurately determined by droplet digital PCR in all experimental blood pools (pools 1.0–1.5). The concentration and copy number of the *DO* allele in droplets can be visualized in the 2D projection in Figure 2e–j. The ratios of FAM fluorescence channels to VIC fluorescence channels in blood pools 1.1–1.5 were 100, 44, 38, 19 and 15, respectively (Table 3; Figure 2k). It can be seen that as the number of *DO*(*A*+*B*+) donors in the blood pool increases, that is, the number of *DO*A* alleles increases, the concentration ratio of FAM fluorescence channel (*DO*B* gene) to VIC fluorescence channel (*DO*A* gene) significantly decreases; the ratio of *DO*A* allele in each blood pool can be roughly estimated by this ratio. Results can be used to calculate the frequencies of rare alleles of *DO*A* and to guide the search for rare *DO*A* blood donors.

Inference of *DO* gene frequency by droplet digital PCR

The results showed that droplet digital PCR was highly consistent with the actual gene frequencies of *DO*A*. Due to its proven advantages of high sensitivity, high accuracy and absolute quantitation, droplet digital PCR was used to test 20 pools (pool 2.1–2.20) of

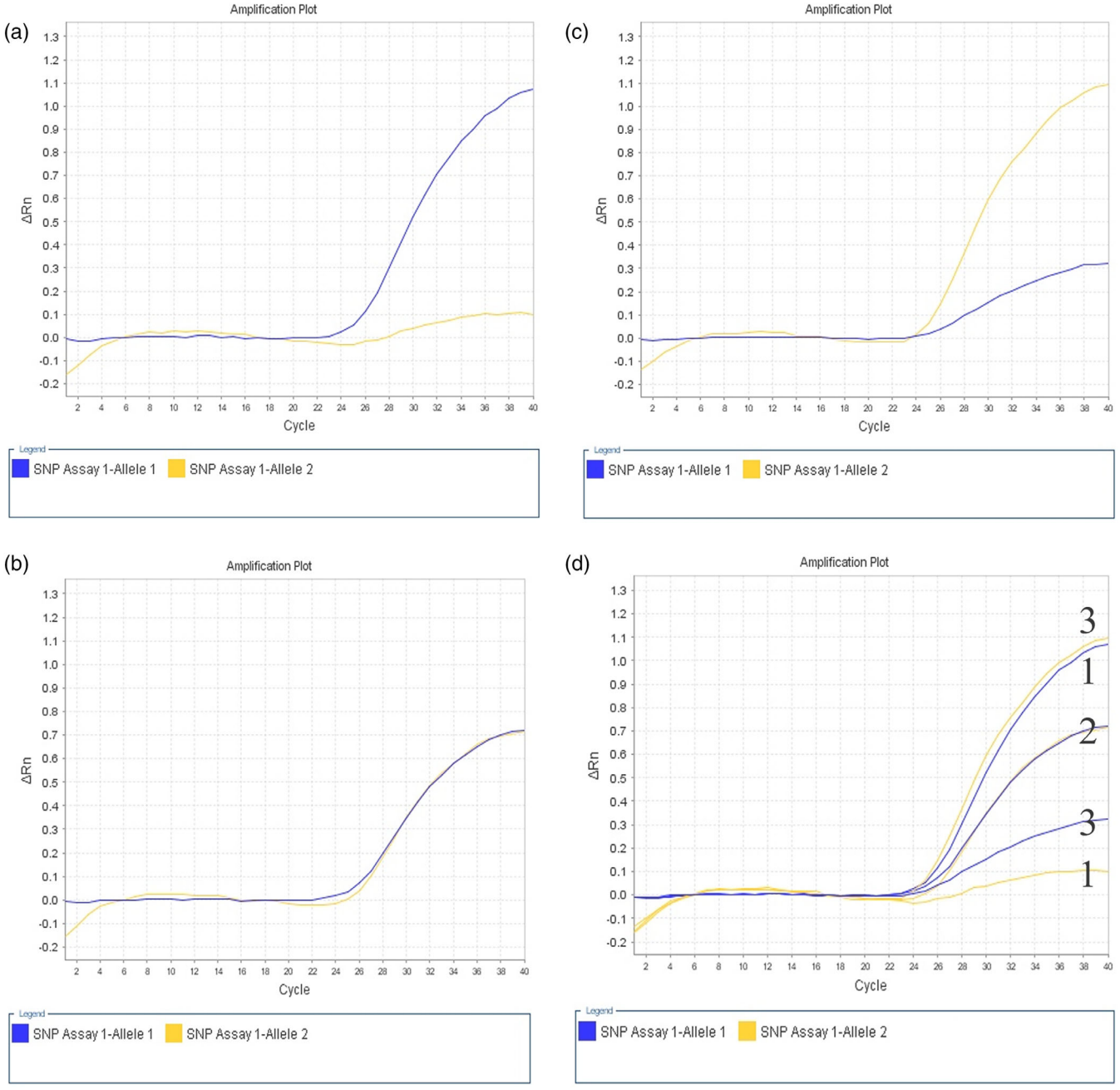

FIGURE 1 TaqMan real-time polymerase chain reaction genotyping results of three representative Dombrock blood group system genotypes. Single-nucleotide polymorphism (SNP) Assay 1-Allele 1 stands for *DO*A* allele, SNP Assay 1-Allele 2 stands for *DO*B* allele. (a) *DO*(*A*+*B*−) genotype. (b) *DO*(*A*+*B*+) genotype. (c) *DO*(*A*−*B*+) genotype. (d) Merge of three genotypes, 1 is for *DO*(*A*+*B*−) genotype, 2 is for *DO*(*A*+*B*+) genotype and 3 is for *DO*(*A*−*B*+) genotype.

TABLE 2 Number and proportion of Dombrock three genotypes among 202 blood donors.

DO genotype	Dombrock expression	Donors	Proportion (%)
*DO*A*, *DO*A*	*DO*(*A*+*B*−)	3	1.49
*DO*A*, *DO*B*	*DO*(*A*+*B*+)	34	16.83
*DO*B*, *DO*B*	*DO*(*A*−*B*+)	165	81.68

50 blood samples randomly drawn from 1000 individual blood donors to calculate the *DO* allele frequencies and search for rare blood donors. The droplet digital PCR detection results and gene frequency calculation results are shown in Table 4. The original ratios of FAM fluorescence channel (*DO*B* gene) to VIC fluorescence channel (*DO*A* gene) were 11, 5.3, 8.2, 3.8, 9.2, 7.6, 17, 9.4, 6.2, 6, 18.7, 10.6, 10.7, 4.1, 7.6, 4.1, 13.8, 6.9, 20.6 and 6.5, respectively. Combined the droplet digital PCR results of 1000 blood donors with the TaqMan results

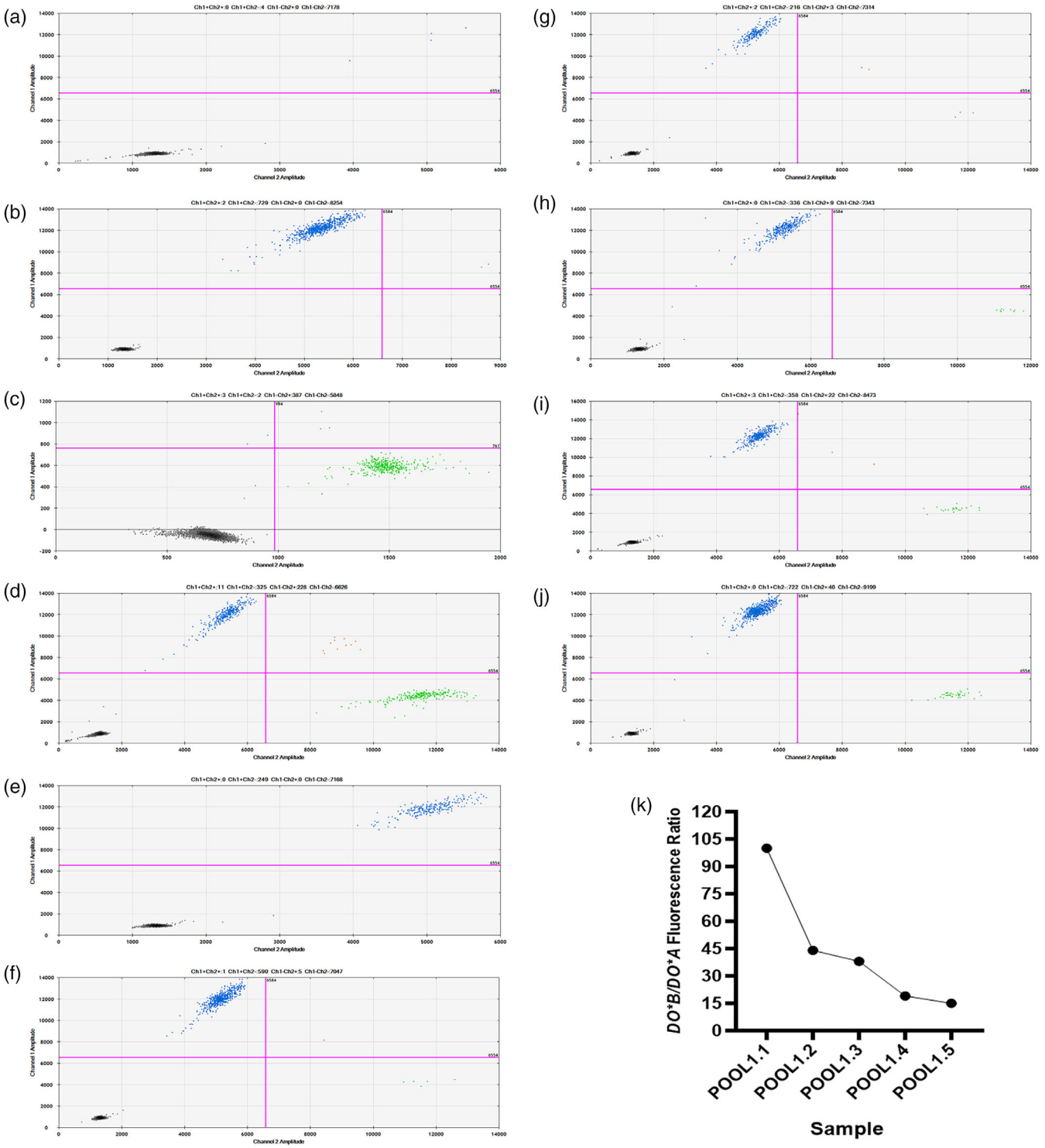

FIGURE 2 Visualization of the 2D projection of the droplets from the QuantaSoft software. The vertical axis shows the FAM fluorescence channel amplitude and the horizontal axis shows the VIC fluorescence channel amplitude. (a) Control group, ddH_2O. (b) *DO*(*A*−*B*+) homozygous. (c) *DO*(*A*+*B*−) homozygous. (d) *DO*(*A*+*B*+) heterozygous. (e) Pool 1.0 contains 50 *DO*(*A*−*B*+) donors. (f) Pool 1.1 contains 1 *DO*(*A*+*B*+) and 49 *DO*(*A*−*B*+) donors. (g) Pool 1.2 contains 2 *DO*(*A*+*B*+) and 48 *DO*(*A*−*B*+) donors. (h) Pool 1.3 contains 3 *DO*(*A*+*B*+) and 47 *DO*(*A*−*B*+) donors. (i) Pool 1.4 contains 4 *DO*(*A*+*B*+) and 46 *DO*(*A*−*B*+) donors. (j) Pool 1.5 contains 5 *DO*(*A*+*B*+) and 45 *DO*(*A*−*B*+) donors. (k) Ratio of fluorescence concentration of FAM to VIC fluorescence channel in pool 1.1–1.5.

of 202 blood donors and the Sanger sequencing results, based on the 1202 samples, the allele frequencies of the Dombrock blood group in Northwest China were *DO*A* = 0.1128 and *DO*B* = 0.8872.

DISCUSSION

Determining antigens and antibodies in donor blood before transfusion is essential to reduce acute haemolytic transfusion reactions. This study showed that TaqMan real-time PCR has high specificity and accuracy for Dombrock blood group genotyping. However, there was no method for batch detection of *DO*A* rare allele genotyping in the Dombrock blood group system. The detection results of droplet digital PCR used in this study in a total of 26 blood pools of 50 donors showed that it can be simple to screen *DO*A* rare genes in a batch population with high sensitivity and accuracy.

Compared with other Dombrock blood group allele detection methods, which have high false-positive rates, low sensitivity, long processing times and high cost, TaqMan real-time PCR has the advantages of simple operation, high accuracy, high sensitivity and low cost. Hence, it has been increasingly applied in allele detection.

In Asian populations, Do^a is a low frequency blood group antigen. Therefore, it is very necessary to screen and detect the rare antigen allele in blood donors. TaqMan real-time PCR technology can detect this, but only a single sample can be determined each time. To reduce the cost of DNA extraction and determination, batch DNA extraction in the form of blood pools and droplet digital PCR can be used to detect rare alleles, with high accuracy and sensitivity, and also with reduced cost.

As droplet digital PCR is more sensitive and accurate than TaqMan real-time PCR, the method can also identify other rare alleles by analogy, detect blood pools containing a larger number of donors, and accurately characterize multiple samples simultaneously to monitor the frequency of population genetic variants in a more cost-effective method. Therefore, droplet digital PCR can also be applied in the fields of population genetics, immunohematology, genetic diseases and so on.

TABLE 3 Expected and obtained ratio of *DO*B*/*DO*A* in six blood pools.

Sample pool	*DO*A*% in blood pool	Expected *DO*B*/*DO*A* ratio	Obtained *DO*B*/*DO*A* ratio	Obt./exp.
1.1	1%	99	100	1.01
1.2	2%	49	44	0.90
1.3	3%	32.3	38	1.18
1.4	4%	24	19	0.79
1.5	5%	19	15	0.79

TABLE 4 Allele frequency, allele number, genotype frequency and the number of donors in 20 blood pools detected by droplet digital polymerase chain reaction.

		Allele frequency		Allele number		Genotype frequency		
Pool	Ratio (*DO*B*/*DO*A*)	*DO*A*	*DO*B*	*DO*A*	*DO*B*	*DO*(*A*+*B*−)	*DO*(*A*+*B*+)	*DO*(*A*−*B*+)
2.1	11 ± 5.28	0.083	0.917	8.33	91.67	0.0069	0.1522	0.8409
2.2	5.3 ± 2.08	0.159	0.841	15.87	84.13	0.0253	0.2674	0.7073
2.3	8.2 ± 4.44	0.109	0.891	10.87	89.13	0.0119	0.1942	0.7939
2.4	3.8 ± 2.13	0.208	0.792	20.83	79.17	0.0433	0.3295	0.6273
2.5	9.2 ± 4.29	0.098	0.902	9.80	90.20	0.0096	0.1768	0.8136
2.6	7.6 ± 3.19	0.116	0.884	11.63	88.37	0.0135	0.2051	0.7815
2.7	17 ± 6.65	0.056	0.944	5.56	94.44	0.0031	0.1057	0.8911
2.8	9.4 ± 4.62	0.096	0.904	9.62	90.38	0.0092	0.1736	0.8172
2.9	6.2 ± 2.02	0.139	0.861	13.89	86.11	0.0193	0.2394	0.7413
2.10	6 ± 2.23	0.143	0.857	14.29	85.71	0.0204	0.2451	0.7344
2.11	18.7 ± 7.05	0.051	0.949	5.08	94.92	0.0026	0.0964	0.9011
2.12	10.6 ± 2.65	0.086	0.914	8.62	91.38	0.0074	0.1576	0.8350
2.13	10.7 ± 2.72	0.085	0.915	8.55	91.45	0.0073	0.1563	0.8364
2.14	4.1 ± 0.71	0.196	0.804	19.61	80.39	0.0384	0.3153	0.6463
2.15	7.6 ± 1.62	0.116	0.884	11.63	88.37	0.0135	0.2055	0.7810
2.16	4.1 ± 0.90	0.196	0.804	19.61	80.39	0.0384	0.3153	0.6463
2.17	13.8 ± 3.25	0.068	0.932	6.76	93.24	0.0046	0.1260	0.8694
2.18	6.9 ± 1.76	0.127	0.873	12.66	87.34	0.0160	0.2211	0.7629
2.19	20.6 ± 5.55	0.046	0.954	4.63	95.37	0.0021	0.0883	0.9096
2.20	6.5 ± 1.39	0.133	0.867	13.33	86.67	0.0178	0.2311	0.7511
Total donors	–	–	–	–	–	15–16	200–201	784–785

Combining the advantages of the above two technologies, this study aimed to determine the frequency of Dombrock blood phenotype alleles encoded by the *DO* gene in Northwest China using a new droplet digital PCR technique, to prove that it can be applied to identify rare *DO*A* alleles in a large population and to guide the search for rare *DO*A* blood donors.

This study describes the application of droplet digital PCR combined with TaqMan real-time PCR for genotyping and gene frequency determination. This method is highly precise and accurate [27] for the inference of allele frequencies in the Dombrock blood group system, indicating that it can be extended to other blood groups with SNPs. In contrast to the conventional method of detecting the genotype of each donor, the use of blood pools can remarkably improve the efficiency of estimating gene frequency in large populations.

In conclusion, a droplet digital PCR screening method with high specificity, high accuracy and low cost for Dombrock blood group genotyping is described. The high accuracy and sensitivity of this technique in identifying rare *DO*A* alleles allow it to be applied to screen for rare *DO*A* phenotypes in a large number of donors and to guide searching for donors with rare blood types. The results of droplet digital PCR showed that the allele frequencies of the Dombrock blood group system in Northwest China were $DO^*A = 0.1128$ and $DO^*B = 0.8872$. This method can replace traditional methods and improve the efficiency of gene frequencies measurement in the population. Furthermore, it can be applied to screen other rare erythrocyte phenotypes.

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K.C. and Y.W were involved in conceptualization; Z.L. and Y.W were involved in methodology; Z.L. and Y.W worked on the software; Z.L., Y.W., M.S. and T.C. were involved in validation; Z.L. and Y.W. were involved in formal analysis; Z.L. and Y.W. were involved in investigation; Y.W. and K.C. collected the resources; M.S. and T.C. were involved in data curation; Z.L. and Y.W. were involved in writing—original draft preparation; M.S., T.C., K.C. and Y.W. were involved in writing—review and editing; M.S. and T.C. were involved in visualization; K.C. and Y.W. were involved in supervision; K.C. and Y.W were involved in project administration; Y.W. was involved in funding acquisition.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All the data will be available upon request to the corresponding authors.

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SHORT REPORT

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Direct antiglobulin test positivity in Serbian blood donors in the era of gel card technology

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Abstract

Background and Objectives: In the era of gel cards, we aimed to determine the incidence of a positive direct antiglobulin test (DAT) in a population of blood donors in Serbia and to compare results with the period when a conventional tube technique was used.

Materials and Methods: A retrospective study was conducted on 184,171 blood donors over 10 years (2012–2021). Positive DAT results initially were detected after positive result of the compatibility test. After a DAT had been initially performed on fresh blood samples, a control DAT was performed 6 months later.

Results: Using gel cards, we found a low incidence (0.09%) of DAT positivity among 55,254 female and 128,917 male blood donors, with no difference found in the occurrence of a positive DAT between them. Positive DAT moderately positively correlated with increasing age of blood donors, but without statistical significance. The vast majority of blood donors (98.86%) were IgG positive. All donors were in good health, did not take medication or had anaemia or haemolysis. During the period 1980–1989, when the test tube technique was used, we found 0.05% DAT-positive blood donors.

Conclusion: The incidence of positive DAT results in Serbian blood donors is higher compared with that found using the previous test tube technique, but the incidence is consistent with the findings of other studies.

Keywords

direct antiglobulin test, gel cards, tube test

Highlights

- The incidence of positive results from direct antiglobulin tests (DATs) in Serbian blood donors is increased compared with previous test tube technique, but the incidence is consistent with the findings of other studies that used gel cards and the test tube method.
- After an initial DAT-positive test, the DAT test and laboratory analysis (complete blood count, reticulocyte count, lactate dehydrogenase and bilirubin tests) should be performed again after 6 months. If the positivity persists, blood donors should be referred for further clinical examination, as the finding of a positive DAT in an apparently healthy blood donor may be a risk marker for malignancy.

- DAT-positive red blood cell units should be excluded from use in order not to interfere with pre-transfusion testing and selection of compatible units for the patient, although there is no evidence of adverse reactions after transfusion of such units.

INTRODUCTION

In 1946, Coombs, Mourant and Race introduced the direct antiglobulin test (DAT) into routine work in immunohaematology and established its purpose in demonstrating non-agglutinating antibodies of the IgG class and/or complement components bound to erythrocytes in vivo (direct Coombs test) [1]. The reaction between the erythrocyte antigen and the corresponding antibody can occur without a visible agglutination reaction, while the secondary antibody in the form of Anti-Human Globulin (AHG) reacts with antibody-coated erythrocytes, thus giving a visible agglutination reaction [1, 2]. The visibility of the reaction also depends on the sensitivity of the test technique, so the gel technique is more sensitive than the test tube technique, while flow cytometry is an even more sensitive technique [1]. A positive DAT with or without a shortened erythrocyte lifespan can be present in many diseases and clinical conditions [1, 3]. Voluntary blood donors are completely healthy persons who meet the criteria for donating a unit of blood. The finding of a positive DAT in blood donors is extremely rare, so screening of blood donors for DAT has traditionally not been recommended. It is most often detected as the cause of interference during the compatibility test within the pre-transfusion testing of the patient. The objective of the work was to determine the incidence of positive DAT in the Serbian population of blood donors during a 10-year period and to compare results obtained with gel technique (GT) with the results for the period when DAT was performed using a conventional tube technique (CTT).

MATERIALS AND METHODS

A retrospective study analysed notifications of positive DAT among blood donors in the Blood Transfusion Institute of Vojvodina (BTIV) in the period from 1 January 2012 to 31 December 2021 and from 1 January 1980 to 31 December 1989. The Ethics Committee of BTIV approved the study on 29th November 2022, number of the clearance is 01–12/22. Informed consent was obtained from all subjects involved in the study.

Notification of a positive DAT

In accordance with the standard operating procedure of pre-transfusion testing of the BTIV, after a positive result of the compatibility test, the unit of erythrocytes is tested for the DAT. In the case of a positive DAT, a notification is sent to the Blood typing and antibody screening laboratory, which invites blood donors: first time for an interview and DAT testing and second time for control testing after 6 months from the notification.

Testing

Fresh blood sample (3 mL) was taken from the blood donors with positive DAT in a vacuum tube with dipotassium ethylenediaminetetraacetic acid (K2EDTA). The sample was tested for DAT using gel ID LISS/Coombs cards (BioRad, DiaMed GmbH, Switzerland) with the polyspecific AHG reagent (anti-human globulin anti-IgG and C3d; BioRad, DiaMed GmbH, Switzerland). To differentiate the reaction, further testing was performed on all samples with monospecific AHG reagent (configuration: IgG, IgA, IgM, C3c, C3d and ctl; BioRad, DiaMed GmbH, Switzerland). The degree of agglutination was measured on a scale from 0 to 4+. Sample was screened for antibodies with indirect antiglobulin test (IAT) by gel ID LISS/Coombs cards (BioRad, DiaMed GmbH, Switzerland) using two cell screening panels (BioRad, ID-DiaCell I-II, DiaMed GmbH, Switzerland) on the fully automated immunohematology analyser (IH-1000).

During the interview, data on previous illnesses, current health problems and medication intake were taken from the blood donors. All blood donors underwent further testing: Complete blood count (CBC), reticulocyte count, lactate dehydrogenase and bilirubin tests.

Blood donors with initially positive DAT underwent control DAT testing after 6 months from notification. DAT-positive blood donors were referred for further evaluation by a haematologist while blood donors with negative anamnesis, DAT-negative and IAT-negative were approved for further blood donation.

RESULTS

During the 10 years of the study, 331,523 units of blood were collected from 184,171 blood donors. Among 55,254 women who donated blood, 51 (0.09%) were initially DAT-positive. Among 128,917 male blood donors, 125 (0.1%) were initially DAT-positive. There were no statistically significant differences between two groups of blood donors regarding that variable.

Among the 176 blood donors with initially positive DAT, the largest number was in the age groups 36–45 and 46–55. Age and DAT positivity in blood donors were found to be moderately positively correlated (Pearson correlation), which means there is a tendency for high X variable scores (age) to go with high Y variable scores (number of DAT-positive blood donors) and vice versa. There was a positive correlation between blood donor age and DAT-positive results without evidence of statistical significance (Table 1).

TABLE 1 Distribution of blood donors with positive DAT by gel method (2012–2021) according to years of life.

	Number of blood donors with positive DAT in each year between 2012 and 2021										
Years of life	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	*N* (%)
18–25	3	3	4	3	4	2	/	2	1	1	23 (13.07)
26–35	2	2	5	3	/	5	1	1	3	4	26 (14.77)
36–45	3	5	9	3	/	12	6	3	2	5	48 (27.27)
46–55	5	6	2	2	1	5	4	6	5	7	43 (24.43)
56–65	2	5	2	2	/	1	6	3	7	8	36 (20.46)
Total	15	21	22	13	5	25	17	15	18	25	176 (100)

Abbreviation: DAT, direct antiglobulin test.

TABLE 2 DAT-positive blood donors using gel cards: initially and after 6 months.

		Initially DAT-positive		After 6 months DAT-positive	
Year of testing	Number of blood donors	*N*	%	*N*	%
2012	15,476	15	0.10	9	0.06
2013	15,944	21	0.13	17	0.11
2014	14,722	22	0.15	19	0.13
2015	14,714	13	0.09	12	0.08
2016	15,309	5	0.03	5	0.03
2017	14,873	25	0.17	19	0.13
2018	20,983	17	0.08	10	0.05
2019	26,483	15	0.06	10	0.04
2020	21,744	18	0.08	11	0.05
2021	23,931	25	0.10	22	0.09
Total	184,171	176	0.09	134	0.07

Abbreviation: DAT, direct antiglobulin test.

The percentage of blood donors with initially positive DAT ranged from 0.03% to 0.15% (Table 2).

Initially, the percentage of blood donors with positive DAT was 0.09% (176 of 184,171) with incidence from 1:775 to 1:3062. Only 2 of 176 (1.13%) blood donors had IgG+, C3d+, while 174 of 176 (98.86%) had IgG+. In all 176 blood donors, IAT was negative. All 176 blood donors had a negative medical history, that is, they denied the existence of any disease, health problems or medication intake. The laboratory finding in none of the blood donors indicated signs of anaemia or haemolysis.

The number of blood donors with positive DAT 6 months later decreased to 134 of 176 (76.14%). The percentage of permanently rejected blood donors due to DAT positivity was 0.07% (134 of 184,171; Table 2). The study found a significant difference in the proportion of blood donors who were DAT-positive after the first and second tests, X^2 (1, $N = 184{,}171$) = 5.6951, $p = 0.017012$.

Control testing showed positive DAT in 40 of 51 (78.4%) women and in 94 of 125 (75.2%) men. A chi-square test of independence was performed to examine the relation between gender and DAT positivity, but the relation between these variables was not significant.

In the period 1980–1989, when CTT was used to detect DAT-positive blood donors, register data showed the incidence ranged from 1:1100 to 1:5590 or from 0.02% to 0.07% (average 0.05%) per year. All DAT-positive blood donors had only IgG+.

DISCUSSION

The study showed that a positive DAT detected by GT was found on average in 0.09% of blood donors, which is higher than results obtained by CTT. It was also determined that there was no difference in the occurrence of positive DAT between men and women both at the first and at the control testing. It has been proven that the occurrence of a positive DAT moderately positively correlates with increasing age of blood donors, but without statistical significance. All blood donors tested had a negative IAT. After the study results showed that the vast majority of blood donors (98.86%) are IgG-positive only, we modified routine work so that extensive testing with the IgG, IgA, IgM, C3c and C3d, but we perform only on the IgG-negative sample.

All donors were in good health, did not take medication or had anaemia or haemolysis.

A positive DAT is a significant indicator of possible potentially immune destruction of erythrocytes in vivo, if it is accompanied by clinical and laboratory signs so that it does not have to be related to immune-mediated haemolysis [2, 3].The binding of antibodies and complement components to the erythrocyte membrane does not in itself mean a shortening of their lifespan. If the number of IgG molecules bound to erythrocytes is greater than 1000, clinically evident destruction of erythrocytes occurs [1, 4]. Therefore, for the correct interpretation of a positive DAT, it is necessary to know the patient's case history, clinical data and the results of other laboratory tests. In healthy individuals, 5–90 IgG and 5–40 C3d molecules can be found per erythrocyte, and these levels are below the detection threshold in routine testing [3, 5]. Depending on the technique and reagents used, DAT can detect 100–500 IgG molecules and 400–1100 C3d molecules on erythrocytes [6, 7]. A positive DAT is rarely found in blood donors primarily because it is not part of the mandatory immunohaematological screening. In the various studies conducted, the incidence of positive DAT in the blood donor population ranges from 1:1000 to 1:14,000 [4, 7]. The incidence varies due to differences in the reading of the strength of DAT positivity, because if weak positivity (+/−) were also taken into account, the incidence would increase [8]. In the study, the incidence was 1:1046 (ranges from 1:775 to 1:3062), although the gel method was always used to carry out the test. Only two blood donors had mixed IgG and C3d specificity, while the others had only IgG-specific DAT. Garraty discovered that in people with positive DAT, about two-thirds of people have only IgG-specific DAT and the rest have mixed IgG and C3d specificity [3, 8]. DAT is detected more often in older blood donors compared with younger ones [1, 5], which is also the case in our research and can be explained by the fact that older people are at a higher risk of developing certain diseases or using medicines.

The incidence of positive DAT in Serbian blood donors is increased compared with the period when CTT was used, which is result of increased sensitivity of GT. Weak agglutination reactions by CTT may pass undetected. GT provides clear grading system with higher sensitivity, which enables the detection of weakly positive DAT and consequently increases the number of positive blood donors.

In a study carried out by Issitt and Anstee by examining and monitoring the health status of blood donors with positive DAT and IgG coating erythrocytes present, 3%–10% develop AIHA, 20%–25% become DAT-negative over time and 60%–70% remain DAT-positive, but with normal haematological findings [8]. In our study, 76.14% of blood donors remained DAT-positive with normal haematological findings. The result is slightly higher compared with the mentioned study, which indicates the need to follow up donors for longer than 6 months. Rottenberg and his collaborators determined that blood donors with positive DAT have a significantly increased risk for the development of malignancies, especially haematological ones, and suggested that a positive DAT may precede the clinical diagnosis of malignancy months to years in advance [9, 10]. For this reason, it is considered that further studies are necessary in terms of confirming this association. Also, all of the above-mentioned leads to the question of whether blood donors with positive DAT can continue donating blood or not. There are no clear literature guidelines regarding the monitoring of this blood donor. Blood donors are usually detected when performing a compatibility test, and the practice in our institution is to retest them 6 months later. In the case of still present positive DAT, the blood donor is permanently excluded from donating blood and referred to a haematologist for further clinical evaluation. Blood donors with positive DAT can be included in blood donation again if they become DAT-negative within a certain time interval after the initial DAT and if they also meet other criteria for blood donation.

In the blood bank, DAT-positive blood units are identified by performing an AHG cross-match. There are no data on adverse consequences of transfusion of DAT-positive blood. Institutions that use only saline phase cross-match or computerized cross-match cannot detect DAT-positive units in this way, so it happens that such units are used in patients [11]. Based on literature data, IgG-coated erythrocytes are not expected to have reduced survival after transfusion [12]. Although there are no data on the adverse consequences of transfusion of DAT-positive blood, such a unit is permanently excluded from use in our institution.

In conclusion, the incidence of DAT positivity in healthy blood donors is low despite the introduction of a more sensitive technique in routine testing. Such persons should be examined in detail for clinical and laboratory signs of haemolysis and monitored over a longer period for the possible occurrence of malignancy.

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J.G. and N.B. designed the study, performed the research, analysed the data and wrote the first draft of the manuscript. Z.B.O and G.M. designed the study, supervised the research and reviewed and edited the manuscript. J.G and N.B. performed the research and reviewed and edited the manuscript. Z.B.O. and G.M. performed the research, assisted in interpretation of the data and reviewed and edited the manuscript. J.G. designed the study, supervised the research and reviewed and edited the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The datasets used and analyses during the current study are available from the corresponding author on reasonable request.

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