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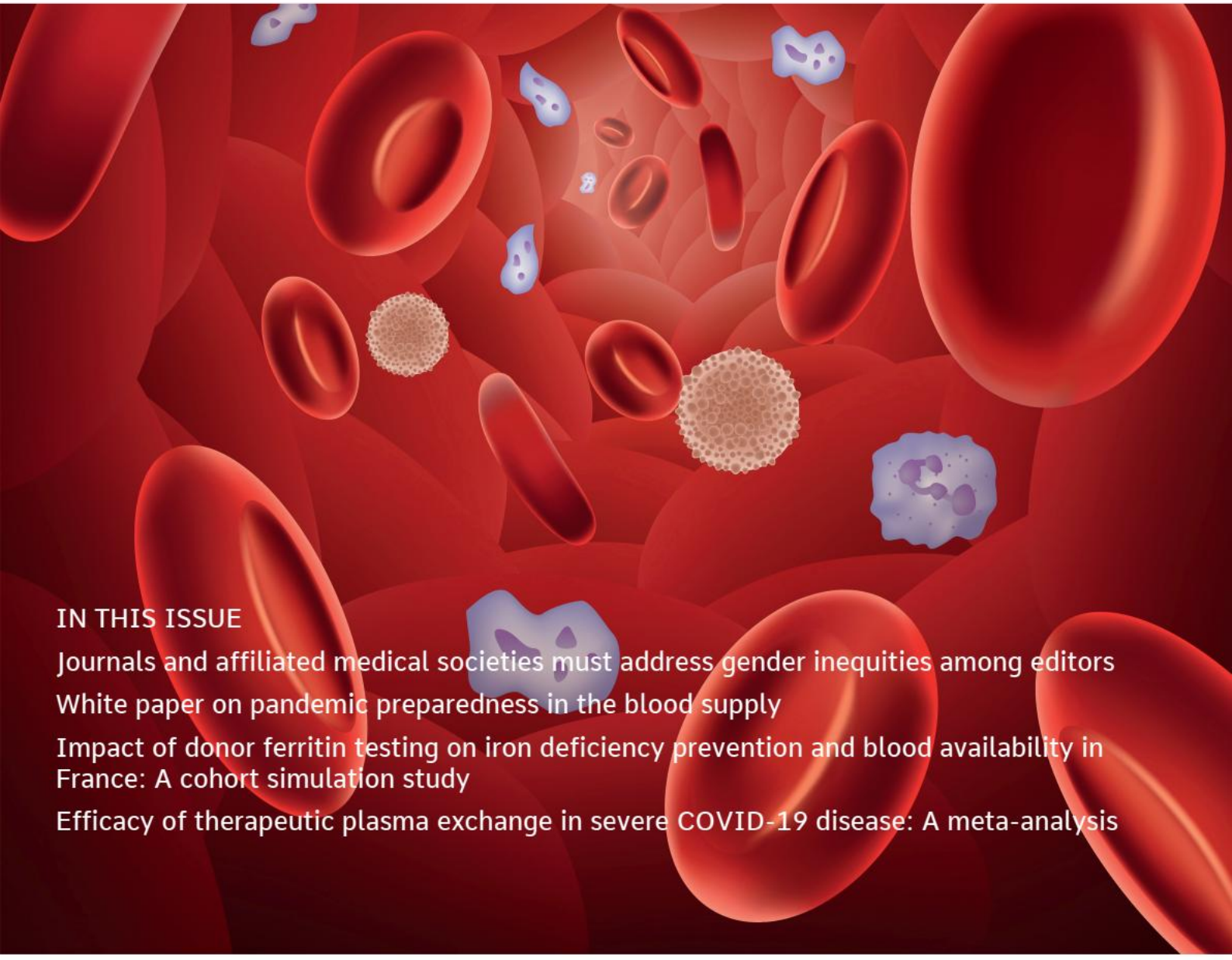

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International Journal of Blood Transfusion

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Vox Sanguinis

International Journal of Blood Transfusion

Aims and Scope

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

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

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

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REVIEW

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Current state of gene therapy in sickle cell disease

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Abstract

Sickle cell disease (SCD) is a type of hemoglobinopathy due to an autosomal recessive genetic defect, causing significant red cell sickling, multi-organ damage and long-term severe morbidities. Due to its complicated care and the impact on quality of life, a curative treatment for SCD is highly desirable. In recent years, gene therapy is emerging as a curative option for SCD, where autologous haematopoietic stem cells are collected from SCD patients and genetically modified ex vivo to reduce its sickling tendency before reinfusion. Although still largely investigational, a limited number of gene therapy options have been recently granted approval for SCD patients. Published data are still currently limited, but early studies have so far demonstrated the intended outcomes of less vaso-occlusive crisis and haemolysis. Nonetheless, despite its curative potential, larger clinical trials and longer follow-up period are still necessary to evaluate the safety of this treatment option, especially the risk of unintended genetic modifications. Furthermore, SCD patients frequently have limited access to specialty care; hence, the issues of affordability and accessibility to SCD gene therapy must also be addressed for it to benefit the appropriate patient population.

Keywords

gene editing, gene therapy, haematopoietic stem cells, sickle cell disease

Highlights

- Gene therapy is potentially a curative treatment for sickle cell disease.
- Early findings show lower occurrence of vaso-occlusive crisis and reduced haemolysis, although longer follow-up is still necessary to evaluate adverse effects.
- The cost of gene therapy will be a significant barrier to its accessibility.

INTRODUCTION

Sickle cell disease (SCD) is a type of hemoglobinopathy with an autosomal recessive genetic inheritance. The required genetic defect in SCD is the HbS allele, which arises from a point mutation in the *HBB* gene that results in the substitution of glutamate (hydrophilic) to valine (hydrophobic) at the sixth amino acid residue of the β-globin chain. In addition to the HbSS genotype, clinical manifestations of SCD are also seen when HbS is inherited with other *HBB* mutations in a compound heterozygous manner, such as HbSC, HbSβ^0-thalassemia or HbSβ^+-thalassemia. HbS haemoglobin tends to polymerize under low oxygen tension, leading to sickled red cells with reduced deformability and survival. When a significant proportion of red cells sickle, this can occlude blood vessels, especially in microvasculature, causing either severe pain episodes known as vaso-occlusive crisis (VOC) or end organ damage. Any organ can be affected by SCD; when significant occlusion occurs in pulmonary or brain microvasculature, it can respectively lead to acute chest syndrome or acute stroke, which are medical emergencies. SCD patients also experience chronic haemolysis due to the abnormal red cells with shortened lifespan—the

resulting free heme leads to production of cytokines and chronic systemic inflammation [1]. Overall, SCD affects multiple organ systems, often with long-lasting morbidity.

CURRENT TREATMENT OPTIONS FOR SCD

Treatments for SCD have been limited, with only four drugs currently Food and Drug Administration (FDA)-approved for SCD (Table 1) [2]. Of these, hydroxyurea is the first-line treatment and is effective in reducing SCD pain symptoms, although continuous long-term use and careful dose escalation are required for clinical response [3]. Due to its myelosuppressive effect, patients on long-term hydroxyurea must be monitored appropriately. Notably, the benefit of hydroxyurea is unclear with the HbSC or HbSβ+thalassemia genotypes. Furthermore, it is insufficient in preventing other complications, such as stroke [4, 5]. Therefore, many SCD patients still rely on frequent red cell transfusions for symptom control; for example, effective stroke prevention in SCD requires long-term chronic red cell transfusion therapy [6, 7]. Because simple chronic red cell transfusions can lead to iron overload with its associated organ damages, many SCD patients need to go on automated exchange transfusion programme to avoid iron overload. Chronic red cell exchange transfusions require large number of red cell units, exposing patients to a high risk of alloimmunization to red cell antigens that can lead to increased difficulties with obtaining compatible red cell units over time. Altogether, SCD care and treatment can be complicated and costly, with significant impact to the patient's quality of life; as such, a curative treatment is highly desirable.

TABLE 1 Mechanism of action and clinical effects of the currently FDA-approved drugs for SCD.

Drug	Mechanism of action	Clinical effect
Hydroxyurea	Stimulation of fetal haemoglobin production	Reduced frequency of VOC [4, 5]
L-glutamine	Exact mechanism unclear, but L-glutamine uptake is increased in sickle cells and oral administration of L-glutamine may reduce adherence of sickle cell to endothelium	Reduced frequency of VOC [42]
Crizanlizumab	Humanized monoclonal antibody to P-selectin; expression of P-selectin on endothelium mediates adherence of sickle cells and development of VOC, which is inhibited by crizanlizumab	Reduced frequency of VOC [43]
Voxelotor	Inhibitor of HbS polymerization by reversibly binding to haemoglobin and stabilizing its oxygenated state	Increased haemoglobin levels and reduced haemolysis [44]

Abbreviations: FDA, Food and Drug Administration; SCD, sickle cell disease; VOC, vaso-occlusive crisis.

Allogeneic haematopoietic stem cell transplant (HSCT) is one way to correct the genetic deficiency in SCD. Although post-transplant GVHD remains a concern, an international retrospective survey reported excellent 5-year and overall survival rates at 91.4% and 92.9%, respectively, when performed with an HLA-identical donor sibling [8]. The current guidelines from the American Society of Haematology conditionally recommend HSCT only for SCD patients with severe complications (such as stroke, recurrent acute chest syndrome) and have HLA-identical donor siblings [9]. Unfortunately, only a minority of HSCT recipient candidates are expected to have HLA-identical donor siblings [10], while alternative haploidentical or matched unrelated donors for SCD treatment are only used in the context of clinical trials and may be associated with worse survival outcome when compared with HLA-identical sibling [11]. In addition to GVHD, development of donor-specific red cell antibodies, which could have clinical effects ranging from asymptomatic to severe haemolysis, should also be considered as a post-transplant complication [12]. This is because post-transplant mixed chimerism has been frequently observed for SCD patients who underwent HSCT with either myeloablative [13] or non-myeloablative conditioning [14] and may lead to development of donor-specific red cell antibodies because red cell antigen matching is not usually considered in HSCT.

GENE THERAPY STRATEGIES IN SCD

In recent years, gene therapy has emerged as an alternative to allogeneic HSCT for curative treatment of SCD. Using this strategy, autologous haematopoietic progenitor cells (HPCs) from an SCD patient are collected and genetically modified ex vivo to reduce its sickling tendency (Figure 1). Patients undergo conditioning chemotherapy before infusion of these genetically modified cells to repopulate the marrow. The use of autologous HPCs mitigates the risk for GVHD. While this treatment option was previously only available through participation in clinical trials (Table 2), a limited number of gene therapy options have been recently approved for SCD patients with history of VOC events including exagamglogene autotemcel (exa-cel) and lovotibeglogene autotemcel (lovo-cel) in the United States, exa-cel in the United Kingdom. However, accessibility to these approved options remains limited to highly specialized treatment centres.

Different genetic modification strategies may be used for gene therapy and may include gene addition (where additional genetic material is introduced into the cells), gene editing (where genetic material in the cells is directly modified) or gene correction (where genetic material in the cells is both directly modified and new genetic material is introduced). The interim analysis of 35 patients who received lovo-cel during its investigation phase is by far the largest peer-reviewed published cohort study on gene therapy for

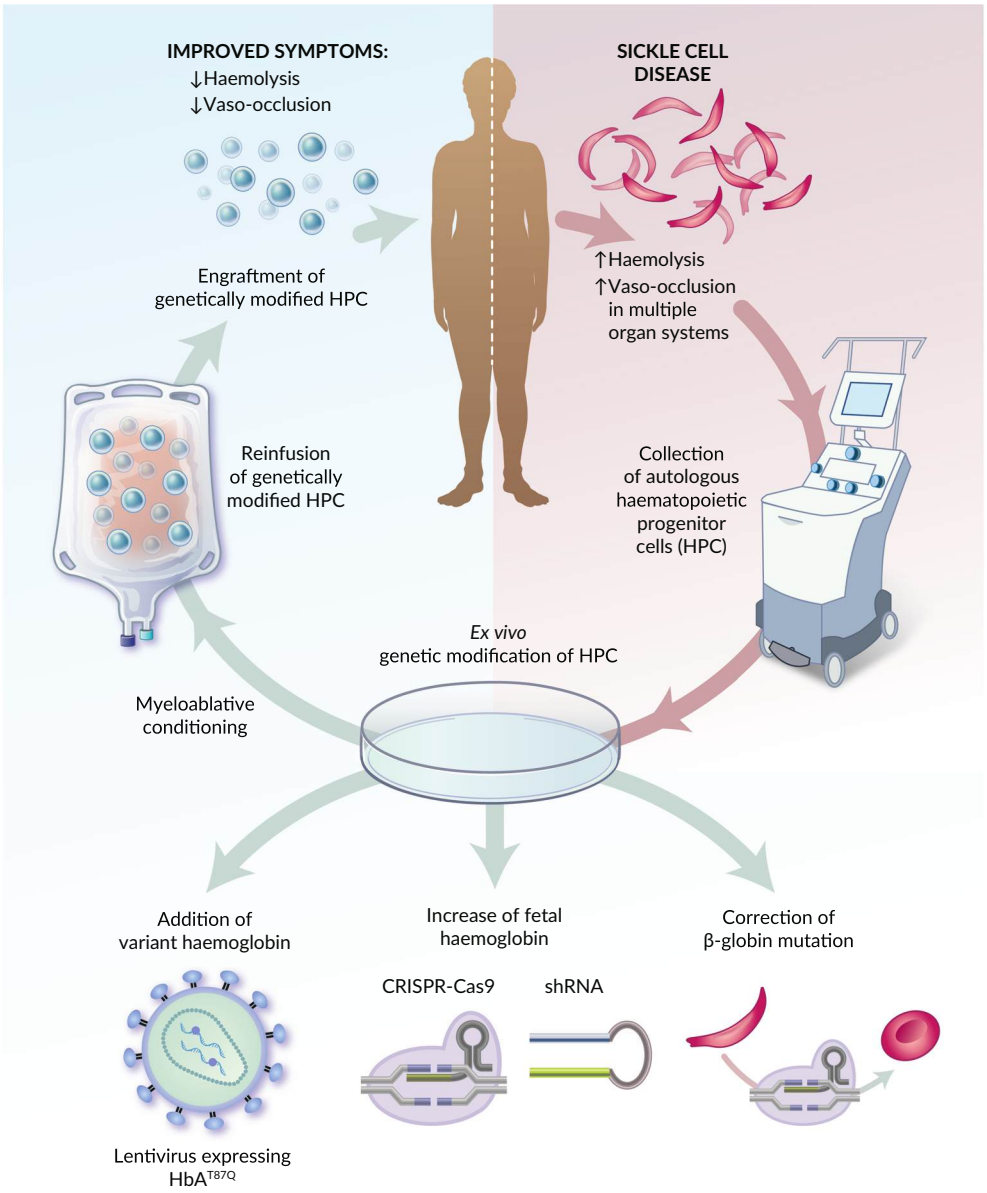

FIGURE 1 Key stages in the process of gene therapy. Genetic modification strategies used for sickle cell disease treatment have included: (1) lentiviral expression of variant haemoglobin with reduced sickling tendency; (2) disruption of fetal haemoglobin repression using CRISPR-Cas9 or shRNA in order to increase its expression; (3) direct correction of the β-globin gene mutation using CRISPR-Cas9.

SCD [15]. Lovo-cel is based on a gene addition strategy, where a copy of the modified β-globin gene HbAT87Q, which sterically prevents red cell sickling, is introduced into the cells by lentiviral transduction. Eligibility criteria to the non-randomized, open label, single-dose trial included SCD with genotypes of either HbSS, HbSβ^0 or HbSβ$^+$, and recurrent VOC episodes; the primary outcome evaluated was resolution of VOC. All 35 patients described in the interim analysis achieved engraftment with the genetically modified cells. Among the 25 patients who could be evaluated for VOC for at least 6 months post-infusion, all but two patients had no recurrence of VOC episodes; the two patients who continued to have VOC experienced these episodes with reduced severity and frequency.

Gene editing is a different genetic modification strategy and has been used to disrupt haemoglobin F (HbF) repression, which consequently increases the expression of HbF. This may be achieved either by preventing the expression of BCL11A (HbF repressor) or by preventing repressor binding at the *HBG1* and *HBG2* (γ-globin) gene promoters. Although hydroxyurea may be used to increase HbF expression, the increase achieved through gene therapy happens at a greater magnitude and without the hydroxyurea-associated myelosuppression effect. Furthermore, while the induction of HbF by hydroxyurea tends to be uneven across circulating red cells, gene editing ensures a more even and broader distribution of HbF per red cell. Instead of total HbF concentration, the proportion of HbF-expressing

TABLE 2 SCD gene therapy clinical trials listed on ClinicalTrials.gov as of 8 January 2024.

Trial number	Status	Phases	Mechanism of action	Age (years)
NCT05456880 (BEACON)	Recruiting	1, 2	Gene editing on the γ-globin gene promoter to induce HbF, using a CRISPR-Cas9 protein coupled to a base-editing deaminase enzyme	18–35
NCT05353647 (GRASP)	Recruiting	2	Gene editing using a lentiviral vector with short hairpin RNA that suppresses BCL11A expression to induce fetal haemoglobin	13–40
NCT04853576 (RUBY)	Recruiting	1, 2	Gene editing on the γ-globin gene promoter to induce HbF, using a proprietary CRISPR-Cas system	18–50
NCT04293185	Recruiting	3	Gene addition using a lentiviral vector that expresses an anti-sickling β globin HbA^{T87Q} [15]	2–50
NCT02140554	Active, not recruiting	1, 2		12–50
NCT03964792 (DREPAGLOBE)	Active, not recruiting	1, 2	Gene addition using a lentiviral vector that expresses an anti-sickling β globin AS3 [45]	12–20
NCT03745287	Active, not recruiting	2, 3	Gene editing to suppress BCL11A expression and induce HbF, using CRISPR-Cas9 [17, 18]	12–35
NCT05477563	Recruiting	3		12–35
NCT05329649	Recruiting	3		2–11
NCT03282656	Active, not recruiting	1	Gene editing using a lentiviral vector with short hairpin RNA that suppresses BCL11A expression to induce HbF [19]	3–40
NCT02247843	Recruiting	1, 2	Gene addition using a lentiviral vector that expresses an anti-sickling β globin AS3	≥18
NCT02186418	Active, not recruiting	1, 2	Gene addition using a lentiviral vector that expresses a variant HbF	18–45
NCT04443907	Active, not recruiting	1	Gene editing on the γ-globin gene promoters to disrupt repressor binding and induce HbF, using CRISPR-Cas9 [20]	2–40

Note: The initial search criteria used were: condition = sickle cell disease; intervention = gene; study type = interventional; only trials investigating gene therapy with a status of 'Recruiting' or 'Active, not recruiting' are included.
Abbreviations: HbF, haemoglobin F; SCD, sickle cell disease.

cells and individual cellular HbF content are better predictors of anti-sickling effect [16]. Two different gene editing methods to eliminate BCL11A expression have been evaluated in clinical trials—one method utilized the CRISPR-Cas9 system to knock out *BCL11A* (CLIMB-SCD 121 trial, NCT03745287), now approved as exa-cel for use in the United States and United Kingdom [17, 18], while another used short hairpin RNA interference to inactivate *BCL11A* transcription (NCT03282656) [19]. Participants of both studies were found to have increased HbF expression per cell, reduced frequency of VOC episodes and decreased transfusion needs that persisted after 1 year. Nonetheless, because both studies have only published detailed findings from a very small number of initial trial participants, it is difficult to conclude whether one gene editing approach is superior to the other—the allelic editing frequency by CRISPR-Cas9 in the CLIMB-SCD trial was reported to be 79–83% [17], while the transduction efficiency of the short hairpin RNA was reported at 62%–100%, with five of the six study participants having a transduction efficiency of >90% [19]. Another method to increase HbF expression is by disrupting repressor binding sites at *HBG1* and *HBG2* (γ-globin) gene promoters using CRISPR-Cas9 systems (NCT04443907, NCT05456880) [20, 21]. Of the two phase 1/2 studies evaluating this strategy, peer-reviewed published data are available for NCT04443907 (OTQ923)—multiple editing outcomes could be simultaneously generated from this strategy, but the most common was an intergenic deletion producing a hybrid gene with the *HBG2* promoter sequence fused to the downstream *HBG1* [20]. During its phase 1 study, OTQ923 was infused into three patients and the allelic editing frequencies of the infused product were approximately 80%. Across a 12-month follow-up period, all three patients achieved persistent bone marrow engraftment of the genetically modified cells, with increased and stable expression of HbF that were broadly distributed among red cell (69.7%–87.8% of red cells). All three patients continued to have at least one VOC episode and mild haemolysis post-infusion, but the severity of the clinical symptoms was reduced.

Although no peer-reviewed data so far are available, some clinical trials are also investigating the possibility of correcting the β-globin gene mutation directly using different methods. One method uses CRISPR-Cas9 in combination with an adeno-associated virus type 6 (AAV6) DNA template—this first induces DNA breakage at the β-globin gene mutation, followed by insertion of the donor AAV6 DNA template in order to correct the gene mutation [22, 23]. In a mouse model carrying a humanized globin gene cluster, this method of gene correction produced stable HbA production; the CEDAR trial was initially planned to evaluate its safety and efficacy in human but was voluntarily paused and now discontinued due to prolonged cytopenia in its first trial participant [24]. Another method of β-globin gene correction is to use a modified CRISPR-Cas9 protein coupled with a base-editing deaminase enzyme—this enables editing of the point

mutation without inducing DNA breakage and is thought to be more precise. This gene correction method has been applied to the β-globin gene to induce a non-pathogenic haemoglobin variant that is compatible with HbA function [25, 26].

TREATMENT PROCESS FOR GENE THERAPY

Collection of autologous haematopoietic stem cells

The first step in gene therapy is the collection of autologous HPCs. This can be challenging in SCD patients due to their underlying disease, necessitating modifications to the collection procedure. Given the likelihood of cell loss during ex vivo genetic modification, the target collection yield for gene therapy is typically higher than that in standard-of-care autologous HSCT to ensure successful engraftment.

The three anatomic sources for HPCs are peripheral blood, bone marrow and umbilical cord. Although some of the SCD gene therapy protocols used bone marrow harvest to collect HPCs, peripheral HPCs collected by apheresis have become the most common source of HPCs these days. However, as HPCs rarely circulate peripherally under physiological condition, they must be mobilized from the bone marrow to the periphery using mobilization agents. While granulocyte colony stimulating factor (G-CSF) is widely used as a mobilizing agent, it should be avoided in SCD patients due to increased risk of severe adverse reactions, which include severe VOC, multi-organ failure and death [27]. These may represent consequences of G-CSF-induced hyperleukocytosis that are worsened by the underlying chronic inflammation in SCD.

Instead, plerixafor has emerged as an alternative mobilizing agent for SCD patients. Plerixafor is a CXCR4 inhibitor and mobilizes HPC by disrupting the interaction between HPCs and bone marrow stromal cells [28]. It is currently approved for HPC mobilization in patients with multiple myeloma or non-Hodgkin's lymphoma who will be undergoing autologous HSCT, but its safety and efficacy as a mobilizing agent for SCD patients have been demonstrated in several phase I clinical studies [29–32]. Specific steps were also implemented in these studies to reduce the risk of adverse reactions during mobilization and HPC collection, which included hydroxyurea discontinuation and regular red cell transfusions to decrease HbS level to <30% in the few months prior to mobilization. Hydroxyurea has myelosuppressive effect and a washout period of at least 1 month has been proposed, although some studies discontinued hydroxyurea for a longer period of 2–3 months prior to mobilization. Pre-mobilization red cell transfusions may be performed either as simple or exchange transfusions; in addition to reducing stress erythropoiesis, scheduled red cell transfusions may also help reduce vaso-occlusive symptoms after hydroxyurea discontinuation prior to mobilization. Peripheral HPC collection by plerixafor mobilization and apheresis had lower adverse reaction rates when compared with bone marrow HPC harvest [31].

In addition to optimizing patient-specific factors, the HPC collection process will also require certain optimization specific to SCD patients. Esrick et al reported poor collection yield in SCD patients when apheresis was performed at the collection interface recommended by manufacturer for routine HPC collection; however, this was improved when collecting at a deeper interface [30]. The mechanism for improved yield when collecting at a deeper interface is still unknown but is thought to be related to altered cell shape and sedimentation in SCD patients. However, while modification to the collection interface is required, HPCs collected from SCD patients by plerixafor mobilization are similar to HPCs collected from SCD patients by bone marrow harvest and HPCs collected from healthy donors by G-CSF mobilization, when compared by gene expression profiles [29].

In addition to SCD symptoms severity and length of hydroxyurea discontinuation, the other factors predictive of HPC collection yield among SCD patients include patient's age and bone marrow reserve at time of mobilization and collection, which are similar to findings from the general population [32]. Interestingly, HbS level did not correlate with HPC collection yield [32]. Some of these factors may not be easily modifiable and the likelihood of successful mobilization should be taken into consideration before enrolling a patient into gene therapy study.

Conditioning

In allogeneic HSCT for haematological malignancies, myeloablative conditioning has a therapeutic anti-leukaemic effect and reduces risk of graft rejection. While there is no concern for graft incompatibility in gene therapy that uses autologous cells and the anti-leukaemic effect is not indicated in SCD patients, optimization efforts from the lovo-cel trial suggest that myeloablative conditioning may increase the expression of the therapeutic gene product [15]. Currently, most gene therapy trials are performed with myeloablative conditioning, with the exception of NCT02186418 [33], which uses reduced intensity conditioning and a lentiviral vector to produce the anti-sickling HbF^{G16D}—this trial may provide insight on how to optimize a gene therapy treatment process without myeloablation, which may be more appropriate for patients with existing end organ damage. Early results of this trial suggested that engraftment of the genetically modified cells was possible with reduced intensity conditioning and production of HbF comparable with other trials with myeloablative conditioning, although one of the three patients experienced lower engraftment and HbF production that were attributed to sub-therapeutic melphalan [34].

Infusion and engraftment

Gene therapy trials with published findings reported engraftment in patients approximately 1 month post-infusion. During the engraftment period, patients may require transfusion support and irradiated blood products should be used, due to the immunocompromised state and risk for transfusion-associated GVHD. Once engraftment occurs, the therapeutic gene products have been shown to persist throughout the study follow-up periods, which ranged between 1 and 3 years currently [15, 17, 19, 20]. Nonetheless, many SCD patients receiving allogeneic HSCT were previously found to have mixed chimerism with

variable proportion of donor cells [13, 14]; whether this would occur with genetically modified autologous cells and affect long-term persistency of the therapeutic gene product is still unknown due to the limited follow-up period with gene therapy trials.

CHALLENGES IN GENE THERAPY

Safety and adverse events from gene therapy

The limited safety analysis available from current gene therapy studies showed that most adverse reactions were effects of myeloablative therapy [15, 19, 20]. While commonly reported adverse reactions (such as stomatitis and cytopenias) are reversible, one long-term consequence that is still not well documented in SCD gene therapy trials is gonadal toxicity from high-intensity myeloablative conditioning, which can pose a significant risk for infertility—most patients who receive busulfan-based myeloablative conditioning would be infertile and post-pubertal patients are at higher risk of gonadal failure after myeloablative conditioning [35].

In addition, there is also concern for the possibility of new haematological condition following gene therapy. Two patients, who were among the earlier group A participants of the lovo-cel trial, developed anaemia after infusion and were also found to have abnormal erythroid precursors and chromosome 8 trisomy; this constellation of clinical findings was initially concerning for myelodysplastic syndrome, but no predisposing new mutation was detected in either patient and the aetiology for these findings remained unclear [15]. Two different patients from the lovo-cel trial developed new acute myeloid leukaemia 3–6 years post-infusion, although it was subsequently determined that insertional mutagenesis from lentiviral transduction was unlikely to have been the cause [36]. Off-target editing is also a concern with CRISPR-Cas9-based gene therapy, although the limited published data so far have not reported this phenomenon [17, 20]. Nonetheless, detection of low-frequency off-target editing can be challenging. Most recently, the CEDAR trial, which uses CRISPR-Cas9 with AAV6 to perform β-globin gene correction, has been voluntarily paused and ultimately terminated due to prolonged cytopenia in the first enrolled patient [24]. It is unclear what may have caused this, although no myelodysplastic changes have been reported. Overall, the limited cohort and short follow-up period from gene therapy trials so far cannot definitively rule out the possibility of haematological abnormality as an unintended consequence of genetic modification and patients who have received gene therapy should be appropriately monitored in the long term. Patients receiving exa-cel will be enrolled into a 15-year follow-up study (NCT04208529) to monitor for new malignancies and serious adverse events post-infusion.

Barriers to access

Even with the recent approvals granted to exa-cel and lovo-cel, the availability of gene therapy remains limited to specialized treatment centres. Unfortunately, within the United States, approximately 60% of SCD patients rely on Medicare or Medicaid as their primary healthcare payer, which limits access to specialized healthcare [37]. In contrast, for patients with haemophilia and von Willebrand disease, both of which are diseases that require long-term specialty care, this rate is approximately twofold to threefold lower [37]—this highlights the healthcare inequity that must be addressed for gene therapy to bring benefit among patients with severe SCD. The lack of access to sickle cell specialists and specialized transplant centres poses a significant barrier to bring gene therapy to the SCD patient population. In addition, gene therapy for SCD is currently not available in low-income countries, such as Sub-Saharan Africa where SCD is prevalent, and none of the clinical trials for gene therapy in SCD are recruiting from these low-income countries [38]. Furthermore, the price of a gene therapy product is estimated to range between $1.4 and 2.1 million in the United States; according to a budget impact analysis, this can significantly limit the immediate affordability of this treatment option and may force healthcare payers to adopt restrictive policies on eligibility [39]. Hence, developing innovative strategies to finance SCD gene therapy is also an important area of research. One proposal is to adopt a value-based payment model [40], where the manufacturer will only be paid in full if the treatment benefits the patient. Nonetheless, this represents a change from the current fee-for-service payment model in the United States and may not be readily adopted. Academic and research institutions are also encouraged to increase the cost-efficiency of their production process and to change their licensing methods for innovation to be less exclusive, especially when the discoveries were supported by public funding [41].

In conclusion, early studies among patients with severe SCD demonstrated that the various gene therapy strategies investigated in clinical trials might help reduce frequency of VOC episodes, increase total haemoglobin and decrease haemolysis. However, longer follow-up is still necessary to evaluate the safety and risk of this treatment option more comprehensively. In addition, strategies to broaden specialty care coverage in SCD patients and innovative healthcare financing strategies to improve the affordability of gene therapy must also be developed.

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The authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

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REFERENCES

1. Nader E, Romana M, Connes P. The red blood cell—inflammation vicious circle in sickle cell disease. Front Immunol. 2020;11:454.

2. Food and Drug Administration. FDA approves first targeted therapy to treat patients with painful complication of sickle cell disease. Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-first-targeted-therapy-treat-patients-painful-complication-sickle-cell-disease. Last accessed 15 Apr 2021.
3. Yawn BP, Buchanan GR, Afenyi-Annan AN, Ballas SK, Hassell KL, James AH, et al. Management of sickle cell disease: summary of the 2014 evidence-based report by expert panel members. JAMA. 2014; 312:1033–48.
4. Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB, Eckert SV, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. N Engl J Med. 1995;332:1317–22.
5. Steinberg MH, Barton F, Castro O, Pegelow CH, Ballas SK, Kutlar A, et al. Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia. JAMA. 2003;289:1645–51.
6. Adams RJ, McKie VC, Hsu L, Files B, Vichinsky E, Pegelow C, et al. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial doppler ultrasonography. N Engl J Med. 1998;339:5–11.
7. Adams RJ, Brambilla D. Discontinuing prophylactic transfusions used to prevent stroke in sickle cell disease. N Engl J Med. 2005;353: 2769–78.
8. Gluckman E, Cappelli B, Bernaudin F, Labopin M, Volt F, Carreras J, et al. Sickle cell disease: an international survey of results of HLA-identical sibling hematopoietic stem cell transplantation. Blood. 2017;129:1548–56.
9. Kanter J, Liem RI, Bernaudin F, Bolaños-Meade J, Fitzhugh CD, Hankins JS, et al. American Society of Hematology 2021 guidelines for sickle cell disease: stem cell transplantation. Blood Adv. 2021;5: 3668–89.
10. Besse K, Maiers M, Confer D, Albrecht M. On modeling human leukocyte antigen–identical sibling match probability for allogeneic hematopoietic cell transplantation: estimating the need for an unrelated donor source. Biol Blood Marrow Transplant. 2016;22:410–7.
11. Eapen M, Brazauskas R, Walters MC, Bernaudin F, Bo-Subait K, Fitzhugh CD, et al. Effect of donor type and conditioning regimen intensity on allogeneic transplantation outcomes in patients with sickle cell disease: a retrospective multicentre, cohort study. Lancet Haematol. 2019;6:e585–96.
12. Allen ES, Srivastava K, Hsieh MM, Fitzhugh CD, Klein HG, Tisdale JF, et al. Immunohaematological complications in patients with sickle cell disease after haemopoietic progenitor cell transplantation: a prospective, single-centre, observational study. Lancet Haematol. 2017; 4:e553–61.
13. Bernaudin F, Dalle J-H, Bories D, Latour RP, Robin M, Bertrand Y, et al. Long-term event-free survival, chimerism and fertility outcomes in 234 patients with sickle-cell anemia younger than 30 years after myeloablative conditioning and matched-sibling transplantation in France. Haematologica. 2020;105:91–101.
14. Hsieh MM, Fitzhugh CD, Weitzel RP, Link ME, Coles WA, Zhao X, et al. Nonmyeloablative HLA-matched sibling allogeneic hematopoietic stem cell transplantation for severe sickle cell phenotype. JAMA. 2014;312:48–56.
15. Kanter J, Walters MC, Krishnamurti L, Mapara MY, Kwiatkowski JL, Rifkin-Zenenberg S, et al. Biologic and clinical efficacy of LentiGlobin for sickle cell disease. N Engl J Med. 2022;386:617–28.
16. Steinberg MH, Chui DHK, Dover GJ, Sebastiani P, Alsultan A. Fetal hemoglobin in sickle cell anemia: a glass half full? Blood. 2014;123: 481–5.
17. Frangoul H, Altshuler D, Cappellini MD, Chen Y-S, Domm J, Eustace BK, et al. CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. N Engl J Med. 2021;384:252–60.
18. Frangoul H, Locatelli F, Sharma A, Bhatia M, Mapara M, Molinari L, et al. Exagamglogene autotemcel for severe sickle cell disease. Blood. 2023;142:1052.
19. Esrick EB, Lehmann LE, Biffi A, Achebe M, Brendel C, Ciuculescu MF, et al. Post-transcriptional genetic silencing of BCL11A to treat sickle cell disease. N Engl J Med. 2020;384:205–15.
20. Sharma A, Boelens J-J, Cancio M, Hankins JS, Bhad P, Azizy M, et al. CRISPR-Cas9 editing of the HBG1 and HBG2 promoters to treat sickle cell disease. N Engl J Med. 2023;389:820–32.
21. Mayuranathan T, Yen JS, Newby GA, Yao Y, Porter SN, Woodard KJ, et al. Adenosine Base editing of γ-globin promoters induces fetal hemoglobin and inhibit erythroid sickling. Blood. 2020;136:21–2.
22. Kanter J, DiPersio JF, Leavey P, Shyr DC, Thompson AA, Porteus MH, et al. Cedar trial in Progress: a first in human, phase 1/2 study of the correction of a single nucleotide mutation in autologous HSCs (GPH101) to convert HbS to HbA for treating severe SCD. Blood. 2021;138:1864.
23. Wilkinson AC, Dever DP, Baik R, Camarena J, Hsu I, Charlesworth CT, et al. Cas9-AAV6 gene correction of beta-globin in autologous HSCs improves sickle cell disease erythropoiesis in mice. Nat Commun. 2021;12:686.
24. Larkin HD. Sickle cell disease gene therapy trial paused. JAMA. 2023;329:364.
25. Chu SH, Lam D, Packer MS, Olins J, Liquori A, Marshall J, et al. Adenine base editing of the sickle allele in CD34+ hematopoietic stem and progenitor cells eliminates hemoglobin S. Blood. 2020; 136:47.
26. Chu SH, Ortega M, Feliciano P, Winton V, Xu C, Haupt D, et al. Conversion of HbS to Hb G-Makassar by adenine base editing is compatible with normal hemoglobin function. Blood. 2021;138:951.
27. Fitzhugh CD, Hsieh MM, Bolan CD, Saenz C, Tisdale JF. Granulocyte colony-stimulating factor (G-CSF) administration in individuals with sickle cell disease: time for a moratorium? Cytotherapy. 2009;11: 464–71.
28. Mozobil (plerixafor injection). Cambridge, MA: Genzyme Corporation; 2010.
29. Lagresle-Peyrou C, Lefrère F, Magrin E, Ribeil J-A, Romano O, Weber L, et al. Plerixafor enables safe, rapid, efficient mobilization of hematopoietic stem cells in sickle cell disease patients after exchange transfusion. Haematologica. 2018;103:778–86.
30. Esrick EB, Manis JP, Daley H, Baricordi C, Trébéden-Negre H, Pierciey FJ, et al. Successful hematopoietic stem cell mobilization and apheresis collection using plerixafor alone in sickle cell patients. Blood Adv. 2018;2:2505–12.
31. Tisdale JF, Pierciey FJ Jr, Bonner M, Thompson AA, Krishnamurti L, Mapara MY, et al. Safety and feasibility of hematopoietic progenitor stem cell collection by mobilization with plerixafor followed by apheresis vs bone marrow harvest in patients with sickle cell disease in the multi-center HGB-206 trial. Am J Hematol. 2020;95:E239–42.
32. Leonard A, Sharma A, Uchida N, Stroncek D, Panch SR, West K, et al. Disease severity impacts plerixafor-mobilized stem cell collection in patients with sickle cell disease. Blood Adv. 2021;5:2403–11.
33. Gene Transfer for Patients With Sickle Cell Disease. Available from: https://clinicaltrials.gov/ct2/show/NCT02186418. Last accessed 1 Jun 2022.
34. Grimley M, Asnani M, Shrestha A, Felker S, Lutzko C, Arumugam PI, et al. Safety and efficacy of Aru-1801 in patients with sickle cell disease: early results from the phase 1/2 momentum study of a modified gamma globin gene therapy and reduced intensity conditioning. Blood. 2021;138:3970.
35. Shenoy S, Angelucci E, Arnold SD, Baker KS, Bhatia M, Bresters D, et al. Current results and future research priorities in late effects after hematopoietic stem cell transplantation for children with sickle cell disease and thalassemia: a consensus statement from the second pediatric blood and marrow transplant consortium international conference on late effects after pediatric hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2017;23:552–61.

36. Goyal S, Tisdale J, Schmidt M, Kanter J, Jaroscak J, Whitney D, et al. Acute myeloid leukemia case after gene therapy for sickle cell disease. N Engl J Med. 2021;386:138–47.
37. Lee L, Smith-Whitley K, Banks S, Puckrein G. Reducing health care disparities in sickle cell disease: a review. Public Health Rep. 2019; 134:599–607.
38. Cornetta K, Patel K, Wanjiku CM, Busakhala N. Equitable access to gene therapy: a call to action for the American Society of Gene and Cell Therapy. Mol Ther. 2018;26:2715–6.
39. DeMartino P, Haag MB, Hersh AR, Caughey AB, Roth JA. A budget impact analysis of gene therapy for sickle cell disease: the Medicaid perspective. JAMA Pediatr. 2021;175:617–23.
40. Jensen K. Bluebird proposes installment plan for LentiGlobin gene therapy. Available from: https://www.biopharmadive.com/news/jpm19-bluebird-proposes-installment-plan-for-lentiglobin-gene-therapy/545646/. Last accessed 12 May 2022.
41. Vokinger KN, Avorn J, Kesselheim AS. Sources of innovation in gene therapies — approaches to achieving affordable prices. N Engl J Med. 2023;388:292–5.
42. Niihara Y, Miller ST, Kanter J, Lanzkron S, Smith WR, Hsu LL, et al. A phase 3 trial of l-glutamine in sickle cell disease. N Engl J Med. 2018; 379:226–35.
43. Ataga KI, Kutlar A, Kanter J, Liles D, Cancado R, Friedrisch J, et al. Crizanlizumab for the prevention of pain crises in sickle cell disease. N Engl J Med. 2017;376:429–39.
44. Vichinsky E, Hoppe CC, Ataga KI, Ware RE, Nduba V, El-Beshlawy A, et al. A phase 3 randomized trial of voxelotor in sickle cell disease. N Engl J Med. 2019;381:509–19.
45. Magrin E, Magnani A, Semeraro M, Hebert N, Joseph L, Leblanc O, et al. Clinical results of the Drepaglobe trial for sickle cell disease patients. Blood. 2021;138:1854.

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COMMENTARY

Vox Sanguinis ISBT International Society of Blood Transfusion

Limited evidence, lasting decisions: How voluntary non-remunerated plasma donations can avoid the commercial one-way street

INTRODUCTION

In July 2022, the European Commission published their proposal for a regulation on substances of human origin, which has since undergone debate [1]. The core of this debate surrounds whether or not the principle of voluntary non-remunerated donation (VNRD) should be loosened to increase the plasma supply in the European Union (EU). Based on the Committee of Ministers to Member States [2] and in line with the Council of Europe [3], we follow this definition of VNRD: '*Donation is considered voluntary and non-remunerated if the person gives blood, plasma or cellular components of his or her own free will and receives no payment for it, either in the form of cash or in kind which could be considered a substitute for money. This would include time off work other than that reasonably needed for the donation and travel. Small tokens, refreshments and reimbursements of direct travel costs are compatible with voluntary, non-remunerated donation*'. As such, we define remuneration as anything that falls outside of this definition, including financially non-neutral compensation, financial gain, or monetary compensations. Both proponents and opponents of VNRD use ethical or ideological argumentation, not often relying on empirical data to support their arguments [1]. Based on the limited available data, we argue that EU member states should retain VNRD at the core of their policies, even if a new regulation would allow remuneration. Our argumentation uses empirical findings surrounding three themes: safety for donors and donated products, product pricing, and supply resilience.

HIGHER SAFETY FOR DONORS AND DONATED PRODUCTS

In the United States, the maximum plasmapheresis donation frequency is twice per week (maximally 104 donations/year) [4]. Most plasma donors are remunerated 50–75 USD per donation, with certain centres advertising that regular donors can earn 6,000 USD annually [5, 6]. In Austria, Czechia, Germany and Hungary, although there is a lower maximum donation frequency, plasma donors are remunerated between 15 and 30€ [7]. It is reasonable to assume that when donors are remunerated, they will donate more frequently, as donors may depend on these payments as a source of income [8]. This differs from countries with VNRD, such as most countries in the EU [9]. Guidelines of the European Directorate for the Quality of Medicines and HealthCare of the Council of Europe recommend a maximum plasma donation frequency of once per week (maximally 33 donations/year) [10], such as in France, where the minimum interval between two plasma donations is 2 weeks (maximally 24 donations/year) [11].

A randomized controlled trial examined blood parameters across four plasma donation frequencies [12]. During the trial (Day 42) and after (Day 84), immunoglobulin G levels decreased (on average to <6 g/L on Day 84) within the group that donated twice a week (the maximum allowance in the United States [4]), corroborating previous research [13]. Because commercial plasma donation centres are located in areas of greater poverty [14], vulnerable populations are made even more vulnerable concerning their health safety, especially given the aforementioned advertising from these centres to donate frequently [4–6, 8].

Within Lithuania, blood and blood components donations shifted from mostly remunerated to mostly VNRD due to national programmes promoting VNRD following Lithuania's EU membership [15]. A study examining this unique within-country natural experiment showed higher prevalence of transfusion-transmitted infectious (TTI) disease markers for remunerated donations compared to donations based on VNRD from 2013 to 2017 [15]. This is consistent with previous research that found lower TTI disease markers for voluntary compared to paid donors in the Democratic Republic of Congo [16] and Nigeria [17].

In the case of plasma donations specifically, a human immunodeficiency virus/hepatitis C infection outbreak occurred in the 1970s–80s within the haemophilia community, whose individuals depend on plasma-derived clotting factor products [18]. Consistent with the research on Lithuania [15], at that time, higher infection rates were found for products made plasma in countries with a collection system based on remunerated donations than countries with a collection system based on VNRD [19]. The procedure has since been improved to reduce infectious risk, but there remains an infectious burden on donated products.

TABLE 1 Mean price (in euros) of Privigen® (per gram) for 2018 and 2023 in private collection centres compared to public collection centres.

	2018	2023	% change from 2018 to 2023
Private collection	38.95€	73.20€	88%
Public collection	51.73€	64.33€	24%

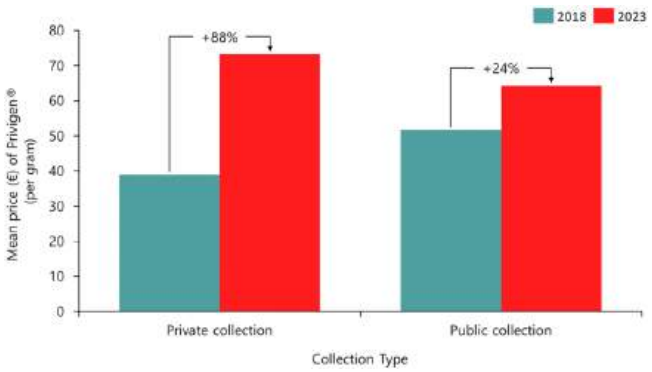

FIGURE 1 Mean price (in euros) of Privigen® (per gram) for 2018 and 2023 in private plasma collection centres compared to public collection centres. Increase in price from 2018 to 2023 is shown as a positive percentage difference with an arrow. Data extracted from personal communication and government websites (see Appendix S1).

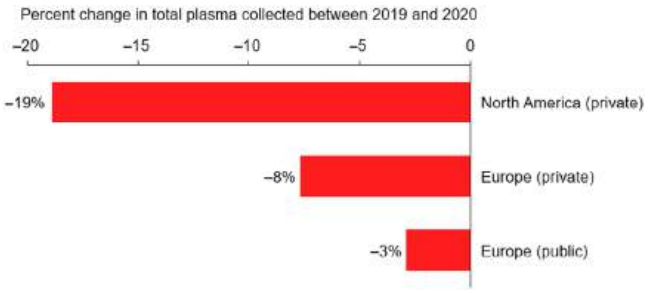

FIGURE 2 The percent change in total plasma collected between 2019 and 2020 for different sectors. Exact percentages and direction of change are reflected directly in front of bars [25, 26].

LOWER PRICES FOR PATIENTS AND THE HEALTHCARE SYSTEM

Plasma-derived medicinal products (PDMPs), such as Privigen® [20–22], an immunoglobulin product, are medicines derived from plasma to treat several medical conditions. We gathered Privigen® price data for several European countries, through personal communication and government websites (see Appendix S1), as publicly available data on PDMP prices is limited. Our data showed that Privigen® prices (per gram) increased less in countries with only public and non-governmental organization (NGO) plasma collection (non-remuneration) compared to countries with private plasma collection (donors are effectively remunerated) from 2018 to 2023 (Table 1; Figure 1). This illustrates seemingly differing pricing mechanisms for PDMPs between these European countries. Moreover, since these dates are before and during the coronavirus disease 2019 (COVID-19) pandemic, these data also show that PDMP prices seem to be better managed in countries with only public and NGO plasma collection compared to countries with private plasma collection.

MORE RESILIENT SUPPLY

Ensuring plasma supply is maintained if a crisis arises is crucial [23, 24]. By comparing the plasma supply before (2019) and during (2020) the COVID-19 pandemic, we measured supply resilience across the private sector (donors are effectively remunerated) and public sector (non-remuneration). Results showed that the plasma supply collected through the private sector decreased more drastically than the plasma supply collected through the public sector from 2019 to 2020 (Figure 2) [25, 26].

Moreover, research on remunerated donations in Germany found 77% of remunerated donors would discontinue donating if remuneration ceased [27]. This further demonstrates the issues with remunerated donations: once remuneration is expected by donors, the decision is irreversible without a major loss of donors (i.e., 50% [27]), thus causing a significant supply shortage.

CONCLUSION

The empirical evidence presented here complements the ethical and ideological argumentation surrounding the importance of VNRD. Research indicated that when donors are remunerated and therefore donate very frequently, donor safety is at risk and the infectious burden of the donated products is higher [11–19]. Moreover, PDMP prices increased less over time when plasma was collected in countries with collection system based on VNRD as opposed to remunerated donations (Figure 1). In addition, once countries allow remuneration, they enter a 'one-way street': the supply of plasma—and potentially also whole blood and components—will be at risk when re-introducing VNRD policies [27]. It should be noted that the data on pricing and resilience are limited due to a lack of transparent data and do not allow for establishing a causal mechanism between implementing a system based on VNRD and the impact on price or resilience, especially since different countries have different pricing procedures for medicines. We encourage future research to explore the causal links between VNRD and outcomes such as pricing of PDMPs and reslience of supply. In conclusion, based on the above empirical evidence, we argue that EU members should retain VNRD at the core of their policies and refrain from introducing remuneration, even if the new regulation enables it.

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K.J.M. wrote the first draft, assembled the database, edited the figures and revised and edited the manuscript. F.S. developed the concept, collected and analysed the data, created the figures and reviewed and edited the manuscript. P.V. developed the concept, supervised the research and reviewed and edited the manuscript.

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All authors are employed by Belgian Red Cross–Flanders, responsible and reimbursed for supplying adequate quantities of safe blood products to hospitals in Flanders and Brussels.

DATA AVAILABILITY STATEMENT

Data are available upon reasonable request to the corresponding author, Kelsey J. MacKay.

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REFERENCES

1. Proposal for a REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on standards of quality and safety for substances of human origin intended for huma application and repealing Directives 2002/98/EC and 2004/23/EC.14-Jul-2022. The European Parliament and the Coucil of the European Union. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52022PC0338. Last accessed 10 Oct 2023.
2. Recommendation No.R(95)14 of the Committee of Ministers to member states on the protection of the health of donors and recipients in the area of blood transfusion. Council of Europe. Available from: https://www.coe.int/t/dg3/health/recommendations_en.asp. Last accessed 10 Oct 2023.
3. Guide for the implementation of the principle of prohibition of financial gain with respect to the human body and its parts from living or deceased donors. Council of Europe. Available from: https://rm.coe.int/guide-financial-gain/16807bfc9a. Last accessed 10 Oct 2023.
4. Giving blood and plasma. United States Department of Health Services. Available from: https://www.hhs.gov/oidp/topics/blood-tissue-safety/giving-blood-plasma/index.html. Last accessed 9 Oct 2023.
5. How to donate plasma for money. Money. Available from: https://money.com/donate-plasma-for-money/. Last accessed 9 Oct 2023.
6. Frequently Asked Questions. Life Plasma. Available from: https://lifeplasma.com/. Last accessed 10 Oct 2023.
7. Fortuna G, Peseckyte G. Euractiv. 2022 Available from: https://www.euractiv.com/section/health-consumers/news/plasma-donor-compensation-still-an-open-wound-in-eus-blood-directive-revision/. Last accessed 10 Feb 2024.
8. Shafi S. The dangers of the blood plasma industry's over-reliance on paid for "donations". BMJ. 2023;380:199.
9. Directive 2002/98/EC of the European Parliament and of the Council of 27 January 2003 setting standards of quality and safety for the collection, testing, processing, storage, and distribution of human blood and blood components and amending Directive 2001/83/EC. The European Parliament and the Coucil of the European Union. Available from: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:033:0030:0040:EN:PDF. Last accessed 9 Oct 2023.
10. European Committee & European Directorate for the Quality of Medicines and Healthcare. Chapter 2: Donor selection. Guide to the preparation, use and quality assurance of blood components. 21st ed. Strasbourg, France: Council of Europe; 2023. p. 158.
11. Arrêté du 11 janvier 2022 modifiant l'arrêté du 17 décembre 2019 fixant les critères de sélection des donneurs de sang(Gouvernement de la République française) Article 1. Ministère Des Solidarités et de la Santé. Available from: https://www.legifrance.gouv.fr/loda/id/JORFTEXT000039667225/. Last accessed 10 Feb 2024.
12. Mortier A, Khoudary J, van Doorslaer de Ten Ryan S, Lannoy C, Benoit N, Antoine N, et al. Effects of plasmapheresis frequency on health status and exercise performance in men: a randomized controlled trial. Vox Sang. 2024;119:134–43.
13. Laub R, Baurin S, Timmerman D, Branckaert T, Strengers P. Specific protein content of pools of plasma for fractionation from different sources: impact of frequency of donations. Vox Sang. 2010;99:220–30.
14. Ochoa A, Shaefer HL, Grogan-Kaylor A. The interlinkage between blood plasma donation and poverty in the United States. J Sociol Soc Welf. 2022;48:56.
15. Kalibatas V, Kalibatienė L. Reducing the risk of transfusion-transmitted infectious disease markers in blood and blood component donations: movement from remunerated to voluntary, non-remunerated donations in Lithuania from 2013 to 2020. PloS One. 2022;17:a0277650.
16. Batina A, Kabemba S, Malengela R. Marqueurs infectieux chez les donneurs de sang en République Démocratique du Congo (RDC). Rev Med Brux. 2007;28:45–9.
17. Ekwunife CA, Ozumba NA, Eneanya CI, Nwaorgu OC. Malaria infection among blood donors in Onitsha urban, Southeast Nigeria. Sierra Leone J Biomed Res. 2011;3:21–6.
18. The contaminated blood scandal. The Haemophilia Society. Available from: https://haemophilia.org.uk/public-inquiry/the-infected-blood-inquiry/the-contaminated-blood-scandal/. Last accessed 10 Feb 2024.

19. Simonetti A, Smit C. Europe needs 2 million extra donors of blood and plasma: How to find them? Vox Sang. 2024;119:121–7.
20. Privigen. European Medicines Agency. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/privigen. Last accessed 9 Oct 2023.
21. Plasma-derived medicines are unique: Treatments for patients with rare diseases in Europe. PPTA. Available from: https://www.euneedsmoreplasma.com/plasma-derived-medicines. Last accessed 10 Oct 2023.
22. What is Privigen? Privigen. Available from: https://www.privigen.com/what-is-privigen/pi. Last Accessed 10 Feb 2024.
23. Plasma-derived medicines and the shortage of plasma donations in Europe. European Parliament. Available from: https://www.europarl.europa.eu/doceo/document/E-9-2020-005543_EN.html. Last accessed 10 Oct 2023.
24. Covington ML, Voma C, Stowell SR. The impact of the COVID-19 pandemic on source plasma donations. J Clin Apher. 2023;38:644–6.
25. Plasma collection and manufacturing. PPTA. Available from: https://www.pptaglobal.org/resources/plasma-collection-and-manufacturing. Last accessed 9 Oct 2023.
26. European Blood Alliance. European Blood Alliance Benchmarking group 2021–2022 report. Brussels: Benchmarking Working Group; September 2022.
27. Kretschmer V, Weipper-Kretschmer M, Slonka J, Karger R, Zeiler T. Perspectives of paid whole blood and plasma donation. Transfus Med Hemother. 2004;31:301–7.

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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SARS-CoV-2 immunoassays in a predominantly vaccinated population: Performances and qualitative agreements obtained with two analytical approaches and four immunoassays

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Abstract

Background and Objectives: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) serosurveys are typically analysed by applying a fixed threshold for seropositivity ('conventional approach'). However, this approach underestimates the seroprevalence of anti-nucleocapsid (N) in vaccinated individuals—who often exhibit a difficult-to-detect anti-N response. This limitation is compounded by delays between the onset of infection and sample collection. To address this issue, we compared the performance of four immunoassays using a new analytical approach ('ratio-based approach'), which determines seropositivity based on an increase in anti-N levels.

Materials and Methods: Two groups of plasma donors and four immunoassays (Elecsys total anti-N, VITROS total anti-N, Architect anti-N Immunoglobulin G (IgG) and in-house total anti-N) were evaluated. First-group donors ($N = 145$) had one positive SARS-CoV-2 polymerase chain reaction (PCR) test result and had made two plasma donations, including one before and one after the PCR test (median = 27 -days post-PCR). Second-group donors ($N = 100$) had made two plasma donations early in the Omicron wave.

Results: Among first-group donors (97.9% vaccinated), sensitivity estimates ranged from 60.0% to 89.0% with the conventional approach, compared with 94.5% to 98.6% with the ratio-based approach. Among second-group donors, Fleiss's κ ranged from 0.56 to 0.83 with the conventional approach, compared with 0.90 to 1.00 with the ratio-based approach.

Conclusion: With the conventional approach, the sensitivity of four immunoassays—measured in a predominantly vaccinated population based on samples collected ~1 month after a positive test result—fell below regulatory agencies requirement of ≥95%. The ratio-based approach significantly improved the sensitivities and qualitative agreement among immunoassays, to the point where all would meet this requirement.

Keywords

COVID-19 antibodies, donors, epidemiology, high throughput testing, nucleocapsid immunoassay, ratio-based approach, sensitivity, serological testing, vaccination

Highlights

- When the conventional approach was used to analyse the results of samples collected ~1 month after a positive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) test result, the sensitivity of four immunoassays (which included three commercial immunoassays and one in-house immunoassay) failed to meet the minimum sensitivity of ≥95% required for regulatory agencies.
- The ratio-based analytical approach significantly improved the sensitivities and the degrees of qualitative agreement among immunoassays (both assessed ~1 month after a positive SARS-CoV-2 test result), to the point where all would meet this regulatory requirement.
- These results highlight the need to compare the performance of diagnostic or serological assays in vaccinated populations, especially when no standalone assay can be considered as the gold standard.

INTRODUCTION

Blood services have collaborated with public health authorities for the conduct of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) serosurveys targeting mostly anti-nucleocapsid (N) because of its ability to distinguish infection from vaccination which elicit anti-spike (S) response. This experience has laid the foundations for expanding the scope of collaborations between blood services and public health authorities [1, 2].

However, blood services faced many challenges while conducting these serosurveys, including the fact that the anti-N response to an infection is often blunted and hardly detectable in recipients of a SARS-CoV-2 messenger ribonucleic acid (mRNA) vaccine [3–7]. This issue may be addressed in at least two ways.

First, more sensitive immunoassays or lower cutoff thresholds may be used to allow for the detection of low anti-N levels. However, the performances of existing immunoassays have primarily been characterized in unvaccinated individuals [8], which makes it challenging to select an optimal immunoassay or lower cutoff for a predominantly vaccinated population.

Second, a new analytical approach developed and validated by Bazin et al. (termed 'ratio-based approach') may be used to improve sensitivity [9]. This approach determines seropositivity based on an increase of (or 'ratio' between) the anti-N signal of two longitudinally collected samples, as opposed to applying a hard threshold to a single sample (i.e., 'conventional approach') [9]. However, the performance of this approach has only been characterized with an in-house immunoassay and is therefore unknown for other commercial immunoassays [9].

To fill these knowledge gaps, the performance of various anti-N immunoassays and their qualitative agreement were assessed in a predominantly vaccinated population, and the data were analysed with the conventional and ratio-based approaches.

METHODS

Donors

The study included two groups of plasma donors enrolled in PlasCoV—a Québec-based (Canada) plasma biobank dedicated to coronavirus disease (COVID-19) research [10]. Approximately two thirds of previous PlasCoV donors made at least two donations [10], thus enabling the conduct of longitudinal studies on COVID-19 immunity and, importantly, the use of the ratio-based approach.

The first group was used to assess the sensitivity of different SARS-CoV-2 immunoassays (with two analytical approaches). The donors included in this group met the following criteria (Figure 1): (1) one positive SARS-CoV-2 polymerase chain reaction (PCR) test result recorded between 14 December 2021 and 11 February 2022 and (2) two plasma donations, including one made between 1 November 2021 and 14 January 2022 (i.e., before the positive PCR test) and one between 14 January 2022 and 15 March 2022 (i.e., after the positive PCR test).

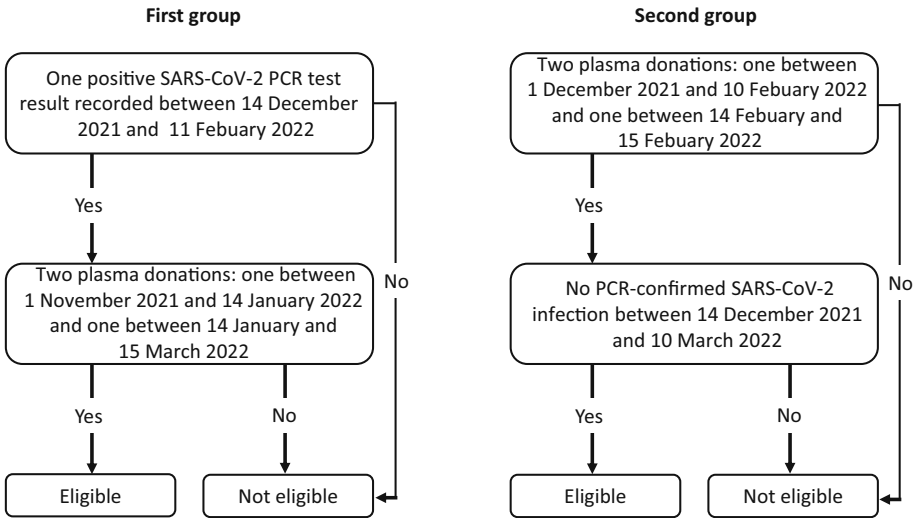

FIGURE 1 Donor selection flowchart. PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

The second group was used to assess the degree of qualitative agreement (as measured by the overall percentage agreement [OPA] and Fleiss's κ) among the results of different SARS-CoV-2 immunoassays. The donors in this group met the following criteria (Figure 1): (1) 2 plasma donations, including one between 1 December 2021 and 10 February 2022 and one between 14 February 2022 and15 February 2022, and (2) no PCR-confirmed SARS-CoV-2 infection recorded in *Trajectoire de Santé Publique* (i.e., a government registry) between 14 December 2021 and 10 March 2022. Of note, the second inclusion criterion aimed to lower the prevalence of SARS-CoV-2 into real life conditions.

Serological testing of plasma samples

Three commercial and one in-house SARS-CoV-2 anti-N immunoassays were evaluated. The three commercial immunoassays were Elecsys® Anti-SARS-CoV-2 immunoassay (total anti-N) from Roche diagnostics ('Elecsys'), VITROS Anti-SARS-CoV-2 Total N Antibody immunoassay from Ortho-Clinical Diagnostics ('VITROS') and Architect anti-SARS-CoV-2 chemiluminescent microparticle N Immunoglobulin G (IgG) immunoassay from Abbott Laboratories ('Architect'). The commercial immunoassays were carried out according to manufacturers' instructions, and the in-house total anti-N immunoassay was carried out as previously described [9].

Analytical approaches

The results of these immunoassays were analysed with the conventional and ratio-based approaches. For the conventional approach, seropositivity was determined according to a hard threshold (which varied among immunoassays) applied to the anti-N signal of the second sample. For the ratio-based approach, positivity was defined by an increase of ≥50% in the anti-N signal of the 'reference sample' (i.e., collected before the positive PCR test) and the 'test sample' (i.e., collected after the positive PCR test), as previously described [9]. Based on the fact that lower absorbance may have higher coefficient of variation, test samples with an absorbance <0.100 were excluded and considered seronegative [9].

Ethics approval

All plasma donors enrolled in PlasCoV have given their informed consent for the use of their samples and personal data for SARS-CoV-2 diseases and immunity studies, including linkage with provincial infection and vaccination registries, as described in the Management and Governance Framework for the Plasma Donor Biobank (https://www.hema-quebec.qc.ca/userfiles/file/media/anglais/rd/Governance_Framework_Plasma_Donor_Biobank_HQ.pdf). All data collection and management have been performed in accordance with the directive and legislation of research in Canada and Quebec. Consequently, all donors, blood donations and patients were identified by a unique anonymous number and no patient identifiers were stored with clinical data. The resulting database is encrypted and stored centrally. Access is restricted and only designated members of the study team have access to the dataset. This project was approved by Hema-Quebec Research Ethical Board (REB # 2021-020).

RESULTS

Donors

The first group included 145 PlasCoV donors who had a PCR-confirmed SARS-CoV-2 infection. These donors were aged (on average) 41.5 years and included 67 (46.2%) female donors and 132 (91.0%) White donors (Table 1). In total, 142 (97.9%) donors had

TABLE 1 Characteristics of donors included in the first and second group.

	First-group donors	Second-group donors
	N = 145	*N* = 100
Age, mean ± SD (median), years	41.5 ± 14.7	56.1 ± 17.2
Female, *n* (%)	67 (46.2)	35 (35.0)
Race, *n* (%)		
White	132 (91.0)	98 (98.0)
SARS-CoV-2 vaccines received, *n* (%)		
≥2	142 (97.9%)	98 (98.0%)
≥3	49 (33.8%)	90 (90.0%)
Anti-N-positive test result on the reference donation, *n* (%)[a]		
In-house immunoassay	0 (0.0%)	NA
Elecsys immunoassay	7 (4.9%)	NA
VITROS immunoassay	7 (4.8%)	NA
Architect immunoassay	0 (0.0%)	NA
Gap between the first donation and the positive PCR test result, mean ± SD (IQR), days	36.5 ± 13.4 (25–47)	NA
Gap between the positive PCR test result and the second donation, mean ± SD (IQR), days	30.3 ± 14.9 (19–39)	NA

[a]The reference sample measurement was unavailable for one donor tested with the Elecsys immunoassay (*N* = 144) and for two donors tested with the Architect immunoassay (*N* = 143).
Abbreviations: IQR, interquartile range; N, nucleocapsid; NA, not applicable; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation.

received ≥2 doses of a vaccine against SARS-CoV-2 before their positive PCR test result.

Among the reference samples (i.e., pre-PCR) of these donors, the same seven (4.8%) tested seropositive with the Elecsys immunoassay (*N* = 144) and with the VITROS immunoassay (*N* = 145); none tested seropositive with the in-house (*N* = 145) or Architect immunoassay (*N* = 143). The gap between the positive PCR test result and the second donation was ~1 month (mean = 30.3 days; median = 27 days) (Figure S1).

The second group included 100 regular plasma donors. These donors were aged (on average) 56.1 years and included 35 (35.0%) female donors and 98 (98.0%) White donors (Table 1). Ninety-eight (98.0%) had received ≥2 doses of a SARS-CoV-2 vaccine when their test sample was collected.

Sensitivity of SARS-CoV-2 immunoassays with the conventional approach

With the conventional approach used on test samples (post-PCR), 94 (64.8%) first-group donors tested seropositive with the in-house immunoassay, 106 (73.1%) tested seropositive with the Elecsys immunoassay, 129 (89.0%) tested seropositive with the VITROS immunoassay and 87 (60.0%) did so with the Architect IgG immunoassay (Figure 2). One donor could not be assessed with the Elecsys immunoassay.

Overall, 75.0% of the samples missed by the VITROS immunoassay, and 76.9% of those that were missed by the Elecsys immunoassay were collected ≤27 days after the positive PCR test (Table S1). These proportions fell to 52.9% for the in-house immunoassay and 50.0% for the Architect immunoassay. Among all samples missed by the VITROS and Elecsys immunoassays (*N* = 16), 4 (25.0%) were captured by either the in-house or Architect immunoassay.

Sensitivity of SARS-CoV-2 immunoassays with the ratio-based approach

With the ratio-based approach, one donor was not tested with the Elecsys immunoassay (*N* = 144), and two were not tested with the Architect immunoassay (*N* = 143), all owing to the unavailability of reference sample measurements.

Using this approach, 136 (94.5%) first-group donors tested seropositive with the in-house immunoassay, 141 (97.9%) tested seropositive with the Elecsys immunoassay, 143 (98.6%) tested seropositive with the VITROS immunoassay and 140 (98.6%) did so with the Architect immunoassay (Figure 2). The relative increase in signal between the reference (pre-PCR) and test samples (post-PCR) as well as the relative increase in ratio was higher with the VITROS immunoassay (Figure S2).

One of the two donors missed by the VITROS immunoassay donated only 7 days after the PCR result. With the conventional approach, this donor tested seronegative with all immunoassays. However, with the ratio-based approach, the donor tested positive with the in-house and Elecsys immunoassays. The other missed donor donated 54 days after the PCR test. With the conventional approach, this donor's reference sample tested seropositive with the VITROS and Elecsys immunoassays but seronegative with the other immunoassays, and the test sample was seronegative with all immunoassays. With the ratio-based approach, the donor tested seronegative with all immunoassays.

Qualitative agreement among anti-N SARS-CoV-2 immunoassays performed on second-group donors

Among second-group donors, the anti-N seroprevalence ranged between 6.1% and 11.6% depending on the immunoassay and analytical approach being used (Table S2).

With the conventional approach, the OPA ranged between 91.9% and 97.0% (Table 2) and Fleiss's κ ranged between 0.56 and 0.83 (Table 3). With the ratio-based approach, the OPA ranged between 97.9% and 100.0% (Table 4) and Fleiss's κ ranged between 0.90 and 1.00 (Table 5). The qualitative agreements appeared largely similar, regardless of the immunoassays being compared.

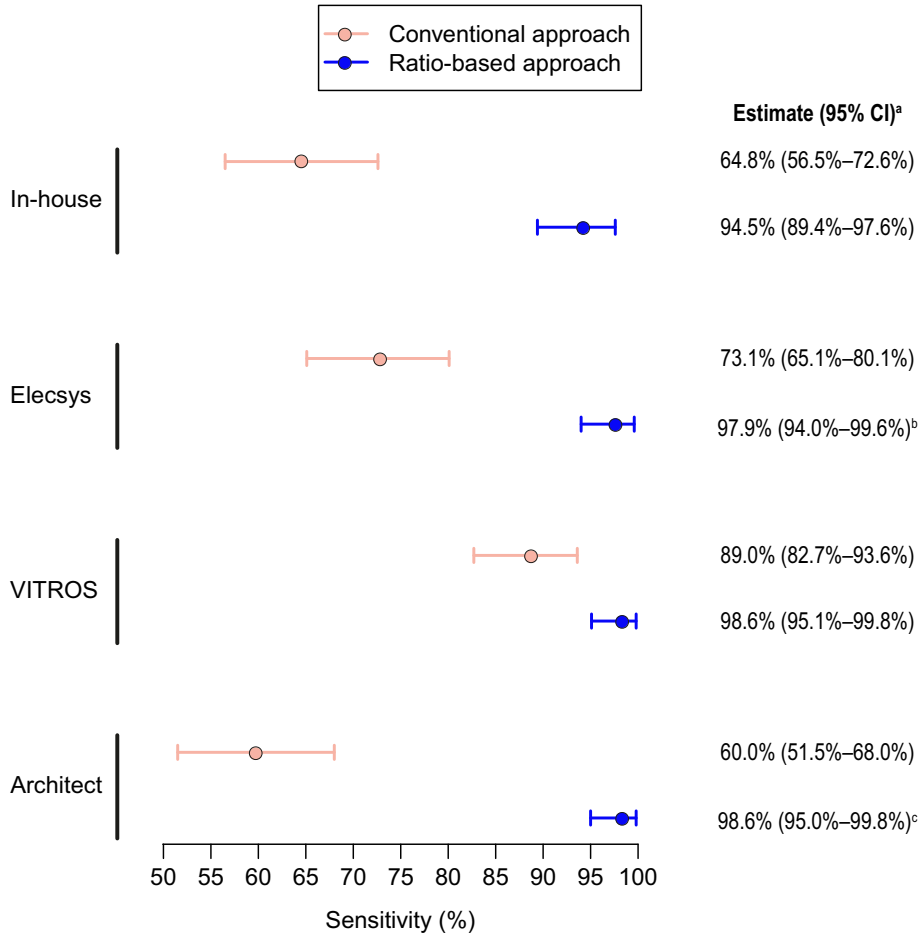

FIGURE 2 Sensitivity estimates for the study immunoassays, measured with the conventional and ratio-based approaches. [a]Clopper–Pearson confidence intervals (CIs). [b]One donor could not be assessed with the ratio-based approach given the unavailability of reference sample measurements ($N = 144$). [c]Two donors could not be assessed with the ratio-based approach given the unavailability of reference sample measurements ($N = 143$).

TABLE 2 Qualitative agreement among the results of the study immunoassays analysed with the conventional approach, as measured by the overall percentage agreement (95% CI)[a] in second-group donors.

	In-house	Elecsys	VITROS	Architect
In-house		92.9% (86.1%–96.5%)	91.9% (84.9%–95.8%)	96.0% (90.1%–98.4%)
Elecsys			97.0% (91.5%–99.0%)	97.0% (91.5%–99.0%)
VITROS				96.0% (90.1%–98.4%)
Architect				

[a]Clopper–Pearson CIs.
Abbreviation: CI, confidence interval.

DISCUSSION

This study estimated the sensitivity of anti-N SARS-CoV-2 immunoassays in a predominantly vaccinated population based on samples collected ~1 month after a positive PCR test. The sensitivity estimates ranged from 60.0% to 89.0% with the conventional approach, compared with 94.5% to 98.6% with the ratio-based approach. Likewise, the qualitative agreement among immunoassays (as measured by Fleiss' κ) ranged from 0.56 to 0.83 with the conventional approach, compared with 0.90 to 1.00 with the ratio-based approach. A similar trend (albeit more modest) was observed when measuring the OPA.

With one exception (i.e., VITROS immunoassay), the observed sensitivities obtained with the conventional approach—and in a predominantly vaccinated population—were lower than those previously reported in unvaccinated populations (Table S3). This observation is consistent with the blunted anti-N response in vaccinated individuals [3–7]. Notably, with samples collected ~1 month after a positive SARS-CoV-2 test result, none of the study immunoassays would meet

TABLE 3 Qualitative agreement among immunoassay results analysed with the conventional approach, as measured by Fleiss's κ (95% CI)[a] in second-group donors.

	In-house	Elecsys	VITROS	Architect
In-house		0.59 (0.32–0.87)	0.56 (0.28–0.83)	0.73 (0.48–0.98)
Elecsys			0.83 (0.63–1.00)	0.78 (0.55–1.00)
VITROS				0.73 (0.48–0.98)
Architect				

[a]Clopper–Pearson CIs.
Abbreviation: CI, confidence interval.

TABLE 4 Qualitative agreement among immunoassay results analysed with the ratio-based approach, as measured by the overall percentage agreement (95% CI)[a] in second-group donors.

	In-house	Elecsys	VITROS	Architect
In-house		99.0% (94.5%–99.8%)	99.0% (94.5%–99.8%)	97.9% (92.6%–99.4%)
Elecsys			100.0% (96.3%–100.0%)	98.9% (94.3%–99.8%)
VITROS				98.9% (94.3%–99.8%)
Architect				

[a]Clopper–Pearson CIs.
Abbreviation: CI, confidence interval.

TABLE 5 Qualitative agreement among immunoassay results analysed with the ratio-based approach, as measured by Fleiss's κ (95% CI)[a] in second-group donors.

	In-house	Elecsys	VITROS	Architect
In-house		0.95 (0.84–1.00)	0.95 (0.84–1.00)	0.90 (0.76–1.00)
Elecsys			1.00 (1.00–1.00)	0.95 (0.84–1.00)
VITROS				0.95 (0.84–1.00)
Architect				

[a]Clopper–Pearson CIs.
Abbreviation: CI, confidence interval.

Canada's regulatory requirement for sensitivity (i.e., ≥95% [11]) if these immunoassays were used in a vaccinated population and the results were analysed with the conventional approach using manufacturer-recommended cutoffs.

Part of this limitation can be addressed with the ratio-based approach, which reliably estimates incidence insofar as a sufficient number of individuals can be longitudinally observed over consecutive periods (something easily achievable with blood donors). This analytical approach substantially enhanced the sensitivity of all immunoassays, to the point where all would meet the sensitivity threshold of ≥95% in Canada [11]. The ratio-based approach would therefore make all of these immunoassays appropriate for public health surveillance and modelling studies, contingent on the availability of test results for many repeat donors. This approach also significantly improved the degree of agreement among the study immunoassays with OPA between 97.9 and 100%. Readers interested in knowing more about the strengths and limitations of the ratio-based approach are referred to the original publication by Bazin et al. [9].

Interestingly, with the conventional approach, the false-negatives obtained with the VITROS and Elecsys immunoassays were associated with shorter gaps between the PCR test and sample collection. This result suggests that these immunoassays—although highly sensitive—are prone to missing cases if the time interval between PCR test and sample collection is short. By contrast, this association was not observed with the in-house and Architect immunoassays, that is, the false-negatives were evenly distributed around the median gap of 27 days (Table S1). However, these two immunoassays appeared slightly less sensitive.

Although reliable, the ratio-based approach did not eliminate all discrepancies among immunoassays. However, those remaining could be resolved by other means, such as Bayesian latent class modelling (BLCM). BLCM is increasingly used to estimate disease prevalence, assay sensitivity and assay specificity when an established reference standard is lacking [12, 13]. With BLCM, an assay's sensitivity and specificity and disease prevalence (without a priori knowledge on the true history of infection) can be inferred, as the approach combines prior information on these parameters with the observed results of several imperfect tests [14]. Our data highlight the need for such an approach, as the study immunoassays seemed (at least partially) complementary.

Interestingly, before testing positive for SARS-CoV-2 (by PCR), seven donors tested seropositive with the Elecsys and VITROS immunoassays (along with the conventional approach); the seven donors identified by both immunoassays fully overlapped. The fact that the Elecsys and VITROS immunoassays were the most sensitive (with the conventional approach) suggests that these seven donors might have had anti-N titres below the seropositivity threshold of the other immunoassays before their PCR test. These donors may, therefore, have been re-infected or may have developed anti-N relatively early, that is, after the onset of the infection but before PCR testing. With the ratio-based approach, all but one of these seven donors tested positive with all immunoassays; the one that tested negative (again with all immunoassays) exhibited stable anti-N levels in the reference and test samples, suggesting potentially a false-positive PCR result or a long viral shedding that could still be detected by PCR, although the infection was acquired a few weeks before.

The mechanism behind the blunted anti-N response of vaccine recipients remains unclear. In a recent retrospective analysis of the COVE trial (i.e., mRNA-1273 to prevent SARS-CoV-2 infection), the anti-N response of vaccine recipients was not only weaker than that of placebo recipients but also highly heterogeneous, with some donors seemingly exhibiting no anti-N response at the study time points [5]. Similar to the current study, the samples were collected ~1 month after symptom onset [5]. According to Follmann et al., the low rate of seroconversion in vaccine recipients (i.e., 37%) 'was not attributable to transient or low-level [antibodies]' [5]. Our results are not, however, consistent with this interpretation. In fact, the ratio-based approach hinges on the premise that breakthrough infections induce a weaker anti-N response that may be undetectable with the conventional approach. Therefore, if Follmann et al.'s interpretation was correct [5]; the ratio-based approach would have a relatively poor sensitivity. Yet it captures nearly all infections in vaccine recipients [9].

Our study is subject to some limitations. To begin, specificity could not be evaluated for the ratio-based approach because no included donors had available pre-pandemic samples. However, the high qualitative agreement among the test results of second-group donors might suggest a high specificity for all study immunoassays, although this is speculative. Likewise, specificity was not evaluated for the conventional approach, as vaccination should not impact this parameter (unlike sensitivity) [15].

Another limitation of the ratio-based approach is that samples collected before and after an infection (or after a reinfection) must be available. Blood services are, therefore, ideally positioned to leverage the ratio-based approach, as they routinely collect samples from repeat donors (who account for 90% of all donors). Moreover, the gap between the collection of the test samples and the PCR test may have impacted the sensitivity of the ratio-based approach. On one hand, the gap of ~1 month may not have been sufficient to enable anti-N seroconversion due to the blunted viral replication and seroconversion in vaccine recipients. On the other hand, longer gaps might have caused anti-N waning [16, 17]. Our previous data showed that the optimal period between the reference and test samples is 3–4 months, which correlates with anti-N waning [9]. Use of longer intervals (e.g., 6–12 months) would decrease the sensitivity of detection of new infections and may miss reinfection that could happen within the interval.

In a predominantly vaccinated population, none of the study immunoassays met Canada's minimum sensitivity requirement of ≥95% [11] when the results were analysed with the conventional analytical approach. Lowering the cutoffs of commercial assays has been evaluated to increase sensitivity but this would be at the cost of reduced specificity [15, 18]. The ratio-based analytical approach significantly improved the sensitivities and the degrees of qualitative agreement among immunoassays, to the point where all would meet the regulatory requirement for sensitivity. These results highlight the need to compare the performance of diagnostic or serological assays in vaccinated populations, especially when no standalone assay can be considered as the gold standard.

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C.R., A.L., M.P.B., M.G. and R.B. conceived the study; Y.G., N.S., E.V., M.P., S.J.D., S.F.O. and C.D.G. acquired the data; A.L. and Y.G. analysed the data; C.R., A.L., Y.G., S.J.D., S.F.O., M.P.B., M.G. and R.B. interpreted the data; C.R., A.L. and R.B. wrote the manuscript, which was critically reviewed by all authors.

CONFLICT OF INTEREST STATEMENT

Authors have no conflict of interest to declare. This work was undertaken without any financial support or contribution from the assay manufacturers involved in this research.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Lewin A, Osiowy C, Erikstrup C, Custer B, Renaud C, Tiberghien P, et al. Research partnerships between blood services and public health authorities: an international, cross-sectional survey. Vox Sang. 2022;117:1368–74.
2. O'Brien SF, Lieshout-Krikke RW, Lewin A, Erikstrup C, Steele WR, Uzicanin S, et al. Research initiatives of blood services worldwide in response to the covid-19 pandemic. Vox Sang. 2021;116:296–304.

3. Allen N, Brady M, Carrion Martin AI, Domegan L, Walsh C, Doherty L, et al. Serological markers of SARS-CoV-2 infection; anti-nucleocapsid antibody positivity may not be the ideal marker of natural infection in vaccinated individuals. J Infect. 2021;83:e9–e10.
4. Follmann D, Janes HE, Buhule OD, Zhou H, Girard B, Marks K, et al. Antinucleocapsid antibodies after SARS-CoV-2 infection in the blinded phase of the randomized, placebo-controlled mRNA-1273 COVID-19 vaccine efficacy clinical trial. Ann Intern Med. 2022;175:1258–65.
5. Follmann D, Janes HE, Chu E, Jayashankar L, Petropoulos CJ, Serebryannyy L, et al. Kinetics of the antibody response to symptomatic SARS-CoV-2 infection in vaccinated and unvaccinated individuals in the blinded phase of the mRNA-1273 COVID-19 vaccine efficacy trial. Open Forum Infect Dis. 2023;10:ofad069.
6. Streibl BI, Lahne H, Grahl A, Agsten P, Bichler M, Büchl C, et al. Epidemiological and serological analysis of a SARS-CoV-2 outbreak in a nursing home: impact of SARS-CoV-2 vaccination and enhanced neutralizing immunity following breakthrough infection. Microorganisms. 2022;10:1809.
7. Dhakal S, Yu T, Yin A, Pisanic N, Demko ZO, Antar AA, et al. Reconsideration of antinucleocapsid IgG antibody as a marker of SARS-CoV-2 infection postvaccination for mild COVID-19 patients. Open Forum Infect Dis. 2023;10:ofac677.
8. Cochrane Infectious Diseases Group, Fox T, Geppert J, Dinnes J, Scandrett K, Bigio J, et al. Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Database Syst Rev. 2022;6:CD013652.
9. Bazin R, Rochette S, Perreault J, Fournier M-J, Grégoire Y, Boivin A, et al. Evaluation of anti-nucleocapsid level variation to assess SARS-CoV-2 seroprevalence in a vaccinated population. Infect Dis. 2023;55:425–30.
10. Germain M, Lewin A, Bazin R, Dieudé M, Perreault J, Boivin A, et al. Cohort profile: a Québec-based plasma donor biobank to study COVID-19 immunity (PlasCoV). BMJ Open. 2023;13:e068803.
11. Health Canada. COVID-19 serological testing devices: notice on sensitivity and specificity values. Available from: https://www.canada.ca/en/health-canada/services/drugs-health-products/covid19-industry/medical-devices/testing/serological/notice-sensitivity-specificity-values.html. Last accessed 27 Jul 2023.
12. Kostoulas P, Nielsen SS, Branscum AJ, Johnson WO, Dendukuri N, Dhand NK, et al. STARD-BLCM: standards for the reporting of diagnostic accuracy studies that use Bayesian latent class models. Prev Vet Med. 2017;138:37–47.
13. Camirand Lemyre F, Honfo SH, Caya C, Cheng MP, Colwill K, Corsini R, et al. Two-phase Bayesian latent class analysis to assess diagnostic test performance in the absence of a gold standard: COVID-19 serological assays as a proof of concept. Vox Sang. 2023;118:1069–77.
14. Cheung A, Dufour S, Jones G, Kostoulas P, Stevenson MA, Singanallur NB, et al. Bayesian latent class analysis when the reference test is imperfect. Rev Sci Tech Int off Epizoot. 2021;40:271–86.
15. Sulaeman H, Grebe E, Dave H, McCann L, di Germanio C, Sanghavi A, et al. Evaluation of ortho VITROS and Roche Elecsys S and NC immunoassays for SARS-CoV-2 serosurveillance applications. Microbiol Spectr. 2023;11:e0323422.
16. Stone M, Grebe E, Sulaeman H, di Germanio C, Dave H, Kelly K, et al. Evaluation of commercially available high-throughput SARS-CoV-2 serologic assays for serosurveillance and related applications. Emerg Infect Dis. 2022;28:672–83.
17. Muecksch F, Wise H, Batchelor B, Squires M, Semple E, Richardson C, et al. Longitudinal serological analysis and neutralizing antibody levels in coronavirus disease 2019 convalescent patients. J Infect Dis. 2021;223:389–98.
18. Whitaker HJ, Gower C, Otter AD, Simmons R, Kirsebom F, Letley L, et al. Nucleocapsid antibody positivity as a marker of past SARS-CoV-2 infection in population serosurveillance studies: impact of variant, vaccination, and choice of assay cut-off. medRxiv. 2021. https://doi.org/10.1101/2021.10.25.21264964

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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Routine results of an algorithm for managing the production of blood components

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Abstract

Background and Objectives: The variability in the number of donations together with a growing demand for platelet concentrates and plasma-derived medicines make us seek solutions aimed at optimizing the processing of blood. Some mathematical models to improve efficiencies in blood banking have been published. The goal of this work is to validate and evaluate an algorithm's impact in the production of blood components in the Blood and Tissues Bank of Aragon (BTBA).

Materials and Methods: A mathematical algorithm was designed, implemented and validated through simulations with real data. It was incorporated into the fractionation area, which uses the Reveos® fractionation system (Terumo BCT) to split blood into its components. After 9 months of daily routine validation, retrospective activity data from the Blood Bank and Transfusion Services before and during the use of the algorithm were compared.

Results: Using the algorithm, the outdating rate of platelet concentrates (PC) decreased by 87.8% in the blood bank. The average shelf life remaining of PC supplied to Transfusion Services increased by almost 1 day. As a consequence, the outdating rate in the Aragon Transfusion Network decreased by 33%. In addition, extra 100 litres of plasma were obtained in 9 months.

Conclusions: The algorithm improves the blood establishment's workflow and facilitates the decision-making process in whole blood processing. It resulted in a decrease in PC outdating rate, increase in PC shelf life and finally an increase in the volume of recovered plasma, leading to significant cost savings.

Keywords

algorithm, optimization, platelet concentrate stocks, whole blood management

Highlights

- In recent years, there has been an increasing demand for platelet concentrates and a shortage of plasma for the production of plasma-derived medicines in some countries.
- Automation contributes to improving the productivity, efficiency and quality of blood components.
- The incorporation of mathematical algorithms can help address the challenging task of managing blood components production.

INTRODUCTION

The mission of blood establishments is to provide timely supply of the products required by Transfusion Services. Moreover, whole blood (WB) fractionation must be optimized to minimize waste and obtain blood components with the highest quality and safety possible. To achieve this goal, blood establishments face two problems: the rising demand for platelet concentrates (PC) [1, 2] and the insufficient collected volume of plasma to satisfy the current need for plasma-derived medicines [3].

PC is a blood component with short shelf life (a maximum of 5 days, extendable to 7 if a bacterial detection method is used or it is treated with a pathogen reduction technology [PRT] [4–6]). This, coupled with a growing and variable demand [7], makes it difficult to achieve a balance between sufficient supply and minimum outdating.

Many methodologies have been reviewed [8] and can be used by PC inventory managers to reduce the level of outdating. Some publications discussed the use of mathematical models to reduce the expiry of PC [9–12], showing that if the PC are subjected to PRT, its shelf life can be extended by 2 days and almost entirely eliminate the outdating rate [13].

Furthermore, increasing the total volume of plasma recovered from WB fractionation may be an important step in helping countries achieve self-sufficiency in the production of plasma-derived medicinal products [14]. Spain has a low level of self-sufficiency in plasma for haemoderivatives, leading to a continuous increase of the import of these medicines. In recent years, self-sufficiency has dropped, due to a fall in WB donations arising from the decrease in use of red blood cell concentrates (RBC) in transfusion and the increased use of haemoderivatives (IVIG and albumin) [15].

Reveos® Automated Blood Processing System (Terumo BCT) can process up to four units of WB within 20 min, into two (plasma and RBC; 2C-protocol) or three components (plasma, RBC and interim platelet unit; 3C-protocol) [16, 17]. This decision may be adopted at the time of processing the unit, as the set of bags is the same for all protocols. Both protocols allow for the WB unit to be separated within 2 and 12 h of collection, so called fresh blood, or after overnight hold, for the units collected between 12 and 24 h before the processing [17]. In this way, it is possible to obtain the platelet units needed with the 3C-protocol maximizing the plasma collection with the 2C-protocol [18]. Moreover, this system provides a platelet yield index (PYI) which estimates the platelet count in the interim platelet unit (IPU) containing approximately 30 mL of plasma. In order to make the final PC it is necessary to add additive solution or more plasma [19].

A software called T-Pool IPU select tool selects the units according to ABO/Rhesus blood groups and PYI. It produces PC with a minimum yield [20] in accordance with internal procedures (3.0×10^{11} platelets per unit), national procedures (Committee of Transfusion Accreditation—more than 2.4×10^{11} platelets per unit) [21] and international procedures (European Directorate for the Quality of Medicines & HealthCare—more than 2.0×10^{11} platelets per unit) specifications [22]. Besides, it proposes the number of units (four or five depending on IPUs availability) in each pool.

The Blood and Tissues Bank of Aragon (BTBA) is a public service that collected and processed 41,183 WB units and 2349 apheresis donations (plasmapheresis and plateletpheresis), suppling 6255 PC, 38,515 RBCs and 2083 plasma units in 2023 to 19 Transfusion Services (public and private hospitals) in the region of Aragon (Spain), with more than 1.3 million of inhabitants [23]. The Transfusion Services together with the BTBA compose the Transfusion Network of Aragon.

The BTBA implemented the Reveos system in 2013 and started using the Mirasol PRT (Terumo BCT) [5] in 2015 for improving the safety of platelet transfusions, and since March 2020, all produced PC are treated with PRT, extending their shelf life from 5 to 7 days.

In this scenario, this study presents the results of applying a mathematical algorithm in a blood establishment using Reveos and treating all PC with PRT.

Our objective is to show that a mathematical algorithm for blood processing:

1. Achieves minimum outdating rates of PC while avoiding stock-outs.
2. Gets the highest plasma recovery.
3. Reduces wastage during the process of WB fractionation.
4. Improves the planning of WB collections to avoid blood shortages for PC.
5. Facilitates the delivery of fresher PC to Transfusion Services.

MATERIALS AND METHODS

Mathematical algorithm and data used

To design the mathematical algorithm, the BTBA provided historical data on estimated donations and number of units collected,

production of PC, supply of PC to Transfusion Services per day and per hour and PC units transfused and discarded from 1 January 2018 to 30 November 2020. The data were divided into two sets. All data from 2018 were used to design and develop the algorithm, whereas data from 1 January 2019 to 30 November 2020 were used to validate the algorithm through mathematical simulations.

Statistical analyses were carried out on the first dataset to calculate key parameters and identify trends in the data. Then a mathematical algorithm was proposed. The algorithm uses a mathematical model with nonstationary stochastic demand. To design it, the characteristics of the Reveos automatic fractioning system, the collection days, the production days and the expired PC discarded at the end of the day were taken into account. In addition, extraordinary holidays were also considered to avoid the problem of overproduction or insufficient stocks. It automatically adapts to periods of uneven demand and production such as holidays by changing the input parameters. The algorithm was implemented in the programming language Python.

To facilitate its routine use, the algorithm has been integrated into a user-friendly software application. The software consists of an Excel sheet (Figure 1), linked to a Python script. In order to prevent unintended changes, the Excel sheet has protected cells allowing users to modify only those designated for input data. Except for the data to be entered manually, the software code is compiled into an executable so that users cannot access or modify its contents.

To use the software, eight data fields must be completed daily. Once all the WB donations for a given day have been collected by the blood establishment, the staff introduces into the software: the total number of WB donations received (column 3 [REAL] in Figure 1), the number of platelet apheresis received and pending analysis (column 4 [APHERESIS]) and the stock of PC categorized by their expiry at the time of decision-making (column 5 [S1] to column 10 [S6], from the number of PC expiring tomorrow to the number of PC expiring in 6 days). In addition, the estimated number of donations for each day must be completed at the beginning of each month (column 2 [ESTIMATED]) at least 1 week before using the software. Finally, by clicking on the executable, the software generates the production recommendations based on the algorithm's calculations. More specifically, the software indicates the number of PC to be produced (column 12 [PC]) and the number of WB units to be fractionated with the 3C-protocol (column 11 [WB INTO 3C PROTOCOL]). The remaining WB units are fractioned with the 2C-protocol. If the software detects a potential blood shortage to meet the demand for PC, it generates a warning message 1 week in advance (column 13 [WARNING]), indicating the exact number of additional WB donations needed to prevent such a shortage.

Mathematical simulations performed

Due to the importance and potential impact of the software output, we decided to perform an initial validation of the algorithm using mathematical simulations with real data, prior to its incorporation into the BTBA. The dataset from 1 January 2019 to 30 November 2020 was split into two subsets. The subset of data from 1 January 2019 to 30 June 2019 was used to validate the algorithm against the usual BTBA-specific trends prior to the onset of the coronavirus disease 2019 (COVID-19) pandemic (first simulation). The subset of data from 1 January 2020 to 30 November 2020 was used to validate the algorithm against abrupt changes in trends, both in donations and demand of PC, such as those present during the COVID-19 pandemic (second simulation).

In both simulations, the activity flow of the BTBA was modelled to determine what results would have been obtained if the recommendations of the software had been followed. Then, those results were compared with the real data of the BTBA without using the software. The parameters studied to assess the performance of the algorithm were the number and percentage of produced and expired PC in the BTBA, the number of PC stock-outs, the number of WB units fractionated with the 3C-protocol and those with the 2C-protocol.

Validation in a real environment

In May 2021, following the good results obtained in the simulations, the software was incorporated into the fractionation and distribution area of the BTBA, independently of any software or medical device present there.

DATE	DONATIONS			STOCK						PC		WARNNG
	ESTIMATED	REAL	APHERESIS	S1	S2	S3	S4	S5	S6	WB units into 3C-protocol	PC	
11 May 2022	123	156	3	0	2	10	7	10	9	95	19	NO WARNING
12 May 2022	155											
13 May 2022	143											
14 May 2022	79											
15 May 2022	98											
16 May 2022	0	0	0	*	*	*	*	*	*	0	0	Holiday
17 May 2022	78											

FIGURE 1 Excel sheet illustrating the software installed in Blood and Tissues Bank of Aragon (BTBA). PC, platelet concentrates; WB, whole blood.

TABLE 1 Data used in the development and validation of the algorithm.

	Start date	End date
Design and development of the algorithm		
	1 January 2018	31 December 2018
Validation of the algorithm via simulation		
Normal situation	1 January 2019	30 June 2019
Extraordinary situation (COVID-19)	1 January 2020	30 November 2020
Evaluation of the algorithm BTBA		
Period 1 (without software)	1 May 2020	31 January 2021
Period 2 (with software)	1 May 2021	31 January 2022

Abbreviations: BTBA, Blood and Tissues Bank of Aragon; COVID-19, coronavirus disease 2019.

To ensure proper use, BTBA professionals received 1 h of training, and a 15-day time limit was set to become proficient with the software. At the end of the trial period, the staff started using the tool on a regular basis.

After 9 months of daily routine use, retrospective activity data from the BTBA and Transfusion Services was collected. On the one hand, the BTBA provided data from 1 May 2020 to 31 January 2021 on processed PC (pools and apheresis), supplied and expired PC in the BTBA and the Transfusion Services, the number of PC stock-outs, the outdating rate of PC in the BTBA, the average shelf life of PC sent to Transfusion Services, the number of WB units processed, the percentage of these units fractionated with each of the protocols, the number of IPUs discarded and the volume of plasma obtained from WB units. During this period, no tool was used to support production decision-making. We call this period 'Period 1' (no software). On the other hand, the BTBA provided the same data from 1 May 2021 to 31 January 2022, when the software was being used. This period is referred to as "Period 2" (with software). The phases of the algorithm development and evaluation are shown in Table 1.

RESULTS

This section shows the results of the mathematical simulations (Tables 2 and 3) and the results of the algorithm validation (Table 4).

Table 2 compares the results of the mathematical simulations with real data from 1 January to 30 June 2019. Table 3 compares the results of the mathematical simulations with real data from 1 January to 30 November 2020 (in the midst of the COVID-19 pandemic). In both simulations, the mathematical algorithm improves the real data of the BTBA, reducing the outdating rate of PC, avoiding stock-outs and increasing the percentage of WB units fractionated with the 2C-protocol. The results in Table 3 show that the mathematical algorithm performs well in the presence of large trend changes such as those faced during the COVID-19 pandemic.

TABLE 2 Results of the simulation and real data from 1 January to 30 June 2019.

	BTBA (real data)	Simulation (estimate data)
PC expired	75	7
PC stock-outs	1	0
3C-protocol fractionated WB	19,350	12,400
2C-protocol fractionated WB	1207	8157

Abbreviations: BTBA, Blood and Tissues Bank of Aragon; PC, platelet concentrates; WB, whole blood.

TABLE 3 Results of the simulation and real data from 1 January to 30 November 2020.

	BTBA (real data)	Simulation (estimate data)
PC expired in BTBA	70	58
PC stock-outs	0	0
3C-protocol fractionated WB	31,527	23,670
2C-protocol fractionated WB	3124	10,981

Abbreviations: BTBA, Blood and Tissues Bank of Aragon; PC, platelet concentrates; WB, whole blood.

Table 4 shows the results of the algorithm validation in the BTBA from May 2021 to January 2022 compared with the same period a year before. The demand for PC from the Transfusion Services increased from May 2021 to January 2022, compared with the same period 1 year prior (May 2020 to January 2021), so the number of PC produced in the BTBA was 12.9% higher in Period 2. Using the algorithm, the PC outdating rate decreased significantly (87.8%) and 65 PC less were discarded due to outdating in 9 months, only 0.18% of those produced because the PC production better adjusted to demand. The outdating rate decreased not only in the BTBA but also in the Transfusion Services from 4.35% to 3.80%. The algorithm also helped to increase the remaining PC shelf life supplied by nearly 1 day. Using the algorithm, the units processed with the 2C-protocol (higher volume plasma and RBC) increased to 41.6% (1313 more units in 9 months). As a result, the algorithm led to a reduction of the percentage of discarded IPUs from 25.8% in Period 1 to 11.0% in Period 2. Furthermore, 100 L of extra plasma were recovered from WB donations. No stock-outs occurred in both periods. However, the software warned of a possible shortage of 85 donations for the supply of PC and a stock-out was avoided by improving collection planning in time.

DISCUSSION

Good Manufacturing Practices [24] requires the optimization of the processing of WB units. This requirement is in line with the resolution of two of the problems faced by the blood establishments: a balanced production of PC and the maximum collection of plasma.

TABLE 4 Results of the validation in the BTBA.

	Period 1	Period 2
	May 2020–January 2021 (without software)	May 2021–January 2022 (with software)
PC produced (pools + apheresis)	4083 + 365 = 4448	4604 + 420 = 5024
Expired PC at BTBA	74	9
PC sent to Transfusion Services	4374	5015
PC stock-outs	0	0
PC outdating rates (%)		
BTBA	1.66	0.18
Transfusion Services	4.35	3.80
Aragon Transfusion Network	5.94	3.98
Average remaining shelf-life of PC sent to Transfusion Services (days)	3.02	3.73
WB units	28,941	29,315
WB units fractionated with 2C-protocol (%)	10.90	15.24
WB units fractionated with 3C-protocol (%)	89.10	84.76
Discarded IPUs	7458	3224
Volume of plasma recovered (L)	7541	7641

Abbreviations: BTBA, Blood and Tissues Bank of Aragon; IPU, interim platelet unit; PC, platelet concentrates; WB, whole blood.

The gradual increase in automation of WB processing over the last four decades has resulted in continued improvements in product quality, productivity, yield, processing time and product safety, as well as reducing costs through reduced wastage and resources [25].

Reveos System (Terumo BCT) avoids many manual steps. The possibility of several working protocols together with the PYI and the T-pool Select software for selecting and combining the IPUs in the production of PC helps to optimize the production of PC [18]. In addition, it was found that treating PC with PRT, which allows for a two-day extension in shelf life, resulted in a decreased rate of PC expiration [13]. However, the number of PC that have to be produced each day, as well as the number of units that can be processed with the 2C-protocols to obtain more plasma are ultimately determined by production managers based on demand trends, their experience and quality indicators. This is where automated decision-making tools can be useful in streamlining the process.

In this article, we present a software tool based on a mathematical algorithm that has proven to be effective for this purpose. The algorithm considers the stochastic nature of demand, as production orders for a day are established before knowing the PC demand. Moreover, it is well suited to handle periods of uneven demand and production. For instance, Christmas holidays fell within Period 2 of validation, and the algorithm performed exceptionally well throughout the period. It showed that it is possible to optimize the production of blood components, getting the highest possible yield from each WB donation and improving the production of PC, while maintaining an adequate stock without expiry or stock-outs. Thus, this software is another step in the automation of blood processing.

There have been numerous studies attempting to develop models for managing PC inventory [26]. This algorithm has been able to reduce the percentage of PC discarded due to expiration, without any stock-out (see Table 4). Although the outdating rate of PC without using the algorithm was 5.94%, which is less than the national average (10% in Spanish Transfusion Network [27]), the use of the algorithm further reduced the percentage of PC that expired. Using this tool, the outdating rate of PC in the Transfusion Network of Aragón decreased to 3.98%. Note that the increase in demand in the second period naturally led to a higher production, so the outdating rate should not have seen a significant reduction if the system operated similarly in both periods. Thus, the substantial decrease in outdates in the second period can be attributed, at least in large part, to the implement of our algorithm. This conclusion is reinforced by the findings in Tables 2 and 3, where the algorithm's impact on outdating was evaluated under identical daily demands.

The fact that during the period of use of the software PC had been supplied to Transfusion Services with longer remaining shelf-life (from 3.02 to 3.73 days) contributed to a reduction in the outdating rate of the Transfusion Services and, consequently, in the Transfusion Network of Aragon. This aspect is also important because the patient receives a fresher and therefore more effective PC [28]. Also, from an economic point of view, the cost of manufacturing an inactivated platelet pool in the BTBA was 447.58 euros [29]. Therefore, the reduction in outdated PC units resulted in significant savings. Additionally, each PC contains approximately 150 mL of plasma, contributing further to the overall savings.

With this software, it was possible to optimize the WB fractionation process. During the period of use of the software, the number of discarded IPUs decreased by 56.7%, and the software proposed to fractionate more units with the 2C-protocol (not to elaborate IPUs). The ratio 3C/2C decreased from 8.17 in 2020 (without software) to

5.56 in 2021 with the software. Hence, there was an increase of 100 L in plasma recovery in Period 2 compared with Period 1, as one IPU contains approximately 30 mL of plasma. Although this increase might appear modest, it is important to consider that it is derived from a comparable number of WB units and under a significantly higher demand for PC (14.6%). This indicates that the algorithm has successfully enhanced the efficiency of plasma extraction from each WB donation. Plasma is considered a strategic resource [30]. However, in 2017, the level of self-sufficiency for albumin and immunoglobulins was 62.4% and 42.9%, respectively [15]. From a logistical, economic and ethical point of view, it is mandatory to obtain as much plasma as possible from WB donations. In 2021, in Spain, 1,622,610 WB were collected, producing 235,677 PC from WB donations. If each PC was derived from four to five donations, approximately 1,000,000 WB units were used for their production. This means that around 600,000 platelet units (buffy coat and IPU) were discarded for several causes, mainly because of outdating (e.g. 87% in the BTBA). Assuming this percentage and since each platelet unit contains 30 mL of plasma, this means that more than 16,000 L of plasma have been lost in buffy coat and IPU—otherwise valid—that were not used in the production of PC. Furthermore, during 2021 in Spain, 26,925 PC expired [27]. Assuming an approximate volume of 150 mL of plasma per PC, this would amount to over 4000 L of plasma. In summary, we estimate that the annual plasma volume discarded due to the expiration of buffy coats, IPU and PC was around 20,000 L. Given that one apheresis donation contains approximately 600 mL of plasma, this is equivalent to more than 33,000 plasmapheresis donations. Therefore, the usefulness of an algorithm to recover part of this plasma is evident.

In conclusion, the successful results of the BTBA validation demonstrate that, despite having approximately the same number of WB units but facing a 14.6% increase in demand for PC in Period 2, the algorithm not only met the demand without experiencing any stock-outs of PC but also provided PC of higher quality. Additionally, it successfully recovered more plasma.

Aside from the obvious economic savings resulting from the reduction in the outdating rates of PC (the unitary cost of manufacture is around 450 euros [29]) and the increase in the volume of recovered plasma, our algorithm is beneficial from an ethical standpoint. By minimizing waste, we ensure effective utilization of WB donations, given freely by donors, contributing positively to ethical considerations.

The software has the capability to forecast, with a one-week lead time, situations where there may not be sufficient WB donations to produce the required number of PC. For this forecasting, it is necessary to include WB collection forecasts for at least the following week. This early warning allows the blood establishment to increase the number of planned WB collections.

The tool is very intuitive and easy to use as shown by the fact that 1 hour of training is enough to learn how to use it. This, coupled with the speed of data calculation, makes its routine application very straightforward.

The tool facilitates and guides production decisions regardless of the experience of the blood establishment staff. It facilitates the incorporation of new professionals and decision-making in the absence of the head of the area in the blood establishment. If the staff are very experienced, they can still use their judgement but with the help of software, which would give even better results.

The software has been applied in a blood establishment with an automated fractionation system, but it could be implemented in blood establishments that use the buffy coat method for WB fractionation, in which case the 2C protocol would be equivalent to dry-buffy coat. It can also be applied in those centres that do not treat PC with PRT or treat only a part of them. Other particular conditions of the blood establishment may require minor modifications to the algorithm, but those such as the size of the blood establishment or the distance to hospitals are not relevant in this case. Although the current algorithm does not explicitly account for blood groups, it can be adapted for individual blood groups. Some modifications would be needed in the algorithm to take into account the groups and their compatibilities in a unified way. In this situation, it would be necessary to re-validate the new version of the algorithm in one blood establishment to ensure an optimal result.

In summary, the software meets the objectives by improving PC inventory management, optimizing WB fractionation, recovering more volume of plasma as well as providing guidance on collection planning.

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A.P. and I.A. conceived and designed the study; A.P. executed data collection; I.A. and F.L. performed the statistical analysis; I.A. and F.L. developed the mathematical algorithm; I.A. implemented the algorithm; I.A. and J.S. installed the algorithm in the BTBA; A.P. and I.A. took the lead in writing the manuscript. All authors provided feedback and helped shape the research and analysis, critically reviewed and contributed to the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

Ana Isabel Pérez-Aliaga has collaborated as guest speaker in Terumo BCT. Irene Ayerra founded, holds shares in and works for Hemotic.

DATA AVAILABILITY STATEMENT

Raw data that support the findings of this study are available from the corresponding author, upon reasonable request.

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REFERENCES

1. Stubbs JR, Homer MJ, Silverman T, Cap AP. The current state of the platelet supply in the US and proposed options to decrease the risk of critical shortages. Transfusion. 2021;61:303–12.

2. Smethurst P, Cardigan R. Current challenges in platelet transfusion. Platelets. 2022;33:5–13.
3. Kluszczynski T, Rohr S, Ernst R. White paper key economic and value considerations for plasma-derived medicinal products (PDMPs) in Europe. 2020. Available from: https://www.vintura.com/wp-content/uploads/2020/03/White-paper-key-economic-and-value-considerations-for-plasma-derived-medicinal-products-PDMPs-in-Europe_Vintura-and-PPTA.pdf. Last accessed 25 May 2023.
4. Alter HJ. Pathogen reduction: a precautionary principle paradigm. Transfus Med Rev. 2008;22:97–102.
5. Terumo BCT. Mirasol. 2023. Available from: https://www.terumobct.com/mirasol. Last accessed 25 May 2023.
6. van der Meer PF, Bontekoe IJ, Daal BB, de Korte D. Riboflavin and UV light treatment of platelets: a protective effect of platelet additive solution? Transfusion. 2015;55:1900–8.
7. Fedele PL, Polizzotto MN, Grigoriadis G, Waters N, Comande M, Borosak M, et al. Profiling clinical platelet and plasma use to inform blood supply and contingency planning: PUPPY, the prospective utilization of platelets and plasma study. Transfusion. 2016;56:2455–65.
8. Flint AWJ, McQuilten ZK, Irwin G, Rushford K, Haysom HE, Wood EM. Is platelet expiring out of date? A systematic review. Transfus Med Rev. 2020;34:42–50.
9. Blake JT. Determining the inventory impact of extended-shelf life platelets with a network simulation model. Transfusion. 2017;57:3001–8.
10. Pérez Vaquero MA, Gorria C, Lezaun M, López FJ, Monge J, Eguizabal C, et al. Optimization of the management of platelet concentrate stocks in the Basque Country using mathematical simulation. Vox Sang. 2016;110:369–75.
11. Gomez AT, Quinn JG, Doiron DJ, Watson S, Crocker BD, Cheng CK-W. Implementation of a novel real-time platelet inventory management system at a multi-site transfusion service. Transfusion. 2015;55:2070–5.
12. Schilling M, Rickmann L, Hutschenreuter G, Spreckelsen C. Reduction of platelet outdating and shortage by forecasting demand with statistical learning and deep neural networks: modeling study. JMIR Med Inform. 2022;10:e29978.
13. Gorria C, Labata G, Lezaun M, López FJ, Pérez Aliaga AI, Pérez VM. Impact of implementing pathogen reduction technologies for platelets on reducing outdates. Vox Sang. 2020;115:167–73.
14. Council of Europe, Committee of Ministers. Recommendation No. R (90) 9 of the Committee of Ministers to Member States on plasma products and European self-sufficiency. 1990. Available from: https://www.sanidad.gob.es/profesionales/saludPublica/medicinaTransfusional/recomendaciones/docs/Rec90_9.pdf. Last accessed 25 May 2023.
15. Puig RL. Plasma self-sufficiency in Spain. Transfus Apher Sci. 2020;59:102700.
16. Terumo BCT. Reveos®: Automated blood processing system. 2023. Available from: https://www.terumobct.com/reveos. Last accessed 25 May 2023.
17. Johnson L, Winter KM, Kwok M, Reid S, Marks DC. Evaluation of the quality of blood components prepared using the Reveos automated blood processing system. Vox Sang. 2013;105:225–35.
18. Pérez Aliaga AI, Labata G, Aranda A, Cardoso M, Puente F, Domingo JM, et al. Improvement of blood processing and safety by automation and pathogen reduction technology. Transfus Med Hemother. 2021;48:290–7.
19. Cid J, Magnano L, Molina P, Diaz-Ricart M, Martínez N, Maymó RM, et al. Automated preparation of WB-derived platelets suspended in two different platelet additive solutions and stored for 7 days. Transfusion. 2014;54:426–33.
20. Malvaux N, Schuhmacher A, Defraigne F, Jacob R, Bah A, Cardoso M. Remodelling WB processing through automation and pathogen reduction technology at the Luxembourg red cross. Tranfus Apher Sci. 2021;60:103195.
21. CAT Foundation. Quality certification body in transfusion, cell and tissue therapy. Standards in Hemotherapy 5th ed. 2019 Review in 2022. 2022. Available from: http://www.catransfusion.es/media/upload/arxius/documentos/estandares/estandars/EST%C3%81NDARES_CAT_2022%20(ALTA).pdf. Last accessed 25 May 2023.
22. European Directorate for the Quality of medicines & HealthCare of the Council of Europe (EDQM). Guide to the preparation, use and quality assurance of blood components. 20th ed. Strasbourg: Council of Europe; 2023.
23. Government of Aragón. Official population figures. 2023. Available from: https://www.aragon.es/-/cifras-oficiales-de-poblacion. Last accessed 25 May 2023.
24. EudraLex. The rules governing medicinal products in the European Union. Volume 4 EU Guidelines for good manufacturing practice for medicinal products for human and veterinary use. Annex 14 manufacture of medicinal products derived from human blood or plasma. 2014. Available from: https://health.ec.europa.eu/medicinal-products/eudralex/eudralex-volume-4_en. Last accessed 25 May 2023.
25. Cid J, Comasòlivas N, Pérez-Aliaga A, Illingworth N, Cardoso M. Comparison of automated versus semi-automated whole blood processing systems: a systematic review. Vox Sang. 2023;118:263–71.
26. Gorria C, Lezaun M, López FJ. Performance measures of nonstationary inventory models for perishable products under the EWA policy. Eur J Oper Res. 2022;303:1137–50.
27. Ministry of health. Activity report. 2021. Available from: https://www.sanidad.gob.es/profesionales/saludPublica/medicinaTransfusional/publicaciones/docs/informeActividad2021.pdf. Last accessed 25 May 2023.
28. Kreuger AL, Caram-Deelder C, Jacobse J, Kerkhoffs JL, van der Bom JG, Middelburg RA. Effect of storage time of platelet products on clinical outcomes after transfusion: a systematic review and meta-analyses. Vox Sang. 2017;112:291–300.
29. Official journal of Aragón. ORDER/928/2019, of 12 July, determining public prices by the provision of services or activities of the BTBA. 2023. Available from: https://www.boa.aragon.es/cgi-bin/EBOA/BRSCGI?CMD=VEROBJ&MLKOB=1083816463939. Last accessed 25 May 2023.
30. Strengers P. Plasma is a strategic resource. Transfusion. 2016;56:3133–7.

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ORIGINAL ARTICLE

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Feasibility evaluation of a blood rotation system for efficient blood product utilization in remote island settings

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Abstract

Background and Objectives: Geographical limitations in remote island medical facilities result in excessive wastage of blood products. To address this, we explored the feasibility of a novel blood rotation system, which enables the return and redelivery of blood products to/from the blood bank while ensuring the management of product quality, including temperature control. This study aimed to enhance the supply of blood products to these facilities.

Materials and Methods: The Japan Red Cross Nagasaki Blood Center, Nagasaki Goto Chuoh Hospital (NGCH) and Nagasaki University Hospital collaborated to coordinate the transport and supply of red blood cell (RBC) products. Type O, RhD-positive, irradiated RBC products were stored at a precise 4.0 ± 2.0°C in an active transport refrigerator (ATR). After transport from the Japan Red Cross Nagasaki Blood Center to NGCH, RBC products were held for 1 week in the ATR, and unused products were returned. Eligible returned products were reissued to the Nagasaki University Hospital.

Results: All the returned RBC products met the redelivery criteria. Among the 103 redelivered RBC preparations, 101 bags (98.1%) were successfully used. NGCH utilized 597 RBC products and discarded 80 samples. The ATR supplied 107 type O RBC bags without any wastage. The overall wastage rate was 10.2% during the study period compared with 24.2% in the same period in the previous year.

Conclusion: This innovative supply and operation system ensures a consistent and secure RBC product supply to remote islands while maximizing blood product use.

Keywords

blood rotation, quality management, red blood cell product, remote island

Highlights

- The use of an active transport refrigerator to transport and store red blood cell products ensured appropriate temperature maintenance.
- Reshipped red blood cell products can be utilized effectively after being returned from a hospital on a remote island, where strict temperature control is ensured.
- This innovative blood rotation system ensures a consistent and secure supply of red blood cell products to remote islands while maximizing their use.

INTRODUCTION

In medical institutions located in remote island areas, the management of transfusion medicine presents unique challenges owing to the time-consuming transportation and delivery of blood products [1, 2]. In Japan, the Japan Red Cross Nagasaki Blood Center (JRCNBC) serves as the sole source of blood products for transfusions, and a network of medical institutions with blood bank capabilities is absent in daily practice. Additionally, blood products are supplied to remote islands through regular transport services conducted directly via sea or air routes from the blood centre (BC) in the mainland to various medical facilities. However, emergency transport via sea or air routes is carried out in cases of urgent demand that cannot be met by an on-site inventory. Factors such as transportation delays and interruptions caused by the time required for shipment or adverse weather conditions affecting maritime transport result in excessive stockpiling of blood products, leading to higher wastage rates than in mainland facilities. The 2020 National Survey investigated the status of blood transfusions in remote islands in Japan. Among the surveyed hospitals, 78% indicated that they would transport patients requiring massive emergency transfusions to core regional hospitals. However, only 17% of these institutions were regularly prepared. Furthermore, the time required for product delivery, including routine and emergency deliveries, from the BC exceeded 4 h in 68.6% of hospitals [3].

This study focused on the remote islands of Nagasaki Prefecture, situated in the westernmost part of Japan, with the Goto Islands approximately 102 km from the mainland (Figure 1). The transportation of blood products to Nagasaki Goto Chuoh Hospital (NGCH) in Goto City typically takes approximately 4 h via the sea route. Consequently, the prolonged transit time from the blood bank in Nagasaki City to the NGCH, which takes approximately 16 h, contributes to the annual wastage rates of unutilised blood products, ranging from 10% to 20% in these hospitals. In contrast, the wastage rate of red blood cell (RBC) bags in Japan stands at 1.76%, as per the nationwide survey results [4]. Therefore, improving the supply system of blood products to medical institutions in regions with limited geographical supply constraints is imperative to optimize the efficient use of these valuable resources [5, 6]. However, it is worth noting that research on this issue is limited, with only a few reports available [7].

To address these challenges, we investigated the feasibility of implementing a novel operational framework, the blood rotation (BR) system, which employs the active transport refrigerator (ATR) 705 developed by Toho Pharmaceutical Co., Ltd., Tokyo [8, 9]. This system is designed to enable the efficient return and redelivery of

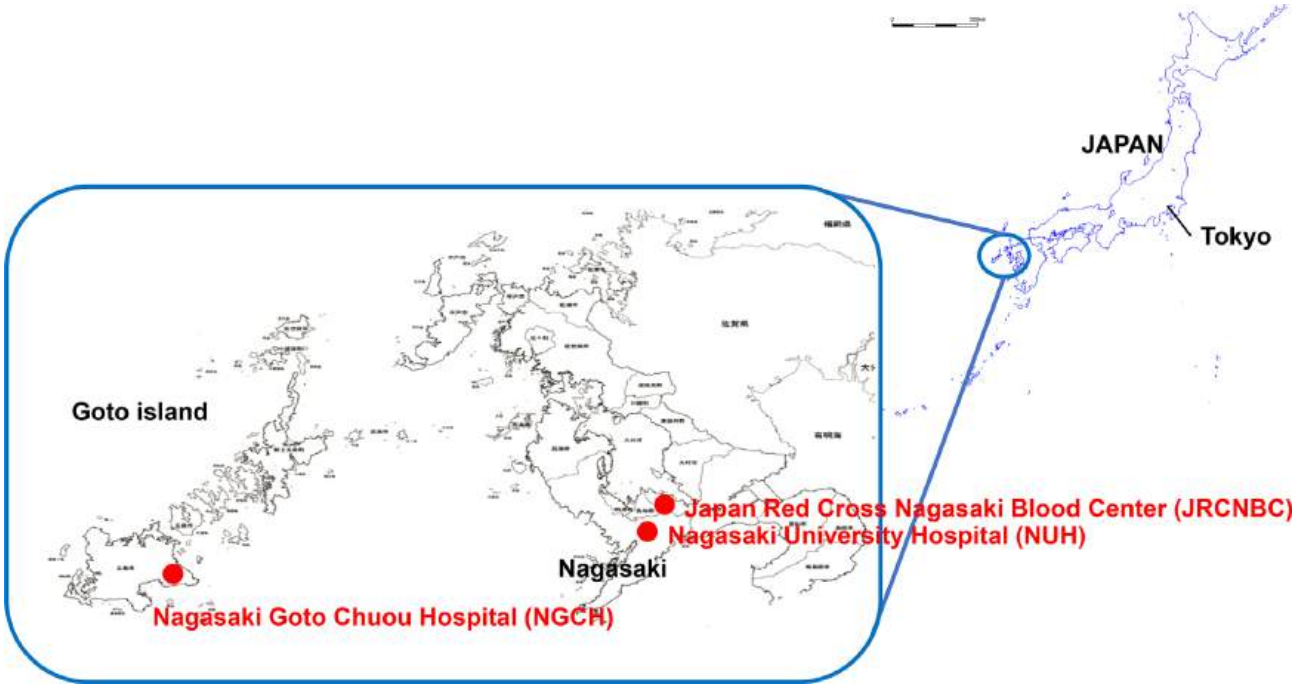

FIGURE 1 Geographical relationships of the relevant institutions in this study. Nagasaki Prefecture, in the westernmost part of Japan, has many remote islands. The Goto Islands in Nagasaki Prefecture are situated approximately 102 km from the mainland, and usually, it takes approximately 4 h via sea to and from Goto City, where Nagasaki Goto Chuoh Hospital (NGCH) is located. NGCH is a core general hospital in the secondary healthcare region of the Nagasaki Prefecture. It has 304 inpatient beds and is a hub hospital for secondary emergency medical care, healthcare in remote areas and disaster medicine. The annual usage of red blood cell products is approximately 850–900 bags, with a 10%–20% wastage rate. Nagasaki University Hospital (NUH) is the only university hospital in the Nagasaki Prefecture responsible for advanced medical care and tertiary emergency medical services. The annual usage of red blood cell products exceeds 2000 units, with a wastage rate of approximately 1%. The Japan Red Cross Nagasaki Blood Center (JRCNBC) serves as a supply base for blood products used for transfusion and blood donation services in the Nagasaki Prefecture.

blood products while maintaining optimal temperature control, aiming to streamline the supply chain (SC) for medical institutions in remote islands. Specifically, in this study, we evaluated whether the BR system could establish an effective SC that maximizes the utilization of red blood cell RBC products, ensuring their proper use in these challenging settings.

MATERIALS AND METHODS

Blood products

The Japanese Red Cross Society produces RBC products in Japan. One unit of the RBC product is derived from 200 mL of whole blood collected from a single donor. At the time of the study, the expiration period of the RBC products was 21 days after donation. Type O RhD-positive RBC products are universally used in emergencies, such as for patients with massive bleeding before blood typing is confirmed.

Temperature-controlled transportation by ATR705-RC05

ATR705-RC05 (Toho Pharmaceutical, Ltd., Tokyo, Japan) is an ATR capable of maintaining RBC products at a precise temperature of 4.0 ± 2.0°C, regardless of the ambient temperature [8, 9]. This device, powered by an internal lithium-ion battery, enables the transport of RBC products for >7 h, even without routine power. The internal temperature and events can be recorded continuously for an extended period, and an alarm is issued if the internal temperature exceeds the specified limits (Figure 2).

BR system

The NGCH in Goto City, Nagasaki Prefecture, is a flagship hospital with 304 beds and is responsible for emergency medical care in the Goto Islands. Typically, blood products, including RBCs, are transported daily via the sea route from the JRCNBC to NGCH. Additional shipping via the sea or aeroplanes is conducted in response to temporary requests. In clinical practice, blood products are used based on proper judgement following the Guideline for The Use of Blood Products released by the Ministry of Health, Labor and Welfare's Pharmaceutical and Living Hygiene Bureau, Japan [10].

In this study, RBC products were specially coordinated among the JRCNBC, NGCH, and Nagasaki University Hospital (NUH), in addition to the regular supply system described above. An overview of the BR system is shown in Figure 3. Type O, RhD-positive, irradiated RBC products were stored in an ATR and transported from the JRCNBC to NGCH via a scheduled ship (blue arrow). RBC products in the ATR were stored for 1 week and used in emergencies or shortages of other blood type products in the NGCH. After 1 week of storage, the unused RBC products were returned to the JRCNBC in the ATR

ATR705-RC05 (Toho Pharmaceutical Co., Ltd.)

FIGURE 2 Active transport refrigerator, the ATR705-RC05 (Toho Pharmaceutical, Ltd., Tokyo, Japan). ATR705-RC05 is a mobile refrigerator designed for red blood cell (RBC) products. RBC products are maintained under precise control at 4.0± 2.0°C. The internal temperature and events can be recorded for extended intervals. Its internal battery enables transportation for over 7 h. In the event of the internal temperature surpassing specified limits, the system promptly issues an alarm. The main body of the refrigerator weighs 6.6 kg.

(indicated by the red arrow). Only bags that met the shipping criteria (not deviating from the standard temperature range in the temperature log data of the returned RBC bags, with no abnormalities during the visual inspection of the bag and label for damage, haemolysis, or clot formation) were reshipped from the JRCNBC to NUH and utilized (indicated by the green arrow). To enhance inventory management operations at the NGCH, we implemented a three-phase BR system as follows: Phase 1: 22 January 2019–18 February 2019. This phase served as a test for the continuous operability of the ATR system and the efficiency of the transport route. We introduced three additional bags into the ATR, supplementing the hospital's standard inventory of seven bags of O-type blood; Phase 2: 19 February 2019–25 April 2019. During this phase, we reduced the number of conventional type O RBC products in the inventory by two bags and augmented the ATR storage, aiming to maintain the overall inventory at 10 bags; Phase 3: 4 June 2019–4 December 2019. In this phase, we reverted to the conventional inventory of seven type O RBC products. Five bags were allocated to the hospital inventory, and two bags were reserved for emergency use within the ATR system.

Endpoints

The primary endpoint of this study was the utilization rate of products redelivered to NUH during the planned period. The secondary endpoints included the wastage rate of stored blood products delivered to the NGCH and the frequency of temporary transportation from the JRCNBC to the NGCH. A comparative analysis was conducted using data from before the start of the study as a historical reference.

	specification	period	stock in NGCH (bags) Conventional*	in ATR
phase 1	Test for the feasibility of the transport route. Maintaining the conventional type O RBC products.	22 January 2019–18 February 2019	7	3
phase 2	Conducted with 2 bags reduced from the conventional type O RBC products.	19 February 2019–25 April 2019	5	5
phase 3	Conducted with 3 bags reduced from the stock of type O RBC products in the ATR.	4 June 2019–4 December 2019	5	2

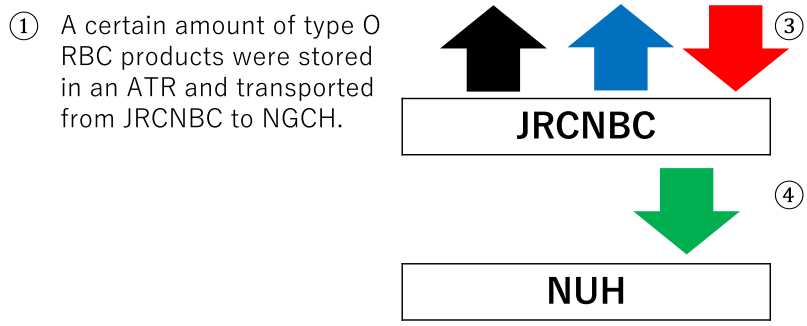

FIGURE 3 Overview of the blood rotation system. First, as indicated by the blue arrow, type O, RhD-positive, irradiated red blood cell (RBC) products were stored in an active transport refrigerator (ATR) and supplied from Japan Red Cross Nagasaki Blood Center (JRCNBC) to Nagasaki Goto Chuoh Hospital (NGCH) by a scheduled ship. The RBC products in the ATR were reserved for a week and used in cases of emergency or shortages of other blood type products in the NGCH. After a 1-week storage period, as indicated by the red arrow, the unused RBC products were returned to the JRCNBC in the ATR. If the shipping criteria were met, as indicated by the green arrow, the recovered RBC products were reissued and used at the Nagasaki University Hospital (NUH).

Ethical approval

This study was approved by the Clinical Research Ethics Committee of NUH and NGCH.

Statistical analyses

Data are expressed as mean ± standard deviation. The cumulative chi-squared test was used to analyse the usage and wastage rates. Statistical significance was set at $p < 0.05$. All analyses were performed using R 2.14.1 (CRAN: the Comprehensive R Archive Network at http://cran.r-project.org/).

RESULTS

Validation of storage conditions for RBC products using ATR

We monitored the temperature conditions of the ATR used to store and transport the RBC products between NGCH and JRCNBC. Representative data are shown in Figure 4. The external temperature fluctuated widely between 2.9 and 24.9°C owing to the installation environment of the ATR. However, the internal temperature of the ATR remained within the appropriate range of 3.2–4.4°C. Furthermore, transportation in the ATR allowed us to maintain the temperature of the RBC products using a thermometer placed between two RBC bags as a surrogate marker of the core temperature. The temperature range observed for this marker was 3.0–3.7°C, indicating proper temperature control. Thus, the use of ATR to transport and store RBC products ensured appropriate temperature maintenance.

Blood utilization at NUH

Of the 107 RBC product preparations stored in the ATR and shipped to NGCH, 4 were used for patients, resulting in 103 products being returned to JRCNBC (Figure 5). A re-evaluation of their quality at the JRCNBC revealed that they all met shipping standards. Among the 103 returned products reissued to the NUH during the study period, 101 (98.1%) were transfused into patients. Two bags were discarded because of expiration. Additionally, the wastage rate of type O RBC products at NUH during the study period was 24 out of 2033 bags (1.18%), nearly equivalent to 19 out of 1854 bags (1.02%) during the same period in the previous year. The remaining expiration date at the time of warehousing to the NUH was 4–11 days, but 84 out of 101 bags (83.2%) could be used within 2 days. No adverse reactions

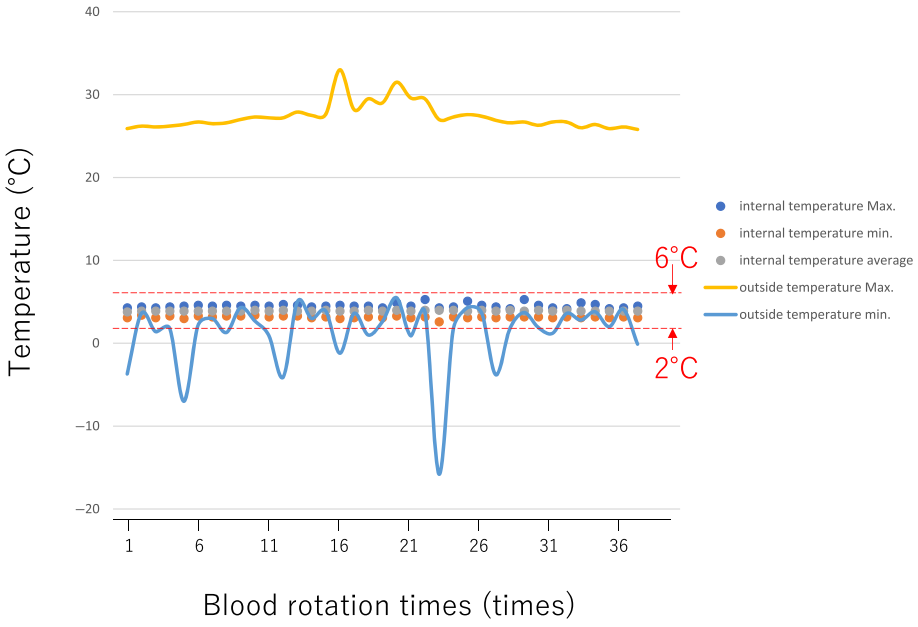

FIGURE 4 Temperature variation record of red blood cell products stored in active transport refrigerator (ATR). Continuous temperature data for each operation were obtained from the logs recorded in the ATR. Through the operation period, as indicated by coloured dots indicating maximum (blue), minimum (orange), and average (grey) temperature in this figure, we could confirm that the temperature inside ATR was stably controlled within the appropriate temperature range of 2.6–5.3°C, regardless of the ambient temperature; yellow line, maximum; blue line, minimum.

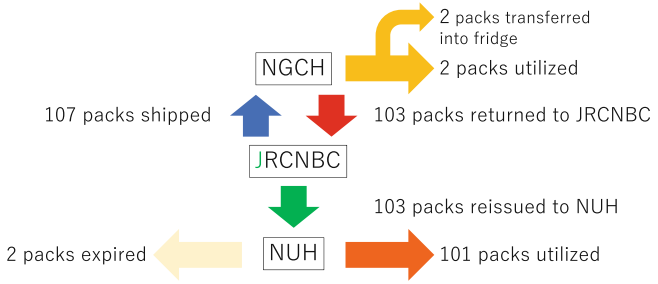

FIGURE 5 Summary of the utilization of red blood cell products. A total of 103 red blood cell products were returned to Japan Red Cross Nagasaki Blood Center (JRCNBC), as indicated by the red arrow, and all were reissued to Nagasaki University Hospital (NUH), as indicated by the green arrow. Of the 103 products returned and stored at the NUH, 101 (98.1%) were used, as indicated by the orange arrow. Two discarded bags were used until expiration. NGCH, Nagasaki Goto Chuoh Hospital.

or transfusion-associated hyperkalaemia were observed among patients who received reshipped RBC products.

Blood utilization at NGCH

At NGCH, 597 RBC products were used, whereas 80 products were discarded during the entire study period. The ATR supplied 107 type O RBC bags without any wastage. Consequently, the overall wastage rate was 10.2% over the entire study period compared with 24.2% in the previous year (Table 1). The discard rate during Phase 3 was notably lower than that in Phases 1 and 2, whereas the discard rate in the corresponding period of 2018 was significantly higher than that in any other phase of this study, as well as over its entire duration ($p < 0.001$).

Furthermore, the results for each blood type are presented in Figure 6. Two hundred and fifteen bags were used for type O RBC products, and 13 bags were discarded. Based on the same methodology, the wastage rate for 331 type O bags supplied, including 103 ATR-stored and returned products, was 3.93% (compared with 12.9% in the same period in the previous year). During the research period, 74 scheduled and 103 quick deliveries of RBC products to the NGCH (excluding ATR transport) were similar to 76 and 110 deliveries, respectively, during the same period in the previous year.

Three type O RBC products stored in ATR were used in NGCH. One was used in an artificial bone-head replacement surgery because of the unavailability of replenishment products owing to bad weather, resulting in only ATR stock being available for type O blood within the hospital. Additionally, two products were used for patients with gastrointestinal bleeding because replenishment would require >2 h via the sea route to arrive. Furthermore, one bag was temporarily stored in the hospital inventory and used for blood transfusion to a urological patient the following day.

DISCUSSION

In the present study, we revealed that reshipped RBC products can be effectively utilized after their return from a hospital on a remote

TABLE 1 Blood utilization at Nagasaki Goto Chuou Hospital.

	Phase 1 (22 January 2019–18 February 2019)	Phase 2 (19 February 2019–25 April 2019)	Phase 3 (4 June 2019–4 December 2019)	Corresponding period in 2018
Bags used	60	129	408	550
Return bags in ATR	12	45	50	-
Bags discarded	12	27	41	176
Discard rate (%)	14.3	13.4 10.2	8.2[a]	24.2[b]

[a]The discard rate during Phase 3 was significantly lower than that in Phases 1 and 2.
[b]While the discard rate in the corresponding period of 2018 was significantly higher than that in any other phase of this study, as well as over its entire duration (chi-squared test, $p < 0.001$).
Abbreviation: ATR, active transport refrigerator.

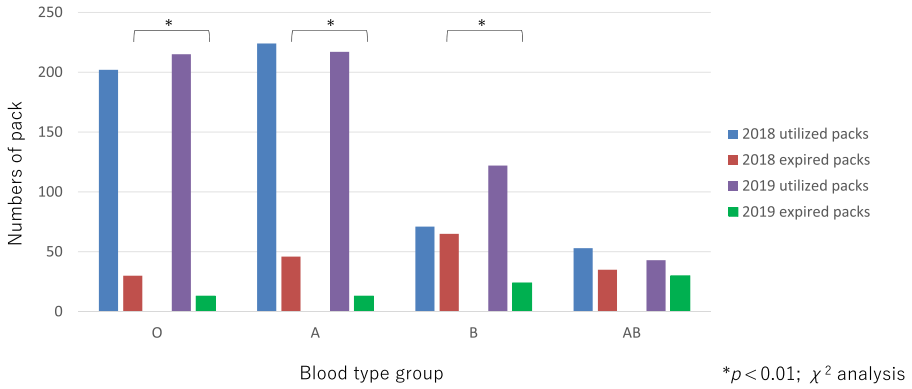

FIGURE 6 Impact on the wastage rate of red blood cell products based on blood type in Nagasaki Goto Chuoh Hospital. The histogram shows the usage and wastage of red blood cell products based on blood type in the Nagasaki Goto Chuoh Hospital. The research period corresponds to 2019, and data from the same period in 2018 are presented as data when the blood rotation system was not in operation. The utilized pack numbers are indicated by blue (2018) and violet (2019) bars, and wastage numbers are indicated by red (2018) and green (2019) bars.

island, where strict temperature control during storage in an ATR is ensured.

Previous research demonstrated that unused RBC bags in hospitals on the Ogasawara Islands, where transfusion therapy is not routinely performed, could be effectively reused (returned and redelivered) with an efficiency rate of 87.5% through the BR system in hospitals in mainland Tokyo, Japan [7]. These studies demonstrated the effectiveness of implementing the ATR system in medical institutions on the Ogasawara Islands. Our study shows the feasibility of implementing the BR system for the cyclical operation of blood products, even in a remote island hospital that requires routine transfusion therapy and inventory management, through collaboration with BC and hospitals in the mainland. Ayyalil et al. [11] reported that although they do not explicitly mention transportation methods, temperature management or quality assurance, the system successfully reduces the disposal of blood products by efficiently distributing them to medical facilities based on the remaining shelf life of the products. However, hospitals in Japan are prohibited from supplying blood products to other medical institutions as businesses operate under the regulations of the Act on Securing Quality, Efficacy, and Safety of Products, Including Pharmaceuticals and Medical Devices [12].

Additionally, once blood products are shipped from the BC, they are sold to medical institutions, and no returns are accepted. The establishment of the BR system aims to address issues related to the SC of transfusion medicine and blood products while considering the future of regional healthcare, including the prediction of population dynamics and changes in healthcare infrastructure. Therefore, extensive consultations with medical institutions, BCs and administrative agencies are essential for realizing the operational flow of blood products in BR systems. It is recommended that the regional transfusion therapy committee engage in the successful implementation of this system [13].

Our study confirmed that the temperature of RBC bags during the storage period in the ATR consistently remained within the appropriate range during transportation and when delivered at the NGCH. Additionally, the temperature variations resulting from the opening of the lid during inspections at the medical facilities and in the quality control processes at the BC and formulation retrieval remained within the limits specified by quality management regulations. All the unused bags returned to the BC met the reshipment criteria. Most of the reshipped products were properly utilized at the NUH. In this study, a significant portion of RBC products shipped within the ATR for NGCH

had a shelf life of approximately 14 days. The wastage rate was also low because of the high usage of RBC bags at NUH. In practical implementations, operational considerations regarding shelf life and use at the reshipping destination significantly affect the effectiveness of the BR system.

Appropriate management of RBC products is necessary for ATR-equipped medical facilities to enhance their effectiveness in remote island hospitals. One crucial aspect is proper control of the quantities of non-ATR stock bags and the number of ATR-stored units. In this study, at NGCH, the inventory of blood products other than type O was managed as usual, whereas the number of type O RBC products was reduced from seven to five in the conventional stock. Nevertheless, the wastage rate of these products during the study period showed a general decrease compared with the corresponding period of the previous year. However, various confounding factors, such as an increase in product usage and remaining expiration dates of incoming products, cannot be ignored. Furthermore, the introduction of the ATR system resulted in a net increase in round-trip transportation costs of €32 per visit, totalling €1202 over 38 trips. This cumulative cost is equivalent to the acquisition cost of approximately 10.7 units of RBC products in Japan, valued at €112 each. The initial investment required to implement the ATR system is approximately €3100 per unit, with the estimated annual expenses for operation and maintenance ranging between €310 and €370. Considering the observed reduction in RBC solution waste during the same period in the previous year, these additional costs can be effectively offset, and the initial costs will be depreciated over time. This evaluation highlights the enhancement in operational efficiency and the possibility for cost recovery associated with the ATR system, indicating its significance extends beyond transportation facilitation.

This study had several limitations. First, because single-arm operations using ATR were conducted, the impact on factors such as the use and wastage of blood products at NGCH and NUH, as well as the frequency of unscheduled (quick) deliveries to NGCH, was limited to comparisons with data from the corresponding period of the previous year. Regarding waste reduction at NGCH, we acknowledge that the retrospective analysis and the potential influence of confounding factors may limit the interpretation of our findings. Nevertheless, waste reduction was observed across all phases compared with that in the same period in the previous year. This reduction held true even in Phase 2, despite an increase in total inventory to 10 bags. We believe that a more detailed examination is necessary to optimize the balance between hospital inventory and ATR storage, considering institutional function, size and location variables.

Second, owing to restrictions on the use of ATR-preserved products at NGCH, this study did not reveal the impact on the frequency of transfusions using ATR-preserved type O RBC bags with compatible but ABO-different types during periods of increased in-house demand, including cases of massive bleeding [14–16]. A continuous stock of type O RBC bags in ATR is believed to contribute to the life-saving treatment of patients in emergency situations. It also enables the medical staff to confidently and safely perform compatible transfusions. Therefore, it is necessary to elucidate the contribution of BR systems to this process.

In conclusion, the establishment of a BR system supported by robust quality management and transportation technology demonstrated in this study can contribute to the effective utilization of valuable blood products, even when they are not used after being dispatched to medical institutions, by reintroducing them into an effective SC within their expiration dates and ensuring a stable supply of blood products in remote island regions. Further investigation is warranted to establish a sustainable and innovative blood product SC, including cost–benefit and application to platelet or plasma products, which may require different storage and handling methods.

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All authors had access to the data and participated in data collection and interpretation. K.N. and A.T. conceived and designed the study; S.E., N.T., Y.K. and D.I. operated the BR system among NGCH, NUH and JRCNBC; S.E. and I.K. performed supplementary and quality management of RBC products; K.N., S.E., H.I. and S.Y. analysed and interpreted the data; K.N. performed the statistical analysis and wrote the manuscript; S.Y., Y.M. and A.T. supervised the project and revised the draft critically; all authors approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

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REFERENCES

1. Nagai K. Research on efficient blood product management in remote island areas [in Japanese]. Research on establishing comprehensive blood transfusion management system in the region. Integrated and collaborative research report for the years 2017 to 2019 (Tanaka A). Grants for health and labor sciences research expenses: regulatory science policy research project for pharmaceuticals, medical devices. 2020:53–60. [in Japanese]. Available from: https://mhlw-grants.niph.go.jp/system/files/2019/193041/201925002A_upload/201925002A0008.pdf. Last accessed 24 Oct 2023.
2. Takanashi K. Establishment of a rational transportation and management system for blood products in the blood business [in Japanese]. Research on establishing comprehensive blood transfusion management system in the region. Integrated and collaborative research report for the years 2017 to 2019 (Tanaka A). Grants for health and labor sciences research expenses: regulatory science policy research project for pharmaceuticals, medical devices 2020;116–20. [in Japanese]. Available from: https://mhlw-grants.niph.go.jp/system/files/2019/193041/201925002A_upload/201925002A0011.pdf. Last accessed 24 Oct 2023
3. Tanaka A, Kitazawa J, Kon M, Satou T, Nagura Y, Fujiwara S, et al. The report on the actual use of blood products in the Reiwa 2 fiscal year comprehensive survey on blood transfusion operations

conducted by the JSTMCT. Available from: 893323d7134252d899c08ea0ab7bd7d9.pdf (jstmct.or.jp). Last accessed 24 Oct 2023.
4. Makino S, Kanno H, Okamoto Y, Kitazawa JI, Yamamoto K, Yasumura S, et al. Current status and future issues in blood transfusion therapy in Japan: a nationwide questionnaire survey [in Japanese]. Jpn J Transfus Cell Ther. 2020;66:619–28.
5. Yazer MH, Abraham S, Beckman N, Folléa G. International Society for Blood Transfusion international survey on blood product wastage in hospitals. ISBT Sci Ser. 2016;11:24–31.
6. Anthes E. Evidence-based medicine: save blood, save lives. Nature. 2015;520:24–6.
7. Igarashi T, Fujita H, Asaka H, Takada Y, Ametani R, Naya I, et al. Patient rescue and blood utilization in the Ogasawara blood rotation system. Transfusion. 2018;58:788–94.
8. Kitada K, Onodera H, Kurihara K. Quality of red blood cell components packed in the ATR700 transported by a ship [in Japanese]. Ketsuekijigyo. 2015;38:755–8.
9. Toho Pharmaceutical Co. Ltd. The ATR700-RC05/ATR705-RC05 mobile red blood cell refrigerator [in Japanese]. Available from: https://www.tohoyk.co.jp/medical-total-support/transport/. Last accessed 1 Nov 2023.
10. The Guideline for The Use of Blood Products the Ministry of Health. Labor and Welfare's Pharmaceutical and Living Hygiene Bureau [in Japanese]. 2019 Available from: http://www.mhlw.go.jp/new-info/kobetu/iyaku/kenketsugo/5tekisei3b.html. Last accessed 24 Oct 2023.
11. Ayyalil F, Irwin G, Ross B, Manolis M, Enjeti AK. Zeroing in on red blood cell unit expiry. Transfusion. 2017;57:2870–7.
12. Ministry of Health, Labour and Welfare. Act on securing quality, efficacy and safety of products including pharmaceuticals and medical devices [in Japanese]. Available from: https://www.mhlw.go.jp/web/t_doc?dataId=81004000&dataType=0&pageNo=1. Last accessed 24 Oct 2023.
13. Seki Y, Sato K, Abe T, Komata T, Fuse I. A research on the effective use of blood products in Niigata prefecture through wide-area blood rotation using the ATR blood transport system. Jpn J Transfus Cell Ther. 2022;68:496–501.
14. Hardwick J. Blood storage and transportation. ISBT Sci Ser. 2008;3:177–96.
15. Metcalf RA, Baker SA, Goodnough IT, Shah N. Transportation cooler interventions reduce plasma and RBC product wastage. Am J Clin Pathol. 2016;146:18–24.
16. Bots M, de Grouw EP, van Rooyan-Schreurs IH, van den Akker GJ, Sturk A, Klinkspoor JH, et al. Strategies to reduce wastage of red blood cell units. Vox Sang. 2016;110:143–9.

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ORIGINAL ARTICLE

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Evaluation of new haematology analyser, XN-31, for malaria detection in blood donors: A single-centre study from India

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Abstract

Background and Objectives: Malaria continues to be a significant public health concern in India, with several regions experiencing endemicity and sporadic outbreaks. The prevalence of malaria in blood donors, in India, varies between 0.02% and 0.07%. Common techniques to screen for malaria, in blood donors and patients, include microscopic smear examination and rapid diagnostic tests (RDTs) based on antigen detection. The aim of this study was to evaluate a new fully automated analyser, XN-31, for malaria detection, as compared with current practice of using RDT.

Materials and Methods: Cross-sectional analytical study was conducted to evaluate clinical sensitivity and specificity of new automated analyser XN-31 among blood donors' samples and clinical samples (patients with suspicion of malaria) from outpatient clinic collected over between July 2021 and October 2022. No additional sample was drawn from blood donor or patient. All blood donors and patients' samples were processed by malaria rapid diagnostic test, thick-smear microscopy (MIC) and the haematology analyser XN-31. Any donor blood unit incriminated for malaria was discarded. Laboratory diagnosis using MIC was considered the 'gold standard' in the present study. Clinical sensitivity and specificity of XN-31 were compared with the gold standard.

Results: Fife thousand and five donor samples and 82 diagnostic samples were evaluated. While the clinical sensitivity and specificity for donor samples were 100%, they were 72.7% and 100% for diagnostic samples.

Conclusion: Automated haematology analysers represent a promising solution, as they can deliver speedy and sensitive donor malaria screening assessments. This method also has the potential to be used for pre-transfusion malaria screening along with haemoglobin estimation.

Keywords

blood donors, haematology analyser, malaria, rapid detection test, thick-smear microscopy

Highlights

- A new haematology analyser, XN-31, evaluated for malaria detection in whole blood donors, was found to be 100% sensitive and specific in over 5000 donor samples.

- In diagnostic samples of patients with suspicion of malaria, the sensitivity and specificity were 100% and 72.7%, respectively. [Corrections were added on 4 April 2024: After the first online publication, the preceding sentence was corrected.]
- This haematology analyser, XN-31, also has the potential to be used for pre-transfusion malaria screening, along with haemoglobin estimation.

INTRODUCTION

Blood transfusions are critical and life-saving medical interventions that are commonly employed in various healthcare settings. Safety of blood transfusions relies on rigorous screening procedures to identify potential infections and reducing the risk of transmission of pathogens. In India, these screening procedures are governed by strict regulatory guidelines defined in the Drugs and Cosmetic Act, specifically Part X11 B of Schedule F [1]. These guidelines have been put in place to safeguard the health of both recipients and blood donors, and to maintain the safety of the national blood supply.

According to these regulatory provisions on blood screening procedures, five transfusion transmissible infection (TTI) tests are mandated on all donated blood units, before they can be released for transfusion. These essential tests are provisioned to detect the presence of specific pathogens and disease markers that poses significant risks to the recipient's health. The five TTI tests include screening for antibodies to human immunodeficiency virus (anti-HIV 1 and 2), antibodies to hepatitis C virus (anti-HCV), hepatitis B virus surface antigen (HBsAg), serological tests for syphilis and test for malaria.

Malaria continues to be a significant public health concern in India, with several regions experiencing endemicity and sporadic outbreaks [2]. The prevalence of malaria in blood donors, in India, varies between 0.02% and 0.07%, in few published studies [3–5], with cumulative sample size of 393,730 whole blood donors. It is therefore important to ensure detection of malaria in blood donors and prevent transfusion-transmitted malaria (TTM). To date, various techniques have been employed by blood transfusion services (BTS) across India to screen for malaria. These techniques include microscopic smear examinations and rapid diagnostic tests (RDTs) based on antigen detection; the latter seems to be overtaking the former with more widespread use, across BTS. While RDTs are commonly used for malaria detection, they have certain disadvantages, such as being manual, difficult to batch, quality control and document, which limit their usefulness.

This study was undertaken to evaluate a new fully automated analyser XN-31 (Sysmex Corporation, Kobe, Japan) for malaria detection, as compared with the current practice of using RDT.

MATERIALS AND METHODS

Study design and settings

A cross-sectional analytical study was conducted to evaluate the clinical sensitivity and specificity of a malaria screening technique among consecutive blood donors in the department of transfusion medicine, in a tertiary care hospital in the National Capital Region (NCR), India. Blood donor samples were collected over a period of 15 months (from July 2021 to October 2022).

Subject groups

Blood donors

The study population primarily consisted of prospective whole blood (WB) donors who registered, provided basic demographic information, completed a health history questionnaire, underwent a brief physical examination and granted their consent for donation. The brief physical examination encompassed measurements of weight, blood pressure, pulse rate, body temperature and an overall assessment of their health condition. Donors meeting the selection criteria outlined in the Drugs and Cosmetic Act of 1940 [1] were eligible. Individuals with a recent history of malaria infection within the last 3 months or a body temperature exceeding 37.5°C were deferred from blood donation.

Clinical samples

Besides blood donor samples, few clinical samples from patients with suspicion of malaria were also used, because the probability of finding a positive result in donors was very low, as evidenced by historical published data [3–5]. These clinical samples were sourced from patients visiting outpatient clinics of the hospital, where these individuals sought medical attention due to symptoms such as fever, headache, fatigue, muscle and joint pain, and other symptoms suggestive of possible malaria infection.

Routine testing for malaria in blood donors/clinical samples

Routine testing for blood donors

For donor samples, as per institutional standard operating procedures (SOP), three blood samples were collected from each blood donor: two in EDTA tubes (3 mL and 6 mL, respectively) and one in a clot activator tube (4 mL). These tubes were labelled with unique donation identification numbers (UDIN) and intended for blood grouping, nucleic acid amplification tests (NAT) and screening for TTIs, respectively. Testing for malaria was performed by malaria RDTs (mRDTs) in

the blood centre of the hospital, using the residual sample from 6-mL EDTA tube, after the NAT assay. If the test result was non-reactive, no further tests were performed and it was released for transfusion. As part of study protocol, reactive mRDT was further submitted to thick-smear microscopic examination (MIC) for confirmation in the haematology section of the laboratory in the hospital. Any incriminated WB unit on mRDT/MIC was discarded as per institutional SOP.

Routine testing for clinical samples (patients' samples)

Clinical samples (patients' samples with suspicion of malaria) collected in 3-mL EDTA sample collection tube was submitted simultaneously to both mRDT and thick-smear examination in the haematology section of the laboratory in the hospital. Any sample positive by mRDT and/or MIC was considered positive and treated clinically.

Testing protocol followed in the study

In the study protocol, besides routine testing, all blood donor and patients' samples were additionally processed using the new haematology analyser XN-31. The haematology analyser used the residual sample from the blood group sample tube, which was used for initial ABO and RhD blood group test. Testing on XN-31 included complete blood counts and malaria parasite testing, which was performed within 12 h of venipuncture. No additional sample was drawn from blood donor or patient. Laboratory diagnosis using MIC was considered gold standard in the present study. Sensitivity and Specificity of tests results from XN-31 and the gold standard were calculated and tabulated.

Equipment/testing techniques employed in the study

XN-31 haematology analyser

XN-31 (Sysmex Corp., Kobe, Japan) uses a combination of optical fluorescence techniques, flow cytometry and laser-optical recognition by a violet semiconductor 405 nm laser beam that analyses the resultant forward-scattered light (FSC) and side fluorescent light (SFL). The blood cells are initially treated automatically with reagents such as Lysercell M and Fluorocell M (Sysmex Corporation). Lysercell M shrinks the red blood cells (RBCs), and the size of these shrunken cells reflects the parasite cell size within the RBCs. Treatment with the Fluorocell M stains the nucleic acids inside the cells. After these treatments, the Plasmodium-infected RBCs are effectively analysed according to the intensity of the FSC and SFL of each cell. The intensity of the FSC signal mainly reflects the size of the cells, whereas that of the SFL reflects the amount of each cell's nucleic acids. These signals are consequently used to detect malaria-infected RBCs (MI-RBC) with the help of unique proprietary algorithms [6]. There were two modes of testing: the LM (low malaria) mode and the PD (pre-dilution) mode. The LM mode used 60 µL WB samples and was optimized to count low numbers of Plasmodium-infected RBCs (analytical limit was quantification of 20 parasites/µL). XN-31 also has a PD mode (analytical limit was quantification of 40 parasites/µL), which uses 70 µL blood sample, which is diluted seven times with Cellpack DCL (Sysmex Corporation) [6]. This mode can be used to test capillary blood at the time of screening of donor. In the present study, blood samples were measured by only LM mode of the XN-31 within 12 h of blood collection from patients. LM mode was preferred, because low positivity rate was anticipated in blood donors, who are apparently healthy individuals and do not exhibit signs or symptoms of malaria infection.

XN-31 output included parasite count, both as a percentage of infected RBCs and absolute parasite density, expressed as 'MI-RBC%' and 'MI-RBC#', respectively. Information on the species of malaria parasites was suggested by various flags of the algorithm used in the XN-31 haematology analyser. These flags were, namely, 'Malaria? (P. f)' suggesting *Plasmodium. falciparum* and 'Malaria? (Others)' suggesting Plasmodium species other than *P. falciparum*. When the malaria parasite could not be classified, flagging it as 'Malaria? (UNC)' meant unclassified parasites. Moreover, in rare instances, the detection of unknown particles in the blood sample sometimes causes indeterminant results, and then flagging of 'MI-RBC Abn Scattergram' was suggested, which meant abnormal scattergram. The analyser also provided a concurrent complete blood count measurement for each sample analysed.

Malaria rapid diagnostic test

An mRDT was performed on EDTA-anticoagulated venous blood within 12 h of venipuncture, according to recommendations from the manufacturer. Malaria antigen test kit PAN pLDH (SureTest, Microgene Diagnostic Systems (P) Ltd. India) was a rapid immunochromatography based test. The kit format contains two bands: the first band (test band) detects PAN pLDH (Plasmodium lactate dehydrogenase), while the second acts as a procedural control, validating the individual test. The test used WB sample for testing. PAN pLDH is present if a person is infected from any of the four species, viz. *Plasmodium vivax*, *P. falciparum*, *Plasmodium. malariae* and *Plasmodium. ovale*. The manufacturer claimed detection level of 100–200 parasites/µL and a specificity of >99% for the test kit [7].

Thick-smear MIC

Two thick blood films were prepared from capillary samples from all participants and stained with Giemsa according to international guidelines [8]. Two expert malaria microscopists read the thick-film slides to establish the malaria diagnosis. A third blind reader resolved any discrepancies (positive vs. negative). Both microscopists had 8 years of demonstrated expertise in malaria diagnosis and were classified as level expertise 1 according to World Health Organization (WHO).

A stained, thick blood smear was used to establish positive and negative judgement; the smear was considered negative if, after completing examination of 500 fields at 100× magnification, no parasites (asexual or sexual) were found. A second thick smear was used as a backup for cases where there was any problem with staining or doubts with judgement.

Statistical analysis

Clinical sensitivity and specificity of using the XN-31 was compared with the gold standard. All recorded variables were presented as means and SD. The results were compiled using Microsoft Excel (Microsoft Inc., WA, USA), and descriptive statistics were analysed using PRISM 10.0 (GraphPad Inc., CA, USA).

Ethical clearance

This study was approved by the institutional review board and additional study-specific informed consent was waived. Recorded data from participants were anonymized using a code. Participants who were diagnosed by mRDT/MIC were treated according to the national malaria treatment guidelines. The results from the XN-31 were not used to manage patients because this instrument was under evaluation in this study.

RESULTS

Demographics of donors and diagnostic samples

Five thousand and five donor samples were examined to evaluate the performance of the XN-31 in routine testing. XN-31 equipment's clinical sensitivity was also evaluated using clinical samples ($n = 82$). The demographic summary of the recruited subjects is mentioned in Table 1. The WB donor group comprised of 96% (4803 of 5005) males and 4% (202 of 5005) of females, while patients who provided diagnostic malaria samples consisted of 62% ($n = 51$) males, 38% ($n = 31$) females. While the median age of blood donors was 32 years (interquartile range [IQR] 18–65), the median age of patients with suspected malaria was 40 years (IQR 17–60).

TABLE 1 Demographic details of blood donors (donor samples) and patients (clinical samples).

Description	Donor samples ($N = 5005$)	Clinical samples (patient samples) ($N = 82$)
Age (median ± SD)	32 ± 8.5	40 ± 17.49
Gender		
Male	4803	51 (62)
Female	202	31 (38)

Sensitivity and specificity of blood donor samples

Among the 5005 donor samples analysed, overall findings were consistent with the established laboratory testing protocols for identifying malaria infection in blood donors. It is worth noting that both sensitivity and specificity for donor samples was 100%. A summary of the evaluation results is collated in Table 2. Remarkably, among the 5005 samples, only one blood donor sample that tested positive by the gold standard was also identified by XN-31. The instrument raised an MI-RBC positive flag for this sample. Figure 1 illustrates a representative scatterplot generated by the analyser, displaying clear evidence of infected RBCs (MI-RBCs). The instrument also provided parasitaemia counts and a potential identification of the malaria parasite species.

Instrument flags

For all negative donors ($n = 5004$), we observed the instrument XN-31 with negative flags for any infection. For clinical samples, the scattergram patterns that triggered related flag messages are outlined in Table 3. While all 71 true positives were flagged, three false positives were flagged, too. In the positive clinical samples, the most common instrument flag was *P. vivax* cases, categorized under the flag 'others' category ($n = 41$), with instrument flag corresponding to *P. falciparum* infections, were the second most common ($n = 6$). These 47 falciparum and vivax also corroborated with MIC observations.

Though these features may not be applicable to donor services when screening blood donations, they may provide supporting diagnostic value when linking infected positive cases to routine care.

Sensitivity and specificity of clinical samples

Among the 82 clinical samples that warranted a suspicion of malaria infection, we observed a remarkable agreement in measurements between gold standard and XN-31 as illustrated in Table 2: clinical sensitivity of 100% and clinical specificity of 72.7%. Importantly, in this randomly selected group, we did not encounter any false negatives. [Corrections were added on 4 April 2024: After the first online publication, the preceding sentence was corrected.]

DISCUSSION

TTM is a relatively infrequent occurrence, with reported frequencies ranging from 0.2 cases per million in non-endemic regions to 50 or more cases per million in malaria-endemic areas [9]. However, the continuous quest for ideal diagnostic tools that are simple, rapid and reliable is more crucial than ever before. The advent of newer haematology instruments has presented an opportunity to streamline and automate the detection of malaria-positive infections, ultimately enhancing laboratory efficiency [10].

TABLE 2 Sensitivity and specificity of XN-31 in detecting malaria in blood donors and diagnostic samples.

	Donor samples ($N = 5005$)			Clinical samples ($N = 82$)		
	Gold standard			Gold standard		
XN-31	Positive	Negative	Total	Positive	Negative	Total
Observed positive	1 (TP)	0 (FP)	1	71 (TP)	03 (FP)	74
Observed negative	0 (FN)	5004 (TN)	5004	0 (FN)	8 (TN)	8
Total	1	5004	5005	71	11	82
Sensitivity	100%			100%		
Specificity	100%			72.7%		

Abbreviations: FN, false negative; FP, false positive; TN, true negative; TP, true positive. [Corrections were added on 4 April 2024: After the first online publication, Table 2 was corrected.]

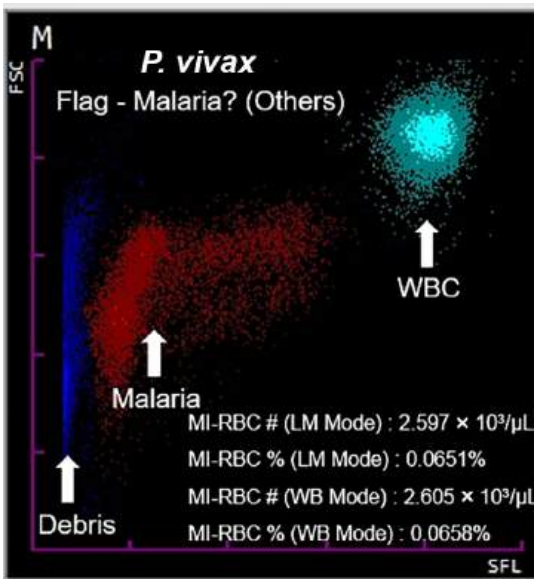

FIGURE 1 XN-31 scattergram plot and accompanying instrument flags identifying the positive blood donor sample. FSC, forward-scattered light; LM, low malaria; MI-RBC, malaria-infected red blood cell; SFL, side fluorescent light; WB, whole blood; WBC, white blood cell.

TABLE 3 Different instrument flags of XN-31 in positive diagnostic samples.

XN-31 instrument flag	Positive diagnostic samples ($N = 74$)
MI-RBC Abn Scattergram + Malaria? (others)	11
Malaria? (P.f)	6
Malaria? (others)	41
Malaria? (UNC)	2
Malaria? (P.f) + Malaria? (others)	9
Malaria? (UNC) + MI-RBC Abn Scattergram	1
MI-RBC Abn Scattergram + Malaria? (P.f) + Malaria? (others)	2
MI-RBC Abn Scattergram	2

Abbreviation: MI-RBC, malaria-infected red blood cell.

Automated haematology analysers represent a promising solution, as they can deliver speedy, sensitive and cost-effective assessments for suspected malaria infections. These analysers not only reduce analytical time but also enhance accuracy, with a quantification limit of 20 infected RBCs per microliter (iRBCs/µL) [11]. In our study, we evaluated the performance of the XN-31 automated haematology analyser, which demonstrated impressive sensitivity and specificity. It achieved a sensitivity of 100% for both donors and patients, coupled with a specificity of 100% for donors and 72.7% for patients. [Corrections were added on 4 April 2024: After the first online publication, the preceding sentence was corrected.] All 75 true-positive samples (one donor sample and 74 clinical samples), which were positive on MIC (gold standard), were also positive on mRDT. These results underline the potential of the XN-31, a new automated technology with a limit of quantification of 20 iRBCs/µL holds promise as an effective tool for the detection of common Plasmodium species, offering high sensitivity and specificity. Its automated design facilitates rapid and high-throughput analysis, making it well-suited for transfusion malaria screening. Pillay et al. found that the results reported by similar haematology analyser XN-30 correlated with microscopy and the analyser demonstrated 100% sensitivity and specificity. They concluded that haematology analyser XN-30 provided a robust, rapid, automated and accurate platform for diagnosing malaria in a clinical setting [12].

In the clinical samples, there were three false-positive results that were reported by XN-31. It has been speculated that triggering of false positive and indeterminate in XN-31 results is correlated with diseases and conditions associated with the presence of immature cells of the erythrocyte lineage or mature RBCs with abnormal morphology. Few of these diseases/conditions are sickle cell disease, beta thalassemia, haemochromatosis premature newborns, etc. [13]. Though these individual diseases/conditions were not evaluated in the present study, it is possible that few of the samples in the present study cohort of 'clinical samples' had one of these underlying disease conditions, triggering false-positive results in XN-31.

In the field, malaria screening in blood donors primarily relies on two modalities: RDTs and MIC. However, both methods have their respective drawbacks. The mRDT assay format employs either lactate dehydrogenase (pLDH) or histidine-rich protein 2 (HRP2), both of which are expressed by human malaria parasites [14]. RDTs are often

preferred due to their ease of use, minimal infrastructure requirements, the capability to detect parasite densities exceeding 100 parasites/μL and rapid results delivery within 15–30 min [15] However, despite the advantages of malaria RDTs, they are not without limitations. They have a relatively high limit of detection, typically in the range of 100–200 parasites/μL [16]. Environmental factors such as humidity and extreme temperatures can adversely affect their performance. Furthermore, false-negative results can occur in regions where certain variants of *P. falciparum* do not express HRP2 [17]. Moreover, HRP2-based RDTs have an additional limitation that they may give false-positive result in donors with recent infection (though the donor may have had infection earlier than '3 months of deferral period') because HRP2 antigens can persist in circulation for weeks following parasite clearance [18]. Additionally, the emergence of parasite gene deletions in pfhrp2 and pfhrp3, which enable parasites to evade detection by RDTs, underscores the evolving challenge of diagnosing malaria [19] using RDT. However, in the present study, HRP2 antigen based were not used; pLDH-based RDTs were used. MIC continues to be another commonly employed and dependable method for malaria diagnosis in several centres. It is cost-effective and provides the potential for identifying other pathogens. Nevertheless, MIC has inherent subjectivity [20], is manual, time-consuming, necessitates trained personnel, depends on slide quality and possesses an operator-dependent limit of detection. Expert microbiologists can identify parasite densities as low as 10–50 parasites/μL, whereas non-experts typically have detection limits in the range of 100–500 parasites/μL [21]. Consequently, novel approaches are urgently needed to overcome the existing obstacles in the global effort to eradicate malaria. Malaria detection by newer haematology analyser seems to be one such approach, as evidenced in the present study.

One of the possible and interesting applications could be the use of XN-31 at the time of brief physical examination prior to WB donation. During this physical examination, haemoglobin (Hb) estimation is performed to ensure that donors with Hb of 12.5 g% or higher are bled, while anyone with lower Hb is counselled and temporarily deferred. In the present study, only LM mode was used, companion complete blood count (CBC) results did reveal that all blood donors had haemoglobin higher than 12.5 g%. All 5005 donors were considered eligible for blood donation with existing institutional practice. While this finding does enhance our confidence in the currently used POC (point-of-care) equipment for Hb estimation, it also makes us think whether we could replace the current POC with XN-31 doing twin functions of Hb estimation and malaria detection. The PD (pre-dilution) mode of XN-31 has an analytical limit of quantifying 40 parasites/μL and uses only 70 μL blood sample (capillary blood), which can be drawn from the finger tip. Besides Hb estimation, XN-31 output offers the added benefit of obtaining comprehensive CBC parameters, which may be useful in even suspecting thalassemia minor donors, though identification would involve additional confirmatory tests like Hb electrophoresis or high-performance liquid chromatography (HPLC) [22]. These additional features enhance our ability to provide donors with access to their health status and refer them for appropriate care. The additional benefit of a CBC with every malaria analysis offered by XN-31 would be helpful for donor health management and quality control testing of blood products. Wataru Kagaya et al used paired capillary and venous blood samples collected from 169 malaria-suspected outpatients in Homa Bay County Referral Hospital, Kenya. Malaria infections were diagnosed with the XN-31p, microscopy, RDT and PCR. Identical results in malaria diagnosis were observed between venous and capillary blood samples processed immediately after collection with the Sysmex XN-31p (prototype of XN-31). Relative to PCR, the sensitivity and specificity of the XN-31p with capillary blood samples were 0.857 and 1.000, respectively. Short-term storage of capillary blood samples at chilled temperatures had no adverse impact on parasitaemia and CBCs measured by the XN-31p [23].

Targeting the human reservoir of infection, including individuals who are infected but do not exhibit symptoms, such as healthy blood donors, is necessary to eradicate malaria. Asymptomatic malaria is possible in healthy adult blood donors, and the percentage of donors who are infected with the disease generally reflects the region's overall malaria burden, according to research done in areas where malaria is endemic [24, 25]. Blood donors should be routinely screened for malaria, which would be a significant data source for surveillance. Because blood donation is a continuous round-the-year process, screening donors for malaria would produce data continually and in real time, enabling the quick identification of shifting trends.

In conclusion, XN-31 was found to have 100% sensitivity and specificity in malaria screening of donors. This method also has the potential to be used for pre-transfusion malaria screening along with Hb estimation.

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A.K.T. and S.G. designed the study, performed the research and wrote the first draft of the manuscript, G.S. and P.K.G. carried out the tests (new haematology analyser XN-31, and malaria rapid detection tests) and entered data in excel sheet, S.G. and G.S. did the preliminary thick-smear microscopy, R.S. was the third expert microscopist for confirmation of thick-smear malaria examination, when required, V. J. and M.S. edited the manuscript and all contributing authors reviewed the manuscript. [Corrections added on 8 April 2024, after first online publication: Percentage values were corrected in Abstract (Results) and Discussion (Paragraph 2, 4th sentence).

CONFLICT OF INTEREST STATEMENT

Mr. Vaibhav Jadhav and Ms. Monisha Sethi are employee of Sysmex, India.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Malik V. Drugs and cosmetics act, 1940. 16th ed. New Delhi: Eastern Bank Company; 2003.
2. Rahi M, Sharma A. Malaria control initiatives that have the potential to be gamechangers in India's quest for malaria elimination. Lancet Reg Health Southeast Asia. 2022;1:2.
3. Makroo RN, Agrawal S, Chowdhry M, NL R. Prevalence of malaria in blood donor population from a tertiary care centre and effective donor notification and counselling policy. ISBT Sci Ser. 2018;13:131–5.
4. Rawat A, Diwaker P, Gogoi P, Singh B. Seroprevalence & changing trends of transfusion-transmitted infections amongst blood donors in a Regional Blood Transfusion Centre in north India. Indian J Med Res. 2017;146:642.
5. Sharma DC, Rai S, Bharat S, Iyenger S, Gupta S, Jain B. A 10 years comparative study to assess trends in seroprevalence of transfusion transmitted infections among blood donors at Gwalior, India. Open J Blood Dis. 2014;4:24–32.
6. Komaki-Yasuda K, Kutsuna S, Kawaguchi M, Kamei M, Uchihashi K, Nakamura K, et al. Clinical performance testing of the automated haematology analyzer XN-31 prototype using whole blood samples from patients with imported malaria in Japan. Malar J. 2022;21:1–13.
7. Suretest Malaria Pan Test Device [package insert]. Faridabad: Medsource Ozone. 2021.
8. Ba EH, Baird JK, Barnwell J, Bell D, Carter J, Dhorda M, et al. Microscopy for the detection, identification and quantification of malaria parasites on stained thick and thin blood films in research settings: procedure: methods manual. Geneva: World Health Organization; 2015.
9. Oh JS, Kim JS, Lee CH, Nam DH, Kim SH, Park DW, et al. Evaluation of a malaria antibody enzyme immunoassay for use in blood screening. Mem Inst Oswaldo Cruz. 2008;103:75–8.
10. Campuzano-Zuluaga G, Hänscheid T, Grobusch MP. Automated haematology analysis to diagnose malaria. Malar J. 2010;9:1–5.
11. Post A, Kaboré B, Reuling IJ, Bognini J, van der Heijden W, Diallo S, et al. The XN-30 hematology analyzer for rapid sensitive detection of malaria: a diagnostic accuracy study. BMC Med. 2019;17:1–2.
12. Pillay E, Khodaiji S, Bezuidenhout BC, Litshie M, Coetzer TL. Evaluation of automated malaria diagnosis using the Sysmex XN-30 analyser in a clinical setting. Malar J. 2019;18:1–4.
13. Khartabil TA, de Rijke YB, Koelewijn R, van Hellemond JJ, Russcher H. Fast detection and quantification of plasmodium species infected erythrocytes in a non-endemic region by using the Sysmex XN-31 analyzer. Malar J. 2022;21:119.
14. Mouatcho JC, Goldring JD. Malaria rapid diagnostic tests: challenges and prospects. J Med Microbiol. 2013;62:1491–505.
15. Mbabazi P, Hopkins H, Osilo E, Kalungu M, Byakika-Kibwika P, Kamya MR. Accuracy of two malaria rapid diagnostic tests (RDTS) for initial diagnosis and treatment monitoring in a high transmission setting in Uganda. Am J Trop Med Hyg. 2015;92:530–6.
16. WHO. Malaria rapid diagnostic test performance: results of WHO product testing of malaria RDTs: round 6 (2014–2015). Geneva: World Health Organization; 2015.
17. Cheng Q, Gatton ML, Barnwell J, Chiodini P, McCarthy J, Bell D, et al. Plasmodium falciparum parasites lacking histidine-rich protein 2 and 3: a review and recommendations for accurate reporting. Malar J. 2014;13:1–8.
18. Plucinski MM, McElroy PD, Dimbu PR, Fortes F, Nace D, Halsey ES, et al. Clearance dynamics of lactate dehydrogenase and aldolase following antimalarial treatment for *Plasmodium falciparum* infection. Parasit Vectors. 2019;12:1–6.
19. Feleke SM, Reichert EN, Mohammed H, Brhane BG, Mekete K, Mamo H, et al. *Plasmodium falciparum* is evolving to escape malaria rapid diagnostic tests in Ethiopia. Nat Microbiol. 2021;6:1289–99.
20. Moody A. Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev. 2002;15:66–78.
21. Mathison BA, Pritt BS. Update on malaria diagnostics and test utilization. J Clin Microbiol. 2017;55:2009–17.
22. Tiwari AK, Chandola I, Ahuja A. Approach to blood donors with microcytosis. Transfus Med. 2010;20:88–94.
23. Kagaya W, Takehara I, Kurihara K, Maina M, Chan CW, Okomo G, et al. Potential application of the haematology analyser XN-31 prototype for field malaria surveillance in Kenya. Malar J. 2022;21:252.
24. Owusu-Ofori AK, Parry C, Bates I. Transfusion-transmitted malaria in countries where malaria is endemic: a review of the literature from sub-Saharan Africa. Clin Infect Dis. 2010;51:1192–8.
25. Ahmadpour E, Foroutan-Rad M, Majidiani H, Moghaddam SM, Hatam-Nahavandi K, Hosseini SA, et al. Transfusion-transmitted malaria: a systematic review and meta-analysis. Open Forum Infect Dis. 2019;6:283.

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Transfusion Camp Rwanda 2023: A train-the-trainer workshop establishing locally driven leadership in knowledge translation and sustainability in transfusion medicine education

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Abstract

Background and Objectives: Blood transfusion is performed daily in hospitals. Gaps exist between transfusion guidelines and day-to-day clinical care. These gaps are prevalent in resource-limited settings due to scarce continuing medical education. *Transfusion Camp* Rwanda aims to bridge this gap by (1) delivering context-appropriate up-to-date education, (2) teaching participants how to independently deliver a case-based curriculum and (3) identifying strategies to promote change in transfusion practice in Rwanda.

Materials and Methods: In May 2023, a multidisciplinary team from Canada and Rwanda carried out a *Transfusion Camp* train-the-trainer workshop for clinicians from all five provinces in Rwanda. Participants attended in-person lectures, seminars and workshop group discussions on the implementation of the Rwanda National

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Directives on Rational Use of Blood and Blood Components. Course feedback was based on the Kirkpatrick Model of Training and Evaluation.

Results: Fifty-one physicians and laboratory technicians participated in the course. Confidence in caring for patients based on transfusion guidelines was self-rated as 'excellent' by 23% of participants before and 77% after, while 84% reported they planned to teach *Transfusion Camp* to others and 100% responded that they will apply course content to clinical practice. Workshop groups recommended strategies to improve transfusion medicine practice in Rwanda in four domains: Communication, Institutional Approval, Practice Audits and Education.

Conclusion: Transfusion medicine education in Rwanda using a train-the-trainer approach was well-received by participants and allowed for a more detailed understanding of the local medical and educational environment. These observations can inform the further expansion of the *Transfusion Camp* Rwanda project.

Keywords

global health, medical education, Rwanda, training of trainers, transfusion medicine

Highlights

- *Transfusion Camp* Rwanda train-the-trainer course participants gained transfusion medicine knowledge and confidence in the ability to teach colleagues and trainees.
- Workshop groups focused on four domains: Communication, Institutional Approval, Practice Audits and Education, and generated recommendations to disseminate and implement the Rwanda National Directives on Rational Use of Blood and Blood Components.
- Both the train-the-trainer course and the workshop together aim to improve adherence to transfusion medicine practice guidelines.

INTRODUCTION

Blood transfusion is a life-saving therapy and access to blood is on the World Health Organization (WHO) list of essential medications [1]. Transfusion medicine (TM) education is essential as physicians and nurses in almost every specialty prescribe and manage transfusions and associated reactions [2]. Significant deficits in baseline TM knowledge have been described in postgraduate trainees [3, 4].

Transfusion Camp is a longitudinal curriculum, originally designed in Canada and now scaled internationally, that aims to provide a strong baseline level of TM knowledge for non-transfusion specialty trainees [2]. *Transfusion Camp* has demonstrated the capacity to increase trainee TM knowledge and confidence in their transfusion practice [2, 5–7]. In 2022, *Transfusion Camp* was piloted in Rwanda, with the ultimate goal of improving the appropriate use of blood products in that country [8], and demonstrated similar knowledge and confidence increases from pre- to post-testing in Rwandan postgraduate trainees [8]. However, the lack of Rwandan *Transfusion Camp* trainers and faculty exposure to up-to-date TM guidelines limited the long-term sustainability of this course.

Training of trainers using the 'TRAIN' framework relies on 'talent, resources, alignment, implementation, and nurturing' as core concepts to foster sustainable education in LMICs [9]. Another core concept is the evaluation of trainer ability and confidence to teach others using the Kirkpatrick Model of Training Evaluation [10]. This model has four levels—reaction, learning, behaviour and results—measuring skill acquisition, relevance, changes in behaviour and, ultimately, patient outcomes.

The objectives of this project were to (1) bring contextually appropriate TM education and evidence-based practice updates to practitioners in Rwanda, (2) introduce the team-based learning framework of *Transfusion Camp*, guided by TRAIN, so that participants could independently teach their colleagues and medical trainees in the future, and (3) identify specific knowledge-translation strategies through which changes in transfusion practice could be realized. The goal of this report is to describe our experience to date and to provide a foundation upon which future initiatives may be based.

MATERIALS AND METHODS

Local context

In Rwanda, blood components are all separately available (red blood cells, platelets, plasma and cryoprecipitate) through hospital laboratories in tertiary care centres or via Zipline© (2023 Zipline International Inc.), a drone-based blood delivery system [11]. Blood donation is 100% voluntary and order fulfilment exceeded 99% of requests in

2022 [12]. Rwanda has a small geographical area compared with many other countries (26,338 km^2) but travel times remain substantial and cold chain infrastructure is limited [13]. As course participants were invited from all five Rwandan provinces, some travelling in excess of 8 h, they came with varying experience on blood product order fulfilment times along with varying hospital laboratory testing capabilities. Rwandan faculty were provided with a teaching stipend for the preparation and delivery of course content, and meal catering was provided for all course participants. The Rwanda Biomedical Centre/Blood Transfusion Division (RBC/BTD) also provided a travel stipend to course participants as per their standard practice for continuing professional development.

Course planning

Transfusion Camp was first taught in Rwanda to 27 postgraduate trainees in June 2022 [14]. Through course debriefing, participating Rwandan faculty and trainees concluded that practicing clinicians would also benefit from a modified version of *Transfusion Camp*, with the ultimate goal of delivering it themselves to other Rwandan trainees and practitioners. Through the partnership between the University of Rwanda (UR) Department of Anaesthesiology and Critical Care, the University of Toronto's Department of Laboratory Medicine and Pathobiology, Canadian Blood Services (CBS) and the RBC/BTD, a roadmap was defined to address the education-to-practice divide faced by Rwandan clinicians.

A memorandum of understanding between the University Teaching Hospital of Kigali (CHUK) and RBC/BTD was updated to reflect the investment of personnel, time and financial support for future *Transfusion Camp* courses. A concept note was created that defined the objectives and expected outcomes. Additional course funding support was obtained through a grant from the International Society of Blood Transfusion. Initially planned as one course, RBC/BTD support allowed for a second 2-day course to be added due to high demand.

Course content

The workshop offered didactic content in red blood cell, platelet, plasma and cryoprecipitate transfusion, paediatric transfusion, and massive haemorrhage protocols; these lectures were interspersed with case-based small group team-based learning seminars. Lectures and cases were adapted from *Transfusion Camp* Canada for delivery in Rwanda in collaboration with Rwandan physicians specializing in TM, haematology, paediatrics and anaesthesiology, as previously described [14].

Lectures were delivered by both Canadian and Rwandan faculty in English, while team-based learning seminars were led entirely by local Rwandan faculty (in both English and Kinyarwanda as per group preference). Advocating for Rwandan faculty to share teaching roles as much as possible was important feedback from the pilot course of *Transfusion Camp* Rwanda in 2022 [14].

Following delivery of the *Transfusion Camp* curriculum, two workshop sessions were held, each moderated by Rwandan physician leads. In the first, attendees were divided into four groups with the purpose of reviewing recently updated Rwandan national transfusion guidelines for the following: (1) red blood cells, (2) platelets, (3) plasma and cryoprecipitate and (4) massive haemorrhage [15]. As a means of stimulating discussion, differences in content between these guidelines and clinical practice were emphasized.

In the second set of workshop groups, attendees were asked to reflect on how to shift transfusion practice in Rwanda to be in line with these national guidelines. The ideas that emerged were identified and summarized according to the principles of Interpretive Description [16]. Specifically, groups of 5–6 individuals of varying background and a shared interest in TM practice were assigned a moderator who was a local Rwandan faculty. The moderator posed questions and provided reference material to focus the discussion (Table 1). Moderators were encouraged to let discussion evolve naturalistically, in either English or Kinyarwanda. If the discussion was in Kinyarwanda and the note taker was English speaking, permission was sought to record that

TABLE 1 Workshop reference materials and guiding questions for discussion.

Subject	Reference material	Initial questions
Communication	Choosing Wisely Canada, 'Why Give Two When One Will Do' poster [25]	Would a similar communication strategy work in Rwanda? If so, how could it be promoted? If not, what other type of strategies exist?
Institutional Approval	International Society for Blood Transfusion, 'The Role of Transfusion Committees' [26]	How common are hospital transfusion committees in Rwanda? Are they useful, and if so, what are the barriers to creating them?
Practice Audits	Ontario Regional Blood Coordinating Office, 'RBC Audit' [27]	Can such audits be feasible in Rwandan healthcare facilities? What are the barriers? What modifications would make them work better?
Education	University of Rwanda College of Medicine and Health Sciences, 'Bachelor of Medicine and Surgery, Revised 5-Year Curriculum (2015)'	How are medical trainees currently taught about transfusing blood products? Are there ways this can be improved?

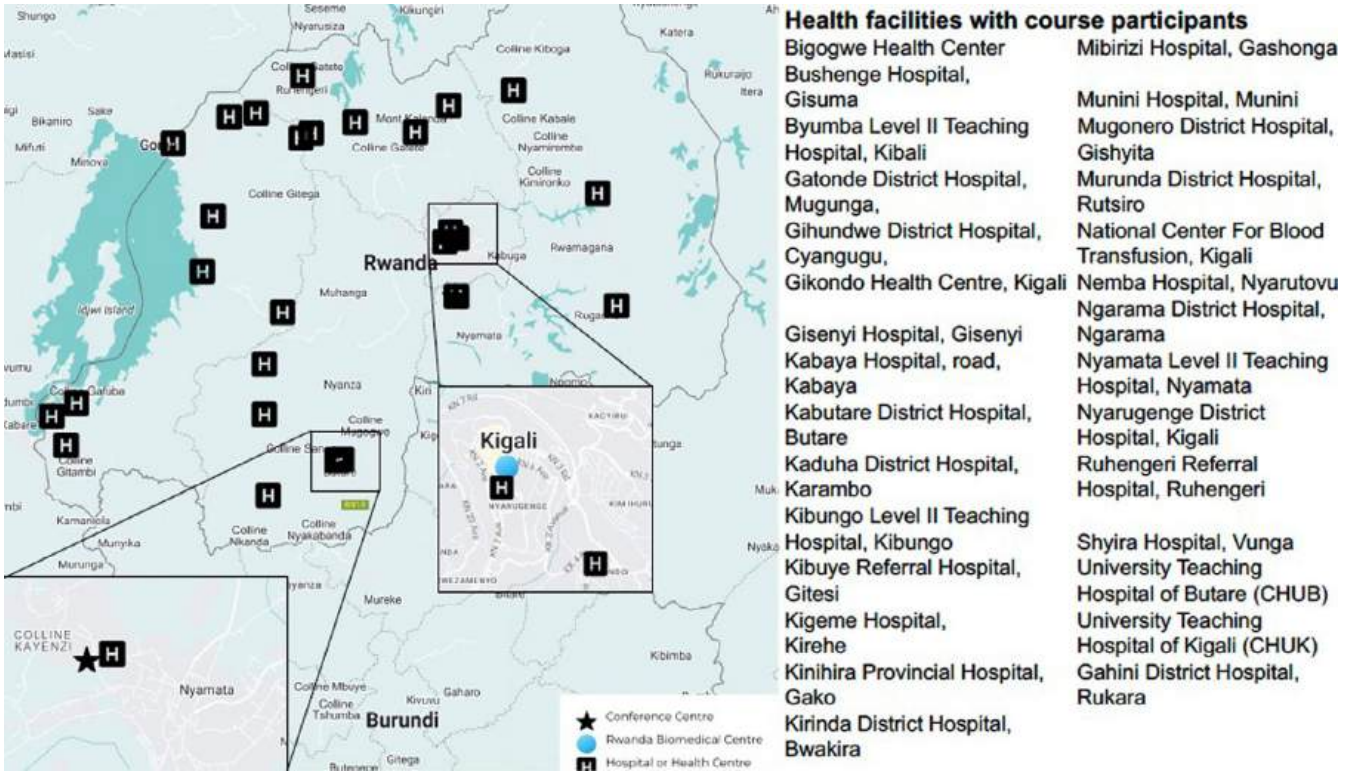

FIGURE 1 Course participants travelled from throughout Rwanda to attend one of two 2-day *Transfusion Camp* Rwanda train-the-trainer courses in Nyamata, Rwanda.

portion of the discussion for translation afterwards. Each workshop was held twice with different attendees.

RESULTS

Participants

Fifty-one Rwandan healthcare workers travelled from across the country to participate in one of two 2-day *Transfusion Camp* train-the-trainer workshops (Figure 1). Approximately three-quarters were physicians, including general practitioners and specialists, while the remainder were either laboratory technicians or quality assurance specialists (Table 2).

Most attendees completed feedback forms. Fewer were received at baseline (68%) than at course completion (86%) as some attendees arrived after the forms had been distributed. Course evaluations were positive (Table 3), with participants reporting that it met their educational needs in TM, and that they would like to participate in more *Transfusion Camp* courses in the future. Specific feedback included an appreciation for learning in an interactive and multidisciplinary environment. When evaluated for teaching efficacy (Table 4), pre- and post-course evaluations demonstrated effectiveness in the Reaction category of the Kirkpatrick model. The largest improvement was seen in self-evaluation of knowledge and skills in TM, with 39% of participants ranking themselves as advanced or expert in this domain before attending, increasing to 91% immediately afterwards (Table 4). Less improvement seen in self-rated capability and confidence in teaching TM, with participants having high baseline confidence (87% were at least fairly confident prior to the course vs. 96% afterwards). Participants endorsed plans to apply guidelines to their daily practice, implement transfusion guidelines at their local institution and teach the TM curriculum themselves.

Transfusion Camp Rwanda workshops

Communication

The group noted that many physicians in Rwanda were not aware that national transfusion guidelines were available, and that guideline dissemination needed improvement. While different strategies were considered (e.g., easy-to-read posters in clinical locations), the most effective approach was believed to be using established hospital communication networks: emphasizing in-person meetings wherever possible. While participants were urged to take the lead in developing communication strategies at their home institution, the most effective delivery of information was thought to be provided by local opinion leaders. In addition, while presentations at the hospital or provincial level were recognized as an efficient means of reaching a large

TABLE 2 Participant demographics.

Number of course participants	51
Specialty	
Anaesthesiology	5
Blood Transfusion Division employee (laboratory technician or quality assurance officer)	12
Emergency and critical care	1
General practitioner	22
General surgeon	2
Internal medicine	1
Non-physician anaesthetist	1
Obstetrician and gynaecologist	3
Orthopaedic surgery	1
Paediatrics	3
Hospital type (total of 28 institutions represented)[a]	
Health Centre	2
District Hospital	18
Level II Teaching Hospital	4
Referral Hospital	2
Tertiary Referral Hospital	2
Years in practice[b]	
<1 year	8
1–5 years	16
5–10 years	7
>10 years	12

[a]Does not include laboratory facilities.
[b]Data available for 43 participants.

TABLE 3 Course evaluation.

	N (%)
This course met my transfusion medicine education needs[a]	
Strongly agree	33 (77)
Agree	10 (23)
Neutral	0 (0)
Disagree	0 (0)
Strongly disagree	0 (0)
Course content was directly applicable to my daily practice	
Strongly agree	29 (66)
Agree	14 (32)
Neutral	0 (0)
Disagree	1 (2)
Strongly disagree	0 (0)
I would like to participate in more *Transfusion Camp* courses	
Strongly agree	40 (91)
Agree	4 (9)
Neutral	0 (0)
Disagree	0 (0)
Strongly disagree	0 (0)
Interactive nature of the course group seminars was beneficial to my learning	
Strongly agree	41 (93)
Agree	3 (7)
Neutral	0 (0)
Disagree	0 (0)
Strongly disagree	0 (0)
I liked learning with my peers from disciplines different than my own[a]	
Strongly agree	36 (84)
Agree	7 (16)
Neutral	0 (0)
Disagree	0 (0)
Strongly disagree	0 (0)

[a]Missing data on one participant.

audience, departmental meetings were favoured, tailoring messaging to the specific audience (e.g., anaesthesia vs. obstetrics, or physician vs. nursing staff); attendance could be encouraged through continuing professional development credits. While the duration of training sessions might vary (e.g., in some departments a bullet-point summary would suffice, while others may prefer a morning seminar), regular outreach was felt to be important. This would provide opportunity to report compliance over time with national guidelines.

For communicating the Rwandan national guidelines for massively bleeding patients, it was deemed desirable to extract the massive transfusion protocol from within the guidelines as its own stand-alone document, post a simplified process flow diagram in areas where massive haemorrhage might occur (e.g., in operating theatres), and perform regular simulation exercises as part of the communication strategy.

Auditing

The group noted in the past year, RBC/BTD hemovigilance visits to the hospital had begun to include reviewing physician practice patterns; previously, the focus had been on product discard rates and adverse transfusion reactions. Prospective transfusion order screening by laboratory staff was occurring to varying degrees (e.g., requests to issue red blood cells to non-bleeding patients). Even after a physician order was accepted by the hospital laboratory, the local blood supplier (either RBC/BTD, or Zipline©) occasionally questioned orders for appropriateness. However, these activities were not felt to represent true auditing, because they did not involve the systematic comparison of current practices against an established standard.

Rwanda was thought to be well-positioned to perform both retrospective and prospective auditing as transfusion orders are universally submitted to hospital laboratories using a standardized paper requisition that prompts the physician to provide the order indication and

TABLE 4 Participant feedback.

	Pre-course	Post-course
	N = 31 (%)	*N* = 44 (%)
I would rate my knowledge of transfusion medicine as[a]		
Expert	1 (3)	10 (23)
Advanced	9 (29)	30 (70)
Intermediate	21 (68)	3 (7)
Beginner	0 (0)	0 (0)
None	0 (0)	0 (0)
I would rate my knowledge of transfusion medicine patient care guidelines as[a]		
Expert	2 (7)	16 (36)
Advanced	8 (27)	24 (55)
Intermediate	20 (66)	4 (9)
Beginner	0 (0)	0 (0)
None	0 (0)	0 (0)
I would rate my knowledge on how to deliver interactive and evidence-based education as[a]		
Expert	1 (3)	11 (25)
Advanced	15 (49)	27 (63)
Intermediate	14 (45)	5 (2)
Beginner	1 (3)	0 (0)
None	0 (0)	0 (0)
I would rate my confidence in teaching transfusion medicine to medical students and residents as[a]		
Completely confident	9 (30)	26 (59)
Fairly confident	15 (50)	15 (34)
Neutral	3 (10)	2 (5)
Somewhat confident	3 (10)	1 (2)
Not at all confident	0 (0)	0 (0)
I would rate my confidence in caring for my patients following Rwanda transfusion medicine guidelines as		
Excellent	7 (23)	34 (77)
Good	20 (64)	10 (23)
Fair	3 (10)	0 (0)
Poor	1 (3)	0 (0)
Very poor	0 (0)	0 (0)
I would rate my confidence in leading my hospital department in adopting transfusion medicine guidelines as		
Completely confident	11 (35)	29 (66)
Fairly confident	18 (58)	13 (30)
Neutral	2 (7)	2 (4)
Somewhat confident	0 (0)	0 (0)
Not at all confident	0 (0)	0 (0)
I would rate the transfusion supplies and resources available at my hospital department to perform transfusions on patients as[b]		
Excellent	10 (34)	18 (41)
Good	14 (48)	19 (42)
Fair	5 (17)	5 (11)
Poor	0 (0)	2 (4)
Very poor	0 (0)	1 (2)
Transfusion Camp has provided me with useful reference materials for my transfusion practice in my hospital department[b]		
Strongly agree		32 (76)
Agree		10 (24)
Neutral		0 (0)
Disagree		0 (0)
Strongly disagree		0 (0)
Do you intend to teach future *Transfusion Camp* courses?[a]		
Yes		36 (84)
No		0 (0)
Not sure		7 (16)
Do you feel like you could now teach a *Transfusion Camp* seminar yourself?		
Yes		33 (75)
No		1 (2)
Not sure		10 (23)
Do you intend to apply the transfusion medicine guidelines from this course in your daily practice?		
Yes		44 (100)
No		0 (0)
Not sure		0 (0)

[a]Missing data on one participant.
[b]Missing data on two participants.

relevant laboratory values. Incomplete forms or verbal orders were not commonly accepted. When emergency requests for blood products were raised (where prospective auditing was recognized as more challenging), laboratory staff within the workshop groups noted that a separate special request form was available, although many clinicians admitted they were unaware of this form. Transfusion orders in Rwandan hospitals are not submitted electronically, but there was confidence that they were routinely translated into laboratory ledgers and could be used for retrospective audits.

Despite being able to capture physician orders, the group concluded that meaningful, systematic auditing could only be pursued after other quality improvement initiatives were in place. Existing national transfusion guidelines would need to be established as the local practice standard through official institutional endorsement and then effectively incorporated into departmental policies. The creation of clinician-led auditing teams was deemed a necessary pre-requisite, rather than relying solely on laboratory or visiting RBC/BTD staff. Nursing unit managers could be tasked with creating such teams. Auditing could also extend to the review of adverse reactions and practice errors. Audit teams would effectively communicate their observations to their hospital's transfusion committee. Currently,

auditing by either laboratory or RBC/BTD staff is generally not communicated to clinicians, while transfusion reactions and errors are largely confined to departmental mortality and morbidity rounds. When provided with the results of audits, hospital transfusion committees (in collaboration with RBC/BTD) would be better able to address gaps in practice. Ideally, the results of audits would be compared between hospitals and between departments to further increase the uptake of transfusion guidelines.

Institutional approval

While representatives from RBC/BTD noted that, several years prior, they had directed all hospitals in Rwanda to establish transfusion committees, members of the workshop group observed that many transfusion committees had become largely non-functional. Some hospitals relied instead on committees whose task was to review and approve any hospital guideline or policy; once passed, this guideline would be sent to the hospital's Director General (DG) for final approval. For hospitals that lacked such an approval process, national guidelines were simply accepted as a practice standard as written. In both models, however, local physicians were often unaware that national transfusion guidelines existed. Having a dedicated hospital transfusion committee, with a defined responsibility of improving local transfusion practice, would be of benefit. Ideas for ensuring transfusion committee sustainability included appointing a local ambassador with accountability for ensuring that such committees were fulfilling their mandate, the nomination of other committee members by the hospital DG, and provision of committee terms of reference by the RBC/BTD. A functioning hospital transfusion committee would not only approve clinical policies but would ensure policies were communicated to hospital staff, review audit results and ensure gaps in practice were addressed.

Education

This workshop group reported that the Rwandan medical school curriculum (currently 5 years in duration) focused most of its TM teaching in fourth year, during which a module in immunology and haematology provided students 10 credits towards their degree. This allots 100 h of instruction (divided into didactic lectures, seminars, and laboratory work), with little time dedicated to how to appropriately prescribe blood products. While there was some inclusion of transfusion practice in other modules (e.g., surgery and paediatrics), the discussion there was deemed superficial. Due to time constraints, it would not be possible to fully incorporate the *Transfusion Camp* curriculum into the fourth-year haematology module (although the existing curriculum might be more effectively delivered using similar pedagogical techniques, such as team-based learning). In addition, the content of *Transfusion Camp* was felt to be of greatest benefit to individuals who were engaged in some clinical work within hospitals.

The ideal time to provide the *Transfusion Camp* curriculum was therefore felt to be in the first year of postgraduate training, the internship year, during which many residency programmes share common educational rotations. An annual 1-week retreat would be most effective, noting a similar model was already in place for the teaching of paediatric medicine. One proposal was that attendees of *Transfusion Camp* Rwanda could subsequently serve as educational ambassadors at the hospital level, in the form of mentorship, administrative leadership and continuing professional development initiatives. A separate series of *Transfusion Camps* targeted towards practicing physicians was also proposed. While the need for long-term funding of *Transfusion Camp* Rwanda was acknowledged, this group was confident that both hospitals and governmental structures such as RBC/BTD would offer support if the teaching resulted in the more judicious use of blood products and better patient outcomes.

DISCUSSION

The *Transfusion Camp* Rwanda train-the-trainer course demonstrated that Rwandan physicians and laboratory technicians from across the country are willing and interested in teaching TM, and after participating in the course, felt well-equipped in their knowledge and their ability to teach others. Other strengths of this course included raising awareness of the RBC/BTD role in the availability of blood components and their expertise to clinicians, along with bringing laboratory technicians and clinicians together to understand one another's perspectives.

While previous studies have shown that educational support in resource-limited settings can improve transfusion practice (e.g., in the post-war Balkan region, [17]) and decrease the need for transfusion for conditions such as post-partum haemorrhage (e.g., in Colombia, [18]), we believe our course workshop is the first to explore the feasibility of introducing a TM curriculum into such an environment.

The primary goal of the train-the-trainer initiative in Rwanda was to build a broader framework within which any future educational initiatives could be most effectively pursued. Following the principles of Interpretive Description [16], the authors leveraged their knowledge of strategies for improving transfusion practice to create a theoretical scaffolding upon which to begin the inquiry, which was pursued through the workshop group discussions. The summary reports from these discussions provide a detailed foundation upon which to plan. One future strategy might be to incorporate the *Transfusion Camp* Rwanda curriculum as a 1-week educational retreat during the internship year. Invitations would be extended to the DG of each participant's home hospital, tasked with supporting a hospital transfusion committee, and other local opinion leaders who would help communicate the national transfusion guidelines via departmental rounds. Uptake could be assessed through before-and-after audits of blood transfusion orders. As previously described [19], audit results could guide broader quality indicators of good transfusion practice and be

used to identify opportunities for further intervention. The feasibility of such a strategy is supported by the institutional culture observed within the Rwandan medical system, one in which RBC/BTD oversees transfusion operations within the country's hospitals. Importantly, while the Rwandan government is operating in an environment of relative economic scarcity compared with more highly resourced countries such as Canada, it has made significant investments towards providing an adequate and safe blood supply to its citizens. The prioritization of transfusion safety is perhaps the most important indicator that further advancement of the *Transfusion Camp* curriculum in Rwanda is achievable.

An important limitation of this study is its reliance on subjective feedback from attendees to demonstrate course efficacy. Such feedback is prone to bias (e.g., attendees may have exaggerated the value of the course as a means of expressing appreciation for its delivery) and does not necessarily portend a change in more objective measures. In the current study, only the Reaction category of the Kirkpatrick model was assessed, although sustained improvement in test scores (the Learning category) had previously been demonstrated during the pilot phase of the *Transfusion Camp* Rwanda project [8], and an intention to both modify future transfusion practices and teach others to do the same was reported by the current cohort of course attendees. Future projects will aim to determine whether delivery of *Transfusion Camp* curricula has or will change transfusion practice behaviour [20] and will be informed by concepts drawn from the field of implementation science [21].

The TRAIN framework for training of trainers in global health partnerships guided this course, creating more capacity for sustainable education delivery. However, to change practice, the workshops highlighted that cultural and contextual nuances must be considered and could impede a sustainable training cascade as highlighted in the TRAIN framework [9]. One model that addresses this is the Consolidated Framework for Implementation Research [22]. This model conceptualizes five major domains: (1) the intervention, (2) the external setting (e.g., the economic, political, and social context), (3) the internal setting (e.g., the structural, political, and cultural environment of the hospital), (4) the individuals involved and (5) the process of the implementation [23]. As was noted in the workshops, the specific context and organizational culture must also be addressed to change transfusion practice [24].

In conclusion, continuing professional development in TM was well-received in Rwanda using a train-the-trainer approach. Course participant self-evaluation of knowledge and skills improved, and participants reported a commitment to teach *Transfusion Camp* in the future. Course workshops noted that effecting change in transfusion practice requires a detailed understanding of the local medical and educational environment. Future efforts should study whether these educational initiatives effect change in practice and patient outcomes.

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CONFLICT OF INTEREST STATEMENT

Jacob Pendergrast: Research funding from CBS and consulting fees from GBT, Novartis, CSL Behring and Novo Nordisk. Yulia Lin: Research funding from CBS and Octapharma; Consultant with Choosing Wisely Canada. Remaining authors: None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Weimer A, Tagny CT, Tapko JB, Gouws C, Tobian AAR, Ness PM, et al. Blood transfusion safety in sub-Saharan Africa: a literature review of changes and challenges in the 21st century. Transfusion. 2019;59:412–27.
2. Lin Y, Khandelwal A, Kapitany C, Chargé S. Transfusion Camp: successes and challenges in scaling and expanding a transfusion medicine education program. Transfus Apher Sci. 2023;62:103629.
3. O'Brien KL, Champeaux AL, Sundell ZE, Short MW, Roth BJ. Transfusion medicine knowledge in postgraduate year 1 residents. Transfusion. 2010;50:1649–53.
4. Alvarado-Navarro DM, Cázares-Tamez R, Pérez-Chávez F, Díaz-Olachea CG, Lee-González BA, Ramos-García AG, et al. Assessment of physicians in training's knowledge of transfusion medicine at a university hospital. Med Univ. 2016;18:139–47.
5. Lin Y, Haspel RL. Transfusion medicine education for non-transfusion medicine physicians: a structured review. Vox Sang. 2017;112:97–104.
6. Yeung KCY, Kapitany C, Chargé S, Callum J, Cserti-Gazdewich C, D'Empaire PP, et al. Transfusion camp: a retrospective study of self-reported impact on postgraduate trainee transfusion practice. Transfusion. 2023;63:839–48.
7. Lin Y, Tilokee E, Chargé S, Alam A, Cserti-Gazdewich C, Lau W, et al. Transfusion Camp: a prospective evaluation of a transfusion education program for multispecialty postgraduate trainees. Transfusion. 2019;59:2141–9.
8. Hagumimana J, Skelton T, Pendergrast J, Nizeyimana F, Masaisa F, Kanyamuhunga A, et al. Transfusion Camp Rwanda: a prospective feasibility study evaluating the delivery of Transfusion Camp to a multidisciplinary group of postgraduate medical trainees in Rwanda. Transfusion. 2023;63:2170–8.
9. Mormina M, Pinder S. A conceptual framework for training of trainers (ToT) interventions in global health. Global Health. 2018;14:1–11.
10. The Kirkpatrick Model of Training Evaluation: Guide. Available from: https://www.valamis.com/hub/kirkpatrick-model. Last accessed 18 Oct 2023.
11. Nisingizwe MP, Ndishimye P, Swaibu K, Nshimiyimana L, Karame P, Dushimiyimana V, et al. Effect of unmanned aerial vehicle (drone) delivery on blood product delivery time and wastage in Rwanda: a retrospective, cross-sectional study and time series analysis. Lancet Glob Health. 2022;10:e564–9.

12. Rwanda Biomedical Centre Biomedical Services Department Blood Transfusion Division. Annual Report 2022. 2022.
13. Twilley N. Africa's cold rush and the promise of refrigeration. The New Yorker. 2022. Available from: https://www.newyorker.com/magazine/2022/08/22/africas-cold-rush-and-the-promise-of-refrigeration. Last accessed 18 Oct 2023.
14. Skelton T, Nizeyimana F, Pendergrast J, Hagumimana J, Masaisa F, Kanyamuhunga A, et al. Transfusion medicine education delivery in Rwanda: adapting Transfusion Camp to a resource-limited setting. Transfusion. 2023;63:2159–69.
15. National Directives on Rational Use of Blood and Blood Components in Rwanda. Republic of Rwanda Ministry of Health. Version 03. Kigali. 2018.
16. Hunt MR. Strengths and challenges in the use of interpretive description: reflections arising from a study of the moral experience of health professionals in humanitarian work. Qual Health Res. 2009;19:1284–92.
17. Kovacevic P, Dragic S, Kovacevic T, Momcicevic D, Festic E, Kashyap R, et al. Impact of weekly case-based tele-education on quality of care in a limited resource medical intensive care unit. Crit Care. 2019;23:220.
18. Escobar MF, Echavarria MP, Vasquez H, Nasner D, Ramos I, Hincapié MA, et al. Experience of a telehealth and education program with maternal and perinatal outcomes in a low-resource region in Colombia. BMC Pregnancy Childbirth. 2022;22:604.
19. Spradbrow J, Cohen R, Lin Y, Armali C, Collins A, Cserti-Gazdewich C, et al. Evaluating appropriate red blood cell transfusions: a quality audit at 10 Ontario hospitals to determine the optimal measure for assessing appropriateness. Transfusion. 2016;56:2466–76.
20. Davis D. Does CME work? An analysis of the effect of educational activities on physician performance or health care outcomes. Int J Psychiatry Med. 1998;28:21–39.
21. Price DW, Wagner DP, Kevin Krane N, Rougas SC, Lowitt NR, Offodile RS, et al. What are the implications of implementation science for medical education? Med Educ. 2015;20:20.
22. Carney PA, Crites GE, Miller KH, Haight M, Stefanidis D, Cichoskikelly E, et al. Building and executing a research agenda toward conducting implementation science in medical education. Med Educ. 2016;21:32405.
23. Damschroder LJ, Aron DC, Keith RE, Kirsh SR, Alexander JA, Lowery JC. Fostering implementation of health services research findings into practice: a consolidated framework for advancing implementation science. Implement Sci. 2009;4:50.
24. Ryan CT, Rosengart TK. Commentary: culture trumps (transfusion) guidelines. J Thorac Cardiovasc Surg. 2022;163:1025–6.
25. CWC Blood Conservation Poster. Choosing Wisely Canada. 2020. Available from: https://www.transfusion.ca/getmedia/7d6c1622-1cdc-4fa1-8109-0c645923f05d/CWC-Blood-Conservation-Poster_March-2020.pdf.aspx. Last accessed 20 Oct 2023.
26. Moss R. The Role of Transfusion Committees. International Society of Blood Transfusion Patient Blood Management Resources. Available from: https://www.isbtweb.org/isbt-working-parties/clinical-transfusion/resources/patient-blood-management-resources/12-the-role-of-transfusion-committees.html. Last accessed 20 Oct 2023.
27. ORBCoN Red Blood Cell Audit. Ontario Regional Blood Coordinating Network. Available from: https://transfusionontario.org/wp-content/uploads/2020/06/Red-Blood-Cell-Audit.pdf. Last accessed 20 Oct 2023.

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Characteristics of red blood cell transfusion among very preterm infants in China

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Abstract

Background and Objectives: National-level data on the incidence of red blood cell (RBC) transfusions and outcomes among very preterm infants (VPIs) are lacking in China. This study aims to describe the use and variation of RBC transfusion among VPIs in China.

Materials and Methods: This cohort study was conducted among 70 tertiary hospitals participating in the Chinese Neonatal Network (CHNN) from 2019 to 2020 across China. All VPIs admitted to the CHNN neonatal intensive care units (NICUs) were included.

Results: A total of 13,447 VPIs were enrolled, of whom 7026 (52.2%) received ≥1 RBC transfusions. The mean number of transfusions per infant was 2 (interquartile range [IQR] 1–4 times) and the median age at first transfusion was 15 days (IQR 3–27 days). The transfusion rate was higher in critically ill infants compared with non-critically ill infants (70.5% vs. 39.3%). The transfusion rate varied widely (13.5%–95.0%) between different NICUs. The prevalence of death, severe intra-ventricular haemorrhage, necrotizing enterocolitis (NEC) or spontaneous intestinal perforation (SIP), sepsis, bronchopulmonary dysplasia (BPD), severe retinopathy of prematurity (ROP) and cystic periventricular leukomalacia (cPVL) was significantly higher in the transfused group. Among non-critically ill infants, RBC transfusion was independently associated with BPD, severe ROP and cPVL.

Conclusion: Our study, providing the first baseline data on RBC transfusions among VPIs in China, shows an alarmingly high RBC transfusion rate with significant site variations. There is an urgent need for national guidelines on RBC transfusions for VPIs in China.

Keywords

Chinese Neonatal Network, red blood cell transfusion, very preterm infants

Highlights

- National data regarding the current practice of transfusion in Chinese neonatal intensive care units (NICU) are lacking.
- Utilizing the latest data (2019–2020) from the Chinese Neonatal Network, we have mapped Chinese transfusion practice for very preterm infants and provide a valuable starting point for further studies and highlight the need for guideline development.
- To avoid the confounding effect of disease severity, we presented transfusion practice separately in critically ill infants and non-critically ill infants. This may provide a new and rational way to benchmark NICU treatments.

INTRODUCTION

Advancements in neonatal–perinatal care over the recent decades have contributed to the increased survival of very preterm infants (VPIs) (<32-week gestational age [GA]) worldwide, including in China [1]. Red blood cell (RBC) transfusion is an important treatment for VPIs, addressing acute and chronic indications. VPIs have a heightened risk for anaemia due to their immature haematopoietic system, impaired erythropoiesis and frequent iatrogenic laboratory loss [2, 3]. Studies indicated that more than 50% of VPIs receive RBC transfusions during hospitalization [4–6].

While RBC transfusion can be life-saving for VPIs, inappropriate RBC transfusions may lead to adverse outcomes. Previous studies have associated RBC transfusion with increased rates of complications, including intra-ventricular haemorrhage (IVH) [7–9], necrotizing enterocolitis (NEC) [10–12], retinopathy of prematurity (ROP) [13, 14], and bronchopulmonary dysplasia (BPD) [15]. Moreover, global guidelines for RBC transfusion lack consistency [16, 17], resulting in substantial practice variations across countries, regions and centres. Notably, two large randomized clinical trials reported similar short- and long-term outcomes among extremely low birth weight infants in higher threshold and lower threshold RBC transfusion groups [18, 19].

Despite the critical role of RBC transfusions, there is a scarcity of data regarding their utilization in neonatal intensive care units (NICUs) in China. Furthermore, there is an absence of a national transfusion guideline for VPIs in the country. The objective of this study was to describe the incidence and frequency of RBC transfusions for VPIs in different NICUs across China and to assess the association between RBC transfusions and clinical outcomes.

METHODS

Chinese Neonatal Network and participating hospitals

The Chinese Neonatal Network (CHNN) is a national network of Chinese tertiary NICUs. CHNN collects and reports outcomes and clinical practice data from neonatal intensive care in participating NICUs, and conducts collaborative quality improvement initiatives and research studies. Enrolled hospitals in CHNN are tertiary referral hospitals with large neonatal services and recognized expertise in caring for high-risk neonates. Since 1 January 2019, CHNN has maintained a standardized clinical database, capturing data on preterm infants <32-week gestation or <1500 g at birth in participating NICUs. A total of 57 hospitals contributed data in 2019, and the number increased to 70 hospitals in 2020.

This study was approved by the Ethics Review Board of Children's Hospital of Fudan University (approval no. 2018-296), which was recognized by all participating hospitals. Waivers of consent were granted at all sites.

Data collection

Trained data abstractors were responsible for the data acquisition in each hospital. Data were directly entered into a customized database with built-in error checking and a standard manual of operations and definitions. De-identified data were electronically transmitted to the CHNN coordinating centre in Children's Hospital of Fudan University. Site investigators were responsible for data quality control in each site. Data such as haemoglobin or RBC specific volume prior to RBC transfusion, transfusion volume, type of RBC and specific indications for RBC transfusions were not available in our database.

Study population

All preterm infants <32-week gestation, who were admitted to CHNN NICUs from 1 January 2019 to 31 December 2020, were included in this study. Infants with incomplete treatment in the NICUs or with major congenital anomalies were excluded. Re-admissions and transfers between participating hospitals were tracked as data from the same infants. Infants were followed until NICU discharge/transfer or death.

Outcomes

Neonatal outcomes included death and major morbidities of severe IVH (grade III or IV), NEC (Bell stage 2 and 3) or spontaneous intestinal perforation (SIP), blood culture positive sepsis, BPD, ROP (grades III–V)

and cystic periventricular leukomalacia (cPVL). Severe IVH was classified as ≥grade 3 according to Papile's criteria [20]. NEC was classified according to Bell's criteria [21]. Sepsis was defined as positive blood or cerebrospinal fluid culture and antibiotic therapy or intent of antibiotics therapy ≥5 days [22]. ROP was diagnosed according to the International Classification of ROP [23]. BPD was defined as either requiring invasive mechanical ventilation or oxygen dependency at 36 weeks post-menstrual age or at the time of discharge/transfer/death if it occurred before 36 weeks [24]. cPVL was defined as the presence of periventricular cysts on cranial ultrasound or magnetic resonance imaging (MRI).

Definitions

GA was determined by obstetric estimate based on prenatal ultrasound, menstrual history, obstetric examination or all of the above. If the obstetric estimate was not available or was inconsistent with the postnatal estimate of gestation for more than 2 weeks, the GA was estimated using the Ballard score [25]. Small for gestational age (SGA) was defined as birth weight <10th percentile for the GA according to the Chinese neonatal birth weight values [26]. Prenatal care was defined as ≥1 pregnancy-related hospital visit during pregnancy. Intensive delivery room resuscitation was defined as endotracheal intubation, cardiopulmonary resuscitation and/or epinephrine to support circulation.

Statistical analysis

Descriptive analysis was used to describe the rate, frequency and initial age of transfusion. The descriptions of RBC transfusion were presented by GA and the severity of illness. The latter was categorized into two subgroups: critically ill group and non-critically ill group. The critically ill group included infants with any cause of death, intensive delivery room resuscitation, severe IVH, sepsis, NEC or SIP, any surgery, ventilation for more than 7 days or inotropic treatment. To describe the variation among participating sites, both crude and adjusted rates of transfusion were calculated. The adjusted rate of transfusion was calculated using a logistic model, adjusting for GA, Apgar score at 5 min, inborn/out born status, sepsis, NEC, BPD, severe IVH and any surgery. The Cochran–Armitage test was used for testing the trend of transfusion rate across GA.

To investigate the relationship between RBC transfusion and neonatal outcomes, demographic characteristics were compared between the transfusion group and non-transfusion group using *t*-test for continuous variables and chi-square test for categorical variables. Logistic regression models were generated to obtain both crude odds ratio and adjusted odds ratio, with the latter one adjusted for GA, SGA, male, multiple birth, inborn, Apgar score at 5 min and any antenatal steroids.

A *p* value of less than 0.05 was considered statistically significant. All analyses were conducted using SAS 9.4 software.

RESULTS

During the study period, a total of 16,016 VPIs were admitted to CHNN NICUs. After excluding 2569 infants due to incomplete treatment ($n = 2484$) or the presence of major congenital anomalies ($n = 85$), the remaining 13,447 were enrolled in our study (Figure 1).

Rate of RBC transfusion

As shown in Table 1, 7026 (52.2%) infants received ≥1 RBC transfusions. The RBC transfusion rates decreased with increasing GA, ranging from 84.9% at 25-week GA to 30.9% at 31-week GA. Even among more mature VPIs born ≥29-week GA, 42.9% (4145 of 9653) received RBC transfusion. The number of RBC transfusions also decreased with increasing GA, while the age of the first RBC transfusion increased. The mean number of transfusions during hospitalization per infant was 2 (interquartile range [IQR] 1–4 times). The median age at the first transfusion for all VPIs was 15 days (IQR 3–27 days), increasing from 2 days at ≤23-week GA to 18 days at 31 weeks; 76.4% of all VPIs received RBC transfusions before 28 days of life.

RBC transfusion in critically ill infants and non-critically ill infants

Table 2 shows RBC transfusion stratified by critically ill infants and non-critically ill infants. Among critically ill infants, 70.5% received RBC transfusion, with a median of 3 (IQR 2–4) transfusions per infant and a median age at first RBC transfusion of 11 days (IQR 2–23). In non-critically infants, although the RBC transfusion rate was 39.3%, a considerable 33.6% (2177 of 6479) of the more mature VPIs born ≥29-week GA received RBC transfusion. The average number of transfusions per non-critically ill infant was two times (IQR 1–2), and the median age at first RBC transfusion was 19 days.

Site variation of RBC transfusion rate

The transfusion rate varied widely among different NICUs, ranging from 13.5% to 95.0%. Only four NICUs had a transfusion rate <20%, while transfusion rates were 20.6%–28.3% in 3 NICUs, 31.0%–37.1% in 7 NICUs, 40.3%–50.0% in 18 NICUs, 51.2%–58.1% in 5 NICUs, 60.5%–69.4% in 8 NICUs, 70.4%–79.0% in 15 NICUs and 10 NICUs had a transfusion rate exceeding 80%. The variation remained significant after adjustment, ranging from 12.7% to 69.2% (Figure 2).

Characteristics of infants with and without RBC transfusion

Table 3 shows baseline characteristics of the enrolled infants. The median GA of all infants was 30.0 (IQR 28.7, 31.0) weeks and the

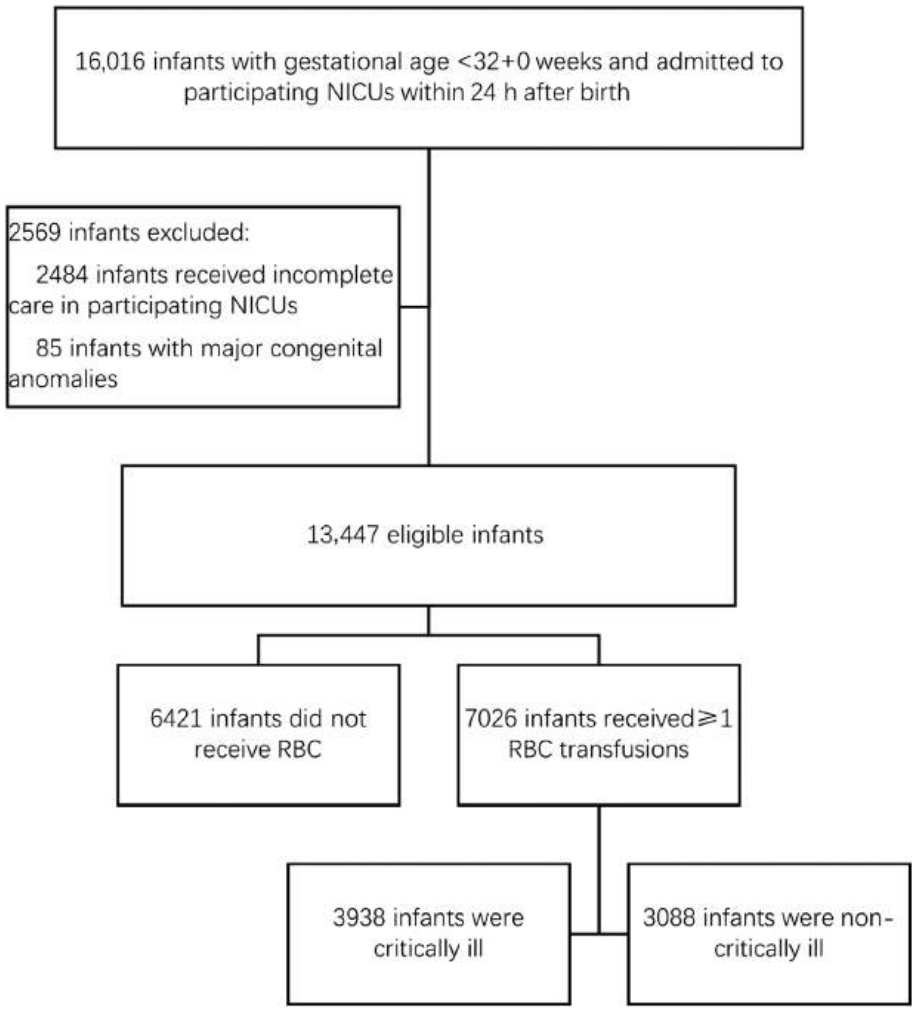

FIGURE 1 Study population. NICU, neonatal intensive care unit; RBC, red blood cell.

TABLE 1 Red blood cell (RBC) transfusion among very preterm infants across gestational age (GA).

GA (weeks)	RBC transfusion rate, *N* (%)[a]	Times of transfusion, median (IQR)[b]	Age at first transfusion, days, median (IQR)
<24	30/49 (61.2)	5.5 (2, 7)	2 (1, 7)
24	64/80 (80.0)	4 (2, 7)	2 (1, 10)
25	202/238 (84.9)	4 (2, 7)	6 (1, 16)
26	390/473 (82.5)	4 (2, 5)	9 (2, 20)
27	807/1017 (79.4)	3 (2, 5)	11 (2, 24)
28	1388/1937 (71.7)	2 (2, 4)	15 (3, 27)
29	1540/2584 (59.6)	2 (1, 3)	17 (4, 28.5)
30	1425/3246 (43.9)	2 (1, 3)	17 (5, 27)
31	1180/3823 (30.9)	2 (1, 2)	18 (6, 28)
Total	7026/13,447 (52.2)	2 (1, 4)	15 (3, 27)

Abbreviation: IQR, interquartile range.
[a]Cochran–Armitage trend test $p < 0.01$.
[b]Calculated among infants received RBC transfusion.

mean BW was 1344.6 ± 310.3 g. GA (29.3 vs. 30.6 weeks, $p < 0.001$) and BW (1225.4 vs. 1475 g, $p < 0.001$) were significantly lower in the transfused group compared with the non-transfused group. SGA, multiple births and intensive delivery room resuscitation were more frequent in the transfused group, while inborn status and delayed umbilical cord clamping were more common in the non-transfused

TABLE 2 RBC transfusion among critically ill and non-critically ill very preterm infants.

GA (weeks)	RBC transfusion rate, *N* (%)[a]		Times of transfusion, median (IQR)[b]		Age at first transfusion, days, median (IQR)	
	Critically ill	Non-critically ill	Critically ill	Non-critically ill	Critically ill	Non-critically ill
<24	30/49 (61.2)	N/A	5.5 (2, 7)	N/A	2 (1, 7)	N/A
24	62/78 (79.5)	2/2 (100.0)	4.5 (2, 7)	3 (3, 3)	2 (1, 10)	8 (8, 8)
25	171/202 (84.7)	31/36 (86.1)	5 (3, 7)	2 (2, 4)	4 (1, 14)	19 (10, 27)
26	301/361 (83.4)	89/112 (79.5)	4 (2, 6)	2 (1, 4)	8 (2, 18)	15.5 (7, 23.5)
27	572/684 (83.6)	235/333 (70.6)	3 (2, 5)	2 (1, 3)	7 (2, 21)	18 (6, 31)
28	834/1041 (80.1)	554/896 (61.8)	3 (2, 5)	2 (1, 3)	11 (2, 23)	19 (9, 30)
29	809/1116 (72.5)	731/1468 (49.8)	2 (1, 4)	2 (1, 2)	13 (3, 25)	20 (9, 33)
30	645/1088 (59.3)	780/2158 (36.1)	2 (1, 3)	2 (1, 2)	12 (3, 25)	20 (8, 29)
31	514/970 (53.0)	666/2853 (23.3)	2 (1, 3)	1 (1, 2)	16 (4, 26)	19 (8, 29)
Total	3938/5589 (70.5)	3088/7858 (39.3)	3 (2, 4)	2 (1, 2)	11 (2, 23)	19 (8, 30)

Abbreviations: GA, gestational age; IQR, interquartile range; RBC, red blood cell.
[a]Cochran–Armitage trend test $p < 0.01$ for both subgroups.
[b]Calculated among infants received RBC transfusion.

group. Significant differences also existed in maternal diabetes, hypertension, premature rupture of membranes >24 h, antepartum haemorrhage, placenta previa and histologic chorioamnionitis between the two groups.

Neonatal outcomes of infants with and without RBC transfusion

Neonatal outcomes in infants with and without RBC transfusion are shown in Table 4. In comparison with the non-transfused group, the prevalence of death, severe IVH, NEC or SIP, sepsis, BPD, severe ROP and cPVL was significantly higher in the transfused group. After adjustment, the risk of all outcomes, except death, remained higher in the transfused group. In the subgroup of non-critically ill infants, RBC transfusion remained independently associated with BPD, severe ROP and cPVL (Table 5).

DISCUSSION

This study provided important data on RBC transfusions in VPIs in China. The findings revealed that more than half of the VPIs received RBC transfusions during their hospitalization, and the use of RBC transfusions exhibited significant variability among different NICUs in China. Notably, even among more mature VPIs born ≥29-week GA or among non-critically ill infants, the RBC transfusion rate remained high (42.9% and 39.3%). We also found significant association between RBC transfusion and adverse neonatal outcomes, especially in non-critically ill VPIs.

In this large multi-centre study of 13,447 VPIs, the overall rate of RBC transfusions was 52.2%. This finding aligns with reports from single-centre studies in United Kingdom and Italy [4, 5], which observed similar RBC transfusion rates and an average of two RBC transfusions per infant. Our results are also consistent with prior studies showing that smaller infants required more transfusions than their more mature counterparts [27, 28]. Furthermore, we confirmed that 75.9% of VPIs born at <29 weeks of GA received RBC transfusions during their hospitalization. This is concordant with a multi-centre study in the United States reporting 78.4% of infants born at 22–28 weeks of GA received RBC transfusions [29]. However, only 49.3% of infants born at 28 weeks of GA received RBC transfusions in the US study, while 71.7% of these infants received RBC transfusions in our study. It is important to note that 42.9% of infants ≥29-week GA, comprising the majority of VPIs in our cohort, received RBC transfusion during their hospital stay. In contrast, in high-income countries, the RBC transfusion rate was only 13.0%–33.7% among infants born at 29- to 32-week GA. These countries also reported a significant decline in RBC transfusion for infants ≥29-week GA after adopting guidelines [9, 30]. A retrospective cohort study in Canada of infants <30 weeks of GA found that clinicians tended to have a higher threshold for transfusion for those with relatively larger gestation [31].

In our study, the most common cause of anaemia in VPIs born at 29- to 32-week GA was anaemia of prematurity. Guidelines to minimize anaemia of prematurity and to identify the transfusion threshold in VPIs born at 29- to 32-week GA are important to reduce RBC transfusions in our NICUs. The difference in RBS transfusion rates between NICUs in China and those in high-income countries indicates that there is substantial room for improvement. We also found that RBC transfusions vary extensively across different NICUs, further indicating an urgent need of the development of a national guideline to promote consistency in RBC transfusion for VPIs and to reduce practice variations in different centres. The benefits of delayed umbilical cord clamping for reducing RBC transfusions had previously been reported [32]. However, only 27.4% of VPIs in our cohort received delayed umbilical cord clamping.

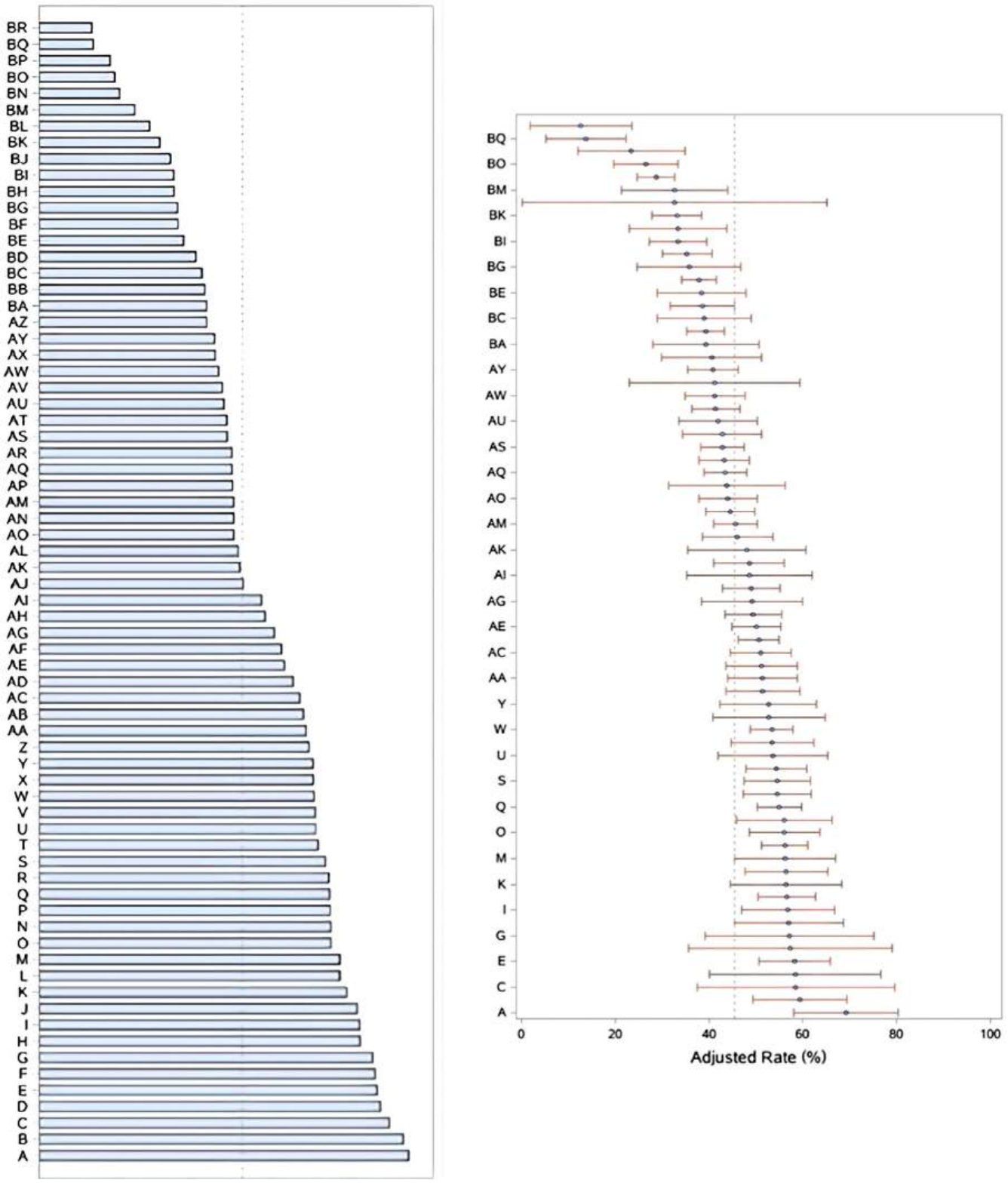

FIGURE 2 Adjusted red blood cell transfusion rate among 70 sites in China. Adjusted for gestational age, Apgar score in 5 min less than 7, Inborn, sepsis, necrotizing enterocolitis, bronchopulmonary dysplasia, severe intra-ventricular haemorrhage and any surgery.

Although RBC transfusions are generally considered to be safe, emerging evidence indicates associations between RBC transfusions and major morbidities of preterm infants. Previous studies have identified several potential complications associated with transfusions, including death [33], IVH, NEC (the so-called TANEC, 'transfusion-associated necrotizing enterocolitis'), ROP and BPD. The underlying

TABLE 3 Baseline characteristics of the total study population and comparison between RBC-transfused and non-transfused group.

	Total ($N = 13,447$)	Non-transfused ($N = 6421$)	RBC-transfused ($N = 7026$)	Test value	*p* value
Maternal characteristics					
Maternal age, mean (SD)	31.0 (4.9)	31.1 (4.9)	31.0 (4.9)	1.340	0.179
Primigravida	6928/13,368 (51.8)	3233/6388 (50.6)	3695/6980 (52.9)	7.231	0.007
Prenatal care	12,882/12,982 (99.2)	6210/6254 (99.3)	6672/6728 (99.2)	0.703	0.402
Diabetics	2541/13,356 (19.0)	1299/6380 (20.4)	1242/6976 (17.8)	14.138	<0.001
Hypertension	2640/13,364 (19.8)	1120/6384 (17.5)	1520/6980 (21.8)	37.683	<0.001
Any antenatal steroids	10,385/12,823 (81.0)	5145/6173 (83.4)	5240/6650 (78.8)	43.039	<0.001
PROM > 24 h	3214/12,886 (24.9)	1737/6215 (28.0)	1477/6671 (22.1)	57.973	<0.001
Caesarean delivery	7940/13,418 (59.2)	3859/6414 (60.2)	4081/7004 (58.3)	4.995	0.025
Antepartum haemorrhage	1339/13,447 (10.0)	577/6421 (9.0)	762/7026 (10.9)	12.935	<0.001
Placenta previa	402/13,447 (3.0)	160/6421 (2.5)	242/7026 (3.4)	10.496	0.001
Antenatal intervention	301/12,937 (2.3)	144/6263 (2.3)	157/6674 (2.4)	0.040	0.841
Histologic chorioamnionitis	1462/10,011 (14.6)	783/4920 (15.9)	679/5091 (13.3)	13.327	<0.001
Infant characteristics					
Gestational age, weeks, median (IQR)	30.0 (28.7, 31.0)	30.6 (29.6, 31.3)	29.3 (28.0, 30.4)	42.282	<0.001
Birth weight, g, mean (SD)	1344.6 (310.3)	1475.0 (288.5)	1225.4 (280.2)	50.790	<0.001
SGA	918/13,398 (6.9)	246/6402 (3.8)	672/6996 (9.6)	173.955	<0.001
Male	7607/13,437 (56.6)	3638/6418 (56.7)	3969/7019 (56.6)	0.026	0.872
Multiple birth	4059/13,447 (30.2)	1875/6421 (29.2)	2184/7026 (31.1)	5.648	0.018
Inborn	10,188/13,447 (75.8)	5055/6421 (78.7)	5133/7026 (73.1)	58.715	<0.001
Delayed umbilical cord clamping	2790/10,199 (27.4)	1512/4988 (30.3)	1278/5211 (24.5)	42.959	<0.001
Intensive delivery room resuscitation (CRP or intubation)	3266/13,172 (24.8)	1033/6327 (16.3)	2233/6845 (32.6)	468.213	<0.001
Apgar score at 1 min, median (IQR)	8 (7, 9)	8 (7, 9)	8 (6, 9)	22.986	<0.001
Apgar score at 5 min, median (IQR)	9 (8, 10)	9 (9, 10)	9 (8, 9)	22.148	<0.001

Abbreviations: CRP, C-reactive protein; IQR, interquartile range; PROM, premature rupture of membranes; RBC, red blood cell; SGA, small for gestational age.

TABLE 4 RBC transfusion and neonatal outcomes among very preterm infants.

Outcomes	Non-transfused ($N = 6421$)	RBC-transfused ($N = 7026$)	*p* value	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
Death	229/6421 (3.6)	328/7026 (4.7)	<0.001	1.32 (1.12, 1.57)	0.52 (0.42, 0.65)
Severe IVH (grade III or IV)	117/5893 (2.0)	499/6557 (7.6)	<0.001	4.07 (3.31, 4.99)	2.96 (2.35, 3.73)
NEC (Bell stage 2 and 3) or SIP	73/6421 (1.1)	401/7026 (5.7)	<0.001	5.26 (4.09, 6.77)	6.31 (4.78, 8.33)
Sepsis	212/6421 (3.3)	859/7026 (12.2)	<0.001	4.08 (3.50, 4.76)	3.63 (3.07, 4.29)
BPD	1792/6211 (28.9)	4821/6853 (70.4)	<0.001	5.85 (5.43, 6.31)	3.23 (2.95, 3.54)
Severe ROP (grade III–V)	66/5151 (1.3)	285/6399 (4.5)	<0.001	3.59 (2.74, 4.71)	1.75 (1.28, 2.38)
cPVL	180/5891 (3.1)	404/6536 (6.2)	<0.001	2.09 (1.75, 2.50)	1.77 (1.44, 2.16)
Hospitalization, days, median (IQR)	36 (28, 46)	55 (43, 69)	<0.001	19.68 (18.99, 20.36)	11.64 (10.97, 12.31)[b]

Abbreviations: BPD, bronchopulmonary dysplasia; CI, confidence interval; cPVL, cystic periventricular leukomalacia; IQR, interquartile range; IVH, intraventricular haemorrhage; NEC, necrotizing enterocolitis; OR, odds ratio; RBC, red blood cell; ROP, retinopathy of prematurity.

[a]Adjust for gestational age, small for gestational age, male, multiple birth, inborn, Apgar score at 5 min and any antenatal steroids.

[b]Risk difference.

TABLE 5 RBC transfusion and neonatal outcomes among non-critically ill very preterm infants.

Outcomes	Non-transfused ($n = 4770$)	RBC-transfused ($n = 3088$)	*p* value	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
BPD	1069/4722 (22.6)	1826/3080 (59.3)	<0.001	4.98 (4.51, 5.49)	3.02 (2.68, 3.39)
Severe ROP (grade III–V)	39/3850 (1.0)	67/2917 (2.3)	<0.001	2.30 (1.54, 3.42)	1.49 (0.95, 2.34)
cPVL	109/4474 (2.4)	94/2891 (3.3)	0.040	1.35 (1.02, 1.78)	1.27 (0.92, 1.74)
Hospitalization, days, median (IQR)	36 (28, 45)	51 (42, 61)	<0.001	15.44 (14.78, 16.11) b	7.68 (7.12, 8.25)[b]

Abbreviations: BPD, bronchopulmonary dysplasia; CI, confidence interval; cPVL, cystic periventricular leukomalacia; IQR, interquartile range; OR, odds ratio; RBC, red blood cell; ROP, retinopathy of prematurity.

[a]Adjust for gestational age, small for gestational age, male, multiple birth, inborn, Apgar score at 5 min and any antenatal steroids.

[b]Risk difference.

pathophysiological reasons and possible causal relationships between transfusion and neonatal morbidities remain unknown. The possible mechanisms include impaired organ blood flow in extreme anaemia, ischaemia/reperfusion injury, increased oxidative injury or the presence of inflammatory mediators in blood products [11, 34]. Two recent randomized clinical trials showed that a higher haemoglobin threshold for transfusion did not improve survival without neurodevelopmental impairment or reduce the likelihood of death or disability at 24 months of age among extremely low birth weight infants [18, 19]. In our study, the prevalence of death, severe IVH, NEC or SIP, sepsis, BPD, severe ROP and cPVL were higher in the transfusion group. However, the design of this investigation precluded the determination of cause-and-effect relationships between these complications and transfusion. The reduction in risk of death in the transfused group after adjustment may be because the majority of deaths occurred before transfusion. Sicker infants required more transfusions to treat their pulmonary or circulatory compromise and were also at higher risk to sustain neurological disabilities and morbidities. In order to reduce the impact of severity of clinical compromise to the outcome, we repeated the analysis among non-critically ill infants, which still demonstrated an independent association between transfusion and adverse outcomes. This result further emphasizes the importance of judicious decision-making regarding RBC transfusion among VPIs.

This study has several limitations. Data such as haemoglobin level or RBC volume prior to RBC transfusion, transfusion volume and indications for transfusions were not available in our database. Our data were collected from large tertiary NICUs with the highest level of neonatal care in China, and may not be representative of the general population. We could not accurately evaluate the relationship between the time of each disease diagnosis and the time of RBC transfusion. We divided infants into critically and non-critically ill groups using self-defined criteria; however, currently there are no standardized criteria to differentiate disease severity for VPIs.

In conclusion, our study provided, for the first time, baseline data on RBC transfusions in VPIs in China. It reveals a relatively high RBC transfusion rate among VPIs in China with significant site variation. Guidelines for nationwide standardization of RBC transfusion in VPIs are required. Quality improvement efforts are needed to improve transfusion practices for VPIs in China, and future prospective studies to investigate the benefits and risks of RBC transfusions in VPIs may improve outcomes in these infants.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Cao Y, Jiang S, Sun J, Hei M, Wang L, Zhang H, et al. Assessment of neonatal intensive care unit practices, morbidity, and mortality among very preterm infants in China. JAMA Netw Open. 2021;4:e2118904.
2. Colombatti R, Sainati L, Trevisanuto D. Anemia and transfusion in the neonate. Semin Fetal Neonatal Med. 2016;21:2–9.
3. Widness JA. Pathophysiology of anemia during the neonatal period, including anemia of prematurity. Neoreviews. 2008;9:e520.

4. Banerjee J, Asamoah FK, Singhvi D, Kwan AW, Morris JK, Aladangady N. Haemoglobin level at birth is associated with short term outcomes and mortality in preterm infants. BMC Med. 2015; 13:16.
5. Fontana C, Raffaeli G, Pesenti N, Boggini T, Cortesi V, Manzoni F, et al. Red blood cell transfusions in preterm newborns and neurodevelopmental outcomes at 2 and 5 years of age. Blood Transfus. 2022;20:40–9.
6. Keir AK, Yang J, Harrison A, Pelausa E, Shah PS. Temporal changes in blood product usage in preterm neonates born at less than 30 weeks' gestation in Canada. Transfusion. 2015;55:1340–6.
7. Christensen RD, Baer VL, Lambert DK, Ilstrup SJ, Eggert LD, Henry E. Association, among very-low-birthweight neonates, between red blood cell transfusions in the week after birth and severe intraventricular hemorrhage. Transfusion. 2014;54:104–8.
8. Baer VL, Lambert DK, Henry E, Snow GL, Christensen RD. Red blood cell transfusion of preterm neonates with a Grade 1 intraventricular hemorrhage is associated with extension to a Grade 3 or 4 hemorrhage. Transfusion. 2011;51:1933–9.
9. D'Amato G, Faienza MF, Palladino V, Bianchi FP, Natale MP, Christensen RD, et al. Red blood cell transfusions and potentially related morbidities in neonates under 32 weeks' gestation. Blood Transfus. 2021;19:113–9.
10. El-Dib M, Narang S, Lee E, Massaro AN, Aly H. Red blood cell transfusion, feeding and necrotizing enterocolitis in preterm infants. J Perinatol. 2011;31:183–7.
11. Mohamed A, Shah PS. Transfusion associated necrotizing enterocolitis: a meta-analysis of observational data. Pediatrics. 2012;129: 529–40.
12. Ghirardello S, Dusi E, Cortinovis I, Villa S, Fumagalli M, Agosti M, et al. Effects of red blood cell transfusions on the risk of developing complications or death: an observational study of a cohort of very low birth weight infants. Am J Perinat. 2017;34:88–95.
13. Hengartner T, Adams M, Pfister RE, Snyers D, McDougall J, Waldvogel S, et al. Associations between red blood cell and platelet transfusions and retinopathy of prematurity. Neonatology. 2020; 117:562–8.
14. Teofili L, Papacci P, Bartolo M, Molisso A, Orlando N, Pane L, et al. Transfusion-free survival predicts severe retinopathy in preterm neonates. Front Pediatr. 2022;10:814194.
15. Patel RM, Knezevic A, Yang J, Shenvi N, Hinkes M, Roback JD, et al. Enteral iron supplementation, red blood cell transfusion, and risk of bronchopulmonary dysplasia in very-low-birth-weight infants. Transfusion. 2019;59:1675–82.
16. New HV, Berryman J, Bolton-Maggs PHB, Cantwell C, Chalmers EA, Davies T, et al. Guidelines on transfusion for fetuses, neonates and older children. Br J Haematol. 2016;175:784–828.
17. Carson JL, Guyatt G, Heddle NM, Grossman BJ, Cohn CS, Fung MK, et al. Clinical practice guidelines from the AABB. JAMA. 2016;316: 2025–35.
18. Kirpalani H, Bell EF, Hintz SR, Tan S, Schmidt B, Chaudhary AS, et al. Higher or lower hemoglobin transfusion thresholds for preterm infants. N Engl J Med. 2020;383:2639–51.
19. Franz AR, Engel C, Bassler D, Rüdiger M, Thome UH, Maier RF, et al. Effects of Liberal vs restrictive transfusion thresholds on survival and neurocognitive outcomes in extremely low-birth-weight infants: the ETTNO randomized clinical trial. JAMA. 2020;324:560–70.
20. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. J Pediatr. 1978;92:529–34.
21. Bell MJ, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Ann Surg. 1978;187:1–7.
22. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. Pediatrics. 2002;110:285–91.
23. The International Classification of Retinopathy of Prematurity revisited. The international classification of retinopathy of prematurity revisited. Arch Ophthalmol. 2005;123:991–9.
24. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med. 2001;163:1723–9.
25. Ballard JL, Novak KK, Driver M. A simplified score for assessment of fetal maturation of newly born infants. J Pediatr. 1979;95:769–74.
26. Zhu L, Zhang R, Zhang S, Shi W, Yan W, Wang X, et al. Chinese neonatal birth weight curve for different gestational age. Zhonghua Er Ke Za Zhi. 2015;53:97–103.
27. Fabres J, Wehrli G, Marques MB, Phillips V, Dimmitt RA, Westfall AO, et al. Estimating blood needs for very-low-birth-weight infants. Transfusion. 2006;46:1915–20.
28. Valieva OA, Strandjord TP, Mayock DE, Juul SE. Effects of transfusions in extremely low birth weight infants: a retrospective study. J Pediatr. 2009;155:331–337.e1.
29. Bell EF, Hintz SR, Hansen NI, Bann CM, Wyckoff MH, DeMauro SB, et al. Mortality, in-hospital morbidity, care practices, and 2-year outcomes for extremely preterm infants in the US, 2013-2018. JAMA. 2022;327:248–63.
30. Patel RM, Hendrickson JE, Nellis ME, Birch R, Goel R, Karam O, et al. Variation in neonatal transfusion practice. J Pediatr. 2021;235:92–99.e4.
31. Keir AK, Yang J, Harrison A, Pelausa E, Shah PS, Network CN. Temporal changes in blood product usage in preterm neonates born at less than 30 weeks' gestation in Canada. Transfusion. 2015;55:2295.
32. Fogarty M, Osborn DA, Askie L, Seidler AL, Hunter K, Lui K, et al. Delayed vs early umbilical cord clamping for preterm infants: a systematic review and meta-analysis. Am J Obstet Gynecol. 2018;218:1–18.
33. dos Santos AM, Guinsburg R, de Almeida MF, Procianoy RS, Leone CR, Marba ST, et al. Red blood cell transfusions are independently associated with intra-hospital mortality in very low birth weight preterm infants. J Pediatr. 2011;159:e1–3.
34. Hirano K, Morinobu T, Kim H, Hiroi M, Ban R, Ogawa S, et al. Blood transfusion increases radical promoting non-transferrin bound iron in preterm infants. Arch Dis Child Fetal Neonatal Ed. 2001;84: F188–93.

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ORIGINAL ARTICLE

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The impact of non-disclosure of HIV status and antiretroviral therapy on HIV recency testing and incidence algorithms

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Abstract

Background and Objectives: Accurate HIV incidence estimates among blood donors are necessary to assess the effectiveness of programs aimed at limiting transfusion-transmitted HIV. We assessed the impact of undisclosed HIV status and antiretroviral (ARV) use on HIV recency and incidence estimates using increasingly comprehensive recent infection testing algorithms.

Materials and Methods: Using 2017 donation data from first-time and lapsed donors, we populated four HIV recency algorithms: (1) serology and limiting-antigen avidity testing, (2) with individual donation nucleic amplification testing (ID-NAT) added to Algorithm 1, (3) with viral load added to Algorithm 2 and (4) with ARV testing added to Algorithm 3. Algorithm-specific mean durations of recent infection (MDRI) and false recency rates (FRR) were calculated and used to derive and compare incidence estimates.

Results: Compared with Algorithm 4, progressive algorithms misclassified fewer donors as recent: Algorithm 1: 61 (12.1%); Algorithm 2: 14 (2.8%) and Algorithm 3: 3 (0.6%). Algorithm-specific MDRI and FRR values resulted in marginally lower incidence estimates: Algorithm 1: 0.19% per annum (p.a.) (95% confidence interval [CI]: 0.13%–0.26%); Algorithm 2: 0.18% p.a. (95% CI: 0.13%–0.22%); Algorithm 3: 0.17% p.a. (95% CI: 0.13%–0.22%) and Algorithm 4: 0.17% p.a. (95% CI: 0.13%–0.21%).

Conclusion: We confirmed significant misclassification of recent HIV cases when not including viral load and ARV testing. Context-specific MDRI and FRR resulted in

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progressively lower incidence estimates but did not fully account for the context-specific variability in incidence modelling. The inclusion of ARV testing, in addition to viral load and ID-NAT testing, did not have a significant impact on incidence estimates.

Keywords
antiretroviral agents, blood donation, HIV disclosure, HIV incidence estimation, HIV recency algorithms

Highlights

- Undisclosed positive human immunodeficiency virus status and antiretroviral (ARV) use among blood donors impact recent infection testing algorithms.
- Context-specific mean duration of recent infection and false recency rate improve accuracy of incidence estimates.
- The inclusion of ARV testing, in addition to viral load and individual donation nucleic acid amplification testing, did not have a significant impact on incidence estimates.

INTRODUCTION

Forty years after the world became aware of HIV, the prevention of transfusion-transmitted HIV infections remains a key focus for blood services. Early in the epidemic, the success of strategies aimed at reducing the risk of transfusion-transmitted HIV could be evaluated by monitoring the prevalence of HIV-positive donations. However, in response to increasingly sensitive HIV testing assays, new methods for assessing HIV transmission risk and the success of prevention strategies were developed including models to calculate the residual risk of HIV transmission [1].

In most instances, these models were based on identifying incident cases which might escape serologic detection during the 'window period'. Initial models focused on 'incident cases, which were defined as donors who donated at least twice during a defined interval and who tested HIV-negative at the first donation and HIV-positive at a subsequent donation, irrespective of the duration between the negative and positive donations' [2]. Such models had significant limitations as they, by definition, excluded first-time donors who are known to confer the most risk [3]. Subsequently, residual risk models were developed that included both repeat and first-time donors with recently acquired HIV infections [4]. Such recently acquired cases included those that were viraemic but HIV-1 seronegative as well as those classified as 'recent' using less-sensitive or expanded dynamic range HIV-1 immunoassays, validated for discrimination of recent from longstanding infection [5]. This marked the beginning of contemporary recent infection testing algorithms (RITA). Simultaneously, such RITA were used in progressively more complex population-level incidence models [6, 7].

Multiple assays for detecting recent versus longstanding infections were developed and evaluated in various settings and populations [8]. For a recency assay to be useful in modelling incidence estimates, it must have a sufficiently long mean duration of recent infection (MDRI) and a sufficiently low 'false recency rate' (FRR) [8]. Factors such as variable immune response and HIV subtypes complicate establishing a 'universal' MDRI, with FRR significantly affected by elite controller status and antiretroviral (ARV) use [9]. Until recently, blood transfusion services were unaware of blood donation by people living with HIV and already on ARV therapy. As a result, little consideration was given to the potential impact of undisclosed HIV status and ARV use on RITA [10]. The identification of 'false elite controllers' and the substantial number of blood donors in South Africa [11, 12] and elsewhere [13] who fail to disclose their HIV status and ARV use means that blood services may need to consider more robust RITA to maximize the precision and accuracy of incidence estimates.

The requirements for accurate and precise HIV incidence estimation models are onerous, especially in resource-constrained settings. Calibrating context-specific MDRI and FRR requires sophisticated statistical knowledge and detailed, recent HIV epidemiologic information. Recency assays, for example, the limiting-antigen avidity enzyme immunoassay (LAg) [14], viral load (VL) assays and ARV testing are expensive and may require specialized laboratory testing. The degree to which uncertainty for each of these factors impacts incidence estimations, especially in the blood donation setting, is unknown. We therefore assessed and compared HIV recency and incidence estimates using increasingly comprehensive RITA.

METHODS

Study setting, design and population

This study was performed in South Africa, known as the country with the largest HIV-positive population in the world. The 2017 South African National Population Based Survey estimated national incidence at 0.48% p.a. among people aged 2 years and above and prevalence at 14.0% [15]. In South Africa, strategies to minimize the risk of transfusion-transmitted HIV include pre-donation education,

the completion of a lifestyle and health questionnaire, one-on-one interviews by trained staff overseen by a registered nurse and testing for HIV antibody by serology and for RNA by individual donation nucleic acid amplification testing (ID-NAT).

Utilizing stored samples from our previous study [9], we performed additional testing to populate increasingly comprehensive RITA. All first-time and lapsed blood donors (those whose last donation was more than 12 months ago) who presented during the 2017 calendar year were included. Ethics approval was obtained from the South African national blood service (SANBS) and the University of Cape Town Institutional Review Boards.

Sampling

The SANBS Biorepository routinely stores plasma aliquots of HIV-positive blood donations at −30°C. For this study, samples from first-time and lapsed donors who donated during 2017 and who tested HIV-positive (RNA, antibody or both) were included in the analysis. Lapsed donors are considered effectively equivalent to first-time donors for the purposes of HIV incidence estimation since any prior HIV screening occurred long before their donation included in this study. During 2017, LAg and ARV testing was performed on all HIV-positive donations as part of an earlier study [12]. Due to budgetary constraints, HIV VL testing was performed on a limited subset of 441 specimens, including all HIV RNA-positive, seronegative (23) as well as randomly selected RNA-positive, seropositive donations (418). VL for NAT-negative, serology-positive specimens was assumed to be below 75 copies/mL, which is higher than the limit of detection of ID-NAT. VL results where thus available for 506 (52.2%) of the 969 HIV-positive donors.

Testing

SANBS routinely performs serological assays and ID-NAT for HIV, Hepatitis B and C in parallel. HIV serology testing was performed using the Abbott Prism HIV1/2® (Abbott Diagnostics, Delkenheim, Germany). The Ultrio Elite® multimarker probe assay (Grifols Diagnostics, Barcelona, Spain) on the Procleix Panther® platform was used for the ID-NAT testing. All HIV-seropositive donations were subjected to recency testing using the Sedia® HIV-1 LAg-Avidity EIA test (Sedia Biosciences Corporation, Portland, Oregon). HIV-positive donations with sufficient residual plasma underwent ARV testing using a validated, high-performance liquid chromatography tandem mass spectrometry assay performed at the Division of Clinical Pharmacology Laboratory, University of Cape Town. The details of these tests have been described previously [12]. Long-acting injectable ARVs such as cabotegravir were not available in South Africa at the time of the study and were therefore not tested for. VL quantification, using the Abbott RealTime HIV-1® VL assay on the m2000 system (Abbott Molecular Inc., Des Plaines, Illinois), was performed on the 441 specimens noted above. HIV-seropositive but ID-NAT-negative donations were assumed to have VL below the lower limit of quantification of the Abbott RealTime HIV-1® VL assay [16, 17].

Donations were classified as follows: antibody-positive (Ab+) if the sample was serology-positive; ID-NAT-positive (NAT+) if HIV RNA was detected; LAg recent if the sample had a normalized optical density <1.5; ARV-positive if it tested positive for one or more ARV. Donations were dichotomized as either high or low VL using a 75 copies/mL cut-off. A relatively low VL cut-off, such as 75 copies/mL used by Kassanjee et al [18] and in this study, ensures a dramatic reduction in the FRR, while minimizing the impact on MDRI and further allows for comparison across different settings. In addition, a cut-off of 75 copies/mL was the lowest cut-off that could be considered, given the limits of quantification of the assays used to measure VL in the RITA calibration specimen panels on which we rely.

Model and model parameters

We constructed four, progressively more comprehensive recency algorithms. Algorithm 1 included only HIV serology and LAg results. Subsequent algorithms added new test results cumulatively: for Algorithm 2, we included qualitative ID-NAT, which allowed for the classification of NAT+/Ab− donations as recent and NAT−/Ab+ donations as longstanding; Algorithm 3 included a quantitative VL cut-off of 75 copies/mL with donations with a VL of <75 copies/mL classified as longstanding. Algorithm 4 included ARV testing with donations with detectable ARV classified as longstanding.

We employed version 3 of the South African Centre for Epidemiological Modelling and Analysis Assay-Based Incidence Estimation (ABIE_V3) toolbox [7] to perform our incidence estimates. The model requires inputs for the post-infection time cut-off *T*, the estimated MDRI and the estimated FRR. The MDRI, given a specific time cut-off *T*, is defined as the average time an HIV-infected person spent both alive and 'recently' infected [7]. The FRR refers to the proportion of persons infected with HIV for longer than *T* who are classified as recently infected by the RITA [6, 7, 19]. The 2022 World Health Organization (WHO) technical guidance on HIV recency assays [9] recommends a 2-year period for time cut-off *T* and that context-specific MDRI and FRR values be derived when employing assay-based incidence estimation.

We therefore estimated context-specific (for South African blood donors during 2017) FRR, MDRI and relative standard errors (RSE) on these parameters for each of the previously described algorithms. MDRI was estimated using HIV-1 subtype C calibration data from the centre for evaluation of public health interventions in Africa (CEPHIA) consortium and a web-based tool from the Joint United Nations Programme on HIV/AIDS (UNAIDS) and WHO (https://worldhealthorg.shinyapps.io/recency_test_properties/). (Table 1) MDRI estimates accounted for the window period associated with the HIV screening strategy employed in each algorithm (ID-NAT, serology), viral load threshold, the estimated median delay between HIV infection and diagnosis in the population and the proportion of HIV-infected donors who are receiving ARV. FRR estimates accounted for ARV coverage

TABLE 1 Model parameters used in the incidence calculations.

Parameters	Kassanjee reference Algorithm	Algorithm 1	Algorithm 2	Algorithm 3	Algorithm 4
Post-infection time cut-off *T*	730	730	730	730	730
HIV screening	Western blot	Ab	Ab and NAT	Ab and NAT	Ab and NAT
Screening adjustment	Base	−17	−3	−3	−3
Recency test	LAg	LAg	LAg	LAg	LAg
LAg Odn	1.5	1.5	1.5	1.5	1.5
VL	Yes	No	No	Yes	Yes
VL threshold	75	None	None	75	75
ARV testing	No	No	No	No	Yes
Adjustment for treatment	No	No	Yes	Yes	Yes
Mean time to treatment	No	N/A	4.9 years	4.9 years	4.9 years
HIV subtype	100% Subtype C	100% Subtype C	100% Subtype C	100% Subtype C	100% Subtype C
MDRI (unadjusted for screening method) (days)	N/A	204.7	204.7	192.3	192.3
MDRI (adjusted) (days)	177	188.7	201.7	188.3	188.3
MDRI RSE	0.05	0.074	0.069	0.069	0.069
FRR untreated[a]	1.3	1.2	1.1	1.1	1.1
FRR treated[a]	Excluded	56.6	N/A	N/A	0
FRR treated and suppressed[a]	Excluded	N/A	0	0	N/A
FRR treated and unsuppressed[a]	Excluded	N/A	56.6	56.6	0
FRR weighted[a]	1.3	6.6	3.5	1.6	1.0
FRR RSE[a]	20	50[b]	50[b]	50[b]	50[b]

Abbreviations: Ab, antibody; ARV, antiretroviral; FRR, false recency rate; LAg, limiting-antigen avidity test; MDRI, mean duration of recent infection; N/A, not applicable; NAT, individual nucleic acid amplification test; ODn, normalized optical density; RSE, relative standard error; VL, viral load.
[a]Expressed as percentage.
[b]Assumed RSE, approximately correct.

and the proportion of HIV-infected donors who are virally suppressed. ARV coverage and viral suppression in the donor population were estimated using ARV testing and quantitative viral load data. In addition, we constructed 'reference' model for each algorithm using the context-specific FRR (1.3%) and MDRI (177 days) values published by Kassanjee et al. [8] for the subset of South African samples included in their study, which excluded patients on ARV and elite controllers. RSE were calculated as a requirement for the above-mentioned software tools. They are also used as a convenient metric to compare the precision of the input parameters and incidence estimates associated with each algorithm. Finally, we used the calculated FRR and MDRI values for each of these algorithms and the number of donations classified as 'recent' using the methods described above to calculate incidence estimates for each algorithm.

Model assumptions

The underlying assumptions applied in this analysis included (1) the majority of people living with HIV in South Africa are diagnosed and take up treatment later than the specific time cut-off *T*; therefore, testing positive for ARV denoted a longstanding infection [20]; (2) viraemic control (VL <75 copies/mL), whether due to natural immune responses or ARV use, would usually only occur after the specific time cut-off *T* and therefore denotes longstanding infection; (3) for Algorithm 3, we computed a viral suppression rate based on VL <75 copies/mL among the ARV-treated group and (4) for Algorithm 4, we assume ARV testing 'perfectly' detects treatment.

RESULTS

A total of 207,768 (99,168 [47.7%] first-time and 108,600 [52.3%] lapsed) donors were accepted for blood donation during 2017 of whom 969 (0.60%) were confirmed as HIV-positive (Figure 1). The LAg, VL and ARV results of the 506 HIV-positive donors for whom a complete set of results were available, are shown in Figure 2. Among the 65 Ab+/NAT− donors, 24 (36.9%) tested LAg recent and 56 (86.2%) had detectable ARV levels. A fifth (87/418) of the Ab+/NAT+ donations tested recent by LAg of whom 11 (12.6% of 87) had VL <75 copies/mL. Seven of these 11 donations had detectable ARV concentrations compared with 3 of the 76 (4.0%) donors with VL

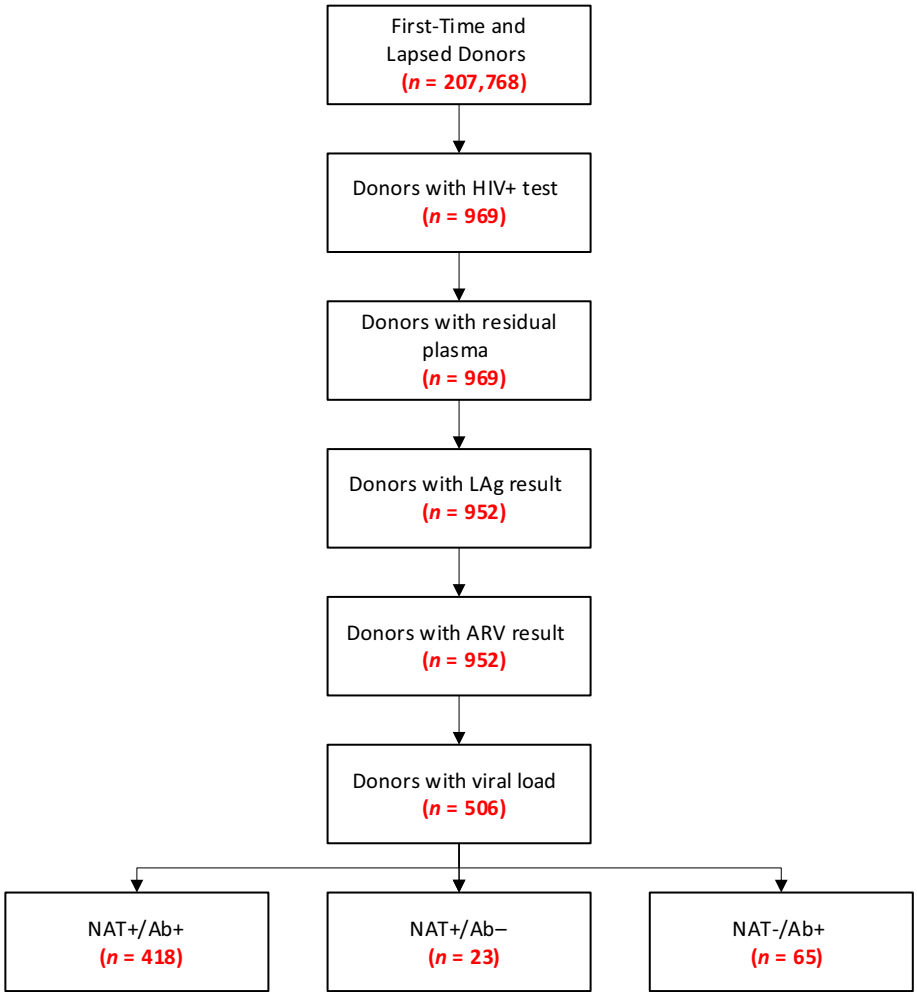

FIGURE 1 Flow diagram of available test results for the 207,768 first-time and lapsed donors who donated from January to December 2017. Ab, antibody; ARV, antiretroviral; LAg, limiting-antigen avidity test; NAT, individual nucleic acid amplification test.

>75 copies/mL. In the Ab−/NAT+ group, four (17.4%) of the 23 donors had VL <75 copies/mL, and none tested positive for ARV.

Algorithm 1 classified 111 (21.9%) of the 506 donors as recent (Figure 2, Table 2). In comparison to Algorithm 4, Algorithm 1 incorrectly classified 24 (36.9%) of the 65 Ab+/NAT− donors and 14 (3.4%) of the 418 Ab+/NAT+ donors as recent. Algorithm 1 also completely failed to detect the 23 Ab−/NAT+ cases as being HIV-positive. Adding ID-NAT (Algorithm 2) results in 110 (21.7%) donors being classified as recent; all 65 Ab+/NAT− donors were correctly classified as longstanding, and all 23 Ab−/NAT+ donors were classified as recent. However, 14 (3.4%) of the 418 Ab+/NAT+ donors were still incorrectly classified as recent. Addition of the VL cut-off in Algorithm 3 reduces the incorrectly classified donors to three (0.6%). Algorithm 4 classified 73 (17.5%) of the 418 Ab+/NAT+ and all 23 Ab−/NAT+ donors, that is, 96 (19.0%) of the 506 donors as recent.

Table 3 compares the incidence estimates for each of the algorithms described above using first the MDRI and FRR published by Kassanjee et al. [8] and then separately the MDRI and FRR derived specifically for each algorithm. The estimated incidence for Algorithm 1, using the algorithm-specific calculated MDRI and FRR compared with the published 'Kassanjee' MDRI and FRR, is slightly lower (0.19% per annum [p.a.] vs. 0.22% p.a.) with a slightly wider 95% confidence interval (CI) (0.13%–0.26% vs. 0.17%–0.26%) but a substantially larger RSE (15.97% versus 10.79%). The same pattern of slightly lower incidence estimates based on calculated input parameters held true for each of the algorithms when compared with its 'Kassanjee' equivalent, although the difference in the RSE decreased with each more comprehensive algorithm, denoting improved precision.

We next compared the incidence estimates when using algorithm-specific calculated MDRI and FRR with each other. When including ID-NAT testing (Algorithm 2) to serology and LAg testing (Algorithm 1), the estimated incidence decreased marginally from 0.19% to 0.18% p.a. but with a tightening of the 95% CI (0.13%–0.26% vs. 0.14%–0.24%) and a decrease in the RSE from 15.97% to 13.39%. (Table 3) Subsequent addition of a VL cut-off (Algorithm 3) decreased the estimated incidence to 0.17% p.a. with a further narrowing of the 95% CI to 0.13%–0.22% and a decrease in the RSE to 12.73%. The addition of ARV testing (Algorithm 4) yielded no change

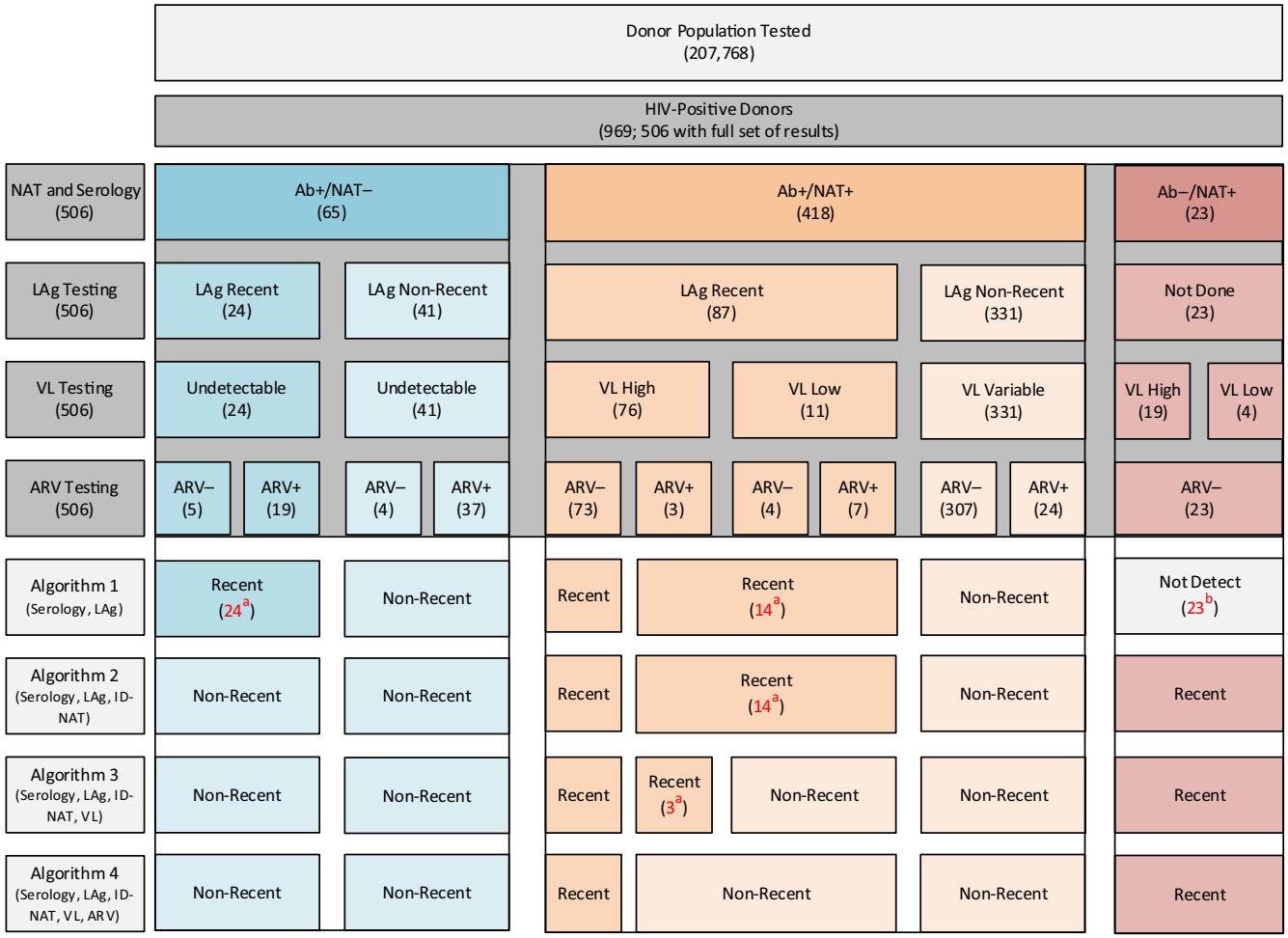

FIGURE 2 Testing algorithms and their outcomes applied to the 506 HIV-positive donations included in the analysis. Ab, antibody; ARV, antiretroviral; ID-NAT, individual donation nucleic amplification testing; VL, viral load. [a]Donations incorrectly classified as 'recent'. [b]Donations not detected as HIV-positive.

TABLE 2 Donor counts for HIV incidence modelling derived from each testing algorithm.

Donor parameters	Algorithm 1	Algorithm 2	Algorithm 3	Algorithm 4
HIV-negative	206,822	206,799	206,779	206,799
HIV-positive	946	969	969	969
Tested for recency	483	506	506	506
Classified recent	111	110	99	96
Total sample size	207,768	207,768	207,768	207,768

in the estimated incidence (0.17%) and almost no change in the 95% CI (0.13%–0.21%) and a marginal decrease in RSE (12.51%).

DISCUSSION

Our study demonstrated a significant misclassification (61 of 506, 12.1%) of the recency status of HIV-positive donors when using a RITA that does not include ID-NAT, VL and ARV testing. Without ID-NAT, the 4.6% ID-NAT-positive but seronegative donors were not identified as HIV-positive. ID-NAT testing reduced the recency misclassification from 12.1% to 2.7% with additional improvement to 0.6% when adding VL testing. The inclusion of ARV testing had a near negligible improvement in the identification of recent cases.

We confirmed a trend of decreasing incidence estimates with each increasingly comprehensive RITA. However, the use of context-specific MDRI and FRR resulted in only marginally lower incidence estimates (none of which were statistically significant) compared with the published 'Kassanjee' estimates. The 'Kassanjee' estimates used in this analysis were also derived from South African blood donors but

TABLE 3 Outcomes of incidence calculations using (a) 'Kassanjee' and (b) context-specific MDRI and FRR.

	'Kassanjee' MDRI and FRR (95% CI)		Context-specific MDRI and FRR (95% CI)	
ALGORITHM 1 (serology and LAg)				
Estimated incidence	0.22% p.a.	(0.17%–0.26%)	0.19% p.a.	(0.13%–0.26%)
RSE of incidence estimate	10.79%		15.97%	
ALGORITHM 2 (serology, LAg, ID-NAT)				
Estimated incidence	0.21% p.a.	(0.16%–0.25%)	0.18% p.a.	(0.13%–0.22%)
RSE of incidence estimate	10.9%		13.39%	
ALGORITHM 3 (serology, LAg, ID-NAT, VL)				
Estimated incidence	0.19% p.a.	(0.14%–0.23%)	0.17% p.a.	(0.13%–0.22%)
RSE of incidence estimate	11.47%		12.73%	
ALGORITHM 4 (serology, LAg, ID-NAT, VL, ARV testing)				
Estimated incidence	0.18% p.a.	(0.14%–0.22%)	0.17% p.a.	(0.13%–0.21%)
RSE of incidence estimate	11.65%		12.51%	

Abbreviations: ARV, antiretroviral; CI, confidence interval; FRR, false recency rate; ID-NAT, individual nucleic acid amplification test; LAg, limiting-antigen avidity test; MDRI, mean duration of recent infection; p.a., per annum; RSE, relative standard error; VL, viral load.

who donated prior to 2014 and for whom an HIV lysate-based western blot assay was used to identify HIV-positive cases. In contrast, this analysis used a chemiluminescent immunoassay (Abbott Prism HIV1/2®) either alone or in combination with ID-NAT to identify HIV-positive cases. The use of ID-NAT resulted in an extended MDRI but simultaneously identified seronegative recent cases. The net effect of which were remarkably, but co-incidentally, similar to that of the Kassanjee group.

Current recommendations [9] include the use of context-specific calibrated MDRI and FRR and RITA aimed at limiting the impact of viral suppression, either due to elite controller status or ARV use, on FRR. The addition of a VL cut-off to differentiate between 'true' and 'false' recent cases has been the backbone of newer RITA [21]. However, the ongoing roll-out of ARV therapy and the not infrequent failure to disclose ARV use [22–24] have raised questions on the need to include testing for ARV in RITA to ensure the exclusion of persons on ARV with incomplete viral suppression [25].

In our setting, a blood transfusion service in a country with a background HIV prevalence of ~13.9% [26] and estimated national incidence of 0.48% (in 2017) [15], ID-NAT testing was the biggest contributor in identifying misclassified cases as it identified both cases that had yet to seroconvert (and therefore assumed to recent) as well as seropositive donations with extremely low VL (below ~18 copies/mL) [4] (and therefore assumed to be longstanding infections), a third of whom tested LAg recent in Algorithm 1. However, in settings with significantly lower prevalence and especially lower incidence, the impact of ID-NAT versus simple VL testing may be less obvious as, once identified as HIV-positive, VL testing would identify the overwhelming majority of HIV-positive donors with low VL, irrespective of the reason for the low VL.

Conversely, the use of ID-NAT only to identify recent cases is also problematic. In their publication on the HIV incidence among South African blood donors, Vermeulen et al [27] essentially applied Algorithm 2 (ID-NAT and LAg) to identify recently infected HIV cases in their 'LAg first-time donor' model and relied solely on ID-NAT to identify recent cases in the 'NAT Yield Window Period' and 'Classic Incidence/Window Period' model. Given the outcome of this study, it is likely that the 'LAg first-time donor' model would have overestimated incidence as it likely did not exclude LAg recent donors who had low, but detectable VL (on highly sensitive ID-NAT testing) and those on ARV with poor viraemic control. In contrast, the two models that rely solely on ID-NAT to identify recent infections, likely underestimated incidence as ID-NAT-positive, seronegative cases accounted for only 23 of the 96 recent cases in our study. Even if the MDRI and FRR of an ID-NAT-only approach were correctly estimated and incidence point estimates were close to correct, precision would suffer severely, given the short MDRI.

In our study, the only benefit of adding ARV testing (in addition to ID-NAT and VL) was the identification of those donors on treatment who were not virally suppressed. Recognizing that NAT testing potentially introduces an overly low VL cut-off, the Transfusion Transmissible Infections Monitoring System program in the USA considered a RITA that included VL (but not ARV assessment) for defining incident HIV cases [28]. Their assessment, based on a cut-off of 1000 copies/mL, was that such cases would be unlikely in their donor population and as a result, did not include it in their final analysis. However, a contemporaneous publication by Custer et al. [13] confirmed that approximately 15% of HIV-positive donors from several regions in the United States were on ARV at the time of donation. Given the overlap between these two studies, there may have been an underappreciation of the impact of low VL and ARV use on identifying recent HIV cases. These findings would suggest that blood services should carefully consider RITA that sufficiently account for potential undisclosed ARV use.

In addition, we demonstrated a decreasing, more precise incidence trend when applying increasingly comprehensive RITA in combination with context-specific MDRI and FRR estimates. A similar decreasing incidence trend was seen when using the RITA outcomes

with the published 'Kassanjee' MDRI and FRR, but in all instances, these incidence estimates were higher than those calculated with the context-specific MDRI and FRR. Although the difference in incidence estimates did not reach statistical significance, likely due to an underpowered sample size (in particular, because HIV infections are relatively rare in blood donors, and therefore 'recent' infections are rare events), the differences have epidemiologic importance. In a country such as South Africa with high HIV prevalence, the 0.05% p.a. difference in estimated incidence between the 'Kassanjee' Algorithm 1 and the 'Calculated' Algorithm 4 may be of programmatic interest.

The outcome of these incidence calculations confirmed that accurate identification of recent infections will impact incidence calculations. However, it also highlighted that previous assertions that introducing a standardized 'cut-off time' and the use of sophisticated, context-specific derived FRR and MDRI should offer 'the opportunity to consistently account for imperfect accuracy and precision of the incidence estimator' [7, 29] did not quite materialize. The fact that it did not, confirms that we do not fully understand the impact of each test on incidence estimation and that there may be other factors affecting recency not yet fully elucidated.

Our study had limitations. We had a full set of results on only 40% of the 969 lapsed and first-time donors who tested HIV-positive during 2017. In particular, the sample included all the RNA-positive, seronegative and a disproportionate number (65 of 75 [86.7%]) of the RNA-negative, seropositive donors (data not shown). This may have impacted the incidence calculations, especially of Algorithm 1 which incorrectly classified nearly 40% of these cases as recent. In addition, the inclusion of this group of donors resulted in a higher ARV use estimation for the overall sample as this group is known to have high levels of undisclosed ARV use [11, 12]. However, this would not have had a material impact on the algorithms other than Algorithm 1 as the remainder of the algorithms correctly classified these cases as longstanding. Furthermore, we assumed that all persons with VL below 75 copies/mL and all those on ARV had longstanding infections. It is conceivable that some true elite controllers may have attained viraemic control within the 'cut-off time *T*' and therefore misclassified. Likewise, donors could have contracted HIV, been diagnosed and started treatment within this same timeframe. Although this was a minor concern in 2017 when South Africa began to adopt the 'test and treat' approach, it may pose a greater challenge in the future. In addition to accounting for the impact of early access to care, future analyses should also consider the impact of poor retention on ARV on MDRI estimation and the impact of poor treatment adherence and associated poor viraemic control on FRR estimation.

In our setting, the biggest gain in accurately identifying recent cases was derived from including ID-NAT testing. The biggest additional improvement in the incidence estimates was from the inclusion of VL, with very limited gain by adding ARV testing. However, these results should be interpreted with caution as they are specific to our setting of high background HIV prevalence, incidence [26] and ARV uptake [30]. Further research is required to identify and clarify other factors that may influence HIV-positive individuals' and populations' progression from 'recent' to 'longstanding' infection.

In conclusion, our work confirmed the need for both accurate identification of recently acquired HIV cases as well as the use of context-specific MDRI and FRR rates to derive epidemiologically useful incidence estimates. Although it appears theoretically feasible to derive MDRI and FRR estimates that can account for all known sources of variability in incidence modelling, we have shown that it is not yet possible to achieve this with sufficient accuracy to eliminate such variability.

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K.v.d.B. performed the research, data collection, initial data analysis and wrote the first draft of the manuscript; E.G. performed extended data analysis; V.J.L. and E.L.M. supervised the research; all authors contributed to the study design, reviewed and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

Eduard Grebe has received consulting income from Sedia Biosciences Corporation for unrelated work.

DATA AVAILABILITY STATEMENT

The study data are not publicly available as it may compromise the privacy of research participants. Reasonable, Institutional Review Board-approved requests may be addressed to the corresponding author.

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REFERENCES

1. Brambilla DJ, Busch MP, Dodd RY, Glynn SA, Kleinman SH. A comparison of methods for estimating the incidence of human immunodeficiency virus infection in repeat blood donors. Transfusion. 2017; 57:823–31.
2. Kleinman S, Busch MP, Korelitz JJ, Schreiber GB. The incidence/window period model and its use to assess the risk of transfusion-transmitted human immunodeficiency virus and hepatitis C virus infection. Transfus Med Rev. 1997;11:155–72.
3. Vermeulen M, Lelie N, Coleman C, Sykes W, Jacobs G, Swanevelder R, et al. Assessment of HIV transfusion transmission risk in South Africa: a 10-year analysis following implementation of individual donation nucleic acid amplification technology testing and donor demographics eligibility changes. Transfusion. 2019;59: 267–76.
4. Weusten J, Vermeulen M, van Drimmelen H, Lelie N. Refinement of a viral transmission risk model for blood donations in seroconversion

window phase screened by nucleic acid testing in different pool sizes and repeat test algorithms. Transfusion. 2011;51:203–15.
5. Rawal BD, Degula A, Lebedeva L, Janssen RS, Hecht FM, Sheppard HW, et al. Development of a new less-sensitive enzyme immunoassay for detection of early HIV-1 infection. J Aquir Immune Defic Syndr. 2003;33:349–55.
6. Welte A. Relating incidence to 'recent infection' prevalence: application to HIV. S Afr J Sci. 2008;104:199–202.
7. Kassanjee R, McWalter TA, Bärnighausen T, Welte A. A new general biomarker-based incidence estimator. Epidemiology. 2012;23:721–8.
8. Kassanjee R, Pilcher CD, Keating SM, Facente SN, McKinney E, Price MA, et al. Independent assessment of candidate HIV incidence assays on specimens in the CEPHIA repository. Aids. 2014;28:2439–49.
9. World Health Organisation. Using recency assays for HIV surveillance: 2022 technical guidance. Geneva: World Health Organisation, Joint United Nations Programme on HIV/AIDS; 2022.
10. Grebe E, Busch MP, Notari EP, Bruhn R, Quiner C, Hindes D, et al. HIV incidence in US first-time blood donors and transfusion risk with a 12-month deferral for men who have sex with men. Blood. 2020;136:1359–67.
11. Sykes W, van den Berg K, Jacobs G, Jauregui A, Roubinian N, Wiesner L, et al. Discovery of "false elite controllers": HIV antibody-positive RNA-negative blood donors found to be on antiretroviral treatment. J Infect Dis. 2019;220:643–7.
12. van den Berg K, Vermeulen M, Louw VJ, Murphy EL, Maartens G. Undisclosed HIV status and antiretroviral therapy use among South African blood donors. Transfusion. 2021;61:2392–400.
13. Custer B, Quiner C, Haaland R, Martin A, Stone M, Reik R, et al. HIV antiretroviral therapy and prevention use in US blood donors: a new blood safety concern. Blood. 2020;136:1351–8.
14. Duong YT, Qiu M, De AK, Jackson K, Dobbs T, Kim AA, et al. Detection of recent HIV-1 infection using a new limiting-antigen avidity assay: potential for HIV-1 incidence estimates and avidity maturation studies. PloS One. 2012;7:e33328.
15. Zuma K, Simbayi L, Zungu N, Moyo S, Marinda E, Jooste S, et al. The HIV epidemic in South Africa: key findings from 2017 National Population-Based Survey. Int J Environ Res Public Health. 2022;19:8125.
16. Abbott Molecular Inc. Abbott RealTime HIV-1. Abbott Laboratories. 2007. https://www.molecular.abbott/us/en/products/infectious-disease/realtime-hiv-1-viral-load. Last accessed 7 Apr 2024.
17. Coleman C, Lelie N, Rademeyer R, van Drimmelen H, van den Berg K, Vermeulen M. Comparison of two nucleic acid amplification technology systems for detection of human immunodeficiency virus, hepatitis B virus, and hepatitis C virus. Transfusion. 2020;60:2929–37.
18. Kassanjee R, Pilcher CD, Busch MP, Murphy G, Facente SN, Keating SM, et al. Viral load criteria and threshold optimization to improve HIV incidence assay characteristics. AIDS. 2016;30:2361–71.
19. McWalter TA, Welte A. Relating recent infection prevalence to incidence with a sub-population of assay non-progressors. J Math Biol. 2010;60:687–710.
20. Johnson L, Dorrington RE. Thembisa version 4.6: a model for evaluating the impact of HIV/AIDS in South Africa. Cape Town: University of Cape Town; 2023.
21. Facente SN, Grebe E, Maher AD, Fox D, Scheer S, Mahy M, et al. Use of HIV recency assays for HIV incidence estimation and other surveillance use cases: systematic review. JMIR Public Health Surveill. 2022;8:e34410.
22. Sithole N, Gunda R, Koole O, Krows M, Schaafsma T, Moshabela M, et al. Undisclosed antiretroviral therapy use at primary health care clinics in rural KwaZulu Natal South Africa: a DO-ART trial sub-study. AIDS Behav. 2021;25:3695–703.
23. Fogel JM, Wang L, Parsons TL, Ou SS, Piwowar-Manning E, Chen Y, et al. Undisclosed antiretroviral drug use in a multinational clinical trial (HIV prevention trials network 052). J Infect Dis. 2013;208:1624–8.
24. Moyo MS, Gaseitsiwe KS, Powis EK, Pretorius Holme EM, Mohammed ET, Zahralban-Steele EM, et al. Undisclosed antiretroviral drug use in Botswana: implication for national estimates. Aids. 2018;32:1543–6.
25. Global HIV Strategic Information Working Group. Recent infection testing algorithm technical update: applications for HIV surveillance and programme monitoring. Geneva: Joint United Nations Programme on HIV/AIDS; 2018.
26. Statistics South Africa. Mid-year population estimates, 2022. Pretoria, Statistics South Africa. 2022. https://www.statssa.gov.za/?page_id=1854&PPN=P0302&SCH=73305. Last accessed 7 Apr 2024.
27. Vermeulen M, Chowdhury D, Swanevelder R, Grebe E, Brambilla D, Jentsch U, et al. HIV incidence in south African blood donors from 2012 to 2016: a comparison of estimation methods. Vox Sang. 2021;116:71–80.
28. Quiner C, Bruhn R, Grebe E, di Germanio C, Kessler D, Reik R, et al. Recently acquired infection among HIV-seropositive donors in the US from 2010-2018. Transfusion. 2020;60:2340–7.
29. Kassanjee R, McWalter TA, Welte A. Short communication: defining optimality of a test for recent infection for HIV incidence surveillance. AIDS Res Hum Retroviruses. 2013;30:45–9.
30. Marinda E, Simbayi L, Zuma K, Zungu N, Moyo S, Kondlo L, et al. Towards achieving the 90-90-90 HIV targets: results from the South African 2017 national HIV survey. BMC Public Health. 2020;20:1375.

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ORIGINAL ARTICLE

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Prediction of the antigenic regions in eight RhD variants identified by computational biology

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Abstract

Background and Objectives: Changes in *RHD* generate variations in protein structure that lead to antigenic variants. The classical model divides them into quantitative (weak and Del) and qualitative (partial D). There are two types of protein antigens: linear and conformational. Computational biology analyses the theoretical assembly of tertiary protein structures and allows us to identify the 'topological' differences between isoforms. Our aim was to determine the theoretical antigenic differences between weak RhD variants compared with normal RhD based on structural analysis using bioinformatic techniques.

Materials and Methods: We analysed the variations in secondary structures and hydrophobicity of RHD*01, RHD*01W.1, W2, W3, RHD*09.03.01, RHD*09.04, RHD*11, RHD*15 and RHD*21. We then modelled the tertiary structure and calculated their probable antigenic regions, intra-protein interactions, displacement and membrane width and compared them with Rhce.

Results: The 10 proteins are similar in their secondary structure and hydrophobicity, with the main differences observed in the exofacial coils. We identified six potential antigenic regions: one that is unique to RhD (R3), one that is common to all D (R6), three that are highly variable among RhD isoforms (R1, R2 and R4), one that they share with Rhce (R5) and two that are unique to Rhce (Ra and Rbc).

Conclusion: The alloimmunization capacity of these subjects could be explained by the variability of the antigen pattern, which is not necessarily recognized or recognized with lower intensity by the commercially available antibodies, and not because they have a lower protein concentration in the membrane.

Keywords

alloimmunization, computational biology, D antigens, Rh system, RhD weak

Highlights

- We propose six different antigenic regions in the analysed models.
- Theoretically, the structural differences among the proteins could be due to changes in interactions or the formation of new interactions.
- The RhD variants have a different physicochemical profile based on the analysis of hydrophobicity, electrostatic potential, secondary structure and comparison of tertiary structures.

INTRODUCTION

Changes in the *RHD* generate variations in protein structure that lead to antigenic variants. The classical model has divided them into two, with fewer antigenic sites and no structural variations they are called quantitative (weak and Del) and qualitative, when the number of antigenic sites is normal, but they have structural differences in the protein (partial D) [1]. The main argument for classifying the D variant is the risk of anti-D alloimmunization: patients with partial D antigens can produce anti-D, while only some with weak D antigens can do so [2].

Because anti-D reagents are designed to show a strong pattern when testing normal D, a weak serologic D phenotype is defined as weak reactivity (≤2+) in the first test; however, it agglutinates slightly to strongly with anti-human globulin [3]. The intensity pattern depends on the combination of 'clones' in each kit.

Weak variants are only detected if they are hemizygous or homozygous mutations. The International Society of Blood Transfusion (ISBT) reports over 175 weak RhD variants. Some, such as types 11, 15 and 21, have immunizing capacity [4].

There are three models that describe the secondary structure of RhD [5–7]. The most widely accepted model is that proposed by Wagner [8]. An idea rooted in transfusion medicine is that only amino acid (AA) changes in the exofacial loops have an influence on the tertiary structure, and therefore, only these alter the recognition of antibodies.

An epitope is the antigenic site to which an antibody binds. There are two types of protein antigens: linear (continuous), which consists of a sequence of 6–10 adjacent AAs in the primary structure, and conformational, which consists of 10–20 AAs that are very close to each other in the 3D structure but scattered in the linear sequence [9, 10]. This second category accounts for 90% of known epitopes [11].

The structural complexity of a protein is due to the remarkable simplicity of the covalent bonds of the AA sequence. From an alphabet of only 20 α-aa arranged in chains of variable sequence and length, innumerable complex folds and functions result, which are the outcome of defined non-covalent contacts within and between the chains [12]. The formation of stable secondary structures and unique tertiary structure proteins is determined by the intramolecular interactions between the individual AA residues along the polypeptide chain and by their interaction with the surrounding medium. During protein folding, the hydrophobic force drives the polypeptide chain into the folded state by overcoming entropic factors, while hydrogen bonds, ion pairs, disulphide bonds and van der Waals interactions determine the shape and prevent it from falling apart. These non-covalent forces, resulting from the interactions between consecutive and adjacent residues and the distant residues in the sequence, together form and stabilize the native structure [13].

The secondary structures of proteins have classically been described in two regular states, the α-helix and the β-sheet, with the remaining unassigned regions described as an irregular state (coil) corresponding to a large number of different conformations. However, the use of only three states oversimplifies the description of protein structures.

The result of the AA substitution can have different effects on the physicochemical properties and thus on the functionality of the protein [14, 15]. The aim of predicting a protein fragment is to reconstruct the atomic coordinates as accurately as possible given the AA sequence. Much information can be obtained from the known structures of fragments with similar features. For this reason, many local structure prediction methods are based on the classification of these fragments based on the so-called structural alphabets or protein blocks (PB) [16]. The PB could define the structural changes in a short region of AAs that alter the global structure of the protein, depending on the hydrophobic conditions in the microenvironment.

Computational biology analyses the theoretical assembly of tertiary protein structures and enables us to identify the 'topological' differences between the isoforms of a protein, for example, the RhD variants.

When a protein sequence changes slowly over time compared with the background mutation rate, we say that it is 'conserved'. In proteins, AAs that play a crucial role in folding, structure, stability and target recognition tend to be conserved or replaced by others with similar properties in order to maintain their biological functions [17]. Conserved regions are often identified by pairwise sequence alignments, structural overlaps and variations thereof [18]. Sequence alignments involve the linear comparison of two or more AA sequences and are typically generated using computer algorithms that attempt to maximize a scoring function based on a substitution matrix in which identical aligned positions are scored most favourably, followed by the alignment of AAs with similar biochemical properties [19]. The degree of structural similarity between two proteins is usually quantified by the root-mean-square deviation (RMSD) of the atomic coordinates, with lower values indicating a higher degree of similarity.

In the Critical Assessment of Techniques for Protein Structure Prediction (CASP) competition, the contact of a pair of residues is defined if the distance between their Cβ atoms is less than or equal to 8 Å, as long as they are separated by at least five residues in the sequence [20]. Contact residues are important for protein folding because they can be far apart in the protein sequence but close to each other in three-dimensional space [21]. Based on the spacing of the residues, contacts are broadly classified as short range (6–11 residues), medium range (12–23 residues) and long range (at least 24 residues). Most contact prediction methods evaluate long-range contacts separately, as they are the most important of the three and also the most difficult to predict [20].

The aim of the present study is to determine the theoretical antigenic differences between weak RhD variants compared with normal RhD based on structural analysis using bioinformatic techniques.

MATERIALS AND METHODS

Based on literature reports, we selected the weak D variants with the highest population frequency. We included RHD*01W.1, W2 and W3 (W1, W2 and W3 respectively), which are common but not associated with anti-D formation. The RHD*09.03.01 (W4.0) and RHD*09.04 (W4.1) variants have reports of probable anti-D formation.

The ISBT identifies RHD*11, RHD*15 and RHD*21 (W11, W15 and W21, respectively), as reported alloanti-D. We used RHCE*01 (Rhce) as a negative control because anti-D does not recognize it (RHD blood group alleles v6.4).

AA sequences were generated using ALM96707.1 (RhD*01) and NP_065231.4 (*RHCE*01*) sequences as standard. We removed the initial methionine [6] and substituted the AA residues indicated in the ISBT catalogue for the analysed variants.

Primary structural analysis

Hydrophobicity analysis of the AA sequence was performed with ProtScale from Expasy (https://web.expasy.org/protscale) using the Kyte and Doolittle scale with a window of nine AAs.

Secondary structure analysis

Secondary structure elements were determined using the prediction algorithm of PBDsum structural analyses [22]. We identified the PBs using the IPBA server [23].

Tertiary structure analysis

The comparative analysis was carried out in five steps:

1. Theoretical construction:
   Modelling: The tertiary structure design was performed using the I-Tasser server [24].
   The evaluation and validation of the 10 structures were performed by uploading the PDB files to three servers with different algorithms.
   a. QMEANDisCo Global (Qualitative Model Energy ANalysis Distance Constraints Applied on Model Quality Estimation): This is a composite assessment function that can derive both global (i.e., for the entire structure) and local (i.e., per residue) absolute quality estimates based on a single model [25].
   b. PDBsum Generate uses the Ramachandran algorithm, which allows visualization of energetically valid regions for the backbone dihedral angles ψ versus φ of the AA residues in protein structure [22].
   c. ModFOLD8 provides a single score and a *p*-value related to the predicted quality of a single 3D model of a protein structure [26].
2. Structural alignment:
   To assess the significance of the sequence alignment and to determine the probability that a pair of biologically significant AA residues occupy the same position within the alignment, we calculated the RMSD of atomic positions in Å using the IPBA web server [23].
   The theoretical tertiary structures were compared using an alignment approach and plotted using Geneious 2015 and Pymol.
3. Analysis of protein position within the lipid bilayer:
   We determined the position of the proteins within the lipid bilayer (hydrophobic thickness, transmembrane steps and exofacial loops) using OREMPRO [27].
   We plotted the transmembrane using the modifications of the OREMPRO and Geneious algorithms to visualize the distribution of AAs along the membrane.
   To compare the space occupied by the proteins in the membrane, we plotted the structures in a 'surface' style.
4. Interactions within the protein structure:
   We determined the probability of interaction between the protein residues using the R2C residue interaction calculator. This is a two-stage hierarchical prediction system in which a raw map of residue contacts is generated in the first step, followed by a Gaussian noise filter to further improve the quality of the prediction [28].
5. Epitopes prediction:
   DiscoTope 3.0 uses a combination of AA composition information, spatial neighbourhood information and a surface area measure to predict epitopes [29].

RESULTS

The tertiary structure of the RhD variants was modelled using the homology of the 3hd6A template (RHCG) with more than 31.36% identity.

The QMEAN quality score ranges from 0 to 1. On average, the value of our models was 0.63 (0.65–0.62, with a modal value of 0.63) and an overall estimated error of ±0.05. Regions of α-helices larger than 10 AAs have average values of 0.77.

The PBDsum server provides the statistical analysis of the Ramachandran diagram. It compares about 118 structures with a resolution of at least 2.0 Å and an R-factor of at most 20.0. A good quality model is expected to have more than 90% in the preferred regions. We calculated RHD 92.0%, Rhce 91.6%, W.1 91.2%, W.2 92.9%, W.3 93.1%, W4.0 92.9%, W4.1 92.6%, W11 90.9%, W15 92.6% and W21 92.9%.

The confidence and *p* values determined by ModFOLD8 were less than $p < 0.001$ for all 10 proteins.

Regarding the content of secondary structures, all models were all-α, that is, 67 ± 1.49% of the structure is formed by α-helices. On average, they have 22 helices, of which 76.92% are H-type and 23.08% are G-type (ratio 3.4, except W4.1, which has a ratio of 2.83).

As reported, the primary AA sequence similarity among RhD, the eight variants and Rhce ranges from 99.76% and 92.067%.

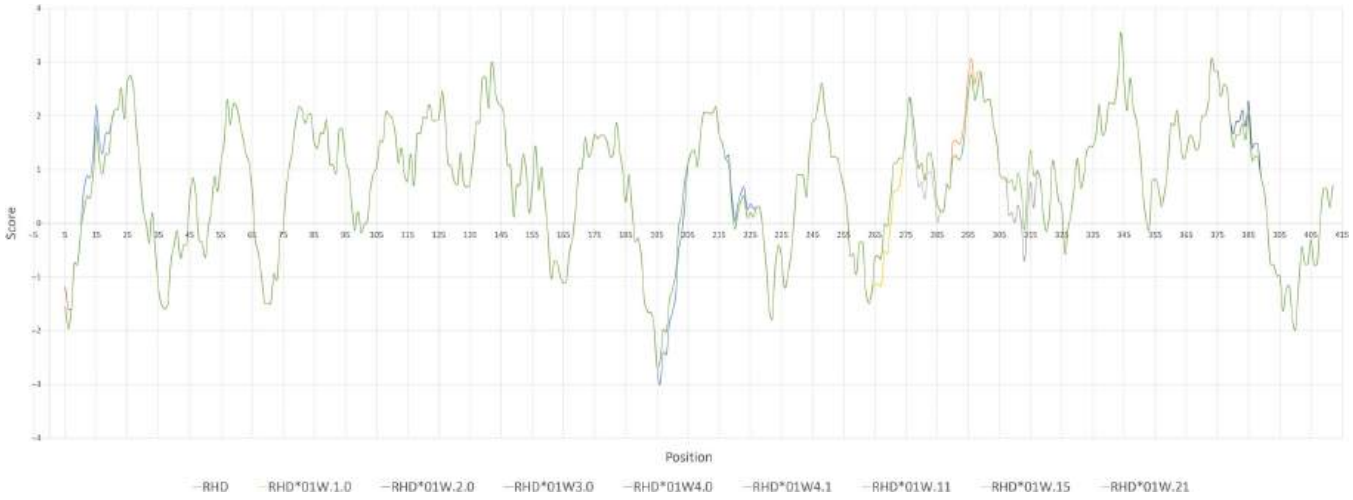

FIGURE 1 Overlay of hydrophobicity diagrams using the Kyte and Doolittle scale with a window of nine amino acids, created with ProtScale from Expasy. The *x*-axis indicates the position of each amino acid and the *y*-axis describes the calculated hydrophobicity score. The identification of each isoform is shown at the bottom of the diagram.

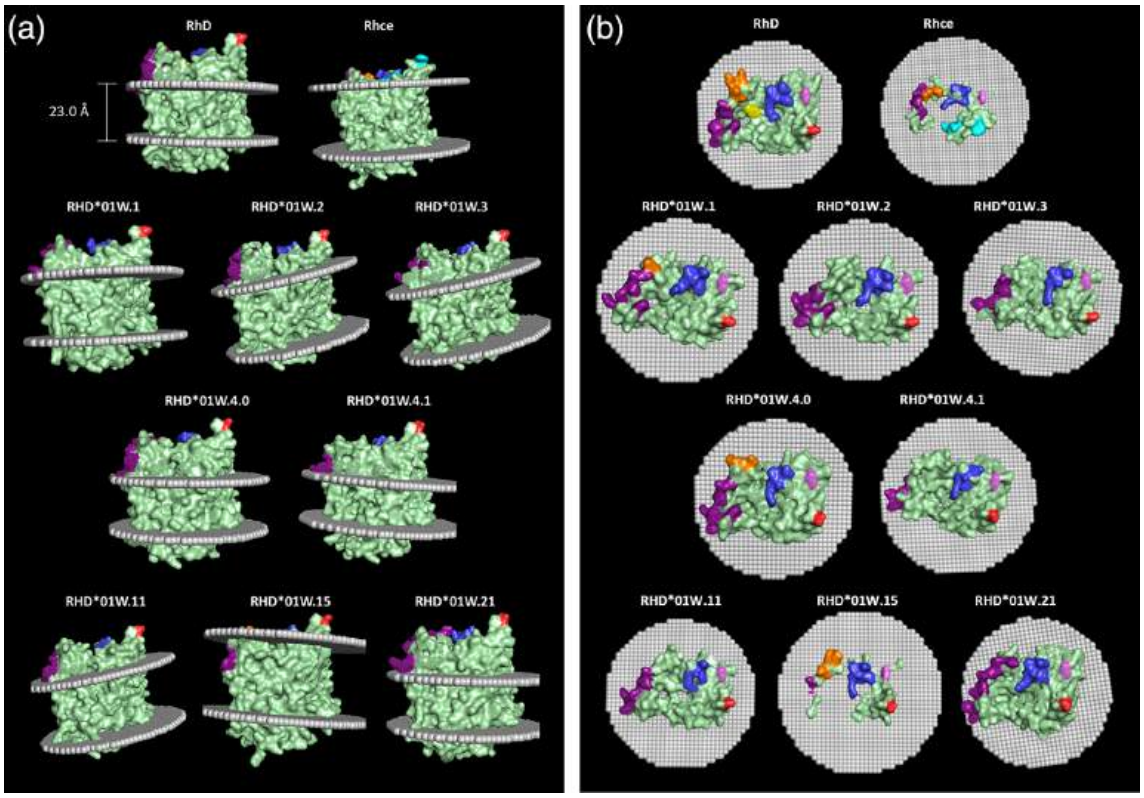

FIGURE 2 Representation of the three-dimensional structures (in surface style) of RhD, RHD*01, RHD*01W.1, W2, W3, RHD*09.03.01, RHD*09.04, RHD*11, RHD*15, RHD*21 and Rhce. (a) Lateral view, one can see the variation in the displacement angle of the hydrophobic layers of the membrane; in the structure of RhD, the width is indicated as 23 Å, varying for each protein. (b) Apical view, six variable antigenic regions are shown: R3 (yellow) exists only in RhD, R6 (red) exists only in D, three is highly variable in RhD isoforms R1 (orange), R2 (deep purple) and R4 (blue), R5 (pink) also exists in Rhce and two only in Rhce (Ra and Rbc, both cyan).

When overlaying the hydrophobicity diagrams (Figure 1), changes are observed in the regions where AA substitution occurs, resulting in hydrophobic changes of approximately ±4 AAs at the site of the substitution. These regions are as follows:

1. 11–19 (p.Trp15Cys) in W4.1, in the transmembrane region, 196–204 (p.Thr200Arg) in W4.0 and W4.1, in a cytoplasmic region, 218–226 (p.Phe222Val) in W4.0 and W4.1, in exofacial 4.

2. 258–273 (p.Val269Gly) W1, 381–396 (p.Gly385Ala) W2, 277–285 (p.Gly281Asp) W15, 291–298 (p.Met294Ile) W11, 308–316 (p.Pro312Leu) W21, all in a transmembrane region.
3. in W3, only changes between 5 and 7 can be seen; the rest of the graph is the same as in RhD.

Regions 21–195, 228–261, 318–379 and 389–412 are the same for all proteins. The hydrophobic regions measure more than 10 AA (Figure 2a). The hydrophobicity pattern is similar for all proteins, which is consistent with the RMSD values (range 0.41–0.55 Å) (Table 1). The conformation of the secondary structures shows a similarity of 94.712%–97.596% (Table 1). However, analysis of the tertiary structure described by the PB sequence shows a similarity between 89.784% and 97.236%.

We calculated the close interactions (aromatic–aromatic interactions within 4.5 and 7 Å, aromatic–sulphur interactions within 5.3 Å, ionic interactions within 6 Å, cation–Pi interactions within 6 Å) and the prediction of long-range residual contacts (L/5) in the 10 proteins and compared them with those of RhD (Table 2). As expected, the percent similarity of Rhce was low (85.54%), but not the lowest. The most similar variants are RhDW1 (92.77%), W2 and W3 (91.57%). The least similar variants are RhDW 4.0, RhDW4.1 and RhDW11 (86.75%, 84.34% and 87.95% respectively). On the other hand, RhDW15 and 21 are 90.36% similar to RhD (Table 1).

To analyse the constructed models, we divided them into three areas, which are discussed next.

Cytoplasmic

This region of RhD consists of 60 AA, compared with 64 AA of Rhce. In the variants, this region comprises between 34 (RhDW2) and 69 (RhDW15) AA. On average, it consists of 56 ± 12 AA, of which 53.33% are Ser (4.74%), Ala (6.32%), Asp (6.52%), Phe (6.72%), Arg (7.9%), Lys (10.27%), Pro (10.86%). The location of the terminal ends is shown in Table 1.

Transmembrane

It consists of an average of 242 ± 43 AA, ranging from 193 (W4.1) to 313 (W15). Five AAs account for 62.47%: Ala (11.89), Gly (8.88), Leu (18.55), Ser (11.71) and Val (11.43). The hydrophobic thickness (Å) is defined by the number of AA within the membrane and by the type of AA, although both conditions create a different microenvironment that changes the three-dimensional conformation and causes variations in the membrane slope (°), they are not directly related. In normal RhD, it measures 23 Å with 209 AA, w15 of 34 Å with 308, but in w21 it has 29.1 Å with 191 AA. The distribution of AAs within the membrane influences the slope of the hydrophobic region (Figure 2a and Table 1). The hydrophobic thickness is shifted towards the exofacial region. With the exception of W15 and W21, the displacement between the two hydrophobic regions of the membrane is parallel.

TABLE 1 Comparison of the number of amino acids distributed in each region cytoplasmic (Cyto), transmembrane (Transm), Exofacial (Exof), amino and carboxyl terminal location, no. α-helix transmembrane, membrane tilt, hydrophobic thickness, root-mean-square deviation (RMSD) and percentage of similarity compared with RhD.

ID	No. aa in Cyto.	No. aa in Transm.	No. aa in Exof.	N-ter	C-ter	α-Helix transm[a]	Membrane tilt	Hydrophobic thickness (Å)	RMSD (Å)	Percentage of similarity compared with RhD (%)			
										1[a]	2[a]	3[a]	Interaction
RHD*1	60	217	139	Cyto	Cyto	12	-	23.0					
RHD*01W.1	59	237	120	Cyto	Trans	12	5.61	26.4	0.53	99.76	96.88	91.35	92.77
RHD*01W.2	34	288	94	Trans	Trans	12	17.61°	30.3	0.43	99.76	97.11	94.11	91.57
RHD*01W.3	36	268	112	Trans	Cyto	12	16.61°	29.4	0.55	99.76	96.63	93.63	91.57
RHD*01W.4.0	61	202	153	Cyto	Cyto	12	2.25°	22.0	0.43	99.52	97.60	97.24	86.75
RHD*01W.4.1	67	193	156	Cyto	Cyto	12	4.61°	22.0	0.51	99.28	95.43	92.19	84.34
RHD*11	45	264	107	Trans	Cyto	12	14.83°	29.1	0.41	99.76	97.60	95.79	87.95
RHD*15	69	313	32	Cyto	Cyto	12	1.43–1.02°	34.0	0.46	99.76	96.15	93.39	90.36
RHD*21	55	196	165	Cyto	Cyto	12	0.31–0.28°	29.1	0.53	99.76	94.71	94.23	90.36
Rhce	64	319	33	Cyto	Cyto	12	1.25–0.09°	33.3	0.46	92.07	96.87	89.78	85.54

Note: 1[a]: Linear sequence of amino acid. 2[a]: Sequence of α-helix and β-sheet. 3[a]: Protein block in each amino acid. Interaction: percentage of the same interaction within the protein.
[a]α-helix greater than 10 amino acids.

TABLE 2 The number of interactions within the protein is displayed.

Interaction type	RHD*1	RHD*01W.1	RHD*01W.2	RHD*01W.3	RHD*01W.4.0	RHD*01W.4.1	RHD*11	RHD*15	RHD*21
Int C–C	4	11	6	5	9	8	5	11	7
Int E–T	17	15	8	3	12	14	6	4	15
Int T–T	68	68	93	90	62	59	92	98	61
Int C–T	3	5	6	5	3	2	9	3	5
Int E–E	26	12	7	16	29	26	11	0	0
Total	118	111	120	119	115	109	123	116	88

Note: Values lower than RhD indicate that some interactions have been lost. Conversely, a higher value means that new interactions have occurred. Although the total number of interactions does not vary significantly, each type of interaction has different values. Amino acid interactions by region.
Abbreviations: C, cytoplasmic; E, exofacial; T, transmembrane.

All variants have AA substitutions within the membrane. Including W3, which is a Cys substitution at the second position but encompasses the entire amino-terminal region.

The regions with the lowest probability of prediction errors are the long α-helices within the membrane. The number of transmembrane α-helices is shown in Table 1.

Exofacial

The AA composition of exofacial region is the most variable, averaging 120 ± 41, with a range of 165–32. W15 has significantly less AA (32) compared with RhD (139).

We identified possible antigenic sites in the 3D structures based on three criteria: the epitope score obtained with the DiscoTope server, the structural differences identified with IPBA (PB), and the association with intra-protein interactions (Figure 2b). We obtained six regions (highlighted in colour) that were highly variable among the nine RhD structures, as well as two particular regions of Rhce. It can be seen that the decrease in exofacial AAs is associated with the decrease in probable epitopes. The values for these regions are shown in Table 3. The column 'Antigenic region' describes the AA of the respective region, each variable has two columns. The PB column describes with letters the structural changes in a short region of the AA. The column ES (epitope score) represents the probability that an AA expresses an epitope, depending on the characteristics of AAs surrounding it.

DISCUSSION

Our hypothesis is that, from a molecular point of view, there are no reports of changes in the transcription and translation of RhD weak and partial genes, suggesting that their expression is decreased; therefore, the difference in antigen exposure is not due to less protein, but to other antigenic sites or with lower affinity to the antibodies of the commercial tests.

To this end, we analysed the consequences of AA substitutions of nine RhD variants and compared them with RhD and Rhce. These changes are known to affect the tertiary structure of proteins, and sometimes their function is not so obvious when the changes have similar physicochemical properties.

In this study, we found that the primary structure of the 10 proteins is more than 99% similar. The hydrophobicity diagrams vary only in the altered regions, and in terms of an RMSD, which averages 0.48 Å, this variation is indeed minimal; furthermore, the tertiary structures overlap significantly. The transmembrane regions, especially the helices, are the structures with the lowest prediction uncertainty and the best conserved. At this point, the reader will agree that there are no significant exofacial changes in the model proposed by Wagner 1999 [8], if the substitution does not occur there, which is consistent with other reports [3, 30]. Most studies do not consider punctuate changes and their structural consequences. However, variations in physico-chemical properties change the torsion angles of the AAs and give rise to other PBs. Although the tertiary structures are spatially similar, changes in the distances between the contact residues may lead to new interactions that cause a number of changes: The protein interaction is modified between 7% and 15% (Table 1), and the membrane tilt and hydrophobic thickness exhibit antigenic patterns with different probabilities (affinity variation), but share some sites that might be weakly recognized by commercial antibodies.

As we show, there is one antigenic region exclusive to RhD (R3), one that only all D have (R6), three highly variable in RhD isoforms (R1, R2 and R4), one shared with Rhce (R5) and two exclusive to Rhce (Ra and Rbc).

Finally, based on the assumption that all variants analysed here are considered RhD positive, we conclude that there are conserved regions that can be recognized by the antibodies and that their affinity would differ due to variations in electrostatic surface potential and torsional dihedral angle, that is, charge and spatial exposure.

We assumed that the antigen patterns of the W1, W2 and W3 variants would be very similar to that of RhD, while those of W11, W15 and W21 would be different. Those of W4.0 and W4.1 were uncertain. Therefore, the alloimmunization capacity of these variants could be explained by the variability of the antigen pattern, which is not necessarily recognized or recognized with lower intensity by the commercially available antibodies, and not because they have a lower protein concentration in the membrane.

TABLE 3 The regions calculated by DiscoTope 3.0 with the highest probability (>0.9) of antigenic sites.

		RHD*1		RHD*01W.1		RHD*01W.2		RHD*01W.3		RHD*01W.4.0		RHD*01W.4.1		RHD*11		RHD*15		RHD*21		Rhce	
Antigenic region	**Residue**	**PB**	**ES**	**PB**	**ES**	**PB**	**ES**	**PB**	**ES**	**PB**	**ES**	**PB**	**ES**	**PB**	**ES**	**PB**	**ES**	**PB**	**ES**	**PB**	**ES**
R1	37*	f	1.38	*k*	2.11											f	0.99	f	1.53		
	40	g	0.95							g	0.97					g	2.38	*n*	2.22		
	41	c	1.85							c	1.11					*i*	2.29				
R2	28	m	2.02	m	1.29	m	1.50	m	1.39	m	1.30	m	1.27	m	1.59	m	1.44	m	1.51		
	29	m	1.51			m	1.14	m	1.29	m	1.13	m	1.28	m	1.36	m	1.05	m	1.11		
	32	c	2.49	c	3.18	c	2.54	c	3.42	c	2.01	c	2.01	c	2.79	c	1.65	c	2.69	c	2.20
	33	d	1.84	d	1.85	d	1.18	d	2.52	d	2.30	d	0.95	d	1.58	d	1.37	d	1.42	d	2.50
	34	d	2.33	d	2.06	d	1.29	d	1.06	d	2.54			d	1.40	d	1.14	d	1.09	d	1.86
	35	d	1.72	d	1.57			d	1.23	d	1.45			d	1.40	d	1.32	d	1.75	d	1.32
	36			*j*	2.08					d	1.34							d	1.42	d	1.54
	100	e	1.14	e	1.44	e	1.11			e	1.00									e	1.10
	103							i	0.93												
	104	a	0.93			a	0.99			a	1.28										
R3	110	l	0.91																		
R4	227											*m*	1.89			*m*	1.16				
	228	b	2.82	b	4.44	b	3.76	b	2.73	b	2.59	b	2.70	b	2.72	b	3.19	b	2.72	*c*	2.51
	229	l	1.80	l	2.07	*f*	1.72	l	1.50	l	1.34	l	1.60	l	1.67	l	1.85	l	1.80		
	230	m	1.94	*k*	3.06	m	1.69	m	1.55	m	1.58					m	1.63	m	1.15	*k*	2.65
	231	l	1.05	l	2.42	l	1.87	l	1.08	l	1.45	l	1.34	l	1.34	l	1.61	l	1.26	l	2.24
	232	m	0.94	m	1.26	m	1.03							m	1.05	m	1.00	m	0.93	m	1.42
R5	288	b	1.00	b	1.88	b	1.55	b	1.18	b	1.03	b	1.07	b	1.10	b	1.59	b	0.98	b	1.54
R6	350	k	1.24	k	1.10	k	1.90	k	1.60	k	1.85	k	1.48	k	1.48	m	1.25	k	1.73	k	0.93
Rce[a]	37*																			f	1.11
	38																			b	1.16
Rce[b]	158																			m	1.11
Rce[c]	161																			p	0.94

Note: Empty cells represent values of <0.90. Residue 37* in the nine forms of RhD (normal and variants) and Rhce: the amino acid sequence from 37 to 40 remains unchanged. Letters in italics represent changes in the protein blocks in relation to RhD.

Abbreviations: ES, epitope score; PB, protein block; R, region.

[a,b,c]Rce: Antigenic region exclusive to Rhce.

The few reports may be due to the low frequency of subjects with these variants, and also because the likelihood of exposure to an antigenic challenge is low.

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R.T.-G. and H.E.-J. designed the research study, performed the research, acquired and analysed the data and wrote the paper. F.R.-M. wrote the first draft of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in figshare.com at https://doi.org/10.6084/m9.figshare.24790563.v3, reference number doi.org/10.6084/m9.figshare.24790563.v3. You can use Pymol to display them in surface format and color the proposed amino acids to distinguish the likely antigenic regions.

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REFERENCES

1. Kulkarni S, Mohanty D, Gupte S, Vasantha K, Joshi S. Flow cytometric quantification of antigen D sites on red blood cells of partial D and weak D variants in India. Transfus Med. 2006;16:285–9.
2. de Brevern AG, Floch A, Barrault A, Martret J, Bodivit G, Djoudi R, et al. Alloimmunization risk associated with amino acid 223 substitution in the RhD protein: analysis in the light of molecular modeling. Transfusion. 2018;58:2683–92.
3. Sandler SG, Chen LN, Flegel WA. Serological weak D phenotypes: a review and guidance for interpreting the RhD blood type using the RHD genotype. Br J Haematol. 2017;179:10–9.
4. ISBT. RHD Blood Group Alleles. Available from: https://www.isbtweb.org/resource/004rhd.html. Last accessed 22 Feb 2024.
5. Avent ND, Ridgwell K, Tanner MJ, Anstee DJ. cDNA cloning of a 30 kDa erythrocyte membrane protein associated with Rh (Rhesus)-blood-group-antigen expression. Biochem J. 1990;271:821–5.
6. Cherif-Zahar B, Bloy C, Le Van KC, Blanchard D, Bailly P, Hermand P, et al. Molecular cloning and protein structure of a human blood group Rh polypeptide. Proc Natl Acad Sci U S A. 1990;87:6243–7.
7. Flegel WA. The genetics of the Rhesus blood group system. Blood Transfus. 2007;5:50–7.
8. Wagner FF, Gassner C, Muller TH, Schonitzer D, Schunter F, Flegel WA. Molecular basis of weak D phenotypes. Blood. 1999;93:385–93.
9. Atassi MZ, Smith JA. A proposal for the nomenclature of antigenic sites in peptides and proteins. Immunochemistry. 1978;15:609–10.
10. Tahir S, Bourquard T, Musnier A, Jullian Y, Corde Y, Omahdi Z, et al. Accurate determination of epitope for antibodies with unknown 3D structures. MAbs. 2021;13:1961349.
11. Hu Y-J, Lin S-C, Lin Y-L, Lin K-H, You S-N. A meta-learning approach for B-cell conformational epitope prediction. BMC Bioinform. 2014;15:378.
12. George KL, Horne WS. Foldamer tertiary structure through sequence-guided protein backbone alteration. Acc Chem Res. 2018;51:1220–8.
13. Gromiha MM, Selvaraj S. Inter-residue interactions in protein folding and stability. Prog Biophys Mol Biol. 2004;86:235–77.
14. Meireles MR, Stelmach LH, Bandinelli E, Vieira GF. Unveiling the influence of factor VIII physicochemical properties on hemophilia A phenotype through an in silico methodology. Comput Methods Programs Biomed. 2022;219:106768.
15. Yousef A, Charkari NM. A novel method based on physicochemical properties of amino acids and one class classification algorithm for disease gene identification. J Biomed Inform. 2015;56:300–6.
16. Salomao M, Zhang X, Yang Y, Lee S, Hartwig JH, Chasis JA, et al. Protein 4.1R-dependent multiprotein complex: new insights into the structural organization of the red blood cell membrane. Proc Natl Acad Sci U S A. 2008;105:8026–31.
17. Kumar R, Jayaraman M, Ramadas K, Chandrasekaran A. Insight into the structural and functional analysis of the impact of missense mutation on cytochrome P450 oxidoreductase. J Mol Graph Model. 2020;100:107708.
18. Baltoumas FA, Theodoropoulou MC, Hamodrakas SJ. Interactions of the alpha-subunits of heterotrimeric G-proteins with GPCRs, effectors and RGS proteins: a critical review and analysis of interacting surfaces, conformational shifts, structural diversity and electrostatic potentials. J Struct Biol. 2013;182:209–18.
19. Wang S, Ma J, Peng J, Xu J. Protein structure alignment beyond spatial proximity. Sci Rep. 2013;3:1448.
20. Monastyrskyy B, Fidelis K, Tramontano A, Kryshtafovych A. Evaluation of residue-residue contact predictions in CASP9. Proteins. 2011;79:119–25.
21. Niggemann M, Steipe B. Exploring local and non-local interactions for protein stability by structural motif engineering. J Mol Biol. 2000;296:181–95.
22. Laskowski RA, Jablonska J, Pravda L, Varekova RS, Thornton JM. PDBsum: structural summaries of PDB entries. Protein Sci. 2018;27:129–34.
23. Gelly JC, Joseph AP, Srinivasan N, de Brevern AG. iPBA: a tool for protein structure comparison using sequence alignment strategies. Nucleic Acids Res. 2011;39:W18–23.
24. Zhou X, Zheng W, Li Y, Pearce R, Zhang C, Bell EW, et al. I-TASSER-MTD: a deep-learning-based platform for multi-domain protein structure and function prediction. Nat Protoc. 2022;17:2326–53.
25. Studer G, Rempfer C, Waterhouse AM, Gumienny R, Haas J, Schwede T. QMEANDisCo-distance constraints applied on model quality estimation. Bioinformatics. 2020;36:1765–71.
26. McGuffin LJ, Aldowsari FMF, Alharbi SMA, Adiyaman R. ModFOLD8: accurate global and local quality estimates for 3D protein models. Nucleic Acids Res. 2021;49:W425–30.
27. Postic G, Ghouzam Y, Gelly JC. OREMPRO web server: orientation and assessment of atomistic and coarse-grained structures of membrane proteins. Bioinformatics. 2016;32:2548–50.
28. Yang J, Jin QY, Zhang B, Shen HB. R2C: improving ab initio residue contact map prediction using dynamic fusion strategy and Gaussian noise filter. Bioinformatics. 2016;32:2435–43.
29. Kringelum JV, Lundegaard C, Lund O, Nielsen M. Reliable B cell epitope predictions: impacts of method development and improved benchmarking. PLoS Comput Biol. 2012;8:e1002829.
30. Flegel WA. Molecular genetics and clinical applications for RH. Transfus Apher Sci. 2011;44:81–91.

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ORIGINAL ARTICLE

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Determining the impact of current Canadian stem cell registry policy on donor availability via dynamic registry simulation

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Abstract

Background and Objectives: When a haematopoietic stem cell registry size is constrained by limits on recruiting, as in Canada, identifying the right person to recruit is a critical determinant of effectiveness. The aim of this study was to evaluate the impact of changes to donor recruitment effort, within ethnic groups, on the matching effectiveness of the Canadian registry as it evolves over time.

Materials and Methods: Simulation methods are applied to create a cohort of donor recruits and patients over a 10-year time horizon. New recruits are added to the registry each year, while some existing donors 'age-out' upon reaching their 36th birthday. In a similar fashion, simulated patient lists are created. At the end of each simulated year, simulated patients are matched against the simulated registry.

Results: There are increased matches in non-White populations when diverse registrants are preferentially recruited, but there are larger decreases in the number of matches for Caucasian patients. Additionally, ethnic communities that have limited registrants in the Canadian registry in 2021 do not benefit from increased recruiting efforts as much as communities with a larger initial number of registrants.

Conclusion: Preferentially recruiting from non-Caucasian populations reduces the number of matches from Canadian sources because increases in non-Caucasian populations will not fully counterbalance decreases to Caucasian patient matches. Nevertheless, more than 80% of all matches are for Caucasian patients, regardless of the donor recruiting effort within ethnic groups.

Keywords

simulation, stem cell recruiting

Highlights

- Using dynamic registry simulation, we studied the impact of donor recruitment effort within ethnic groups in the Canadian Blood Services Stem Cell Registry.
- Recruiting more non-White donors increases the number of matches to non-White donors overall but reduces the number of matches for White patients from Canadian donors.
- Canadian patients will continue to need to draw stem cells for haematopoietic cell transplantation from international sources.

INTRODUCTION

Stem cell registries facilitate haematopoietic transplants by recruiting individuals as potential donors. For patients suffering from blood-related diseases, a haematopoietic stem cell transplant often offers the best, or only, course of treatment. Because less than one in four patients can find a matched donor from a near relative, stem cell registries are an important life-saving tool.

When a registry's size is constrained by limits on recruitment effort, as it is in Canada, identifying the right person to recruit is a critical determinant of effectiveness. Because the distribution of human leukocyte antigen (HLA) profiles varies by ethnicity, strategies must allocate recruiting effort between different ethnic groups to balance the genetic diversity of the registry while recruiting donors who will most likely match a person requiring a transplant. Previous studies have evaluated the impact of recruiting effort on the size of the Canadian registry [1] and optimizing recruiting effort within ethnic groups to maximize the populations that could potentially find a match [2]. More recently, simulated donors were used to estimate the impact of recruiting effort on patient matches and to identify how many new donors would provide novel HLA profiles given an existing registry [3]. In this study, we evaluate the impact of changes to donor recruitment effort on the matching effectiveness of the Canadian registry over a simulated 10-year time horizon.

Literature

The application of simulation is common for evaluating the matching potential of stem cell registries, starting with Beatty et al. [4], who created simulated donor profiles to build a virtual registry against which a matching algorithm is run. This methodology was repeated by Beatty et al. [5] but applied to a larger database of HLA haplotypes. Kollmann et al. [6] estimated the haplotype frequency and used simulated donor profiles to evaluate match probabilities for patients in the United States while including donor availability to arrive at an overall cost effectiveness of recruiting different ethnic groups. Schmidt et al. [7] evaluated the effectiveness of combining German and Polish registries using an EM-estimated haplotype frequency estimation, combined with simulated patients, which were matched against a historical patient profile. There are also other applications of EM haplotype frequency estimation and simulated donor matching [8–12].

Several papers describe studies involving simulated patients matching to either an existing or a simulated registry. Sauter et al. [13] have created both simulated patients and donors to test the impact of incomplete HLA typing on search effectiveness. The use of simulated patients and donors to validate the matching algorithms used by different national or regional stem cell registries, assuming differing levels of ambiguity within the HLA profiles of donors and recipients, has been described by Bochtler et al. [14] Vlachos et al. [15] have created both simulated patients and donors and virtually matched the two to compare the impact of changes to the size of the Greek cord blood registry on match rates. Pappas et al. [16] used simulated patients and donors to evaluate the matching benefit of adding registrants from a population similar to that of California with a high degree of admixture between ethnic groups.

Although the literature contains many studies employing simulated donors, a smaller set employing simulated patients and some involving both simulated patients and donors, there is no report that we are aware of that uses both simulated patients and donors, in a dynamic registry, evolving over time, to evaluate recruiting policies.

STUDY DESIGN AND METHODS

Overview

Simulation methods are applied to create a cohort of donor recruits over a 10-year time horizon, extending from 2021 to 2030, for the Canadian Blood Services Stem Cell Registry (CBSSCR). Starting with the CBSSCR as it existed at the beginning of 2021, the evolution of the registry is simulated over time using existing CBSSCR recruiting policies: 25,000 potential donors are recruited annually, of whom about 16,000 become new recruits and are added to the registry, while existing donors 'age-out' upon reaching their 61st birthday. Both the (fixed) number of recruits and the upper age limit are policy decisions.

In a similar fashion, simulated patient cohorts of between 1144 and 1946 individuals are created: one for each year in the 2021–2030 timeframe according to the expected number of searches in Figure 2. At the end of each simulated year, simulated patients are matched against the simulated registry as it is expected to exist. Matching statistics are collected and the simulation advances. Because simulated donors and simulated patients are stochastic samples from a random distribution, the experiment is repeated over several replications and results are averaged.

The CBSSCR

De-identified donor data, representing the Canadian Adult Stem Cell Registry as it existed in March 2021, was obtained. A total of 444,611 records are included in the dataset, although only 237,836 are under the age of 36 and only 63,357 records represent available individuals under the age of 36 with complete (HLA-A, HLA-B, HLA-C, HLA-DRB1 and HLA-DQB1) profiles. Data available in the records include the individual's sex, self-reported ethnicity and date of birth.

Simulated donors

Similar to our previous analysis [3], simulated donors were created using Monte Carlo methods. Using publicly available data from the U.S. National Marrow Donor Program (NMDP) [17], haplotype frequency lists were created for each of the five broad ethnic categories listed in that source: AFA (African American), API (Asian and Pacific

Islander), CAU (Caucasian [White]), HIS (Hispanic) and NAM (Native American). The source files list a set of haplotypes and expected frequencies within each ethnic group. It has been shown previously that U.S. haplotype frequencies are similar to Canadian frequencies [3] and thus suitable for evaluating recruiting practices.

Simulated donors are created by selecting first a donor ethnicity from an ethnicity distribution. Once the ethnicity is determined, two random selections are drawn from the appropriate haplotype frequency file derived from the NMDP data. Each selection returns a haplotype, and the two are combined to make the donor's phenotype. Sex and age characteristics are also assigned to a donor because the donor's likelihood of completing registration is influenced by both variables. Using the donor's ethnicity, sex and age information, a draw is made from a set of distributions that indicate recruitment effectiveness. Somewhere between 31% and 35% of generated donors do not complete the registration process and therefore do not join the registry, although they are counted as part of the recruiting effort within the ethnic group. See Appendix A for data on recruiting effectiveness.

Simulated donors who complete the registration process are added to a file listing individuals who will join the registry in a particular year. To create a time-phased simulation of the registry, a database routine is executed at each simulated year from 2021 through 2030 to remove donors over the age of 60 from the registry and to add the donors from the list of those who become available in the year.

Simulated patients

Simulated patients are created using the same method as donors. A patient's ethnicity is drawn from a patient ethnicity distribution table. Once assigned an ethnicity, patients are assigned two HLA haplotypes drawn from the appropriate ethnicity frequency file.

Matching

In each of the 10 simulated years of a model replication, patients search the registry for a matching donor. Matches are conducted only against donors with full 5-loci HLA data. The matching algorithm used in this study has been tested and validated in other projects; its details are reported elsewhere [2].

Data

Donor data were derived from a de-identified listing of CBSSCR donors current to January 2021; donor demographic and HLA data were derived from this source. Patient data were derived from a 29-year sample of patient searches conducted against the CBSSCR and its predecessors. Patient data were de-identified but included patient age, gender, partial ethnicity information and full 5-loci HLA data. It should be noted that ethnicity data in the search registry is incomplete because it contains records from non-Canadian patient searches for which an ethnicity field is not always entered.

Patient search data were analysed for trends. The results of this analysis showed that there have been significant shifts in the ethnicity of searches conducted against the CBSSCR over time. See Figure 1.

It may be seen that the proportion of searches conducted for patients belonging to the API community increased from 5%, prior to 2008, to 11% thereafter. However, since 2008 the proportion of searches conducted for patients from the API community has remained stable, with no statistically significant change. Also evident is a change in searches for patients with ethnicity designation of Caucasian and Other, with searches for Caucasians dropping after 2013 and then stabilizing at 38%, while searches for patients designated as Other have increased after 2013, stabilizing at 47%. Analysis shows that the searches for Caucasian and Other are negatively correlated ($R^2 = 0.93$), suggesting that one is the mirror image of the other. This represents a change in ethnicity labelling policies for international searches executed against the CBSSCR. Therefore, for purposes of this analysis, the Caucasian and Other populations are combined as 'Caucasian'. There were no statistically significant trends detected in the proportion of searches conducted for patients from the AFA, the HIS or the First Nations (NAM) community over the time series.

As may be seen from Table 1, the proportion of searches conducted against the CBSSCR is less diverse than either the Canadian census or the composition of the registry in 2021. This may indicate that the patient search population, which is older than the Canadian population (51.4 years vs. 41.4 years), may represent an ethnic distribution from a period when Canada's population was less diverse.

Although recent stem cell search data indicate no trends in the ethnic distribution of searches conducted against the CBSSCR, there is evidence that the absolute number of searches has increased since 1991. The data suggest a polynomial growth rate, with an R^2 value exceeding 97%. Thus, in runs of the dynamic simulation, the number of patient searches conducted is a function of the simulated year. See Figure 2.

Experiments

Experiments were developed to test the impact of recruiting effort within ethnic groups on the number of matches expected over the period from 2021 through 2030. Two parameters are varied in the runs: the ethnic distribution of the recruits to the CBSSCR, and the ethnic distribution of patient searches.

In the first set of runs, it was assumed that patient searches follow the historical patient search distribution detailed in Table 1. We define this as Patient Case P1. Donor recruiting effort within ethnic groups was tested at two levels: C1 and C2. We define Case C1 to have an ethnic distribution similar to the ethnic distribution of the Canadian population in 2021 and Case C2 to have an ethnic distribution within non-Caucasian (White) groups twice that of the Canadian population in 2021.

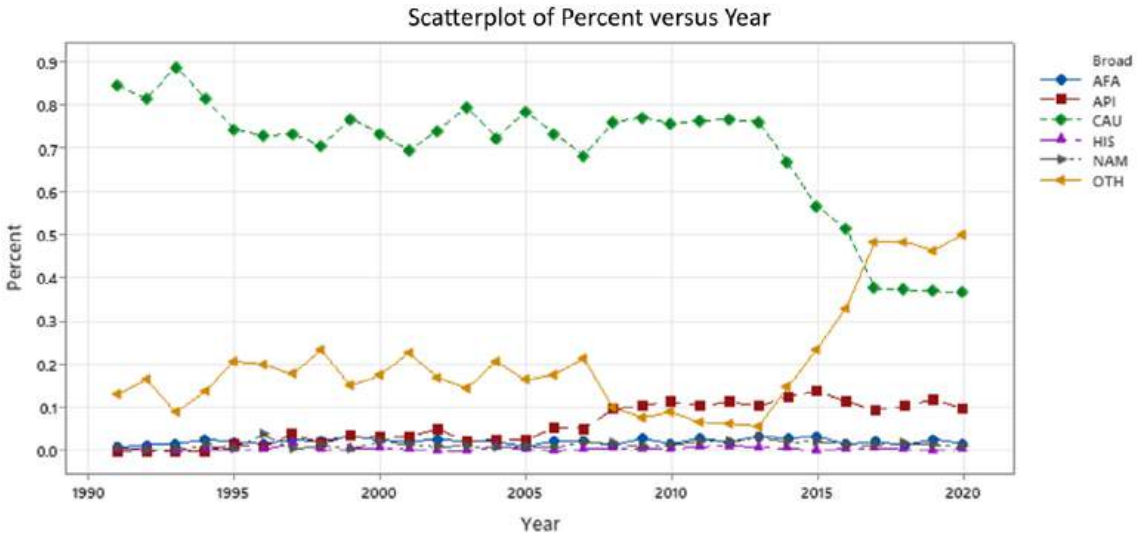

FIGURE 1 Changes in the ethnicity of searches against the Canadian Blood Services Stem Cell Registry from 1991 to 2020. AFA, African American; API, Asian and Pacific Islander; CAU, Caucasian (White); HIS, Hispanic; NAM, Native American; OTH, other.

TABLE 1 Ethnic proportions for the stem cell registry, patient searches and the Canadian population.

	AFA (%)	API (%)	CAU (%)	HIS (%)	NAM (%)
CBSSCR	2.0	25.6	69.4	1.2	1.8
Patient searches	2.0	11.0	85.0	0.5	1.5
Canadian census 2022	4.3	16.1	73.0	1.6	5.0

Abbreviations: AFA, African American; API, Asian and Pacific Islander; CAU, Caucasian (White); CBSSCR, Canadian Blood Services Stem Cell Registry; HIS, Hispanic; NAM, Native American.

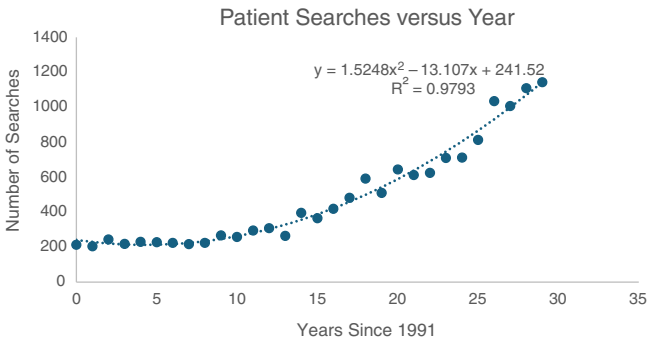

FIGURE 2 The number of searches executed against the Canadian Blood Services Stem Cell Registry over time.

In the second set of runs, the impact of a more diverse patient search population, defined as Patient Case P2, was tested against the same two donor recruitment strategies: equal to the ethnic distribution of the Canadian population in 2021 (Case C1) or with donor recruiting effort doubled within non-Caucasian populations (Case C2).

In all sets of runs, it was assumed that a total of 25,000 individuals begin the recruitment process, with somewhere around 16,000 completing the process and becoming active registrants. The restriction on the number of recruits is made, in this study, to match the expected donor recruitment targets for the CBSSCR.

RESULTS

Registry size

The same simulated patient groups were used in both patient experiment sets. In Table 2 we report the number of active registrants under the age of 36 with the assumption that 25,000 individuals were recruited and that recruitment effort within ethnic groups was proportional to the ethnic distribution of the Canadian census in 2021 (i.e., Recruiting Case R1).

As may be seen from Table 2, recruiting 25,000 individuals from the ethnic groups AFA, API, CAU, HIS and NAM in proportion to the census will result in a small decline in the size of the registry (~2%). There is growth in the number of registrants from the AFA (51% increase), HIS (14% increase) and NAM (84% increase) communities. The number of API registrants declined by 15%, which is not unexpected given that since 2008 the CBSSCR has been successful in recruiting within the community at a rate greater than its census composition. It may also be seen that registrants of the other (OTH) category declined over the time series, but because the NMDP data source do not list an OTH ethnicity, no individuals of type OTH were recruited in the simulation. However, if OTH and CAU ethnicities are combined, a decline of 2% (from 167,700 to 164,241) can be observed. Recruiting from ethnic groups in proportion to the Canadian census will reduce the overall diversity of the registry by 1.7% (from 62,111 to 61,039) from 2021 through 2030. While growth in registrants from AFA, HIS and NAM was observed, there was a net loss of diversity due to the decline in API registrants.

TABLE 2 The U36 registry composition of the CBSSCR under the assumption that 25,000 individuals are recruited annually in proportion to the ethnic composition of the Canadian census in 2022 (i.e., Cas).

	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	Change (%)
AFA	5048	5526	5988	6371	6683	6996	7258	7436	7571	7642	51
API	49,812	49,927	49,880	49,401	48,678	47,748	46,629	45,196	43,775	42,151	−15
CAU	146,514	149,867	152,862	155,210	156,422	156,920	157,192	156,806	156,235	155,031	6
HIS	2953	3047	3163	3254	3323	3345	3390	3375	3387	3354	14
NAM	4299	4832	5324	5781	6173	6553	6921	7286	7596	7891	84
OTH	21,246	20,071	18,965	17,777	16,414	15,031	13,649	12,262	10,738	9210	−57
Total	229,871	233,271	236,183	237,793	237,693	236,593	235,039	232,361	229,303	225,280	−2

Abbreviations: AFA, African American; API, Asian and Pacific Islander; CAU, Caucasian (White); CBSSCR, Canadian Blood Services Stem Cell Registry; HIS, Hispanic; NAM, Native American; OTH, other.

TABLE 3 The U36 registry composition of the CBSSCR under the assumption that 25,000 individuals are recruited annually with double the effort to recruit non-White registrants in the ethnic composition of the Canadian census in 2022.

	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	Change (%)
AFA	5673	6775	7825	8775	9690	10,577	11,345	12,059	12,665	13,205	133
API	52,438	55,176	57,746	59,777	61,463	62,889	63,993	64,756	65,454	65,816	26
CAU	141,828	140,664	139,224	137,316	134,465	131,112	127,648	123,738	119,776	115,360	−19
HIS	3247	3578	3937	4292	4588	4859	5126	5342	5575	5739	77
NAM	5033	6269	7500	8652	9716	10,749	11,749	12,704	13,593	14,434	187
OTH	21,246	20,071	18,965	17,777	16,414	15,031	13,649	12,262	10,738	9210	−57
Total	229,465	232,533	235,197	236,589	236,335	235,217	233,509	230,861	227,800	223,765	−2

Abbreviations: AFA, African American; API, Asian and Pacific Islander; CAU, Caucasian (White); CBSSCR, Canadian Blood Services Stem Cell Registry; HIS, Hispanic; NAM, Native American; OTH, other.

Table 3 shows the registry size and ethnic distribution under the assumption of 25,000 recruits per annum, with recruiting effort in non-Caucasian groups at twice the rate of the Canadian census in 2022 (i.e., Recruit Case R2). The overall registry size is smaller (2.5% decrease) when an increased fraction of non-Caucasian people is recruited, which is due to the lower donor completion rates in these communities. Recruiting non-Caucasian people at an increased rate leads, not surprisingly, to increases in the number of donors in the U36 registry who are from diverse communities; overall, the number of non-Caucasian donors increases by 49%. Donor numbers increased for all communities except CAU and Others, which, combined, saw a decrease of 24%.

Match rates

Experiments tested the impact of the ethnic distribution of both recruits and patients. While 25,000 donors are recruited annually in all runs, the proportion of non-Caucasian donors recruited was set equivalent to the Canadian census in 2022 (Recruit Case R1) or double the rate of the census (Recruit Case R2). The ethnic distribution of patient searches was tested at rates equal to recent historical data (Patient Case P1: 85% Caucasian) and rates more reflective of the census (Patient Case P2: 73% Caucasian). Results are shown in Figures 2 and 3.

Non-Caucasian searches at historic rates (Patient Case P1)

In the first set of experiments, patient searches were assumed to grow over time according to the graph in Figure 1, but to have a stable ethnic distribution reflective of recent historical search rates, by ethnicity, as detailed in Table 1 (i.e., 85% are Caucasian). Simulated patients were matched with simulated patients, assuming that the CBSSCR dynamically evolves over time. Match rates were tested under recruiting strategies that distribute recruiting effort between ethnic groups in proportion to the Canadian census (Recruit Case R1) and/or at twice the rate of the Canadian census (Recruit Case R2) for non-Caucasian communities (8.52% for AFA, 32.26% for API, 46.08% for CAU, 3.2% for HIS and 9.4% for NAM). See Figure 2.

Results suggest that when donor searches are 85% Caucasian, increasing the ethnic diversity of the CBSSCR results in a net decrease of 243.0 ± 16.4 matches over the time horizon between simulated patients and simulated donors. The decrease in total matches is statistically significant at the 95% level over the 10-year time horizon, and changes in matches are also statistically significant for the API, CAU, NAM and OTH communities, but not for the AFA and HIS communities. Thus, expending more effort to recruit from diverse populations does indeed improve the match rate for some, but not all, ethnic groups. Further, the increase in matches in diverse groups does not counterbalance decreases in matches for the Caucasian population,

which sees a 388.0 ± 28.5 decline in matches. Nevertheless, simulated matches for Caucasian patients drops only from 89.2% to 84.9% of all matches under Recruit Case R2 when donor recruitment efforts within non-Caucasian populations are doubled from the rates of the Canadian census.

Non-Caucasian searches at rates equivalent to the Canadian census (Patient Case P2)

In the second set of experiments, patient searches were again assumed to grow over time according to Figure 1 but to have a stable ethnic distribution reflective of the 2021 Canadian census, as also detailed in Table 1 (73% White). Match rates were tested under recruiting strategies that distribute recruiting effort between ethnic groups in proportion to the Canadian census (Recruit Case R1) or that recruiting effort is made at twice the rate of the Canadian census for non-Caucasian communities (Recruit Case R2). See Figure 4.

Match results suggest that when donor searches are 73% Caucasian, increasing the ethnic diversity of the CBSSCR again results in an overall net decrease in matches between patients and simulated donors. The decrease in total matches is statistically significant at the 95% level over the 10-year time horizon. Statistically significant increases in matches were observed for the API and NAM communities. Significant decreases were observed for the CAU and OTH categories. Again, there was no significant change in match rates for the

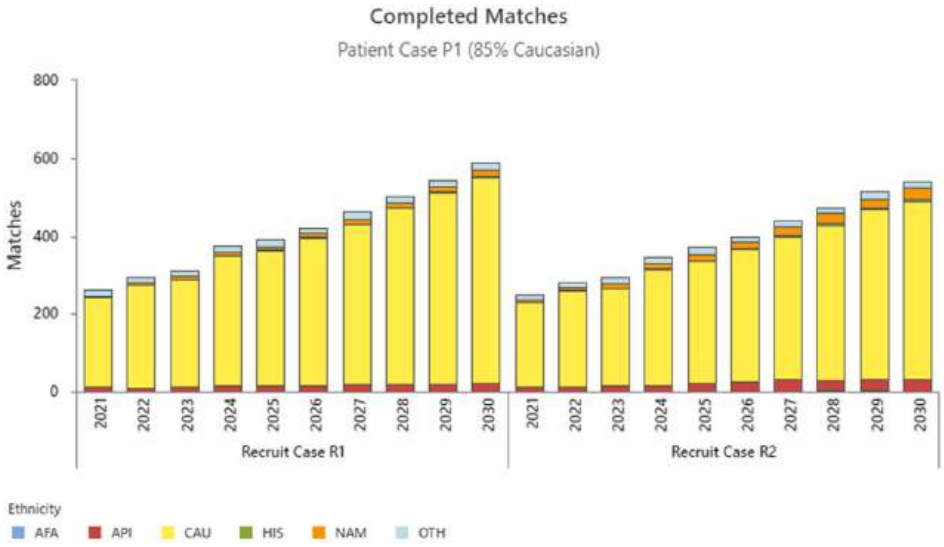

FIGURE 3 Comparison of matches 2021–2030 assuming a patient population that is 85% Caucasian (Patient Case P1). AFA, African American; API, Asian and Pacific Islander; CAU, Caucasian (White); HIS, Hispanic; NAM, Native American; OTH, other.

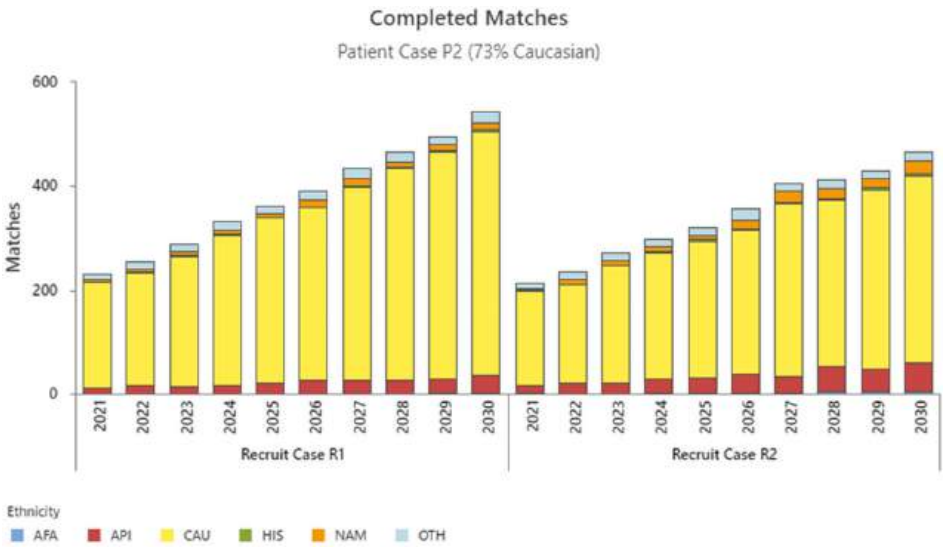

FIGURE 4 Comparison of matches 2021–2030 assuming a patient population that is 73% Caucasian (Patient Case P1). AFA, African American; API, Asian and Pacific Islander; CAU, Caucasian (White); HIS, Hispanic; NAM, Native American; OTH, other.

AFA and HIS communities, both of which have very small numbers in the base registry. Overall, a decrease of 386.4 ± 34.55 matches over the period 2021–2030 is anticipated when diverse donors are recruited at twice the census rate, even if the assumed patient population is 27% non-Caucasian. Although match rates decline when diverse donors are preferentially recruited, the results show that most matches continue to involve Caucasian patients; match rates of 86.8% were observed when non-Caucasian donors were recruited in proportion to the Canadian census (Recruit Case R1) and 80.0% when non-Caucasian donors were recruited at twice the rate of the Canadian census (Recruit Case R2).

DISCUSSION

Altering the ethnic distribution of registrants to the CBSSCR will impact the size of the registry and its underlying ethnic distribution. Changes to the ethnic distribution of the registry, in turn, will affect the number and ethnic distribution of matches that can be made from the registry.

Recruiting 25,000 registrants for the Canadian registry is not quite sufficient for maintaining the number of registrants under the age of 36 listed over the period 2021–2030. However, because of differences in the proportions of individuals completing the registration process between ethnic groups, the registry is smaller after 10 years when non-Caucasian registrants are recruited at rates greater than their proportion of the Canadian census (Recruit Case R2). This result shows that effort to ensure that a larger number of individuals complete the registration process, particularly within non-Caucasian groups, is a necessary strategy to maintaining the effectiveness of the CBSSCR over time.

Preferentially recruiting non-Caucasian donors has an impact on the ethnic distribution of the CBSSCR. Statistically significant increases in the number of registrants of AFA, HIS and NAM descent can be expected when non-Caucaian (White) populations are recruited at twice their rate in the Canadian census (Recruit Case R2). However, there are accompanying decreases in the number of registrants from the API, CAU and OTH communities. The decrease in CAU and OTH registrants is obvious, but the decrease in API registrants is a result of the CBSSCR having been able to preferentially recruit individuals from this community for the period 2010–2020.

Although the diversity of the CBSSCR increases as more non-Caucasian (White) registrants are recruited, the impact on matches is mixed. There are increased matches in non-White populations under Recruit Case R2 when registrants are recruited at twice the rate of their proportions in the Canadian populations, but there are larger decreases in the number of matches for Caucasian (White) patients. Additionally, ethnic communities that have limited registrants in the CBSSCR in 2021 (AFA, HIS) do not benefit from increased recruiting efforts. This result holds if patient searches match the historical ethnic distribution of patient searches (85% Caucasian [White]) or the ethnic distribution of the Canadian census (73% Caucasian [White]).

While matches in the CAU community decrease when ethnically diverse populations are preferentially recruited for the registry, the majority of matches within the simulation continue to be for CAU patients. White patients accounted for more than 80% of all matches in the simulated time horizon even when non-White registrants are preferentially recruited for the registry.

The results from the dynamic registry simulation do not dictate whether an ethnically diverse recruiting strategy is superior to recruiting at rates equivalent to the Canadian population over the period 2021–2030. Because the registry in 2021 had an excess number of Caucasian (White) registrants, oversampling from non-Caucasian (White) populations has less impact over a 10-year horizon than might be expected. Nevertheless, preferentially recruiting from non-Caucasian (White) populations will reduce the number of matches because increases in non-Caucasian (White) populations will not fully counterbalance decreases to Caucasian (White) matches. However, less than 30% of stem cell transplants for Canadians come from Canadian sources [2]. Canadian patients currently make extensive use of stem cells from international sources for transplant. Increasing the size of the recruiting cohort would enhance the CBSSCR's ability to fill domestic demand [1] but at an increased cost. Further, given the size of the Canadian registry relative to international sources, it is unlikely that self-sufficiency in stem cell procurement can be achieved in Canada. Thus, international registries will continue to be an important source of stem cells for Canadian patients for the foreseeable future. Because of the large cohort of Caucasian (White) donors in international sources, there is potential benefit to recruiting from Canada's increasingly diverse population. In our study, we show that such a strategy is not without cost; it will reduce the overall number of domestically source transplants, with large decreases in domestically sourced materials for White patients not being fully balanced by increases in domestically sourced materials for non-Caucasian (White) patients. Canadian patients will make greater use of stem cells from international sources as the registry is made more diverse, over the period 2021–2030, unless an increase in recruiting cohort is also envisioned for the Canadian registry.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Blake J, McTaggart K, Killeen D. Determining recruiting strategies for an adult stem cell registry. Oper Res Health Care. 2016;10:1–12.
2. Blake J, McTaggart K, Killeen D. Modelling the optimal ethnic composition of an adult stem cell registry. Eur J Oper Res. 2018;264:884–93.
3. Blake J, Parmar G, Ganz K, Seftel M, Allan D. Modeling unrelated blood stem cell donor recruitment using simulated registrant cohorts: assessment of human leukocyte antigen matching across ethnicity groups. Transfusion. 2023;63:1060–6.
4. Beatty P, Dahlberg S, Mickelson E, Nisperos B, Opelz G, Martin PJ, et al. Probability of finding HLA-matched unrelated marrow donors. Transplantation. 1988;45:714–8.
5. Beatty P, Mori M, Milford E. Impact of racial genetic polymorphism on the probability of finding an HLA-matched donor. Transplantation. 1995;60:778–83.
6. Kollman C, Abella E, Baitty R, Beatty PG, Chakraborty R, Christiansen CL, et al. Assessment of optimal size and composition of the U.S. National Registry of hematopoietic stem cell donors. Clin Transplant. 2004;78:89–95.
7. Schmidt A, Solloch U, Pingel J, Baier D, Böhme I, Dubicka K, et al. High-resolution human leukocyte antigen allele and haplotype frequencies of the Polish population based on 20,653 stem cell donors. Hum Immunol. 2011;72:558–65.
8. Schmidt A, Baier D, Solloch U, Stahr A, Cereb N, Wassmuth R, et al. Estimation of high-resolution HLA-A, -B, -C, -DRB1 allele and haplotype frequencies based on 8862 German stem cell donors and implications for strategic donor registry planning. Hum Immun. 2009;70:895–902.
9. Buhler S, Nunes J, Nicoloso G, Tiercy JM, Sanchez-Mazas A. The heterogeneous HLA genetic makeup of the Swiss population. PloS One. 2012;7:e41400.
10. Eberhard H, Feldmann U, Bochtler W, Baier D, Rutt C, Schmidt AH. Estimating unbiased haplotype frequencies from stem cell donor samples typed at heterogeneous resolutions: a practical study based on over 1 million German donors. Tissue Antigens. 2010;76:352–61.
11. Schmidt A, Solloch U, Pingel J, Baier D, Böhme I, Dubicka K, et al. High-resolution human leukocyte antigen allele and haplotype frequencies of the Polish population based on 20,653 stem cell donors. Hum Immun. 2011;72:558–65.
12. Maiers M, Halagan M, Joshi S, Ballal HS, Jagannatthan L, Damodar S, et al. HLA match likelihoods for Indian patients seeking unrelated donor transplantation grafts: a population-based study. Lancet Haematol. 2014;1:e57–63.
13. Sauter J, Solloch U, Giani A, Hofmann J, Schmidt A. Simulation shows that HLA-matched stem cell donors can remain unidentified in donor searches. Sci Rep. 2016;6:21149.
14. Bochtler W, Gragert L, Patel ZI, Robinson J, Steiner D, Hofmann JA, et al. A comparative reference study for the validation of HLA-matching algorithms in the search for allogeneic hematopoietic stem cell donors and cord blood units. HLA. 2016;87:439–48.
15. Vlachos D, Iakovou E, Keramyda C, Anagnostopoulos A. On the estimation of the necessary inventory for Hellenic public cord blood banks. Int J Oper Res. 2012;12:57–68.
16. Pappas D, Gourrard PA, Le Gall C, Laurent J, Trounson A, DeWitt N, et al. Proceedings: human leukocyte antigen Haplo-homozygous induced pluripotent stem cell Haplobank modeled after the California population: evaluating matching in a multiethnic and admixed population. Stem Cells Transl Med. 2015;4:413–8.
17. National Marrow Donor Program. Be The Match. Available from: https://frequency.nmdp.org/NMDPFrequencies2011/jsp/haploTypeFrequencies.jsp. Last accessed 26 Aug 2015.

SUPPORTING INFORMATION

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

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SHORT REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

Stigmatizing deferrals disproportionally reduce donor return rates: Evidence from Brazil

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Abstract

Background and Objectives: Prior research has shown that temporary deferrals negatively influence donor return rates, but it remains unknown the extent to which these effects vary across reasons for deferral. We investigate whether deferrals differ in their degree of perceived stigmatization and, if so, how being deferred for stigmatizing (vs. non-stigmatizing) reasons affects subsequent donation behaviour.

Materials and Methods: We examined whether reasons for deferral vary on their perceived level of stigmatization through an online survey ($n = 400$). Furthermore, we used a dataset encompassing 25 years of donation records from the state-run blood collection agency (BCA) from Rio de Janeiro, Brazil, to investigate how stigmatizing (vs. non-stigmatizing) reasons for deferral affected return rates of 82,648 donors over a 60-month follow-up period.

Results: Being deferred for sex- and drug-related reasons was perceived as much more stigmatizing than other reasons for deferral (odds ratio = 3.14, 95% confidence interval [CI]: 2.33–4.25). Controlling for multiple observables, prospective donors were less likely to return to the BCA when deferred for stigmatizing (vs. non-stigmatizing) reasons (adjusted hazard ratio = 0.87, 95% CI: 0.83–0.93).

Conclusion: Donors perceive deferrals motivated by sex- and drug-related reasons as particularly stigmatizing, which is negatively associated with donor return rates. BCAs may want to pay special attention when communicating stigmatizing reasons for deferral to prospective donors.

Keywords

blood donation, donor behaviour, return rates, stigma, stigmatizing deferrals, temporary deferrals

Highlights

- Donors perceive sex- and drug-related deferral reasons as particularly stigmatizing.
- Donors are less likely to return to donate when deferred for stigmatizing versus non-stigmatizing reasons.
- Blood collection agencies should take special care when communicating stigmatizing reasons for deferrals to prospective donors.

Yan Vieites and Claudio M. Ferreira contributed equally to this work.

INTRODUCTION

Millions of prospective blood donors are temporarily deferred worldwide every year [1]. On the one hand, deferrals help preserve blood safety, as many individual behaviours are associated with the increased incidence of infectious diseases [2]. On the other hand, although indisputably valuable, these postponements in donation come at a considerable cost. Deferred donors have been consistently shown to display lower return rates and longer lapses in donation attempts relative to successful donors [3–5]. But not all deferrals are created equal; incipient work shows that some reasons for deferral may have a more negative effect on the donors' careers than others [6–9]. We extend this body of research by examining whether stigmatization can help explain why certain reasons for deferral are particularly detrimental. More specifically, we propose that, while medical in nature, the reasons for deferral vary on the degree of stigma they are perceived to carry. For instance, prospective donors may be temporarily deferred because of their recent intake of fatty foods, use of cannabis or risky sexual conduct, with the latter two being presumably more stigmatizing than the former, as they involve sex- and drug-related aspects, which are generally considered sensitive and even taboo topics in many circles. More importantly, given that people tend to avoid stigmatizing environments and experiences [10], we investigate whether drug- and sex-related reasons for deferral (i.e., presumably stigmatizing deferrals) are associated with lower donor return rates vis-à-vis other reasons for deferral (i.e., presumably non-stigmatizing deferrals).

MATERIALS AND METHODS

Perceived stigmatization—Online survey

To test the assumption that the reasons for deferral vary on stigma, we first selected a pool of supposedly stigmatizing (i.e., drug- and sex-related) and non-stigmatizing reasons available in our donor dataset. Since stigmatizing deferrals are likely to be recurrent and often require behaviour change for future eligibility (e.g., using condoms and quitting drugs), we selected for comparison only non-stigmatizing reasons that would arguably also require comparable effort for behaviour change (e.g., refraining from eating fatty foods). Relatedly, to assure comparability, we only considered reasons for deferral that were comparable in terms of duration and were applied onsite right before the donation. These criteria resulted in a pool of 11 presumably stigmatizing and 6 presumably non-stigmatizing reasons for deferral (see Table 1).

TABLE 1 Selected deferral reasons and proportion of stigma attribution.

Reasons	Coding for stigma	Proportions (%)	*p* value
Included reasons			
Recent intake of fatty foods	Non-stigmatizing	28.5	<0.001
High blood pressure	Non-stigmatizing	29.4	<0.001
Weight over 140 kg (~310 lb.)	Non-stigmatizing	42.4	0.009
Weight–height disproportion	Non-stigmatizing	38.1	<0.001
Risky profession, hobbies or sports	Non-stigmatizing	34.8	<0.001
Recent tattoo, acupuncture or piercing	Non-stigmatizing	43.3	0.022
Recent use of cannabis	Stigmatizing	62.2	<0.001
Recent use of LSD or ecstasy	Stigmatizing	64.9	<0.001
Recent use of injectable contraindicated substances	Stigmatizing	61.8	<0.001
Recent use of inhaled drugs (cocaine)	Stigmatizing	62.1	<0.001
Sex without condom	Stigmatizing	61.5	<0.001
Risky sexual behaviour with condom (e.g., multiple partners)	Stigmatizing	66	<0.001
Sexual partner diagnosed with a blood-borne disease	Stigmatizing	63.6	<0.001
HIV-positive sexual partner	Stigmatizing	68.8	<0.001
Excluded reasons			
Recent intake of alcoholic beverages	Stigmatizing	52.5	0.455
Sexual partner diagnosed with Zika virus	Stigmatizing	55.5	0.104
Sexual partner recipient of organ or tissue transplant	Stigmatizing	49.2	0.832

Note: Proportions refer to the share of participants who indicated the respective reason to be more stigmatizing than the alternative presented in the pair of deferrals. *p* value refers to the statistical significance of the logit models contrasting each reason for deferral against 50%. Significantly higher scores indicate stigmatizing reasons for deferral and significantly lower scores indicate non-stigmatizing reasons for deferral. The three stigmatizing reasons reported above were excluded from further analyses because their coefficients in the logistic analyses were not significant.

We posted a pre-registered online survey on Facebook in February 2021 (for the preregistration protocol, see aspredicted.org/blind.php?x=vt4mw3). Upon providing their consent, respondents were told that although temporary deferrals are always motivated by medical issues, some of the reasons may be perceived as more stigmatizing than others—that is, more strongly associated with personal or behavioural characteristics that are targets of discrimination or prejudice in society in general. They were then presented with 6 pairs of deferral reasons such that one reason in the pair was presumably non-stigmatizing and the other was a randomly selected reason drawn from the set of 11 presumably stigmatizing deferral reasons. Respondents were asked to indicate the reason for deferral they considered more stigmatizing within each pair. The survey was conducted in Portuguese and targeted Brazilian respondents. We ended up unintentionally recruiting 931 participants, 531 more than originally planned. However, to ensure that the preregistration protocol was strictly followed, we analysed the results of the first 400 valid observations only. Importantly, had we examined the full dataset results would remain largely unchanged.

To analyse the data, we first conducted a series of proportions tests contrasting the percentage of times that a reason was considered stigmatizing versus 50%—our presumed point of indifference in the classification. We inferred that the reason was considered stigmatizing (non-stigmatizing) if the observed proportion was significantly greater (lower) than 50%. Next, we conducted a logistic regression analysis to directly compare the overall perceived stigma of stigmatizing and non-stigmatizing reasons for deferral. The model included a dummy variable indicating the presumed classification of the reason for deferral as the independent variable (1 = stigmatizing; 0 = non-stigmatizing) and the participants' assessment of that reason as the dependent variable (1 = reason perceived as more stigmatizing; 0 = reason perceived as less stigmatizing). Clustered standard errors at the individual level were used to assess the association between these two variables.

Donation upon deferral—Blood collection agency data

We then examined longitudinal, de-identified, individual-level data from the state-run blood collection agency (BCA) in Rio de Janeiro, Brazil, to assess the association between stigmatizing (vs. non-stigmatizing) deferrals and donor return rates. Our analysis, which is based on the 14 reasons described in Table 1, used temporary deferrals originally identified in the dataset from 2 January 1995 to 6 May 2020—a unique dataset comprising a 25-year history of donations.

We applied weights from coarsened exact matching to balance stigmatizing deferrals (sex- or drug-related) and non-stigmatizing deferrals on donor age, sex, race, education, prior donation experience, prior donation deferral, donation motivation, year of donation attempt and deferral duration. Matching was exact in categorical and quantitative variables. The latter were coarsened into intervals such that treated and control observations matched exactly within each interval. Table 2 provides a summary of the characteristics of 83,480 matched donation attempts from 82,648 donors and patterns of donation by deferral reason.

To estimate hazard ratios of return after stigmatizing deferrals against non-stigmatizing deferrals, we used Cox proportional hazards models with inverse probability of treatment weighting, controlling for the variables used in matching to reduce error variance and with standard errors clustered at the prospective donor. The outcome of interest was the first donation attempt after the end of the deferral period (when the prospective donor became eligible again). We excluded donation attempts with the end of the deferral period after 1 May 2015 and censored donation history after 60 months to ensure that all participants had the same follow-up period. We repeated the analysis separately by type of stigmatizing deferral (sex- or drug-related), by donor sex, and their interaction.

The survey study was approved by the institutional review board of Fundação Getulio Vargas (approval reference #138/2021), and the donor behaviour study was approved by the national database of research records involving human beings (Plataforma Brasil #2596206). The survey study was pre-registered at AsPredicted. We used Stata MP version 15.1 (StataCorp) to perform the analysis.

RESULTS

Perceived stigmatization

As summarized in Table 1, separate proportions tests revealed that the presumably stigmatizing reasons for deferral were considered as stigmatizing in more than 50% of the occasions, whereas the opposite was true for the presumably non-stigmatizing reasons. However, three presumed stigmatizing reasons were excluded from the list because they were not consistently perceived as such by the respondents: recent intake of alcoholic beverages, sexual partner diagnosed with Zika virus and sexual partner recipient of organ or tissue transplant. We then conducted a logistic regression analysis to directly compare the participants' overall perceptions of stigmatizing and non-stigmatizing reasons for deferral. As expected, the presumably more stigmatizing reasons were perceived as carrying greater stigma than the presumably non-stigmatizing reasons (odds ratio = 3.14, 95% confidence interval [CI]: 2.33–4.25). Importantly, results would remain unchanged had we opted not to remove the three aforementioned reasons for deferral with inconclusive classifications (odds ratio = 2.43, 95% CI: 1.88–3.14).

Donation upon deferral

Compared with those deferred for non-stigmatizing reasons, prospective donors were less likely to return to the BCA when deferred for stigmatizing reasons (adjusted hazard ratio = 0.87, 95% CI: 0.83–0.93). Comparing the effect across stigmatizing reasons, the reduction in return rates was stronger for drug-related (0.61, 95% CI 0.54–0.68)

TABLE 2 Characteristics of prospective donors and deferral reasons.

	Deferral reason					
	Non-stigmatizing		Drug-related stigmatizing		Sex-related stigmatizing	
Characteristic	Did not return ($n = 16,468$)	Returned[a] ($n = 3855$)	Did not return ($n = 3179$)	Returned ($n = 462$)	Did not return ($n = 47,757$)	Returned ($n = 11,759$)
Sex, *n* (%)						
Male	8448 (51.3)	2037 (52.8)	2857 (89.9)	420 (90.9)	36,392 (76.2)	9511 (80.9)
Female	8020 (48.7)	1818 (47.1)	322 (10.1)	42 (9.1)	11,365 (23.8)	2248 (19.1)
Race[b], *n* (%)						
White	7839 (47.6)	1873 (48.5)	1176 (37.0)	174 (37.6)	22,298 (46.7)	5292 (45.0)
Black	1144 (7.0)	237 (6.2)	309 (9.7)	46 (10.0)	2881 (6.0)	772 (6.6)
Mixed race	6877 (41.7)	1561 (40.5)	1390 (43.8)	194 (42.0)	18,849 (39.5)	4726 (40.2)
Others	38 (0.2)	16 (0.4)	7 (0.2)	0 (0.0)	88 (0.2)	21 (0.2)
Missing	570 (3.5)	168 (4.4)	297 (9.3)	48 (10.4)	3641 (7.6)	948 (8.1)
Age, years						
Mean (SD)	37.4 (13.2)	35.5 (12.3)	32.4 (9.0)	31.5 (8.4)	29.5 (9.7)	28.3 (8.6)
Age, *n* (%)						
<21 years	3074 (18.7)	721 (18.7)	199 (6.3)	32 (6.9)	7674 (16.1)	1922 (16.4)
21–30 years	6924 (42.0)	1667 (43.2)	1361 (42.7)	201 (43.5)	23,695 (49.7)	6048 (51.4)
31–40 years	3457 (21.0)	862 (22.4)	1048 (33.0)	161 (34.9)	10,115 (21.2)	2551 (21.7)
41–50 years	2223 (13.5)	468 (12.1)	466 (14.7)	58 (12.6)	4604 (9.6)	980 (8.3)
51 years or more	790 (4.8)	137 (3.6)	105 (3.3)	10 (2.1)	1669 (3.5)	258 (2.2)
Education, *n* (%)						
Incomplete primary	2075 (12.6)	430 (11.2)	1191 (37.5)	128 (27.8)	8531 (17.9)	1641 (14.0)
Complete primary	1495 (9.1)	382 (9.9)	445 (14.0)	75 (16.2)	4590 (9.6)	1160 (9.9)
Incomplete high school	8743 (53.1)	1990 (51.6)	1158 (36.4)	197 (42.6)	22,743 (47.6)	6098 (51.8)
High school or more	4155 (25.2)	1053 (27.3)	385 (12.1)	62 (13.4)	11,893 (24.9)	2.860 (24.3)
Has previous donation, *n* (%)	1332 (8.1)	1124 (29.2)	199 (6.3)	97 (21.0)	3657 (7.7)	3113 (26.5)
Has previous deferral, *n* (%)	146 (0.9)	79 (2.0)	37 (1.2)	12 (2.6)	446 (0.9)	160 (1.4)
Donation attempt year						
Until 2008	5526 (33.6)	1711 (44.4)	1502 (47.3)	268 (58.0)	25,591 (53.6)	7155 (60.1)
2008 or later	10,942 (66.4)	2144 (55.6)	1677 (52.3)	194 (42.0)	22,166 (46.4)	4604 (39.1)

Note: This table presents data for 83,480 donation attempts from 82,648 unique prospective donors.
[a]We observed participants' returns within a 60-month interval after the end of the deferral period.
[b]Race was defined according to self-reports, with options determined in the registration process at the blood centre. We considered race in this study to match prospective donors.

than for sex-related deferrals (0.89, 95% CI: 0.84–0.94). Interestingly, while the decrease following drug-related deferrals was homogeneous across sociodemographic characteristics, sex-related deferrals were associated with a smaller reduction in return rates among male (0.91, 95% CI: 0.85–0.98) than female donors (0.80, 95% CI: 0.75–0.86; Figure 1).

DISCUSSION

Although it is well-established that temporary deferrals have a negative influence over donor return rates [3–5], this research joins an incipient body of work showing that different reasons for deferral have distinct effects on return rates [6–9]. Our studies show that prospective donors perceive deferrals motivated by sex- and drug-related reasons as particularly stigmatizing when compared with other common reasons for deferral. Critically, donors deferred for such stigmatizing (vs. non-stigmatizing) reasons proved to be less likely to try to donate again in a follow-up period of 60 months. When scrutinizing differences across the types of stigmatizing reasons, effects were stronger for drug-related than sex-related deferrals. A plausible explanation for this discrepancy is the fact that the use of drugs such as marijuana and cocaine is illegal in Brazil, which may have reinforced perceptions of stigmatization and created concerns involving the

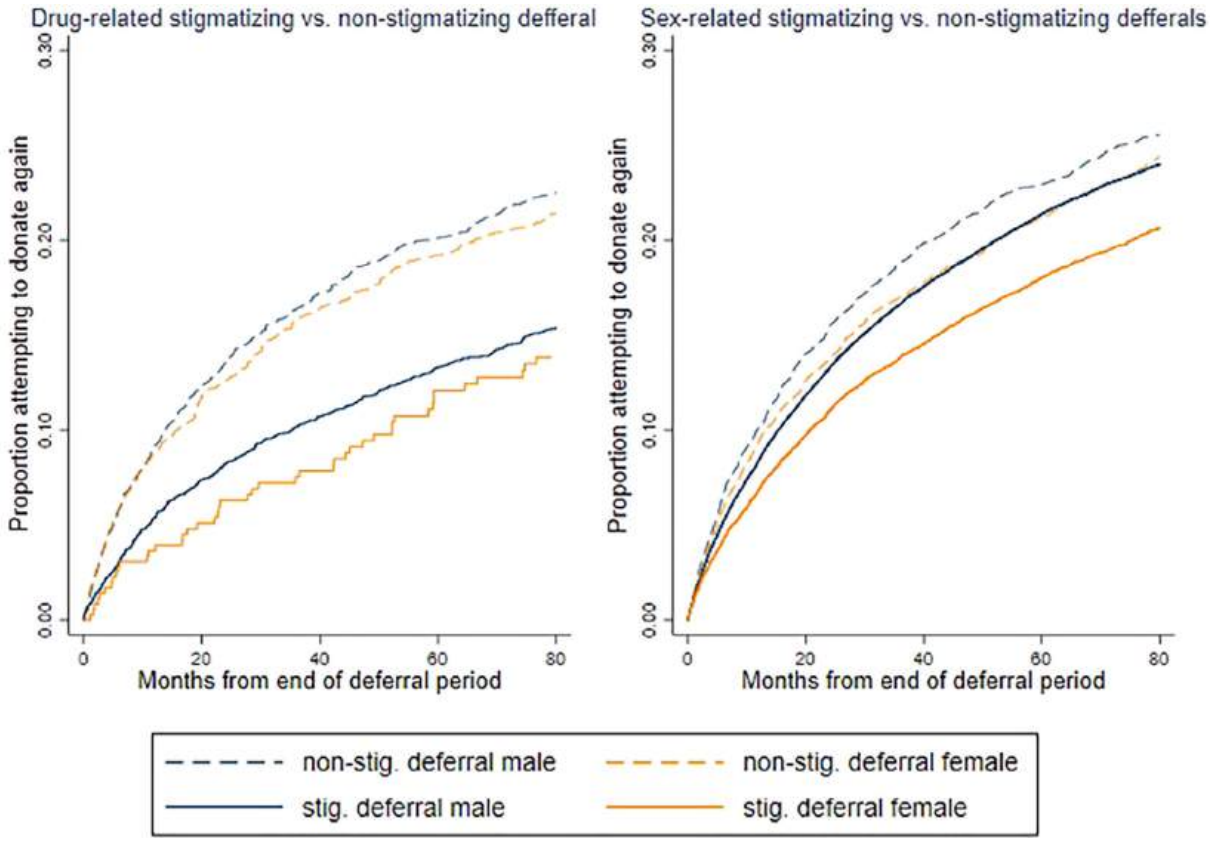

FIGURE 1 Kaplan–Meier curves and hazard ratios for 60-month return rate across prospective donors' sex. (a) Included 21,860 donation attempts: 3641 with drug-related deferrals (3277 males and 364 females) and 18,219 with non-stigmatizing deferrals (10,169 males and 8050 females). We excluded 61,620 donation attempts from the analysis, as they were not matched or were sex-related deferrals. (b) Included 75,149 donation attempts: 59,516 with sex-related deferrals (45,903 males and 13,613 females) and 15,633 with non-stigmatizing deferrals (6295 males and 9338 females). We excluded 8331 donation attempts from the analysis, as they were not matched or were drug-related deferrals. Stig. denotes stigmatizing and non-stig. denotes non-stigmatizing reasons for deferral.

consequences of this misdemeanour. In addition to this average difference, the detrimental consequences of drug-related deferrals were also more homogeneous than those observed for sex-related deferrals. In the latter case, women were more sensitive to the stigmatizing experience than men, as revealed by their especially lower return rates. This finding is consistent with the literature on gender double standards, which suggests that behavioural expectations of sexual restraint for women and freedom for men may lead to unwanted health-related consequences across genders [11].

While an incipient body of work seems to suggest that certain reasons for deferral can unintendedly induce biases and create feelings of stigmatization [12, 13], it has not examined potential differences in perception across types of deferrals. Our studies address this gap and shed light on the importance of taking stigma into consideration when developing donor management and communication strategies. Indeed, as blood donation regulations move from outrightly banning certain groups of donors (e.g., men who have sex with men) to screening individual risky behaviours [14], issues concerning the stigma of temporary deferrals are likely to gain momentum, such as the recent research which investigates strategies for mitigating donor discomfort during screening sessions [15]. Future research could assess how different ways of communicating stigmatizing reasons for deferral to prospective donors influence return rates.

In this article, we examined stigma using a binary approach where reasons are either described as particularly stigmatizing or not. Stigma, however, may be treated in a continuum. Future research could also extend our findings by investigating the extent to which the degree–rather than the presence–of stigmatization influences donation behaviour. Finally, although some deferral reasons described in the current work might be country specific, the finding that deferral reasons vary on stigma is arguably generalizable to other countries. We also do not have information about possible changes in rules and perceptions of stigmatizing deferrals over time. Future research could address these concerns by examining the effect of stigmatizing deferrals on blood donor return rates in other regions and, in doing so, assess the generalizability of our findings while also shedding light on potentially relevant cross-country heterogeneities.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data concerning donation behaviour are proprietary and will thus not be made available. Data concerning prospective donor's perceptions about the stigma of different types of donations, which was derived from a survey conducted by the authors, will be made available using a permanent link at OSF (Open Science Framework).

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REFERENCES

1. World Health Organization (WHO). Blood donor selection: guidelines on assessing donor suitability for blood donation. Geneva: WHO; 2012.
2. Gonçalez TT, Sabino EC, Kaidarova Z, Carneiro-Proietti ABF, Miranda C, Loureiro P, et al. Does deferral for high-risk behaviors improve the safety of the blood supply? Transfusion. 2019;59:2334–43.
3. Ferreira CM, Vieites Y, Goldszmidt R, Andrade EB. The effect of temporary deferrals on donor return: a 26-year assessment in a setting without retention activities. Transfusion. 2022;62:1583–93.
4. Custer B, Chinn A, Hirschler NV, Busch MP, Murphy EL. The consequences of temporary deferral on future whole blood donation. Transfusion. 2007;47:1514–23.
5. Halperin D, Baetens J, Newman B. The effect of short-term, temporary deferral on future blood donation. Transfusion. 1998;38:181–3.
6. Glatt TN, Swanevelder R, Prithvi Raj S, Mitchel J, van den Berg K. Donor deferral and return patterns–a south African perspective. ISBT Sci Ser. 2021;16:178–87.
7. Spekman ML, van Tilburg TG, Merz EM. Do deferred donors continue their donations? A large-scale register study on whole blood donor return in The Netherlands. Transfusion. 2019;59:3657–65.
8. Custer B, Schlumpf KS, Wright D, Simon TL, Wilkinson S, Ness PM. Donor return after temporary deferral. Transfusion. 2011;51:1188–96.
9. Zou S, Musavi F, Notari EP, Rios JA, Trouern-Trend J, Fang CT. Donor deferral and resulting donor loss at the American red Cross blood services, 2001 through 2006. Transfusion. 2008;48:2531–9.
10. Major B, O'Brien LT. The social psychology of stigma. Annu Rev Psychol. 2005;56:393–421.
11. Heise L, Greene ME, Opper N, Stavropoulou M, Harper C, Nascimento M, et al. Gender inequality and restrictive gender norms: framing the challenges to health. Lancet. 2019;393:2440–54.
12. Hofkirchner A, Kohut T, O'Brien S, Fisher W. Assessing unintentional creation of bias against men who have sex with men as a function of exposure to blood donor screening questionnaire: a national randomized controlled trial. Transfusion. 2022;62:1399–407.
13. Armstrong J, Brennan DJ, Collict D, Kesler M, Bekele T, Souleymanov R, et al. A mixed methods investigation of the relationship between blood donor policy, interest in donation, and willingness to donate among gay, bisexual, and other men who have sex with men in Ontario, Canada. BMC Public Health. 2022;22:1–13.
14. Skelly AN, Kolla L, Tamburro MK, Bar KJ. Science over stigma: the need for evidence-based blood donation policies for men who have sex with men in the USA. Lancet Haematol. 2020;7:779–82.
15. Haw J, Woo H, Kohut T, Fisher W. Sexual risk behavior questions: understanding and mitigating donor discomfort. Transfusion. 2022;62:355–64.

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SHORT REPORT

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RhD mismatch does not affect haematopoietic recovery, graft-versus-host disease and survival in allogeneic haematopoietic cell transplantation: A Japanese registry-based study

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Abstract

Background and Objectives: ABO blood group mismatch between the donor and the recipient can affect the success of the transplant as well as problems with the red blood cells during allogeneic haematopoietic cell transplantation (HCT). However, the impact of the Rhesus (Rh) D mismatch on transplant outcomes in allogeneic HCT has been poorly elucidated.

Materials and Methods: We retrospectively evaluated the impact of the RhD mismatch on post-transplant outcomes in 64,923 patients who underwent allogeneic HCT between 2000 and 2021 using a Japanese registry database.

Results: Out of the whole group, 64,293, 322, 270 and 38 HCTs were done when the recipient or donor was RhD-mismatched with (+/+), (−/+), (+/−) or (−/−) combinations. The difference in RhD between recipient/donor (−/+), (+/−) and (−/−) did not affect haematopoietic recovery, acute and chronic graft-versus-host disease (GVHD), overall survival (OS), non-relapse mortality (NRM) or relapse when RhD (+/+) was used as the reference group in multivariate analysis.

Conclusion: Our registry-based study demonstrated that RhD mismatch between recipient and donor did not significantly impact haematopoietic recovery, GVHD, OS, NRM or relapse after allogeneic HCT. These data suggest that RhD mismatches may not need to be avoided for recipient and donor combinations in allogeneic HCT.

Keywords

allogeneic haematopoietic cell transplantation, RhD alloimmunization, RhD antigen, RhD incompatibility, RhD mismatch, rhesus

Highlights

- This is the largest study investigating the impact of Rhesus (Rh) D mismatch on outcomes in 64,923 patients following allogeneic haematopoietic cell transplantation (HCT), using data from the Japanese registry.
- When RhD recipient/donors (+/+) were considered, the reference group in multivariate analysis, RhD mismatches between recipient and donor, (−/+), (+/−) and (−/−), did not affect haematopoietic recovery, acute and chronic graft-versus-host disease, overall survival, non-relapse mortality or relapse.
- These data suggest that RhD mismatches may not need to be avoided for recipient and donor combinations in allogeneic HCT.

INTRODUCTION

ABO blood group mismatch between donor and recipient has been shown to affect transplant outcomes, such as haematopoietic recovery, graft-versus-host disease (GVHD), non-relapse mortality (NRM) and overall survival (OS), as well as erythrocyte-related complications such as haemolysis, delayed erythrocyte engraftment and pure red cell aplasia in allogeneic haematopoietic cell transplantation (HCT) [1, 2]. However, there have been reports of a few cases of immunogenic haemolytic reactions in minor blood group mismatches other than ABO [3–13], but few large-scale reports have been published.

The Rhesus (Rh) D polypeptide is a highly immunogenic antigen, and anti-RhD antibodies can cause clinically immunogenic haemolytic reactions, such as haemolytic disease of the foetus and newborn and acute haemolytic transfusion reactions [14]. In allogeneic HCT, there have been reports of a few cases of immunogenic haemolytic reactions in RhD mismatch settings [4, 6, 7, 13]. The distribution of negative RhD blood groups, the most problematic of the Rh types, varies between populations and races. That is most common in individuals of European and North American descent (15%–17%), is relatively decreased in the regions of Africa and India (3%–8%) and is rare in Asia (0.1%–0.3%) [15]. In Japan, the frequency of negative RhD individuals is very low, at 1 in 200 (0.5%). Even in allogeneic HCT, in which donors are selected mainly according to human leukocyte antigen (HLA), HCTs using RhD-negative donors or HCTs for RhD-negative recipients are sometimes performed. Still, the impact of the RhD mismatch on transplant outcomes in allogeneic HCT has been poorly elucidated [9, 11]. In this study, we evaluated the clinical impact of the RhD mismatch on clinical outcomes in allogeneic HCT using Japanese registry data.

METHODS

Clinical data were provided by the Transplant Registry Unified Management Program of the Japanese Data Center for Haematopoietic Cell Transplantation and the Japanese Society for Transplantation and Cellular Therapy. Patients who underwent allogeneic HCT between 2000 and 2021 in Japan were included in this study. We excluded patients who lacked data on RhD between recipients and donors and survival. Finally, 64,923 patients were eligible for this study. Details for the methods are provided in the Supplementary Methods.

Statistical analysis

To assess differences in baseline characteristics between groups, a chi-squared or Fisher exact test was performed for categorical data,

TABLE 1 Baseline characteristics of patients and transplantations.

Recipient	RhD (+)	RhD (−)	RhD (+)	RhD (−)	
Donor	**RhD (+)**	**RhD (+)**	**RhD (−)**	**RhD (−)**	***p* value**
No. of patients	64,293	322	270	38	
Median recipient age (IQR), years	45 (27–57)	45 (28–58)	47 (26–58)	46 (31–54)	0.804
Recipient gender					**0.049**
Female	26,345 (41.0)	118 (36.6)	96 (35.7)	11 (28.9)	
Male	37,932 (59.0)	204 (63.4)	173 (64.3)	27 (71.1)	
Missing	16	0	1	0	
PS					0.355
0–1	52,913 (86.6)	273 (89.8)	221 (85.0)	33 (86.8)	
2–4	8176 (13.4)	31 (10.2)	39 (15.0)	5 (13.2)	
Missing	3204	18	10	0	
Disease type					0.549
Leukaemia/myelodysplasia/MPN	47,988 (74.6)	250 (77.6)	210 (77.7)	29 (76.3)	
Lymphoma	10,224 (15.9)	43 (13.3)	39 (14.4)	6 (15.7)	
MM/PCD	849 (1.3)	8 (2.5)	2 (0.7)	1 (2.6)	
AA/PRCA/PNH/bone marrow failure	2625 (4.0)	9 (2.8)	8 (2.9)	2 (5.3)	
Others	2607 (4.0)	12 (3.8)	11 (4.1)	0	
Disease risk at HCT					0.205
CR/Benigh	34,864 (54.9)	185 (58.0)	153 (57.1)	26 (68.4)	
NR	28,677 (45.1)	134 (42.0)	115 (42.9)	12 (31.6)	
Missing	752	3	2	0	
HCT period					0.837
2000–2010	24,489 (38.1)	120 (37.3)	96 (35.6)	15 (39.5)	
2011–2021	39,804 (61.9)	202 (62.7)	174 (64.4)	23 (60.5)	
Graft source					**<0.001**
BM	28,592 (44.5)	145 (45.0)	105 (38.9)	11 (29.7)	
PBSC	15,582 (24.2)	70 (21.7)	73 (27.0)	23 (62.2)	
CB	20,111 (31.3)	107 (33.2)	92 (34.1)	3 (8.1)	
Missing	8	0	0	1	
Donor type					**<0.001**
Related	21,248 (33.0)	96 (29.8)	97 (35.9)	32 (84.2)	
Unrelated	43,045 (67.0)	226 (70.2)	173 (64.1)	6 (15.8)	
HLA mismatch					**0.039**
0	34,696 (54.3)	174 (54.5)	139 (51.7)	25 (65.8)	
1	13,360 (20.9)	68 (21.3)	45 (16.7)	2 (5.3)	
≥ 2	15,843 (24.8)	77 (24.1)	85 (31.6)	11 (28.9)	
Missing	394	3	1	0	
Previous history of HCT					0.967
No	52,098 (81.0)	261 (81.1)	218 (80.7)	32 (84.2)	
Yes	12,193 (19.0)	61 (18.9)	52 (19.3)	6 (15.8)	
Sex incompatibility					0.403
Match	32,287 (52.5)	152 (49.7)	141 (55.3)	23 (63.9)	
Male to female	14,133 (23.0)	67 (21.9)	51 (20.0)	5 (13.9)	
Female to male	15,055 (24.5)	87 (28.4)	63 (24.7)	8 (22.2)	
Missing	2818	16	15	2	

TABLE 1 (Continued)

Recipient	RhD (+)	RhD (−)	RhD (+)	RhD (−)	
Donor	**RhD (+)**	**RhD (+)**	**RhD (−)**	**RhD (−)**	***p* value**
ABO incompatibility					0.782
Match	30,684 (47.7)	156 (48.4)	130 (48.1)	22 (57.9)	
Minor mismatch	13,867 (21.6)	73 (22.7)	63 (23.3)	8 (21.1)	
Major mismatch	12,989 (20.2)	61 (18.9)	53 (19.6)	3 (7.9)	
Bidirectional mismatch	6734 (10.5)	32 (9.9)	24 (8.9)	5 (13.2)	
Missing	19	0	0	0	
Conditioning regimen					0.125
MAC	36,005 (56.3)	198 (62.3)	146 (54.3)	24 (63.2)	
RIC	27,925 (43.7)	120 (37.7)	123 (45.7)	14 (36.8)	
Missing	363	4	1	0	
GVHD prophylaxis					0.166
With MTX	46,580 (72.5)	248 (77.0)	186 (68.9)	28 (73.7)	
Without MTX	17,659 (27.5)	74 (23.0)	84 (31.1)	10 (26.3)	
Missing	54	0	0	0	
Use of ATG					0.102
No	55,215 (85.9)	291 (90.4)	228 (84.4)	34 (89.5)	
Yes	9078 (14.1)	31 (9.6)	42 (15.6)	4 (10.5)	
Median follow-up for survivors (IQR), months	62.8 (27.5–112.8)	74.1 (28.2–119.9)	61.3 (27.8–108.0)	60.2 (15.1–122.8)	0.707

Note: The *p* values in bold are statistically significant (<0.05).
Abbreviations: AA, aplastic anaemia; ATG, antithymocyte globulin; BM, bone marrow; CB, cord blood; CR, complete remission; GVHD, graft-versus-host disease; HCT, haematopoietic cell transplantation; HLA, human leukocyte antigen; IQR, interquartile range; MAC, myeloablative conditioning; MM, multiple myeloma; MPN, myeloproliferative neoplasm; MTX, methotrexate; NR, non-remission; PBSC, peripheral blood stem cell; PCD, plasma cell disorders; PNH, paroxysmal nocturnal haemoglobinuria; PRCA, pure red cell aplasia; PS, performance status; RhD, rhesus D; RIC, reduced-intensity conditioning.

and the Kruskal–Wallis test was used for continuous variables. Unadjusted probabilities of haematopoietic recovery, GVHD, NRM and relapse were calculated using cumulative incidence estimates, while competing hazards were taken into consideration. Death prior to each event was a competing event for both GVHD and haematopoietic recovery. Relapse and NRM were opposing phenomena. The Kaplan–Meier method was used to calculate the unadjusted probability of OS. Grey's test for haematological recovery, GVHD, NRM and relapse, as well as a log-rank test for OS, were used in univariate analyses. A Cox proportional hazard model with adjustments for all endpoints was used for multivariate analysis, and the results were presented as a hazard ratio (HR) and 95% confidence interval (CI). The following variables for multivariate analysis were considered: recipient age (0–29 vs. 30–59 vs. ≥60 years), performance status (PS) (0–1 vs. 2–4), disease risk of HCT (standard risk vs. high risk), HCT period (2000–2010 vs. 2011–2021), graft source (bone marrow vs. peripheral blood stem cell [PBSC] vs. cord blood), donor type (related vs. unrelated), HLA disparities based on antigen level HLA-A, HLA-B and HLA-DR in the GVHD direction (match vs. mismatch), previous history of HCT (no vs. yes), sex compatibility between donor and recipient (match vs. male to female vs. female to male), ABO compatibility between donor and recipient (match vs. minor mismatch vs. major mismatch vs. bidirectional mismatch), conditioning regimen (myeloablative conditioning vs. reduced-intensity conditioning), GVHD prophylaxis (with methotrexate [MTX] vs. without MTX), use of antithymocyte globulin (no vs. yes) and RhD mismatch between recipient and donor (+/+ vs. −/+ vs. +/− vs. −/−). The RhD +/+ was considered the reference group in the multivariate analyses. Donor age was not included in the variables of the multivariate analysis because 31.3% of graft sources were cord blood in our cohorts. Statistical analyses were performed with EZR version 1.61 (Saitama Medical Center, Jichi Medical University) [16], a graphical user interface for the R 4.2.2 software program (R Foundation for Statistical Computing). Two-sided *p* values are reported, and $p < 0.05$ was considered to be significant.

RESULTS

The characteristics of patients and transplants are shown in Table 1. Among them, 64,293, 322, 270 and 38 recipients were transplanted in RhD mismatch between recipient/donor (+/+), (−/+), (+/−) and (−/−) groups, respectively. There were no significant differences among the four groups, except for recipient gender, graft source, donor type and HLA mismatch. The RhD recipient/donor (−/−) group contained higher proportions of male recipients, use of PBSC, related donors and HLA match.

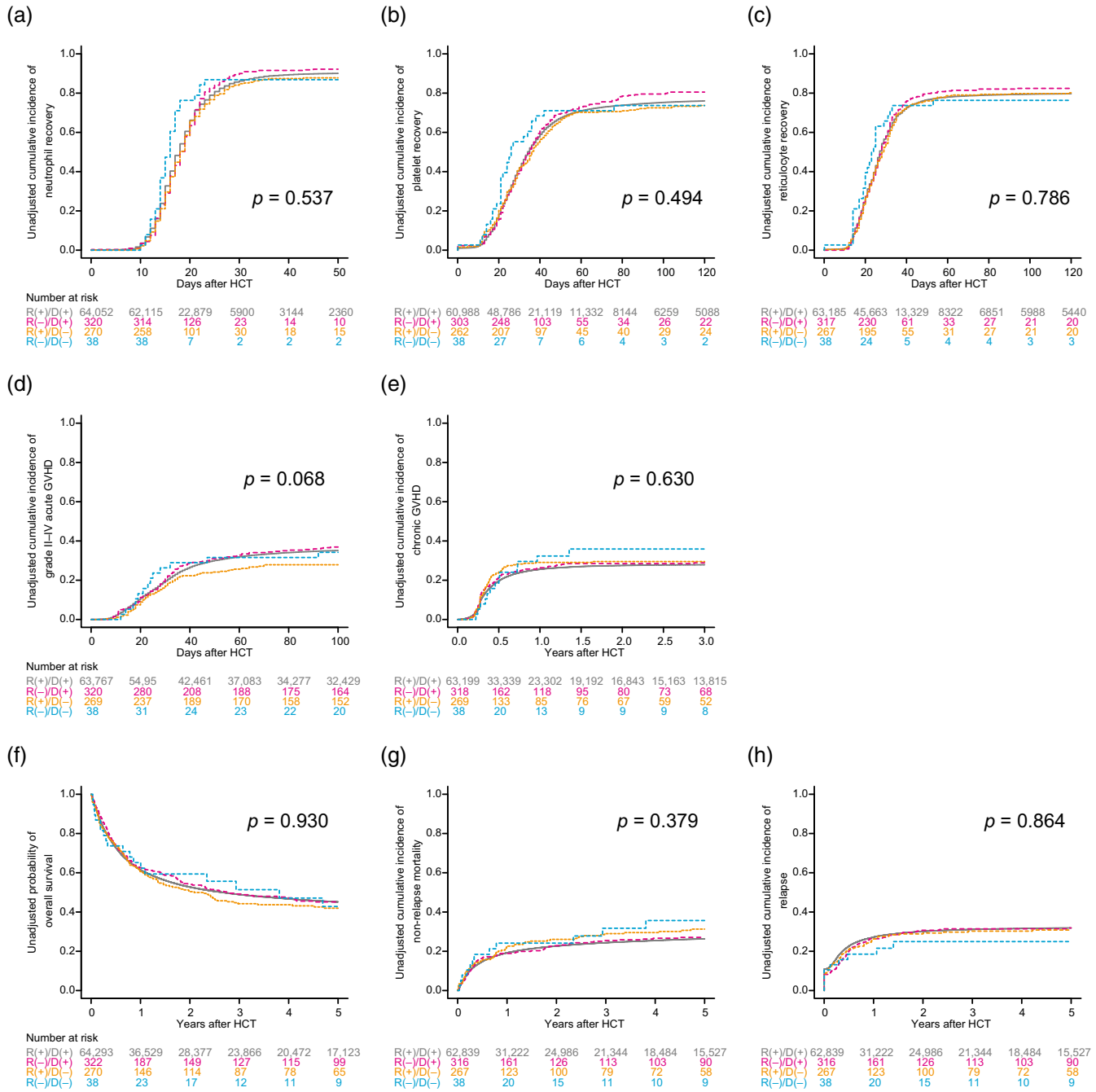

FIGURE 1 Unadjusted cumulative incidence of neutrophil (a), platelet (b), and reticulocyte (c) recovery, grades II–IV acute graft-versus-host disease (GVHD) (d), chronic GVHD (e), unadjusted probability of overall survival (f), non-relapse mortality (g), and relapse (h) according to rhesus D mismatch groups between recipient and donor after allogeneic haematopoietic cell transplantation (HCT). R, recipient; D, donor.

RhD mismatch was not associated with neutrophil, platelet or reticulocyte recovery in univariate and multivariate analyses (Figure 1a–c, Table 2; Table S1). Among evaluable patients who required red blood cell (RBC) transfusion, the median last RBC transfusion days after HCT was 28 (interquartile range [IQR], 18–51) days for the RhD recipient/donor (+/+) group, 31 (IQR, 20–51.5) days for the RhD recipient/donor (−/+) group, 30 (IQR, 18–52) days for the RhD recipient/donor (+/−) group and 22 (IQR, 14–39) days for the RhD recipient/donor (−/−) group, respectively ($p = 0.064$). Among evaluable patients who required platelet transfusion, the median last platelet transfusion days after HCT were 30 (IQR, 17–58) days for the RhD recipient/donor (+/+) group, 30 (IQR, 18–56) days for the RhD recipient/donor (−/+) group, 28 (IQR, 17.5–55) days for the RhD recipient/donor (+/−) group and 20 (IQR, 12.5–33.5) days for the RhD recipient/donor (−/−) group, respectively ($p = 0.095$).

TABLE 2 Multivariate analysis of RhD compatibility for the outcomes after allogeneic HCT.

	Recipient/donor	HR (95% CI)	*p* value
Neutrophil recovery			
	+/+	Reference	
	−/+	0.96 (0.85–1.08)	0.529
	+/−	1.01 (0.88–1.15)	0.836
	−/−	0.90 (0.63–1.29)	0.587
Platelet recovery			
	+/+	Reference	
	−/+	1.00 (0.88–1.14)	0.937
	+/−	0.94 (0.81–1.09)	0.477
	−/−	0.98 (0.67–1.43)	0.928
Reticulocyte recovery			
	+/+	Reference	
	−/+	1.04 (0.92–1.18)	0.487
	+/−	1.01 (0.88–1.16)	0.820
	−/−	0.96 (0.65–1.40)	0.839
Grades II–IV acute GVHD			
	+/+	Reference	
	−/+	1.09 (0.90–1.31)	0.351
	+/−	0.82 (0.65–1.04)	0.110
	−/−	1.15 (0.67–1.99)	0.601
Chronic GVHD			
	+/+	Reference	
	−/+	1.09 (0.88–1.35)	0.404
	+/−	1.16 (0.92–1.47)	0.193
	−/−	1.13 (0.65–1.95)	0.649
Overall mortality (1-OS)			
	+/+	Reference	
	−/+	1.06 (0.90–1.24)	0.458
	+/−	1.02 (0.85–1.21)	0.822
	−/−	1.16 (0.73–1.84)	0.525
NRM			
	+/+	Reference	
	−/+	1.13 (0.91–1.40)	0.252
	+/−	1.09 (0.86–1.38)	0.474
	−/−	1.54 (0.85–2.79)	0.148
Relapse			
	+/+	Reference	
	−/+	0.96 (0.77–1.19)	0.746
	+/−	0.93 (0.73–1.17)	0.544
	−/−	0.99 (0.53–1.84)	0.984

Abbreviations: CI, confidence interval; GVHD, graft-versus-host disease; HCT, haematopoietic cell transplantation; HR, hazard ratio; NRM, non-relapse mortality; OS, overall survival; RhD, rhesus D.

RhD mismatch was not associated with grades II–IV acute GVHD and chronic GVHD, overall mortality, NRM or relapse in univariate and multivariate analyses (Figure 1d–h, Table 2; Tables S2 and S3).

DISCUSSION

Unlike ABO alloantibodies, RhD alloantibodies do not occur naturally and require an immune response through exposure to RhD-positive RBCs through pregnancy, transfusion and transplantation. Therefore, RhD alloimmunization has occurred after allogeneic HCT, which means that the majority of RhD-mismatched HCT represents de novo RhD alloimmunization [17]. However, the incidence of de novo RhD alloimmunization events has been infrequent following RhD-mismatched HCT, ranging from 0% to 43% [3–5, 8–13]. Moreover, recipients with RhD alloimmunization did not always experience haemolysis during and after allogeneic HCT [17]. However, in our study, the incidence of de novo RhD alloimmunization and clinical and laboratory haemolysis following RhD-mismatched HCT could not be evaluated because of incomplete data. Interestingly, although the RhD (−/−) group contained higher proportions of use of PBSC, related donors and HLA match, our data showed that the last transfusion days of RBC and platelets were slightly later in RhD mismatch groups compared with RhD match groups, which suggests that the number of RBC and platelet transfusions could be higher in RhD mismatch groups compared with RhD match groups. This is consistent with a previous study showing that an RhD mismatch led to a significant increase in RBC transfusions in the second month after HCT [9]. Therefore, the frequency and severity of de novo RhD immunization and haemolysis after RhD-mismatched HCT warrant further study with a larger cohort.

Apart from haemolysis after HCT, previous studies demonstrated that ABO incompatibility affected neutrophil and platelet recovery, acute GVHD, NRM or OS after allogeneic HCT [1, 2]. By contrast, there were only two reports evaluating the impact of RhD mismatch on transplant outcomes [9, 11]. Erker et al. [9] in Germany reported that the OS of RhD-mismatched groups was significantly lower than that of RhD-matched groups in 143 allogeneic PBSC transplants. By contrast, Wirk et al. [11] in the United States reported that the RhD mismatch was not associated with OS, NRM or acute and chronic GVHD in 258 allogeneic HCTs, which is consistent with our results. The different effects on transplant outcomes between ABO and RhD mismatch have not been unclear, but the ABO blood group antigens are expressed not only in RBCs but also in neutrophils, platelets and vascular endothelial and epithelial cells, whereas RhD antigens are expressed only in RBCs, which could result in a possible adverse immunological reaction by RBC antigens not in RhD-mismatched HCT but in ABO mismatched HCT.

Our study had several limitations. First, this was a retrospective registry-based analysis. Therefore, several pieces of information were unavailable, such as prior RhD alloimmunization, clinical and laboratory data on haemolysis, indirect and direct antiglobulin tests and the total number of transfusions. Second, the distribution of negative RhD blood groups varies between populations and races. Therefore, our results should be interpreted cautiously when extended to other racial cohorts. Despite some limitations, this study included the largest patient population, which could show no impact of the RhD mismatch on post-transplant outcomes after allogeneic HCT.

In summary, our registry-based study demonstrated that RhD mismatch between recipient and donor did not significantly impact haematopoietic recovery, GVHD, NRM, relapse or OS after allogeneic HCT. Since the frequency of negative RhD individuals is very low in Japan (about 0.5%), it is anticipated that the challenges associated with obtaining allogeneic donors and blood transfusion supplies would raise the risk of allogeneic HCT. However, RhD mismatches may not need to be avoided for recipient and donor combinations in allogeneic HCT.

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T.K. designed the research, analysed the data, performed the statistical analysis and wrote the manuscript; all the other authors contributed to data collection; all authors approved the final version.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data may be available from the corresponding author upon reasonable request and with permission of the JSTCT and JDCHCT.

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REFERENCES

1. Rowley SD, Donato ML, Bhattacharyya P. Red blood cell-incompatible allogeneic hematopoietic progenitor cell transplantation. Bone Marrow Transplant. 2011;46:1167–85.
2. Guru Murthy GS, Logan BR, Bo-Subait S, Beitinjaneh A, Devine S, Farhadfar N, et al. Association of ABO mismatch with the outcomes of allogeneic hematopoietic cell transplantation for acute leukemia. Am J Hematol. 2023;98:608–19.
3. Lasky LC, Warkentin PI, Kersey JH, Ramsay NK, McGlave PB, McCullough J. Hemotherapy in patients undergoing blood group incompatible bone marrow transplantation. Transfusion. 1983;23:277–85.
4. Hows J, Beddow K, Gordon-Smith E, Branch DR, Spruce W, Sniecinski I, et al. Donor-derived red blood cell antibodies and immune hemolysis after allogeneic bone marrow transplantation. Blood. 1986;67:177–81.
5. Ting A, Pun A, Dodds AJ, Atkinson K, Biggs JC. Red cell alloantibodies produced after bone marrow transplantation. Transfusion. 1987;27:145–7.
6. Franchini M, de Gironcoli M, Gandini G, Vassanelli A, Rocca P, Benedetti F, et al. Transmission of an anti-RhD alloantibody from donor to recipient after ABO-incompatible BMT. Bone Marrow Transplant. 1998;21:1071–3.
7. Sokol RJ, Stamps R, Booker DJ, Scott FM, Laidlaw ST, Vandenberghe EA, et al. Posttransplant immune-mediated hemolysis. Transfusion. 2002;42:198–204.
8. Mijovic A. Alloimmunization to RhD antigen in RhD-incompatible haemopoietic cell transplants with non-myeloablative conditioning. Vox Sang. 2002;83:358–62.
9. Erker CG, Steins MB, Fischer RJ, Kienast J, Berdel WE, Sibrowski W, et al. The influence of blood group differences in allogeneic hematopoietic peripheral blood progenitor cell transplantation. Transfusion. 2005;45:1382–90.
10. Cid J, Lozano M, Fernández-Avilés F, Carreras E, Pereira A, Mazzara R, et al. Anti-D alloimmunization after D-mismatched allogeneic hematopoietic stem cell transplantation in patients with hematologic diseases. Transfusion. 2006;46:169–73.
11. Wirk B, Klumpp TR, Ulicny J, Herman JH, Gajewski JL, Martin ME, et al. Lack of effect of donor-recipient Rh mismatch on outcomes after allogeneic hematopoietic stem cell transplantation. Transfusion. 2008;48:163–8.
12. Worel N, Böhm A, Rabitsch W, Leitner G, Mitterbauer M, Kalhs P, et al. Frequency and prognostic value of D alloantibodies after D-mismatched allogeneic hematopoietic stem cell transplantation after reduced-intensity conditioning. Transfusion. 2012;52:1348–53.
13. Chan JY, Tokessy M, Saidenberg E, Giulivi A, Tay J, Allan DS. RhD alloimmunization in allogeneic HSCT. Bone Marrow Transplant. 2013;48:459–60.
14. de Haas M, Thurik FF, Koelewijn JM, van der Schoot CE. Haemolytic disease of the fetus and newborn. Vox Sang. 2015;109:99–113.
15. Bangham J. Blood groups and human groups: collecting and calibrating genetic data after world war two. Stud Hist Philos Biol Biomed Sci. 2014;47:74–86.
16. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013;48:452–8.
17. Cid J, Lozano M, Klein HG, Flegel WA. Matching for the D antigen in haematopoietic progenitor cell transplantation: definition and clinical outcomes. Blood Transfus. 2014;12:301–6.

SUPPORTING INFORMATION

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SHORT REPORT

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Has the frequency of ABO RhD blood groups in Australian blood donors changed as a result of the removal of the variant Creutzfeldt–Jakob disease-based deferral?

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Australian Governments fund Australian Red Cross Lifeblood to provide blood, blood products and services to the Australian community

Abstract

Background and Objectives: Until 25 July 2022, Australians who had spent more than 6 months in the United Kingdom or territories between 1980 and 1996 were deferred from blood donation due to the risk of variant Creutzfeldt–Jakob disease. Removal of this geography-based donor deferral on RhD-negative blood availability has not been reported.

Materials and Methods: All donors who donated at least once from 25 July 2022 to 25 July 2023 were included. UK donor status, first-time donor and ABO RhD data were extracted from the National Blood Management System.

Results: Data from 566,447 blood donors with a valid ABO RhD result were analysed. Of these, 34,560 were new or returning lapsed donors following removal of the UK donor deferral. The median age [range] in years for all donors was 43 [75] with UK donors being older 53 [70]. There was a higher prevalence of RhD-negative status in UK donors (20.2%) compared with first-time blood donors (15.7%).

Conclusion: UK donors were generally older, female and more likely to be RhD-negative. Although UK donors provided a boost to RhD-negative blood collections, the overall prevalence of ABO RhD blood groups in the total Australian blood donor panel remained similar to previous estimates.

Keywords

Australia, blood donation deferral, blood donor, blood groups, UK, variant Creutzfeldt–Jakob disease

Highlights

- Removal of the UK donor deferral policy increased the number of available donors with RhD-negative blood.
- The overall prevalence of blood groups within Australia remained consistent with previous findings.
- UK donors were more likely to be older and female compared with the Australian population.

INTRODUCTION

Before 25 July 2022, individuals who had resided in the UK or its territories for a total of 6 months between 1 January 1980 and 31 December 1996 were ineligible to donate blood in Australia due to the potential transmission risk of variant Creutzfeldt–Jakob disease (vCJD) [1, 2]. Re-evaluation of this geographically based deferral and regulatory approval for it to be discontinued was supported by risk modelling, which indicated that cessation of this deferral would result in negligible risk for vCJD transmission following blood transfusion [1]. The removal of this geographically based deferral was one of the largest recent changes to blood donation eligibility criteria within Australia where the effect on blood collections parameters could be directly attributed. It was estimated that over 737,000 people in Australia would become eligible to donate blood if this restriction was to be removed with the prediction of approximately 58,000 extra donations annually [1]. However, during the first 6 months after lifting this restriction, nearly 68,000 successful donations were made, significantly exceeding estimates. This response from UK donors has had a substantial positive impact on blood collections within Australia, particularly since there is an ever-increasing demand on blood and blood products [2].

Until recently, the national prevalence of each ABO RhD group within Australia had not been reported [3]. This study indicated that prevalence of B RhD-positive and AB RhD-positive individuals had increased within the community [3]. The demand for O RhD-negative blood in Australia is approximately 16% of all issued red blood cell (RBC) units annually, although only 6.5% of the Australian community can provide this type [3, 4]. Because ABO RhD groups are inherited, their prevalence can differ across countries, with RhD-negative blood groups being more commonly found in people with European heritage [5]. Therefore, the current study aimed to determine whether the removal of the UK donor deferral has influenced the availability of RhD-negative blood groups within the Australian blood donor panel.

MATERIALS AND METHODS

The study was reviewed and approved by the Australian Red Cross Lifeblood Human Research Ethics Committee (2023#29-LNR) and was conducted in accordance with the National Health and Medical Research Council's National Statement on Ethical Conduct in Human Research (2007).

Blood donor identification and analysis

Blood donor data from between 25 July 2022 to 25 July 2023 were extracted from the National Blood Management System administered by Australian Red Cross Lifeblood (Lifeblood).

Donors affected by the change in the deferral policy, referred to as UK donors, were identified by extraction of the answer 'Yes' to the following screening question used in section A of Lifeblood's electronic donor questionnaire—From 1 January 1980 through to 31 December 1996 inclusive, have you spent (visited or lived) a total time which adds up to 6 months or more in England, Scotland, Wales, Northern Ireland, the Channel Islands, the Isle of Man or the Falkland Islands? For the study period, UK donors could only be identified from 25 July 2022 to 11 February 2023, after which the question was removed from the donor questionnaire.

UK donors may be first-time donors for Lifeblood but may also be lapsed or returning donors who may have donated blood with Lifeblood before going to the affected geographical areas for a 6-month period. 'First-time' donors are defined as donating blood for the first time with Lifeblood and may or may not have been affected by the geographical deferral. Data for first-time donors as well as identified UK donors are reported since first-time donors broadly represent the community, as previously described [3]. Therapeutic (hereditary haemochromatosis) donors were included in the analysis even though they were not required to complete Section A of the questionnaire.

Only blood donors with a valid ABO RhD blood type recorded on the date of data extraction (15 November 2023) were included in this analysis, and to avoid duplicate records, only the first blood group result recorded for the study period were included. Forward and reverse ABO group and RhD status is determined for each blood donation from a dedicated 6 mL BD Vacutainer® K2 Ethylenediaminetetraacetic acid (EDTA) Crossmatch Tube (catalogue number 367941, Becton, Dickinson and Company Franklin Lakes, NJ) using the NEO blood bank analyser (Immucor, Norcross GA) according to Lifeblood's standard operating procedures.

People under the age of 18 years cannot donate blood in Australia nor can those over 75 years become first-time donors. There are no other age restrictions for current blood donors who fulfil the standard selection criteria.

Donor's country of birth is included in the analysis where available. This is part of the routine questionnaire for first-time blood donors but cannot be directly linked to the donor's heritage. Data on ethnicity are not routinely collected during the blood donation procedure but can be self-reported in the Lifeblood profile app (Donate Blood), with up to two defined categories.

For this study, country names were matched to those that are recognized by the United Nations, and all territories were classified under the sovereign country category [6]. Country of birth was further classified into regions based on Nations Online [7]. Comparisons of reported donors' country of birth within the general Australian community are based on the latest estimates provided from the Australian Bureau of Statistics 2021 data cube [8]. Each country classification for each region is provided in Hirani et al. [9].

All statistical analyses were performed using IBM SPSS version 23 and GraphPad Prism v8.4.3. Descriptive statistics, including the frequencies, are reported with chi-squared and one-way analysis of variance tests conducted to compare some metrics, with a p value of <0.05 considered to be significant.

RESULTS

A total of 566,381 blood donors were included in our analysis. Of these, 114,619 were first-time blood donors; 34,560 were confirmed UK donors, of which 28,301 were first-time blood donors, and 531,821 were non-UK identifiable donors (Table 1). Where the biological sex of the donor was known, 288,210 (50.9%) of the total donor panel, 267,286 (50.3%) of the non-UK identifiable donor panel, 61,541 (53.7%) of the first-time donor panel, 20,924 (60.5%) of the UK donors and 17,255 (61.0%) of the UK first-time donors were female. The median age [range] in years for all donors was 43 [75], for non-UK identifiable donors was 42 [75], for first-time donors was 39 [75], for UK donors was 53 [70] and for UK first-time donors was 51 [64].

Blood group prevalence

First-time blood donors have a lower prevalence of O RhD-negative individuals (7.8%) compared with the total blood donor panel (10.7%), to UK donors (10.8%) and UK first-time donors (10.4%), but this was not significant ($p = 0.207$) (Table 1).

First-time blood donors have more B RhD-positive (11.6%) individuals compared with the total donor panel (9.8%), UK donors (8.1%), UK first-time donors (8.1%) and non-UK identifiable donors (9.9%), but this was not significant ($p = 0.620$).

First-time blood donors have more AB RhD-positive (3.6%) individuals compared with the total donor panel (2.9%), UK donors (2.9%), UK first-time donors (2.9%) to non-UK identifiable donors (2.9%), but this was not significant ($p = 0.355$).

UK donors and UK first-time donors have a higher prevalence of RhD-negative donors (20.2% and 19.8% respectively) compared with the total donor panel (19.0%), to first-time blood donors (15.7%) and to non-UK identifiable donors (18.9%), but this was not significant ($p = 0.754$).

Region of birth

A higher percentage of UK donors and UK first-time donors indicated the country of birth as Europe (64.2% and 68.8%, respectively) compared with the total blood donor panel (10.5%), first-time blood donors (26.3%) and non-UK identifiable donors (7.0%), but this was not significant ($p = 0.079$) (Table S1). For the total blood donor panel and non-UK identifiable donors, most indicated country of birth was Oceania/Pasifika (75.1% and 77.9%, respectively) compared with first-time blood donors (55.2%), UK donors (31.8%) and UK first-time donors (27.4%), but this was not significant ($p = 0.089$).

In UK donors from Europe, 13.0% are RhD-negative, and in UK donors from Oceania/Pasifika, 6.5% are RhD-negative (Table S2). In first-time donors from Europe, 5.2% are RhD-negative, and from Oceania/Pasifika, 9.3% are RhD-negative.

DISCUSSION

Removal of the UK donor deferral in Australia resulted in a response in blood donations that exceeded the modelled predictions [1, 2]. The impact of removing the UK deferral has been a boost to the blood supply that has supported blood sufficiency during periods of high demand as a result of the coronavirus disease 2019 (COVID-19) pandemic. Compared with other countries, the effect of this deferral removal may have a greater influence in Australia since there is a higher rate of UK immigration and also a large number of citizens living and working in the UK for extended periods.

Following the deferral removal, UK donors provided 7.6% of all collections in the study period, with 8.2% of these being whole blood collections, despite consisting of only 6.1% of the total Australian blood donor cohort. Demand for O RhD-negative blood in Australia remains consistently high around 16% of all issued RBC units. Since RhD-negative blood groups are more commonly found in people of European heritage, it was expected that with the removal of the UK deferral that the ability to collect RhD-negative blood might be positively affected.

We found that UK donors, whether first-time or returning lapsed donors, were more likely to be older and RhD-negative and had indicated that their country of birth was either Europe or Australia/New Zealand. However, first-time donors remained more likely to be younger and RhD-positive; therefore, the total donor panel remained at 19% RhD-negative prevalence, which is similar to previous findings from before the UK deferral was removed [3]. Although the overall donor panel has increased in size, the potential for blood group provision remains similar to all previous reported Australian estimates, especially since blood group prevalence in blood donors is subject to selection bias based on clinical demand, such as O RhD-negative for RBC units and group AB blood for plasma, thus resulting in preferential recruitment of donors with these blood groups. Prevalence of other clinically significant blood groups may also have altered based on UK donor deferral being lifted [9]. However when compared with Australian Bureau of Statistics on country of birth, the removal of the UK donor deferral is unlikely to change the overall donor panel phenotypes drastically [9].

The commitment of UK donors is consistent with older donors having higher donation frequencies. Donors aged 40 years and above donate on average 3.35 times a year compared with donors under 40 who donate an average of 2.51 times. Although the country of birth is collected from donors, this is not an indicator for heritage and limits the potential extrapolations that can be made to ABO RhD prevalence within this study. However, the indicated country of birth for first-time donors was similar in composition to the data available from the Australian Bureau of Statistics indicating that they appear to represent the Australian community more broadly. Furthermore, since the question on UK donor status was removed from 12 February 2023, there may be UK donors who donated after this date in the dataset who could not be identified as such.

UK donors have provided a boost to RhD-negative blood provision nationally. However, the overall prevalence of ABO RhD blood groups in the total Australian blood donor panel remained similar to

TABLE 1 Demographics and ABO RhD prevalence in all blood donors, UK donors, non-UK identifiable donors, first-time blood donors and UK first-time donors between 25 July 2022 and 25 July 2023.[a]

Parameter		Australian community ABO RhD prevalence (%)[b]	All blood donors percent of total (*n*)	UK donors percent of total (*n*)	Non-UK identifiable donors percent of total (*n*)	First-time donors percent of total (*n*)	UK first-time donors percent of total (*n*)	*p* value
Percent by biological sex (*n*)	Male	-	49.1 (278,171)	39.5 (13,636)	49.7 (264,535)	46.3 (53,078)	39.0 (11,046)	0.9989
	Female	-	50.9 (288,210)	60.5 (20,924)	50.3 (267,286)	53.7 (61,541)	61.0 (17,255)	
Median age [range] in years		-	43 [75]	53 [70]	42 [75]	39 [75]	51 [64]	0.3761
Percent of total by ABO RhD (*n*)	O RhD+	38.0	38.7 (218,935)	37.8 (13,062)	38.7 (205,873)	38.6 (44,214)	37.8 (10,698)	0.134
	O RhD−	7.0	10.7 (60,667)	10.8 (3724)	10.7 (56,943)	7.8 (8966)	10.4 (2945)	0.207
	A RhD+	32.0	29.7 (168,301)	31.3 (10,742)	29.6 (157,559)	30.5 (34,961)	31.2 (8842)	0.057
	A RhD−	6.0	6.0 (34,171)	7.0 (2427)	6.0 (31,744)	5.7 (6530)	7.1 (2021)	0.134
	B RhD+	12.0	9.8 (55,386)	8.1 (2786)	9.9 (52,600)	11.6 (13,311)	8.1 (2301)	0.620
	B RhD−	2.0	1.7 (9412)	1.7 (595)	1.7 (8817)	1.6 (1865)	1.7 (484)	0.356
	AB RhD+	4.0	2.9 (16,414)	2.9 (1002)	2.9 (15,412)	3.6 (4114)	2.9 (832)	0.355
	AB RhD−	1.0	0.5 (3095)	0.6 (222)	0.5 (2873)	0.6 (658)	0.6 (178)	0.057
Percent of total by RhD status (*n*)	RhD+	86.0	81.0 (459,036)	79.8 (27,592)	81.1 (431,444)	84.3 (96,600)	80.2 (22,673)	0.754
	RhD−	14.0	19.0 (107,345)	20.2 (6968)	18.9 (100,377)	15.7 (18,019)	19.8 (5628)	0.754
Total		**100.0**	**100.0 (566,381)**	**100.0 (34,560)**	**100.0 (531,821)**	**100.0 (114,619)**	**100.0 (28,301)**	

[a]Only data from blood donors who attended during the study period who had a valid ABO RhD result recoded were included. Some UK donors may have attended but may not have been eligible to donate or may not have had venipuncture performed.
[b]Data obtained from Hirani et al. [3].

previous estimates. This data provides further information for evidence-based forecasting of supply and could be used to support removal of this deferral in other countries.

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R.H. designed and performed the research and wrote the manuscript; V.H. contributed to data analysis; I.G. and D.O.I. supervised and directed the research. All authors reviewed and edited the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data can be made available upon request to the corresponding author.

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REFERENCES

1. McManus H, Seed CR, Hoad VC, Kiely P, Kaldor JM, Styles CE, et al. Risk of variant Creutzfeldt-Jakob disease transmission by blood transfusion in Australia. Vox Sang. 2022;117:1016–26.
2. Hoad VC, Seed CR, Kiely P, Styles CE, McManus H, Law M, et al. Removal of UK-donor deferral for variant Creutzfeldt–Jakob disease: a large donation gain in Australia. Vox Sang. 2023;118:891–4.
3. Hirani R, Weinert N, Irving DO. The distribution of ABO RhD blood groups in Australia, based on blood donor and blood sample pathology data. Med J Aust. 2022;216:291–5.
4. Hirani R, Wong J, Diaz P, Mondy P, Hogan C, Dennington PM, et al. A national review of the clinical use of group O D− red blood cell units. Transfusion. 2017;57:1254–61.
5. Mourant A, Kopec A, Domaniewska-Sobczak K. The distribution of the human blood groups and other polymorphisms. Vol 2. London: Oxford Univeristy Press; 1976.
6. United Nations. Member states. Available from: https://www.un.org/en/about-us/member-states. Last accessed 30 Oct 2023.
7. Nations Online. Countries and regions of the world from A to Z. Available from: https://www.nationsonline.org/oneworld/countries_of_the_world.htm. Last accessed 30 Oct 2023.
8. Australian Bureau of Statistics. Australia's population by country of birth. Available from: https://www.abs.gov.au/statistics/people/population/australias-population-country-birth/latest-release#data-downloads. Last accessed 30 Oct 2023.
9. Hirani R, Powley T, Mondy P, Irving DO. The prevalence of selected clinically significant red blood cell antigens among Australian blood donors. Pathology. 2023;S0031-3025:00304-5.

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SHORT REPORT

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Decreasing parvovirus B19 and hepatitis A nucleic acid test positivity rates in Canadian plasma donors following the initiation of COVID-19 restriction in March 2020

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Abstract

Background and Objectives: In Canada, plasma sent for fractionation is tested for both parvovirus B19 (B19V) and hepatitis A virus (HAV). This study compared positivity rates of B19 and HAV nucleic acid tests (NATs) in Canadian plasma samples for the pre-COVID-19 restriction era (2015 to end of February 2020 [Q1] 2020) and the post-COVID-19 restriction era.

Materials and Methods: Pooled EDTA plasma specimens were tested within 24 months of blood draw using the Procleix Panther System (Grifols Diagnostic Solutions Inc, San Diego, CA, USA) for B19V and HAV detection. Reactive pools were resolved by individual specimen testing.

Results: Between 1 January 2015, and 31 March 2022, 3,928,619 specimens from Canadian plasma donors were tested for B19V. For the same period, 3,922,954 specimens were tested for HAV. To account for a lag in specimen testing for up to 24 months, the data were divided into: (1) a pre-pandemic period (1 January 2015–31 March 2020; B19V tested $n = 2{,}412{,}701$, B19V NAT-positive $n = 240$ [0.01%], HAV tested $n = 2{,}407{,}036$, HAV NAT-positive $n = 26$ [0.001%]); (2) a two-year mixed-impact period (1 April 2020–31 March 2022; B19V tested $n = 968{,}250$, B19V NAT-positive $n = 14$ [0.001%], HAV tested $n = 968{,}250$, HAV NAT-positive $n = 2$ [0.0002%]); and (3) a pandemic-impact period (1 April 2022–31 March, 2023; B19V tested $n = 597{,}668$, B19V NAT-positive $n = 3$ [0.0005%], HAV tested $n = 597{,}668$, HAV NAT-positive $n = 1$ [0.0002%]).

Conclusion: The percentage of B19V- and HAV-positive donations was significantly reduced from the pre-pandemic period to the pandemic-impact period.

Keywords

COVID-19, hepatitis A virus, nucleic acid test, pandemic restrictions, parvovirus B19

Highlights

- Parvovirus B19 (B19V) and hepatitis A virus (HAV) nucleic acid test positivity rates decreased significantly in plasma specimens after the implementation of COVID-19 restrictions in Canada.
- COVID-19 restrictions (e.g., social distancing, isolating when ill, hand hygiene, face coverings) could have potentially influenced this reduction.
- As of March 2023, the positivity rates of B19V and HAV have not returned to pre-pandemic levels.

INTRODUCTION

Parvovirus B19 (B19V) is a non-enveloped virus with a linear single-stranded (ss) deoxyribonucleic acid (DNA) genome of approximately 4–6 kb in size. The genetic diversity among B19V isolates was reported to be very low with a single prototype (B19V) until 2002, when new sequence analysis of human erythroparvoviruses showed an organization of three distinct genotypes [1, 2]. Due to uneven public health reporting across the country, the national rates of infection outside of obstetrics settings are poorly understood. B19V is transmitted mainly by the respiratory droplet route, and although there are regional differences in epidemiology, over half the world's adults have been infected with B19V. Outbreaks of B19V occur in school/day care settings among children and susceptible adults. These B19V outbreaks usually occur in winter and spring periods with epidemics occurring every few years [3]. Although there is no available B19V vaccine, studies on safety and efficacy of candidate vaccines are underway [4].

Hepatitis A virus (HAV) is a non-enveloped, spherical, icosahedral virus. The RNA genome is monopartite, single stranded and approximately 7.5 kb. Humans are primarily infected with genotypes I–III [5]. Hepatitis A is an uncommonly diagnosed infection in Canada. Between 2011 and 2015, an average of 236 cases of hepatitis A were reported annually through the Notifiable Disease Surveillance System [6]. In Canada, HAV is transmitted primarily by the faecal-oral route (e.g., interpersonal contact, hand-to-mouth, contaminated food and/or water) among people living in areas with poor sanitation at higher risk for infection, or from travellers returning from endemic regions [7]. Well-established HAV vaccination strategies are established in Canada for persons at increased risk of infection (e.g., high-risk individuals in Canada or travellers outside of Canada), and as a post-exposure or outbreak control strategy. [8].

B19V and HAV are both transfusion-transmissible, are highly resistant to many commonly used pathogen reduction methods (solvent/detergent, heat, nucleic acid cross-linking pathogen reduction) and (because of their small size) also evade filtration. Impacted patient groups include the immunocompromised, patients with haemolytic disorders and pregnant women (including foetuses in utero) [9, 10]. In 2009, the US Department of Health and Human Services Food and Drug Administration recommended that plasma fractionators implement the following procedures to detect the presence of B19V DNA. In general, the viral load of B19V DNA in the manufacturing pools was not to exceed 10^4 international units (IU)/mL [11]. To ensure the safety of the plasma supply, plasma sent for fractionation by Grifols Canada is tested for both HAV and B19V.

This study will compare the rates of B19 and HAV nucleic acid test (NAT) positivity in predominantly Canadian plasma samples for the pre-COVID-19 restriction era (2015 to end of February 2020 [Q1] 2020) and the post-COVID-19 restriction era.

MATERIALS AND METHODS

General characteristics of plasma donors

A list of donation numbers and donor IDs of donations that had plasma (recovered plasma from whole blood or apheresis plasma) sent for fractionation was collated for the time period 1 January 2015, and 31 March 2023. These do not directly align with the Grifols NAT data as they may include donations sent to other fractionators or not yet sent for fractionation.

Numbers of donations and donors were generated from the Canadian Blood Services Epidemiology Donor Database. The mean inter-donation interval was calculated as the sum of the number of days between each donation divided by the total number of donations for donors who gave more than one donation during the study period. Estimates of proportions of source and recovered plasma units sent to the fractionator were estimated by the Canadian Blood Services Inventory Management Centre of Excellence.

Specimen source and study population

Canadian Blood Services collects source and recovered plasma destined for fractionation from across Canada except for Quebec and the northern territories. EDTA plasma specimens were collected by Canadian Blood Services from donors providing source or recovered plasma. EDTA plasma specimens were sent with a larger volume plasma to a plasma fractionator within 24 months of blood draw. EDTA plasma specimen storage and transport temperatures were −20°C.

B19V and HAV NAT

Specimens were generally tested within 2 weeks of receipt by the fractionator. Samples were pooled (16 per pool) for B19/HAV testing

using the Procleix Panther System (Grifols Diagnostic Solutions Inc, San Diego, CA, USA). The B19V quantitative range for the assay is 500–100,000 IU/mL. Any HAV-positive (reactive = above assay cut-off, S/CO ≥ 1.0) and B19 NAT-positive (reactive ≥2000 IU/mL) pools were resolved by individual specimen testing. Individual sample results >500 IU/mL were considered reactive for B19V, and any HAV-positive (reactive = above assay cut-off, S/CO ≥ 1.0) was considered reactive for HAV. The assay does not provide a quantitative HAV value.

Data storage and statistical analysis

Data were collated using Excel (Microsoft, Seattle, WA, USA). Chi-square analyses, column statistics, mean and standard deviation (SD) calculations were performed using GraphPad Prism (Version 9.5.1, GraphPad Software, Boston, MA, USA).

RESULTS

The general characteristics of plasma donors are described in Table 1. These are donation dates and not Grifols report test dates; therefore, they will not align exactly with NAT data. In the pandemic-impact period, plasma donors were slightly more likely to be female, older (e.g., ≥60 years of age) and from far Western regions (British Columbia [BC]&Yukon) compared with pre-pandemic and mixed-impact periods.

Grifols provided Canadian Blood Services with B19V and HAV NAT divided into broad geographic regions based on the month tested for the period 1 January 2015, and 31 December 2022. Because some test pools could potentially include US donations, any test pool data involving US specimens were removed from the analysis. Between 1 January 2015, and 31 March 2022, 3,928,619 specimens from Canadian plasma donors were tested for B19V, and 3,922,954 specimens were tested for HAV.

As B19V and HAV NAT data could not be evenly attributed to the province level across Canada, data were aggregated into one group. No further attempt was made to link B19V and HAV data to subnational or provincial data.

To account for a lag in specimen testing for up to 24 months, the data were divided into three data sets (Tables S1–S3): a pre-pandemic period from 1 January 2015 to 31 March 2020 (Table S1, B19V tested $n = 2{,}412{,}701$, B19V NAT-positive $n = 240$ [0.01%], HAV tested $n = 2{,}407{,}036$, HAV NAT-positive $n = 26$ [0.001%]); a two-year mixed-impact period (specimens may have been from pre-pandemic period or pandemic period) from 1 April 2020 to 31 March 2022 (Table S2, B19V tested $n = 968{,}250$, B19V NAT-positive $n = 14$ [0.001%], HAV tested $n = 968{,}250$, HAV NAT-positive $n = 2$ [0.0002%]); and a pandemic-impact period from 1 April 2022 to 31 March 2023 (Table S3, B19V tested $n = 597{,}668$, B19V NAT-positive $n = 3$ [0.0005%], HAV tested $n = 597{,}668$, HAV NAT-positive $n = 1$ [0.0002%]). The mean percentage data from Tables S1 to S3 is summarized in Figure 1. Reductions in NAT positivity were noted in the pandemic-impact period for both B19V and HAV (Figure 1).

The majority of plasma units sent for fractionation are recovered from whole blood donations and are not considered source plasma (% source plasma, 15% of units [2023 estimate]). Numbers of apheresis and whole blood donations at Canadian Blood Services (regardless of whether they were or were not sent for fractionation) were as follows for the following time periods: pre-pandemic period from 1 January 2015 to 31 March 2020 (apheresis $n = 201{,}387$, whole blood

TABLE 1 Demographics of donors whose donation was used for fractionated products in the pre-pandemic, mixed-impact and pandemic-impact periods.

		Pre-pandemic period		Mixed-impact period		Pandemic-impact period		Chi-squared analysis
Variable		N	%	N	%	N	%	*p* value
Sex	Male	1,043,425	58	373,608	59	180,379	54	<0.0001
	Female	755,442	42	255,202	41	151,215	46	
Age group	17–24	292,450	16	70,098	11	34,975	11	<0.0001
	25–39	504,280	28	185,977	30	92,805	28	
	40–59	661,103	37	224,506	36	118,705	36	
	60+	341,031	19	148,229	24	85,109	26	
Region	BC&Yukon	255,391	14	103,203	16	57,784	17	
	Alberta	364,216	20	126,653	20	58,386	18	<0.0001
	Prairies	194,245	10	64,843	10	33,533	10	
	Ontario	795,996	44	271,672	43	149,742	45	
	Atlantic	188,518	10	62,439	10	32,149	10	
Total		1,798,913		628,830		331,598		
Grand total		2,785,641						

Abbreviation: BC, British Columbia.

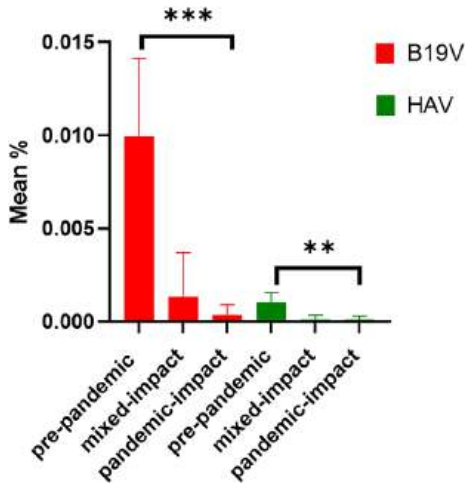

FIGURE 1 Decreased mean percentage of parvovirus B19 (B19V) and hepatitis A virus (HAV) NAT-positive donations in the pandemic-impact period. Error bars represent standard deviations. Values represent mean percentage positivity of B19V and HAV calculated across years, or fractions of years as described in Tables S1–S3. The proportion of positive B19V (two-tailed chi-square 111.3, degrees of freedom [df] = 2, ***$p < 0.0001$) and HAV (two-tailed chi-square 9.8, df = 2, **$p = 0.008$) donations was significantly reduced in the pandemic-impact period.

$n = 4{,}169{,}052$); mixed-impact period from 1 April 2020 to 31 March 2022 (apheresis $n = 60{,}330$, whole blood $n = 1{,}505{,}009$); pandemic-impact period from 1 April 2022 to 31 March 2023 (apheresis $n = 22{,}289$, whole blood $n = 757{,}701$). Overall, 4% source plasma donations were given by a donor who only donated once and of source plasma donors who donated more than once in the period; their mean inter-donation interval was 40.0 days (95% confidence interval 39.7–40.6). Of whole blood donors, 7% were from donors who only gave one donation, and of whole blood donors who donated more than once during the study period, the inter-donation interval was 167.6 days (95% confidence interval 167.4–167.8).

DISCUSSION

Due to variability in how B19V and HAV are tested and reported across Canada, a consistent surveillance approach for relatively healthy individuals is not available in the country. Testing donated plasma for both B19V and HAV from plasma donors provides a unique perspective of viremia in healthy Canadians from the pre-pandemic to the pandemic-impacted period. This unique tool also allowed Canadian Blood Services to assess the impact of sweeping public health controls on the positivity rate of transmissible agents such as B19V and HAV in the Canadian population.

Erythema infectiosum caused by B19V infection occurs in epidemics every 3–4 years, with peaks of activity in the winter and early spring [2]. This study has identified a significant decrease in the percentage of B19V DNA-positive donations from the pre-pandemic period (0.01%) to pandemic-impact period (0.0005%). This is like data signals from the Netherlands where there was a reduction in silent B19V infections from pre-pandemic periods (2013; 0.004% donations) to the pandemic period (0%; 2020 and 2021). The group from the Netherlands suspected that day care and school closures likely reduced the transmission of B19V among children and subsequent infections of parents who were blood donors [12].

Except for Quebec, which was not included in this analysis, provincial/territorial vaccine schedules in Canada do not include routine HAV vaccination. Provinces/territories instead have HAV vaccine strategies, which target higher risk groups based on local risk assessment from local public health authorities [13]. The data generated by this study suggest that hepatitis A is an uncommon infection in Canada and that there was a decrease in tested plasma donations from the pre-pandemic period (0.001%) to the pandemic-impact period (0.0002% in pandemic-impact period). The rarity of HAV infections in plasma donors aligns with data from 2011 to 2015, where a small number of cases ($n = 236$) were reported through the Notifiable Disease Surveillance System [6]. HAV RNAemia rates vary both geographically and over time with rates in US plasma donors increasing from 0% (2016) to 0.04% (2020) [14]. Similarly, French blood donors showed a fivefold increase between 2015–2016 (0.00009%) and 2017 (0.0004%) [15].

This study has several limitations. Plasma donor demographics may have changed in the pandemic-impact period compared with pre-pandemic and mixed-impact periods (Table 1). However, these data could not be directly linked to B19 and HAV NAT data (Figure 1). Storage of plasma and linked EDTA plasma specimens for up to 2 years meant that aggregated data could not be analysed based on time of collection. Aggregation and de-identification of data did not allow for surveys of plasma donors to determine whether donors later developed symptoms for B19V or HAV infection. Pooling of EDTA plasma specimens may have led to a reduction in the detections of low-titre HAV. Similarly, the use of reactive cut-offs ≥2000 IU/mL for pools of 16 specimens would not have allowed for the detection of lower titres of B19V in those pools.

With a mean inter-donation period of 40.0 days for source plasma (167 days in whole blood), it is possible that an asymptomatic or paucisymptomatic HAV-NAT-positive donor could have donated multiple times. A previous study noted that HAV RNA could be detected an average of 17 days prior to a alanine aminotransferase peak with an average persistence of 79 days after the liver enzyme peak (total range 36–391 days) [16]. However, given that these were Canadian plasma donors, and that most adult HAV infections in developed countries are characterized by acute hepatitis and jaundice, which lead to donor deferral, it is unlikely that the data would have been biased to serially detecting HAV RNA in a small subset of asymptomatic/paucisymptomatic plasma donors. In contrast, high levels of B19V DNA-emia (as would be measured in these plasma donors) usually occur about 1 week after infection and generally persist for 5 days [17]. A mean inter-donation period of 40 days in source plasma (167 days in whole blood) donors would thus make B19V NAT-positive serial donations unlikely. Even source plasma donors who

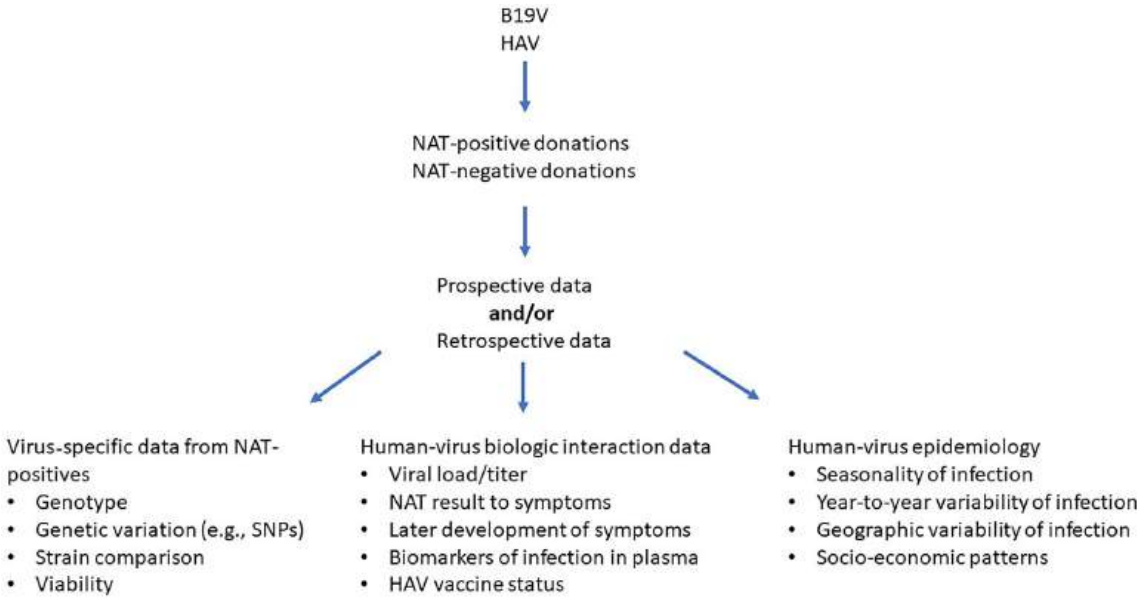

FIGURE 2 How parvovirus B19 (B19V) and hepatitis A virus (HAV) testing of plasma donations can support one health activities. Blood operators and plasma fractionators can generate data on virus characteristics, human–virus interactions and epidemiology. This knowledge can inform public health on B19V/HAV epidemiology in relatively healthy populations. NATs, nucleic acid tests.

returned weekly to donate at Canadian Blood Services would not likely donate two serial high-titre B19V NAT-positive donations.

In Canada, starting in March 2020, a series of public health precautions to reduce the spread of SARS-CoV-2 were implemented. These included public health guidance on precautions to prevent the spread of SARS-CoV-2. Although these public health actions were met with mixed compliance, the restrictions of attendance at schools, day cares, restaurant/bar, places of worship and small-to-medium social gatherings were extensive. Restrictions within Canadian society would continue into the Spring/Summer 2022 when mask mandates began to be lifted on a province-wide basis [18]. As of March 2023, the positivity rates of B19V and HAV had not rebounded to pre-pandemic levels. It is likely that these public health mandates may have reduced B19V (respiratory secretions) and HAV (faecal-oral route) transmission in the general community. As HAV is mainly transmitted via the enteric route, it is suspected that social gathering restrictions and travel restrictions may have reduced opportunities for Canadians to be infected with HAV [19, 20]. Public health communications on the importance of hand hygiene may have additionally reduced the opportunity for faecal-oral transmission in the community, schools and facilities [21]. Masking mandates may have also reduced infection rates by reducing the rate of hand-to-mucosa contacts in individuals [22]. Further analysis of B19V and HAV data from 2023 onwards will identify changing trends in epidemiology as patterns of transmission are likely to be re-established. This further work could also focus on whether other demographic factors (e.g., sex, age, region) are impacting on the changing rates of B19 and HAV in plasma sent for fractionation.

Since the COVID-19 pandemic, the role of blood operators in supporting public health epidemiologic analyses has increased. For the future, blood operators and fractionators generate data that can identify changes in B19V and HAV virology, pathophysiology and epidemiology (Figure 2). At Canadian Blood Services, we are working to obtain and review results in a timely fashion to enable better linkage with an individual donor. In the future, other processes such long-term biobanking or front-end B19V and HAV testing by the blood operator may also allow for direct linking of donor data to specific donation specimens and further genetic analysis of B19V and HAV specimens. At the granular level, B19V and HAV NAT-positive specimens could then be assessed for viral genotype, genetic variability, single-nucleotide polymorphisms (SNPs) and virus viability. With improved linkages between donor health information and laboratory data, data analysis could focus on viral load and time to symptoms, biomarkers of infection and patterns of viremia in HAV-vaccinated individuals. Blood operators and fractionators can also provide further data on the epidemiology of B19V and HAV NAT-positivity in jurisdictions where public health surveillance is uneven (Figure 2).

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G.A.D. and S.B. collected and provided nucleic acid test data for the study; S.J.D. performed data analysis, designed the study and wrote the first draft of the paper; S.F.O. provided statistical review and helped design the study; G.A.D., S.B., C.C. and S.F.O. helped write further paper drafts.

CONFLICT OF INTEREST STATEMENT

Steven J. Drews is a paid consultant to Roche on malaria and emerging arboviruses.

DATA AVAILABILITY STATEMENT

This study makes use of aggregate data. These aggregate data are available from the corresponding author on request.

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REFERENCES

1. Servant-Delmas A, Morinet F. Update of the human parvovirus B19 biology. Transfus Clin Biol. 2016;23:5–12.
2. Heegaard ED, Brown KE. Human parvovirus B19. Clin Microbiol Rev. 2002;15:485–505.
3. Qiu J, Söderlund-Venermo M, Young NS. Human Parvoviruses. Clin Microbiol Rev. 2017;30:43–113.
4. Suzuki H, Noguchi T, Matsugu N, Suzuki A, Kimura S, Onishi M, et al. Safety and immunogenicity of parvovirus B19 virus-like particle vaccine lacking phospholipase A2 activity. Vaccine. 2022;40:6100–6.
5. Smith DB, Simmonds P. Classification and genomic diversity of enterically transmitted hepatitis viruses. Cold Spring Harb Perspect Med. 2018;8:1–15.
6. Government of Canada. Surveillance of hepatitis A. Available from: https://www.canada.ca/en/public-health/services/diseases/hepatitis-a/surveillance.html#a1. Last accessed 3 Feb 2024.
7. Wu J, Zou S, Giulivi A. Current hepatitis A status in Canada. Can J Infect Dis. 2001;12:341–4.
8. Henry B, Baclic O. Summary of the National Advisory Committee on Immunization (NACI) statement update on the recommended use of hepatitis A vaccine. Can Commun Dis Rep. 2016;42:193–4.
9. Chudy M, Budek I, Keller-Stanislawski B, McCaustland KA, Neidhold S, Robertson BH, et al. A new cluster of hepatitis A infection in hemophiliacs traced to a contaminated plasma pool. J Med Virol. 1999;57:91–9.
10. Kleinman SH, Glynn SA, Lee TH, Tobler LH, Schlumpf KS, Todd DS, et al. A linked donor-recipient study to evaluate parvovirus B19 transmission by blood component transfusion. Blood. 2009;114:3677–83.
11. U.S. Department of Health and Human Services FaDA, Center for Biologics Evaluation and Research. Nucleic acid testing to reduce the possible risk of parvovirus B19 transmission by plasma-derived products: Guidance for industry. 2009.
12. Molenaar-de Backer MW, Hogema BM, Koppelman MH, van de Laar TJ, Slot E, Zaaijer HL. Lower incidence of parvovirus-B19 infections in Dutch blood donors during SARS-CoV-2 pandemic. Microbiol Spectr. 2021;9:e0025321.
13. Palaisy D. Hepatitis A immunization and high-risk populations in Canada and internationally: summary and analysis. Winnipeg, MB: University of Manitoba; 2022. p. 1–12.
14. Schoch S, Wälti M, Schemmerer M, Alexander R, Keiner B, Kralicek C, et al. Hepatitis A virus incidence rates and biomarker dynamics for plasma donors, United States. Emerg Infect Dis. 2021;27:2718–824.
15. Gallian P, Barlet V, Mouna L, Gross S, Lecam S, Ricard C, et al. Hepatitis A: an epidemiological survey in blood donors, France 2015 to 2017. Euro Surveill. 2018;23:1–5.
16. Bower WA, Nainan OV, Han X, Margolis HS. Duration of viremia in hepatitis A virus infection. J Infect Dis. 2000;182:12–7.
17. AABB. Human Parvovirus B19. Available from: https://www.aabb.org/docs/default-source/default-document-library/regulatory/eid/human-parvovirus-b19.pdf?sfvrsn=f98853f8_2. Last accessed 3 Feb 2023.
18. Globe Staff. Most provinces have lifted their mask mandates. Where in Canada is the rule still in effect? Toronto, ON: Globe and Mail; 2022.
19. Balogun O, Brown A, Angelo KM, Hochberg NS, Barnett ED, Nicolini LA, et al. Acute hepatitis A in international travellers: a GeoSentinel analysis, 2008-2020. J Travel Med. 2022;29:1–6.
20. O'Reilly KM, Sandman F, Allen D, Jarvis CI, Gimma A, Douglas A, et al. Predicted norovirus resurgence in 2021-2022 due to the relaxation of nonpharmaceutical interventions associated with COVID-19 restrictions in England: a mathematical modeling study. BMC Med. 2021;19:299.
21. Brennan J, Mullins H, Tobey K, Jones C, Kainer MA, Fiscus M, et al. Nosocomial hepatitis A outbreak among healthcare workers and patients in a community hospital during an ongoing statewide outbreak. Infect Control Hosp Epidemiol. 2021;42:139–41.
22. Science M, Caldeira-Kulbakas M, Parekh RS, Maguire BR, Carroll S, Anthony SJ, et al. Effect of wearing a face mask on hand-to-face contact by children in a simulated school environment: the back-to-school COVID-19 simulation randomized clinical trial. JAMA Pediatr. 2022;176:1169–75.

SUPPORTING INFORMATION

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

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EVENTS

See also https://www.isbtweb.org/events.html	
23–27 June 2024	38th International ISBT Congress, Barcelona, Spain. https://www.isbtweb.org/events/isbt-barcelona-2024.html
11–13 September	DGTI & DGI 2024. https://immungenetik.de/index.php/veranstaltungen/dgi-jahrestagungen/jahrestagung-2024
27–29 September 2024	ESPGI 2024 - Platelet and Granulocyte Immunobiology. https://sanquinacademy.nl/en/offers/espgi-2024/
23–26 October 2024	Brazilian Congress of Hematology, Hemotherapy and Cell Therapy (Hemo 2024). https://www.hemo.org.br/2024/index.ingles.html

