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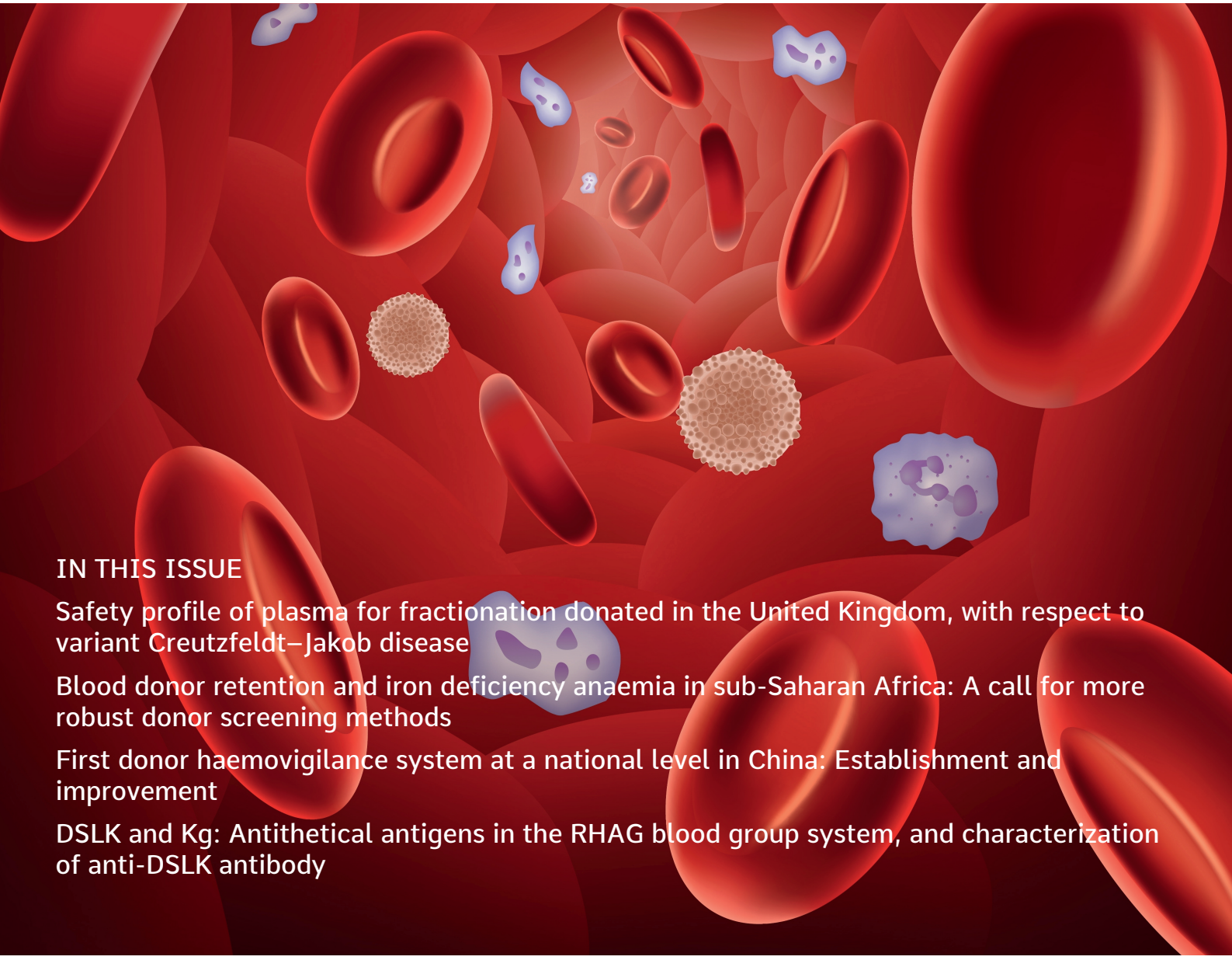

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Aims and Scope

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

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

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

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REVIEW

Vox Sanguinis ISBT International Society of Blood Transfusion

Safety profile of plasma for fractionation donated in the United Kingdom, with respect to variant Creutzfeldt–Jakob disease

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Abstract

Plasma-derived medicinal products (PDMPs) are life-saving and life-improving therapies, but the raw material is in short supply: Europe depends on importation from countries including the United States. Plasma from donors resident in the United Kingdom has not been fractionated since 1999 when a precautionary measure was introduced in response to the outbreak of variant Creutzfeldt–Jakob disease (vCJD). Cases of vCJD have been far fewer than originally predicted in the 1990s. Since the introduction of leucodepletion in 1999, and accounting for the incubation period, more than 40 million UK-derived blood components have been issued with no reports of TT vCJD. In February 2021, the UK Government authorized manufacture of immunoglobulin from UK plasma. Following separate reviews concluding no significant difference in the risk posed, the United States, Australia, Ireland and Hong Kong also lifted their deferrals of blood donors with a history of living in the United Kingdom. Other countries are actively reviewing their position. Demand is rising for PDMPs, and Europe faces a threat of supply shortages. Industry and patient groups are clear that using UK plasma would bring significant immediate benefits to patients and to the resilience of the European supply chain. From this scientific review, we conclude that UK plasma is safe for fractionation and urge blood regulators and operators to take account of this safety profile when considering fractionation of UK plasma, and to revise their guidelines on the deferral of donors who have lived in, or received a transfusion in, the United Kingdom.

Keywords

blood safety, plasma fractionation, prions, vCJD

Highlights

- In 1999, as a precaution against variant Creutzfeldt–Jakob disease (vCJD) transmission, the United Kingdom stopped supplying plasma for fractionation.

- The dietary vCJD outbreak was much smaller than feared, and there have been no transfusion transmissions since 1999. There are numerous and effective risk-reduction steps in the donation and manufacturing process.
- This review concludes that plasma from donors currently or previously resident in the United Kingdom is as safe as any other source of plasma for the manufacture of medicines and concurs with recent reviews in Australia, Ireland, the United States, Hong Kong and by the UK Medicines and Healthcare Products Regulatory Agency.

INTRODUCTION

Europe is dependent on importation from the United States for 38% of the plasma required to meet its patients' needs [1]. Meanwhile, plasma collected from UK donors and from European donors who previously resided in the United Kingdom is not being accepted for fractionation in Europe. This is the result of the precautionary measures implemented in the United Kingdom almost 30 years ago to mitigate the risk of transfusion-transmitted Creutzfeldt–Jakob disease (TT-vCJD). Fortunately, the dietary-related outbreak was much smaller than had been feared and there have been no cases of TT-vCJD since 1999. It is therefore timely to re-assess the current position, and reviews undertaken in the United Kingdom, Australia, United States, Ireland and Hong Kong have concluded that blood and plasma from donors previously resident in the United Kingdom presents no additional risk of vCJD to their respective blood supplies or the manufacture of plasma-derived medicinal products (PDMPs) [2–6].

This paper is an abridged version of a paper reviewing the safety profile of UK plasma, commissioned by the UK Blood Services and written with input from international scientific experts and organizational representatives [7]. The paper considers the epidemiology of vCJD, risk-reduction measures, the latest UK and international decisions, the view of industry and patient groups, ethics and the supply difficulties and demand needs that make reconsideration of these matters so urgently important. The aim of the paper is to inform the Member States of the European Union (EU) and European Economic Area (EEA), and any other interested party, on the safety profile of UK plasma in the context of vCJD and with respect to its fractionation in the EU.

Although the original precautionary decision to cease the use of UK plasma for fractionation was taken by the UK Department of Health following a review by the Committee on the Safety of Medicines in 1998, it was then reiterated by other agencies in Europe, resulting in a lack of clarity on the current position [8]. However, the United Kingdom did not prohibit blood donation and the use of blood components. National, European and world bodies introduced a geographical deferral of their blood donors who lived for a certain time or received a transfusion in the United Kingdom during the bovine spongiform encephalopathy (BSE) epidemic; many did not officially prohibit the use of UK plasma for fractionation—this plasma was simply not available. Now, considering the geographic distribution of fractionation plants, some countries have to take a decision on the acceptability for fractionation of UK plasma, despite never having prohibited its use.

The proposed Regulation on standards of quality and safety for substances of human origin (SoHO) intended for human application encourages EU Member States to 'promote the donation of SoHOs, including plasma, of high quality and safety, thereby also increasing self-sufficiency in the Union' [9]. Although the European Medicines Agency (EMA) has not issued an official position on UK plasma, there is a CJD position statement from the Committee for Medicinal Products for Human Use (CHMP; agreed by the Biologics Working Party), the third revision of which (2018) is currently pending further review [10]. It includes the text from the first version of the document, issued in 2003, which states '… donors who have spent a cumulative period of 1 year or more in the UK between the beginning of 1980 and the end of 1996 are excluded from donating blood/plasma for fractionation'. Although acknowledging the 27 French cases of vCJD, the paper does not recommend the exclusion of donors who have spent any cumulative length of time in France.

In August 2021, the European Centre for Disease Prevention and Control (ECDC) published a risk assessment regarding the potential transmission of vCJD by blood and blood components by donations obtained in the United Kingdom, noting that the absence of a suitable, validated screening test for blood donors makes it difficult to assess the residual risk for transmission in the United Kingdom and Europe [11]. Despite plasma for fractionation being beyond the stated scope of the risk assessment (clarified in July 2022 to be limited to '… the possible transmission of vCJD by blood and blood components from donations obtained in the UK'), it notes the '…risk of… possible transmission… by blood and PDMPs manufactured from donations obtained in the UK'.

The assessment quotes from the MHRA risk assessment, the highly precautionary upper-bound figure of 324 infections per million doses of the highest risk immunoglobulin product if made from non-leucodepleted plasma. The majority of products present a much lower risk, and the use of leucodepletion, which is standard in the United Kingdom and noted to significantly reduce risk, reduces this five-fold to 67 infections per million doses. It is important to note that the MHRA assessment is based on Department of Health and Social Care (DHSC) modelling, which is 'precautionary and uses large input and calibration ranges… [so] will inevitably produce extremely wide estimates with high upper bounds' [12].

The ECDC assessment also notes 'the contrast between the estimated prevalence [as determined from examination of appendix tissue and used in DHSC modelling] … and the reported number of clinical vCJD cases' is such that 'positive' individuals 'may never develop any

symptoms of prion disease, nor may they be capable of transmitting the infection, including through donation of blood and blood products'. This conclusion is supported by findings from a sheep transfusion model which suggest that detectable PrP^{Sc} in lymphoid tissue may be very common following exposure by transfusion, but with no evidence that this leads to detectable PrP^{Sc} in blood, infectiousness or clinical illness, thus supporting the conclusion that the appendix studies do not provide a good estimate for prevalence of an infectious state [13].

Finally, the ECDC assessment comments that the risk of vCJD infection 'is decreased by the safety measures implemented to reduce the risk of donation by exposed donors and during whole blood processing or plasma fractionation'. It presents options for response, suggesting that '…EU/EEA countries may consider assessing their endogenous risks, evaluating product-specific data packages (including the prion-reduction capacities of applied fractionation procedures), and balancing the assessed threat with the supply need for PDMPs and source plasma in their country. Until such data are available, EU/EEA countries may consider, as a precautionary measure, preventing the use of immunoglobulins and other PDMPs derived from UK plasma, as well as the fractionation of UK plasma in EU/EEA facilities'.

It is hoped that this paper provides the relevant background information on the above options, including risk-reduction measures applied during donation and processing of blood and evidence on effectiveness of manufacturing processes to remove prions, and will assist and encourage the relevant regulatory agencies when considering their guidance on the fractionation of UK-sourced plasma.

A HISTORY OF TT-vCJD IN THE UNITED KINGDOM

Since 1995, 178 patients with definite and probable vCJD have been reported in the United Kingdom. Four instances of probable TT-vCJD have been noted, resulting in three clinical cases of vCJD and one asymptomatic infection in a recipient with post-mortem confirmation of abnormal prion protein deposition in the spleen. A fifth individual, who had haemophilia and had received many doses of Factor VIII concentrate, was found to have abnormal prion in his spleen at post-mortem after he died from an unrelated cause in 2008. He had received treatment with the intermediate-purity Factor VIII concentrate 8Y, two batches of which included a donation from a single donor who subsequently died of vCJD in 1997. The Factor VIII treatment was considered the probable cause of the vCJD infection; this remains the only case implicating Factor VIII, with the causal connection unproven and no identified case related to any other PDMP [14].

There is a well-established UK CJD surveillance system that employs multiple overlapping case identification methods, with subsystems relating to possible blood/blood-product-related cases. It is unlikely that a significant number of cases have been missed, a view supported by published studies [15–19]. vCJD has not been reported in anyone in the United Kingdom born after 1989 (the year major dietary protection measures were introduced) and there have been no new cases of TT-vCJD since 2007. Transmissions associated with red blood cell transfusions occurred prior to the 1999 introduction of leucodepletion (the removal of the majority of white blood cells from blood components) [20, 21]. Since then, more than 58 million UK-derived blood components have been issued in the country, 40 million of which were issued more than 8 years ago (the approximate incubation period, based on reported TT cases) with no reports of TT-vCJD [22]. There have been no reports of vCJD transmission via plasma or platelet transfusions, and there have been no reported cases of TT-vCJD anywhere else in the world, even though other countries have had cases of vCJD.

France has reported 27 cases of vCJD, the second highest number after the United Kingdom [23], but has continued to collect plasma for the manufacture of PDMPs. The Établissement Français du Sang (EFS) collects around 850,000 L of leucodepleted plasma for fractionation each year, provided to the Laboratoire Français du Fractionnement et des Biotechnologies (LFB). EFS also issues 3 million blood components each year [24]. There have been no reported transmissions of vCJD by LFB-produced PDMPs or EFS-produced blood components.

UK POSITION ON THE SAFETY OF UK PLASMA

Since the first appearance of vCJD, the UK DHSC has periodically carried out a risk assessment on the predicted number of future infections and associated deaths due to TT-vCJD. There was considerable concern regarding the potential length and magnitude of the outbreak, with predictions of 10 cases per year in the 2020s [25], but the reality has been very different (see Figure 1). Since 2011, when there were five cases, there have only been two further (dietary) cases, one in 2013 and one in 2016 [26].

The risk assessment was reviewed in 2018 by the Advisory Committee on Dangerous Pathogens (ACDP), and in 2019 the advisory committee on the Safety of Blood, Tissues and Organs (SaBTO) considered some of the measures in place to mitigate the risk of TT-vCJD in paediatrics. These included importation of fresh frozen plasma (FFP) and use of apheresis platelets for patients born after the beginning of 1996. The risk of TT-vCJD by UK FFP was predicted to be one in every 5.2 million units of plasma transfused [27]. The requirement to import FFP for these patients was removed and UK FFP is now provided.

In 2020, prompted by the increasing supply risks and the diminishing transmission risk, the MHRA reviewed the evidence on the safety of UK plasma for manufacture of immunoglobulins. In October 2020, evidence was presented to the Commission on Human Medicines (CHM), a committee of the MHRA that advises ministers on the safety, efficacy and quality of medicinal products. The CHM concluded '…the risk of vCJD cases arising from the use of UK plasma for the manufacture of immunoglobulin medicinal products would be negligible. The CHM also noted the clinical need for immunoglobulin

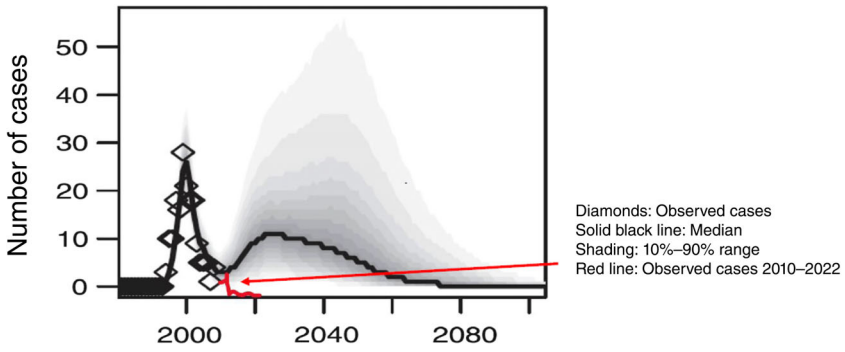

FIGURE 1 Modelling from 2010 predicting a significant second peak of infections. The actual number of cases has been much smaller (adapted from [26]).

products for patients with immunodeficiency and certain autoimmune conditions'. In February 2021, the ban was lifted on the use of UK plasma for manufacture of immunoglobulin for use in the United Kingdom provided that all relevant risk-mitigation measures already in place for blood components for transfusion (the use of leucodepletion, deferral of high-risk donors and traceability between donor and recipient) are applied [2]. In October 2022, the CHM approved the use of UK plasma for the manufacture of albumin medicinal products [28] and may consider reviewing other PDMPs depending on its capacity and demand for the products.

INTERNATIONAL PERSPECTIVES ON GEOGRAPHIC DEFERRAL OF DONORS

The deferral of donors based on time previously spent in the United Kingdom has been recently reviewed in three jurisdictions. In each case, the decision was taken to remove the deferral following analysis of the vCJD risk. There are no known examples of the deferral being maintained following a review.

In May 2022, the US Food and Drug Administration (FDA) updated its guidance around the safety of UK plasma, removing '… previous recommendations to defer blood donors for (1) geographic risk of possible exposure to CJD for time spent in the UK from 1980–1996 and (2) receipt of a blood transfusion in the UK from 1980-present'. [4]. This decision was based on the absence of a significant difference from donors in the United States, where no endogenous cases of vCJD have been reported. Further, the FDA noted that it was '[changing its] geographic deferral recommendations for vCJD risk based on new information in the risk assessments published by UK's SaBTO and MHRA… which FDA has independently evaluated, demonstrate that, in the UK, the current risk of vCJD transmission by blood and blood components would expose transfusion recipients to no or minimal additional risk of vCJD in the future' [4]. This change has also created a paradox where PDMPs manufactured from US plasma could be used in the United Kingdom or Europe, having been derived from donations from individuals who are currently not allowed to donate in the United Kingdom and EU countries because of previous residency or receipt of a transfusion in the United Kingdom. This makes the current European position inconsistent. If, following this change, US imports are considered to remain acceptable, it would appear reasonable that PDMPs derived from UK plasma should also be considered acceptable.

In April 2022, the Australian Therapeutics Goods Administration overturned its ban on former UK residents donating blood in Australia due to the perceived risk of vCJD. This followed a review concluding that the removal of the deferral would have no negative effect on the safety of the Australian blood supply and would be a safe and effective strategy to increase the donor base [29]. Indeed, Australian Red Cross Lifeblood reported 21,000 new donor registrations in the 10 days following the removal of the ban [30].

In 2019, following a review of vCJD risk by the Irish Blood Transfusion Service, Ireland overturned its deferral of donors who had previously resided in the United Kingdom between 1980 and 1996 or possibly been exposed to vCJD via blood transfusion [5].

Hong Kong announced in December 2022 that it would accept blood donors who had stayed three or more months cumulatively in the United Kingdom between 1980 and 1996, and who had stayed five or more years cumulatively in France or Ireland between 1980 and 2001 [6].

The European Blood Alliance (EBA) has published a statement emphasizing that 'Increasing plasma collection by not-for-profit blood establishments in Europe is a priority… to safeguard the supply of safe PDMPs …for patients in Europe while preserving donor health'. and 'EBA notes the change in deferral criteria in countries in which these risk analyses have been performed and calls on all European stakeholders to assess the analyses, with a view to perform a similar risk analysis and, where pertinent, to consider updating their own deferral criteria regarding blood and plasma donation'.

PATIENT AND INDUSTRY PERSPECTIVES

The European Patient Organization for Dysimmune and Inflammatory Neuropathies (EPODIN) and the Platform of Plasma Protein Users (PLUS) represent patients who can suffer major health impacts in the

absence of the appropriate PDMP treatment. Similarly, the International Plasma and Fractionation Association (IPFA) and the Plasma Protein Therapeutics Association (PPTA) are trade organizations that represent, respectively, the not-for-profit organizations engaged in the collection and fractionation of plasma and private sector manufacturers of plasma protein therapies and the collectors of source plasma used for fractionation. CSL Behring is an independent biotechnology company leading in the collection of source plasma and which manufactures a broad range of plasma protein therapies. These organizations concur that a regular and continuous supply of plasma is required as the health independence of Europe is a concern; that initiatives like the MHRA review are welcome as they examine and reassess standards based on accurate scientific reasoning; and that the data presented herein shows that plasma donated by the citizens of the United Kingdom is as safe a raw material for PDMPs as plasma donated anywhere else in Europe.

RISING PATIENT DEMAND FOR PDMPs

The European Union has a shortfall of 3.8 million litres (or 30%) of the plasma needed to manufacture PDMPs for its patients; Europe (including the United Kingdom) depends on US plasma imports for more than 38% of its need; and demand is rising at 6% per year [31, 32]. There are risks associated with this reliance on importation, illustrated by the 2020 fall of 20% in US plasma collection due to the COVID-19 pandemic and the potential for exports to be stopped under the Defence Production Act [33].

The Marketing Research Bureau estimates total immunoglobulin usage in Europe (inclusive of the United Kingdom) to be 64 tons per annum for up to 350,000 patients. To manage the restricted supply, some countries have established usage guidelines and priority lists. Factors such as economic situations, healthcare policies, influence of patient advocacy groups, insurance and other socio-economic factors also vary across EU countries. The UK's National Health Service has implemented prioritization measures to allocate immunoglobulins to patients with the highest clinical need [34]. The United Kingdom needs approximately 1.5 million litres of plasma per year to manufacture sufficient immunoglobulin. The target is to reach 30% self-sufficiency in immunoglobulins by 2025 with a longer term target of 45% self-sufficiency, which would reduce the European plasma demand gap from 38% to 33%. The United Kingdom is currently collecting 240,000 L of recovered plasma per year, which is equivalent to the output of 16 mature and high-performing plasmapheresis centres.

PLASMA RISK REDUCTION METHODS: DONOR SELECTION AND MANUFACTURING PROCESSES

Donor selection criteria in the United Kingdom are stringent and are kept under close review by both the Joint UK Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee (JPAC) and SaBTO. The criteria currently exclude persons who have been told that they have been put at increased risk from surgery, transfusion or transplant of tissues or organs as well as persons who have been told that they may be at increased risk because a recipient of their blood or tissues has developed a prion-related disorder [35]. People known to have received an allogeneic tissue or blood transfusion since 1980 are also deferred from donation in the United Kingdom although it is likely that this measure will be reviewed given the US decision to now accept donations from people who received transfusions in the United Kingdom.

Despite considerable efforts over more than 20 years to develop a blood donor screening assay for vCJD, this has not progressed to the stage where it is sufficiently reliable or practical to be used by blood services and there appears to be little current activity in this area [36, 37]. The United Kingdom has very strong systems in place for donor-to-patient traceability for blood components and this will be applied to collection of plasma for fractionation, through to each manufacturing pool and all resulting products manufactured from that pool.

Leucodepletion of blood components and plasma for fractionation will remain standard practice in the United Kingdom. The CHMP Position Statement comments that 'Despite widespread exposure to potentially contaminated blood transfusions in the UK, Europe and the wider world, confirmed cases of vCJD resulting from exposure to contaminated blood or blood products are small. This may be partly attributed to the rapid introduction of leucodepletion'. [10]. In its review of immunoglobulin manufacture, the MHRA found that 'leucodepletion [of the raw plasma] decreases the risk of infection by a factor of ~5 and the risk of clinical case by a factor of ~3.5' [2].

Regulatory authorities require PDMP manufacturers to carry out 'product-specific investigational studies' and to critically evaluate their manufacturing processes to determine the prion reduction factor (PRF) specific to each individual PDMP [10]. Although the physical and biochemical characteristics of prion agents suggest that they could be removed by separation technologies used in the preparation of PDMPs, suitable experimental data are required to determine the extent to which this is achieved in practice [38].

Nanofiltration with 15- or 20-nm filters is used as a viral reduction step in the manufacture of many PDMPs and has been effective in the removal of prion infectivity using various preparations of scrapie brain homogenate in spike models with in vitro and in vivo read-outs [39, 40]. Cold ethanol precipitation steps, which constitute the upstream part of albumin and immunoglobulin purification processes, provide robust prion removal capability in experimental studies using various spike models and in vitro and in vivo read-outs [39, 41, 42]. Other purification steps such as chemical precipitation steps, low pH depth filtration and chromatography further contribute to the prion reduction capacity of PDMP manufacturing [39, 43, 44].

The PRFs claimed by one manufacturer ranged from 4.8 log to >11 log for a range of PDMPs; another reported 12.9 log reduction for an immunoglobulin preparation, and manufacturer responses

quoted in the MHRA risk assessment for immunoglobulin range from 4.8 log to 10.5 log and from 7.3 to 9.4 for hyperimmune immunoglobulin [2, 40, 45]. Comprehensive reviews of processes and PRFs have been published [39, 46].

Based on currently available experimental studies from manufacturers, it is estimated that PDMPs have >4 log manufacturing process reduction of the vCJD agent [39, 47].

There is a theoretical risk that, should prions causing vCJD be present in a pool of plasma, they may contaminate the manufacturing equipment. Sanitization of manufacturing equipment is a regulatory requirement for inclusion between batches, and the use of sodium hydroxide or other reagents has been shown to be effective for stainless steel decontamination and regeneration of chromatography resins. Manufacturers of PDMPs perform small-scale studies to demonstrate the effective reduction of experimental prion agents by the cleaning regimen of manufacturing lines [43, 48–50]. No additional cleaning or sanitization methods are necessary when processing UK plasma.

CONSIDERATION OF THE RELATIVE RISK OF UK PLASMA

As part of the analysis to support the 2018 SaBTO review of vCJD risk-reduction measures for labile blood components, it was estimated that using UK plasma for these transfusions would create a small additional transmission risk; on average, for every 5.2 million units of UK plasma transfused, there may be one additional death due to vCJD [27].

The risks per unit of plasma used to derive PDMPs will be different from this for two main reasons: pooling of multiple units of plasma and prion reduction during fractionation. The calculations, which may be found in the full paper, show that the risk from each unit of donated plasma that is fractionated, using a process with a 4 log PRF, is over 7000 times less likely to lead to a vCJD transmission than if that unit was used for transfusion [7]. This suggests that there would be less than one death from vCJD for every 36.4 billion units of plasma that are fractionated. It is predicted that approximately 1.1 million units of plasma will be collected each year in the United Kingdom, meaning that there may be one death from vCJD transmission every 33,000 years. It is important to note that there is considerable uncertainty in this modelling and these numbers should be viewed with caution. However, it is clear that the probability of vCJD transmission through the use of UK plasma for fractionation is extremely low.

ETHICAL CONSIDERATIONS OF THE USE OF UK PLASMA FOR PDMP MANUFACTURE

Manufactured medical products such as PDMPs are rightly expected to meet high safety standards, but there can be valid ethical arguments not to apply every safety measure that would further reduce small residual risks [51]. The main ethical argument in favour of allowing the use of UK plasma donations for the production of PDMPs is that increasing the supply of PDMPs seems necessary to avoid shortages and thus to avoid adverse effects for patients whose health relies on PDMPs. Given the extremely low risk of vCJD transmission through UK-sourced PDMPs demonstrated in this paper, this health benefit is expected to outweigh any adverse health effects.

Although arguably it was appropriate, on a precautionary basis, to decide in 1994 to cease fractionation of UK plasma, maintaining this policy under current circumstances cannot be justified by appealing to the precautionary principle. Application of the principle must be consistent and proportionate [52, 53], which implies that it cannot be used to justify retaining the ban on UK-sourced PDMPs: retaining this ban would, in any scenario that is credible today, cause more serious harm than it would prevent. Over the last two decades, it has been found that the risk is much smaller than had been feared, although the deferral policy increases the risk that the supply of essential blood products will be inadequate and that some patients will suffer serious consequences as a result. An evidence-based approach may now be taken to trade off these risks and strongly suggests that retaining the ban will have worse consequences for patients relying on PDMPs than lifting it. Retaining adequate haemovigilance with respect to vCJD to ensure that any unforeseen adverse effects would be identified promptly is arguably still a proportionate precautionary measure.

When considering removing a safety measure, one may ask whether such a policy would be implemented given the latest evidence. Removing safety measures may be more politically sensitive than deciding not to implement them, but it is doubtful that there is any ethical difference [54]. It is unlikely that a ban on UK plasma would be implemented today, knowing that this would not improve safety tangibly but would exacerbate shortages of PDMPs, and removing the ban thus seems ethically warranted.

CONCLUSION

More than two decades have now passed since the precautionary measures were put in place following the outbreak of vCJD. There have been no reported transfusion transmissions by red cells since leucodepletion was introduced in 1999 and no transmissions reported anywhere, ever, through platelets or plasma components. There have been no documented cases of vCJD in the UK population previously treated with UK-sourced immunoglobulin, and no transmissions reported in France, where there were dietary transmissions of BSE/vCJD yet leucodepleted domestic plasma continued to be used for the manufacture of PDMPs.

In countries that have conducted a review of the risk of vCJD transmission, there has been found to be no significant difference in risk posed by the receipt of blood or blood products from UK donors, than from any other donors. The United Kingdom now permits the use of domestic plasma for transfusion to all recipients and for the manufacture of immunoglobulin.

In the United States, where no endogenous cases of vCJD have been reported, the deferral of blood donors transfused in the United Kingdom was lifted recently, also based on the absence of significant difference in risk. This strongly supports the assessment that the risk of transmission of vCJD from UK plasma is not significantly different from that posed by any other plasma for the manufacture of PDMPs. Further, the updated US position means that plasma and products imported from the United States into Europe may already contain UK plasma, making the current European position inconsistent and overdue for review.

It is important to review these precautionary safety measures considering 23 years of epidemiological evidence, which suggests the absence of additional risk, and it is ethical to do so given the opportunity to provide significant benefit to patients currently in need of treatment. The same principle applies to other donor-deferral criteria, which should be kept under regular review to ensure an appropriate balance between safety and sufficiency of supply.

The demand for PDMPs is increasing and there would be significant benefits to EU patients and the resilience of the EU plasma supply chain should fractionation of UK plasma into PDMPs be permitted. This would reduce the current European dependency on importation of US plasma, improving strategic independence and benefiting patients who depend on life-saving treatment with PDMPs, which currently be restricted based on supply.

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CONFLICT OF INTEREST STATEMENT

Stephen Thomas, Barnaby Roberts, Dragoslav Domanović, Koen Kramer, Jean-Philippe Plançon, Lauren Kirkpatrick, Gail Miflin, Janet Birchall, Lorna McLintock and Richard Knight have no conflicts of interest to declare.

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COMMENTARY

Vox Sanguinis ISBT International Society of Blood Transfusion

Blood donor retention and iron deficiency anaemia in sub-Saharan Africa: A call for more robust donor screening methods

Access to sufficient and safe supplies of blood and blood products and safe transfusion practices is an integral component of a strong health system and is necessary to achieve universal health coverage [1]. In Africa, there is an unmatched demand for safe blood and blood products due to several acute and chronic conditions, especially for children and women. These include haemorrhages, severe anaemia as a result of malaria and malnutrition, infectious diseases, abnormalities of the bone marrow, trauma, neoplasms and natural and man-made disasters, which impact millions of people annually [1]. In 2021, the World Health Organization (WHO) African region accounted for 5% of global blood donations despite being home to about 14% of the world's population, compared with the European region where reported donations were 26% of the global donations, yet they account for only 12% of the global population [1]. The estimated blood donations in the African region of 5.4 donations per 1000 population were below the WHO target of 10 donations per 1000 population [1]. By contrast, the European region had 32.1 donations per 1000 population [1]. Therefore, lower rates of blood donations vis-a-vis the high demand may result in avoidable deaths, especially in maternal and paediatric departments. Bridging that gap in Africa requires effective strategies to maintain a pool of regular blood donors. In this commentary, we propose the use of ferritin measurements to safeguard donors against developing iron deficiency and ultimately increase the chances of retaining blood donors in sub-Saharan Africa (SSA).

The backbone of an effective blood transfusion service is recruitment and retention of blood donors. Retention is defined as preventing donors from lapsing and eventually becoming inactive [2]. Retention of regular blood donors is a challenge for many blood collection establishments in SSA [1, 3]. The median percentage of blood donations given by regular donors in Africa is 38% versus 90% given in European countries [1]. Although challenging, blood donor retention ensures adequate, safe and cost-effective blood supplies for two main reasons. Firstly, retained blood donors have a lower risk of transfusion-transmissible infections such as human immunodeficiency virus, hepatitis and syphilis than new recruits [1, 4]. Secondly, it is much cheaper to retain blood donors than to recruit new ones, averaging 7 United States Dollars (USD) for the former and ranging between 22 and 58 USD for the latter [2]. However, retention of blood donors has some challenges.

Iron deficiency is a major complication of repeated blood donation, especially in Africa [5, 6]. A study conducted in Morocco reported iron deficiency of 43% and reduced iron stores of 76% [7]. The prevalence of iron deficiency and reduced iron stores among blood donors in Zimbabwe was reported to be around 12.6% and 38.9%, respectively [4]. Iron deficiency anaemia peaked among regular blood donors who had given at least five donations [4]. Given that the blood donors had passed the copper sulphate ($CuSO_4$) screening test, the findings imply that current haemoglobin screening methods are less sensitive in detecting iron deficiency and are limited in safeguarding blood donors against developing anaemia. This has negative consequences on the health of blood donors and chances of retaining them [6, 8]. The health and well-being of blood donors are crucial in transfusion medicine and blood banks have a responsibility to protect blood donors against iron depletion and anaemia, especially in a region with lower donor retention rates [2, 4].

Africa's blood donor population comprises mostly young individuals [4, 9]. Therefore, alleviating iron depletion in this population is imperative because young people continue to undergo physical and neurocognitive development [4, 9]. The loss of roughly 250 mg of iron with each whole blood donation, along with the limited stores and capacity for iron absorption, generally leads to a high incidence of iron deficiency in regular blood donors, especially women of childbearing age and adolescents [6, 8]. The average recovery period (the time it takes for a blood donor to regain normal haemoglobin levels and become eligible to give blood again) is affected by low iron stores and poor diets [8].

Anaemia is the major reason for blood donor deferrals worldwide [4, 10]. Early detection of iron depletion among blood donors allows for appropriate interventions to be made before blood donors are deferred. If not managed, iron depletion worsens into iron deficiency and ultimately iron deficiency anaemia. The negative consequences of losing a donor to a deferral are impactful. Blood donors who are deferred are less likely to return for blood donation after the deferral period and/or to refer others [11, 12]. For this reason, studies have recommended reviews of donor acceptance guidelines and the incorporation of tests like ferritin, which detect blood-donation-induced iron depletion earlier [5, 6, 13].

Ferritin is recommended by WHO and is strongly positively correlated with bone marrow iron stores——the gold standard for measuring iron stores [4]. Both the $CuSO_4$ and the HemoCue® tests used for screening are cheaper and more convenient point-of-care tests than

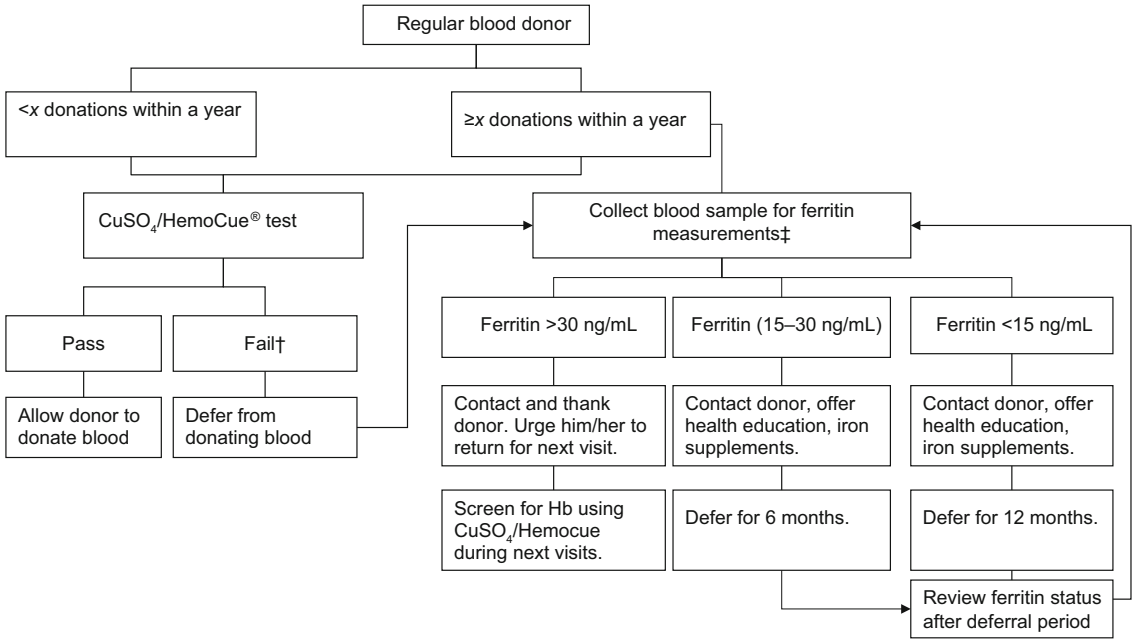

FIGURE 1 Proposed algorithm for ferritin testing in regular blood donors. However, since $CuSO_4$/HemoCue test is a point-of-care test, a blood donor is either deferred or allowed to donate based on $CuSO_4$/HemoCue® test while awaiting ferritin test results. Once ferritin results are out, blood donors are contacted and there are three scenarios depending on the category of ferritin levels to which they belong. Either they are deferred for 6 or 12 months or they are thanked for a recent donation and encouraged to return when they are due for a next donation. Blood donors who would have served deferral periods (6 or 12 months) and those who fail the $CuSO_4$/HemoCue® test are required to undergo ferritin tests before they are allowed to donate. †Hb less than 12.5 and 13.5 g/dL for women and men, respectively; ‡blood samples for ferritin measurements are collected on the same day the $CuSO_4$/HemoCue® test is done; $CuSO_4$, copper sulphate; Hb, haemoglobin; ng/mL, nanograms per millilitre; *x*, cut-off number of donations to be determined using local data.

ferritin. This probably explains their wide use. However, screening tests are based on haemoglobin measurements and cannot accurately estimate iron deficiency anaemia. They underestimate the proportion of blood donors with iron deficiency, thereby depriving donors of early interventions to correct iron deficiency [4]. We propose that iron depletion can be averted by complementing current screening tests with ferritin measurements. Since ferritin is not a point-of-care test and is expensive, two things are crucial to ensure a cost-effective blood service. These are timing of ferritin tests and context-specific data on prevalence of iron deficiency and number of donations at which iron deficiency peaks. Data from Zimbabwe imply that costs of ferritin measurements may be minimized by targeting regular donors, especially those donating five units or more within 2 years [4]. It is important to note that ferritin does not replace current screening tests, but complements them, as shown in Figure 1. In the Netherlands, two cut-off points of ferritin determined deferral periods of 6 months (15–30 ng/mL) or 12 months (<15 ng/mL) [14]. It is encouraging that donor return after low ferritin-induced blood donor deferrals were high in the Netherlands [14]. The extent to which this also applies in low-resource settings is unknown. Blood donation centres will also need to ensure proper donor follow-ups and education on the consequences of repeated blood donation, iron supplementation, the need to maintain healthy diets and varied donation intervals [8, 15]. We hope this comprehensive donor management and care may help maintain adequate and safe blood supplies by improving retention of blood donors in the medium to long term.

In conclusion, the need to invest in blood donor screening methods that detect iron deficiency at an early stage cannot be overemphasized. Though ferritin measurements are relatively expensive, a cost-effective blood service is still possible. Ferritin measurements must be informed by context-specific data on blood donors who should be prioritized for such testing to avert iron deficiency. This may ensure a healthy blood donor pool that guarantees both adequate and safe blood supplies.

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None declared.

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

First donor haemovigilance system at a national level in China: Establishment and improvement

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Abstract

Background and Objectives: No systematic study has measured the incidence of adverse reactions (ARs) to blood donation at the national level in China before 2019. The objective of this study was to establish an effective reporting system to collect information on ARs to blood donation in China.

Materials and Methods: The status of donor haemovigilance (DHV) in blood collection facilities in China was evaluated, and an online DHV system was established to collect data on ARs to blood donation in July 2019. The definitions of ARs were based on the International Society of Blood Transfusion (ISBT) standards. The prevalence and data quality of ARs from 2019 to 2021 were analysed.

Results: A standard online reporting system has been established for ARs to blood donation. In total, 61, 62 and 81 participating sites were included in this pilot study in 2019, 2020 and 2021, respectively. From July 2019 to December 2021, 21,502 cases of whole-blood-related ARs and 1114 cases of apheresis platelet-related ARs were reported, with an incidence of 3.8‰ and 2.2‰, respectively. Data completeness for key reporting elements improved from 41.7% (15/36) in 2019 to 74.4% (29/39) in 2020. Data quality analysis for the year 2021 yielded similar results as for 2020.

Conclusion: The construction and continuous improvement of the blood donor safety monitoring system prompted the establishment of the DHV system. Improvements have been made to the DHV system in China, with a significant increase in sentinels and higher data quality.

Keywords

adverse reactions, blood donation, donor health, haemovigilance

Highlights

- The first donor haemovigilance (DHV) system at national level in China has been established.
- A pilot phase is very useful for starting a DHV system at the national level.
- Hierarchical management according to the importance of adverse reaction elements is helpful in improving data quality.

Junhong Yang and Dinrong Fan contributed equally as joint first authors.

Tao He and Xia Huang contributed equally as joint corresponding authors.

INTRODUCTION

Donor haemovigilance (DHV) is defined as the systematic monitoring of adverse reactions (ARs) and incidents in the whole chain of blood donor care, with a view to improving the quality and safety of blood donors [1]. Internationally, DHV involves four aspects: complications related to blood donation (donor ARs); errors in donor care (adverse incidents); post-donation information (focusing on aspects relating to donor safety); and counselling and procedures related to unexpected findings [2].

The core of DHV is comprised of activities related to blood donation complications [3]. In 2016, the International Haemovigilance Network (IHN) reported the technical issues, results of ARs and events in donors and recipients of blood components [4]. A recent study has reported the complications of blood donation worldwide over 11 years [5]. Nevertheless, no systematic study has measured the incidence at the national and sub-national levels in China before 2019 [6].

Precise definitions of donor ARs are required for any DHV system [7, 8]. The Working Party on Haemovigilance of International Society of Blood Transfusion (ISBT), in collaboration with IHN, published a standard for the surveillance of complications related to blood donation in 2008, which was revised in 2014 [9]. China's Ministry of Health released the guideline for donor ARs (WS/T 551-2017) in 2017 [10].

The Working Party on Haemovigilance of the Chinese Society of Blood Transfusion (CSBT/HV) was set up in 2017 to lead the launch of the haemovigilance system in China. The Working Party aimed to develop a system for all Chinese blood collection facilities that would provide a voluntary, anonymous, non-punitive and easy-to-use digital reporting service focusing on improving donor safety. A DHV system was developed to collect data on all complications of blood donation since July 2019. This study describes the DHV system pilot study from 2019 to 2021 in China. The outcome of this feasibility study would inform the design and applications of the DHV system to promote the safety of blood donors at the national level in developing countries.

MATERIALS AND METHODS

China's blood service

The most common types of blood donation in China include whole blood and apheresis platelets. Blood donors are selected according to China's national standard, namely the Whole Blood and Component Donor Selection Requirements. The age for blood donation advocated by the state is 18–55 years. Donors who have a history of blood donation, have no ARs and meet the requirements of health examination can extend the age to 60 years if they volunteer to donate blood again. Male and female blood donors have different weight requirements, with a minimum weight of 50 kg for men and 45 kg for women. Male donors require haemoglobin levels of at least 120 g/L and female donors of at least 115 g/L. Whole-blood donors can donate 200, 300 or 400 mL each time, and apheresis platelet donors can donate single or double therapeutic doses, or a single therapeutic dose of platelets and no more than 200 mL of plasma. The total quantity of platelets and plasma collected within a year should not exceed 10 L. In addition, approximately 20 mL of blood samples are collected before each blood collection for the detection of any infectious disease. Whole-blood donations should be done at least 6 months apart, and the interval between two apheresis platelet donations should be no less than 2 weeks and no more than 24 times a year. Whole blood can be donated as from 4 weeks following platelet apheresis donation, and platelet apheresis should be performed no less than 3 months after whole-blood donation.

Blood services, which are state-owned public welfare organizations, are responsible for the collection and supply of blood in China. According to their size and operation form, they are divided into provincial blood centres, municipal central blood banks and (a small number of) blood banks affiliated with county-level general hospitals. As regional centres, provincial blood centres are responsible for the quality control and evaluation of blood services in their provinces. Furthermore, some provincial blood centres also act as regional centres for haemovigilance in their regions. There are more than 450 blood services in China, and the total number of blood donors exceeds 11 million each year.

Definitions of complications of DHV

A revision subcommittee of the CSBT/HV was formed, including members from the Chongqing Blood Center, Shanghai Red Cross Blood Center, Anhui Blood Center, and Fujian Blood Center, one of which was also part of the ISBT Haemovigilance Working Party. A draft version of the revised classification scheme and definitions of complications related to blood donation was circulated to members of the CSBT. Comments were obtained from 15 blood centres, and further changes or explanatory comments were made regarding the proposed classification scheme and definitions.

Online system

A web-based database (DHMS, the Donor Haemovigilance Management System, https://hv.cqsbt.org.cn) was established for surveillance of donor ARs by the CSBT/HV. The online surveillance system was designed to collect all complications related to blood donation.

Participants in the pilot study

The participants were recruited through leaflets distributed via group emails and announcements on the CSBT official website. Participation was voluntary, confidential and non-punishing. Participant recruitment lasted from May 2019 until 2021. Each pilot blood collection

establishment or regional centre required a full- or part-time liaison to submit their data. The salaries of all liaisons were provided by the blood services they work for, and this project did not fund the salaries of liaisons.

Data collection

The DHMS online surveillance system enabled the collection of data of the donors, ARs and the donations. The DHMS contained data entry forms for donor information, symptoms and signs, classification, treatment measures, follow-up information and the severity and imputability of each case of AR. The basic information of blood donors was imported from the blood station's own information system, and the classification, symptoms, signs and other information of ARs were first recorded on paper and then manually input or recorded by another staff member in the DHMS at the donation bed. Donor complications included local symptoms, generalized symptoms (vasovagal reactions [VVRs]), complications related to apheresis as well as other serious complications related to blood donation. The imputability of the reported complications was classified as certainly, probably, or possibly related to whole blood or apheresis donation. The severity was classified as severe or non-severe.

Organization users were requested to submit their surveillance data to the CSBT/HV before the 10th of every month via DHMS. Reminder text messages were sent through the information system to the liaison officers of the institutions that had not yet submitted the data. Where applicable, the blood collection facilities provided reasons why the data were not submitted on time. Zero reporting was required to monitor the AR surveillance quality. Blood services were required to report if no AR was recorded in the preceding month. Data were collected by the CSBT/HV Donor Haemovigilance Working Group and analysed statistically every month from July 2019 to December 2021.

Statistics and feedback

The CSBT/HV undertook regular monthly monitoring and auditing of data entry procedures to guide all data management. Data management included regular data checks on key elements for missing data and inconsistencies. When AR data were not reported, liaisons were contacted with a request to supply the missing information. Analyses of data and feedback reports were circulated to the pilot teams quarterly. The feedback reports included the number of pilot blood collection establishments participating in the study, the number of AR cases, the major improvements measured this quarter and the major goals to be achieved in the next quarter. The main contents include the change of data collection, the upgrade of the information system and the delivery of experience and training contents. The data recorded each year were pooled and analysed, and data analysis results were released in an internal feedback report. Feedback reports were provided to all pilot blood collection establishments after data analysis. In addition, yearly meetings were held to provide feedback to data collectors. The presence or absence of each data element was calculated as the reporting rate, which served as an indicator for data quality analysis.

RESULTS

The revised classifications

The CSBT/HV aimed to revise the definitions of complications related to blood donation. The goal of the revision was to align with international definitions and simplify the reports for analysis and international benchmarking. A gap analysis was performed comparing the WS/T 551-2017 classification scheme and the ISBT-2014 classification scheme. Some differences were found between the WS/T 551-201 and ISBT-2014 criteria for classifying ARs. The major difference in the local symptoms category was the categorization of blood vessel injury. The ISBT-2014 criteria included four categories: deep venous thrombosis; arteriovenous fistula; compartment syndrome and brachial artery pseudoaneurysm, whereas the WS/T 551-2017 criteria did not include further categories. Among the generalized symptoms, the major difference was in the subgroups of VVRs with loss of consciousness (LOC). In the ISBT-2014 criteria, the subgroups of VVRs included optional subdivisions for donors with LOC < 60 s and LOC ≥ 60 s. The WS/T 551-2017 criteria did not include subgroups of VVR with LOC. The gap analysis comparing WS/T 551-2017, ISBT-2014 and the CSBT/HV revised criteria is shown in Table 1.

In April 2019, the CSBT proposed a team standard, called the Guideline for Haemovigilance, which described the concepts and working systems of haemovigilance, classification, definition, association, severity and reporting modes for ARs in blood donation, also including an appendix presenting the report form of donor ARs. The guideline facilitated the collection of demographic data, symptoms and signs, treatment and follow-up of blood donors. It was applied to HV systems in blood establishments in China.

Reporting system

The focus of the DHMS was to capture and analyse donor AR information from blood collection facilities. The system monitored the total number of donations of whole blood and apheresis platelets each month. The online system was validated by the Chongqing Blood Center to ensure that the data entry methods, including manual and automated electronic submission, accurately captured and recorded the intended data elements. Subsequently, the database was made accessible to each participating organization.

Authorized users were provided secure access to DHMS via individual usernames and passwords at four levels. The first two levels were users entering their organization's data. Level 1 access was granted to the system administrator in charge of registering organizations and users, issuing their passwords, as well as viewing and

TABLE 1 Gap analysis comparing WS/T 551-2017, ISBT-2014 and the CSBT/HV revised criteria.

WS/T 551-2017	ISBT-2014	The revised classification scheme
A. Complications mainly with local symptoms	A. Complications mainly with local symptoms	A. Complications mainly with local symptoms
A1. Blood outside vessel	A1. Blood outside vessel	A1. Blood outside vessel
Haematoma (bruise)	Haematoma (bruise)	Haematoma (bruise)
Arterial puncture	Arterial puncture	Arterial puncture
Delayed bleeding	Delayed bleeding	Delayed bleeding
A2. Complications mainly characterized by pain	A2. Complications mainly characterized by pain	A2. Complications mainly characterized by pain
Nerve irritation	Nerve injury/irritation	Nerve irritation
Nerve injury	Other pain in the arm	Nerve injury
Tendon injury		Tendon injury
Other pain in the arm		Other pain in the arm
A3. Localized infection/inflammation	A3. Localized infection/inflammation	A3. Localized infection/inflammation
Thrombophlebitis	Thrombophlebitis	Thrombophlebitis
Localized skin allergy	Cellulitis	Localized skin allergy
Localized infection		Localized infection
	A4. Other major blood vessel injury	A4. Other major blood vessel injury
	Deep venous thrombosis	Deep venous thrombosis
	Arteriovenous fistula	Arteriovenous fistula
	Compartment syndrome	Compartment syndrome
	Brachial artery pseudoaneurysm	Brachial artery pseudoaneurysm
B. Generalized symptoms—vasovagal reactions	B. Generalized symptoms—vasovagal reactions	B. Generalized symptoms—vasovagal reactions
Without LOC	Without LOC	Without LOC
With LOC	With LOC	With LOC
	LOC < 60 s; without other signs and symptoms	LOC < 60 s; without other signs and symptoms
	LOC ≥ 60 s; or with complications of convulsive movements, urinary or faecal incontinence	LOC ≥ 60 s; or with complications of convulsive movements, urinary or faecal incontinence
With injury	With injury	With injury
Without injury	Without injury	Without injury
On collection facility	On collection facility	On collection facility
Outside collection facility	Outside collection facility	Outside collection facility
C. Complications related to apheresis	C. Complications related to apheresis	C. Complications related to apheresis
Citrate reaction	Citrate reaction	Citrate reaction
Haemolysis	Haemolysis	Haemolysis
Air embolism	Air embolism	Air embolism
Generalized allergic reaction	Infiltration	Generalized allergic reaction
	D. Allergic reactions	
	Allergy (local)	
	Generalized allergic reaction	
D. Other complications	E. Other serious complications related to blood donation	D. Other complications
	Myocardial infarction	
	Cardiac arrest	
	Transient ischaemic attack	
	Cerebrovascular accident	
	Death	
	F. Other complications	

Abbreviations: CSBT/HV, Haemovigilance of the Chinese Society of Blood Transfusion; ISBT, International Society of Blood Transfusion; LOC, loss of consciousness.

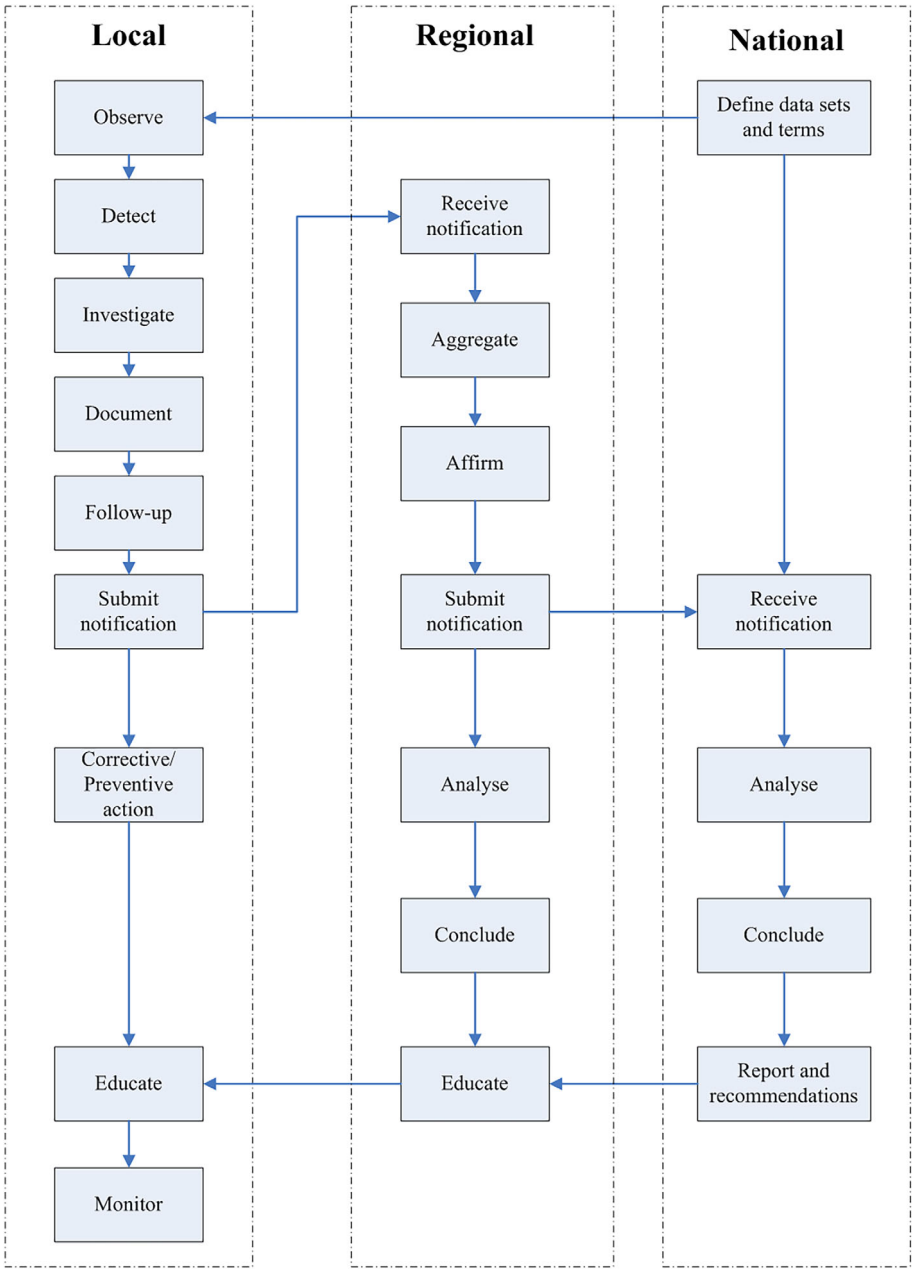

FIGURE 1 Workflow of the donor haemovigilance system.

extracting all DHV data. The system administrator can edit data or unlock the submitted organization's data for correction at the organization's request. Level 2 users comprised members of the CSBT/HV secretariat who can view and extract all data but not edit the data. Level 3 comprised organization users who may enter and edit data, submit and extract organization data, analyse online data and register individual users. Each organization was labelled by a code allocated by the CSBT/HV secretariat, which was known to that participant's representative but not to the representatives of other participating centres. Finally, Level 4 comprised individual users who were permitted to enter, edit and save DHV data but not to submit it to the CSBT/HV secretariat. This function enabled different users within the same organization to enter parts of the data.

Participants in the pilot study

The pilot study included representatives from large, middle and small blood collection organizations. Sixty-one blood collection facilities from 19 provinces initially participated in the pilot study as sentinels. Subsequently, 1 additional centre was included in 2020 and a further 19 in 2021. As a result, 62 and 81 sentinels were included in this pilot study in 2020 and 2021, respectively. The blood services participating in the pilot project for at least 1 year accounted for about one-sixth of the total number of blood services in China, covering about a quarter of the total number of blood donors. A regional centre was formed to review regional DHV data in Chongqing. The workflow of this study is displayed in Figure 1.

TABLE 2 Number and frequency of donor adverse reactions reported from 2019 to 2021.

	No. of donations		No. of adverse reactions		Incidence (‰)	
Year	Whole blood	Apheresis	Whole blood	Apheresis	Whole blood	Apheresis
2019	784,195	75,743	3388	141	4.3	1.9
2020	222,6742	176,764	6157	231	2.8	1.3
2021	2,604,742	257,747	11,957	742	4.6	2.9
Total	5,615,679	510,254	21,502	1114	3.8	2.2

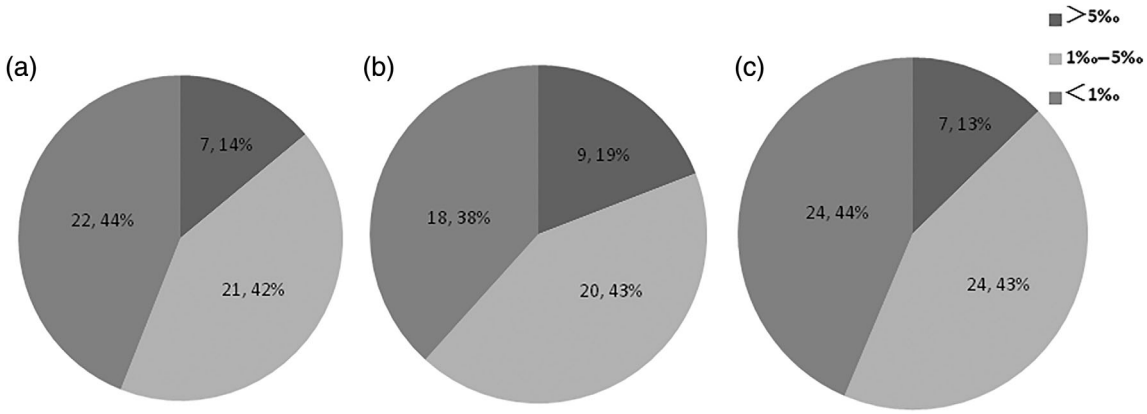

FIGURE 2 Distribution of reported incidence of adverse reactions from 2019 to 2021. (a–c) Distribution of reported incidence of ARs in blood donation from 50, 47 and 55 pilot centres in 2019, 2020 and 2021, respectively. The incidence of adverse reactions in blood donation in each blood collection centre is given in Table S1.

Number and frequency of donor ARs reported from 2019 to 2021

In 2019, 50 blood collection facilities submitted data on ARs to blood donation, followed by 49 in 2020 and 57 in 2021. However, two participating sites in 2020 and 2021 did not submit their denominator data, so their data were not included in the final statistical analysis. The remainder had no DHV data due to zero reporting of ARs.

We investigated ARs to blood donations collected by all participating sites from July 2019 to the end of December 2021. Blood collection facilities reported a total of 6,125,933 blood donations, including 5,615,679 whole-blood donations and 510,254 apheresis platelet donations. Table 2 displays the number of blood donations covered by the DHV system from 2019 to 2021, the number of ARs detected and the incidence distribution. In total, 21,502 cases of whole-blood-related ARs and 1114 cases of apheresis platelet-related ARs were detected, with an incidence of 3.8‰ and 2.2‰, respectively.

The distribution of reported incidence of ARs to blood donation in 2019–2021 is shown in Figure 2. During the study period, 13%–19% of the participating sites reported an incidence of ARs to blood donation of >5‰. In addition, about 40% of the participating sites reported an incidence between 1‰ and 5‰, and about 43% reported below 1‰ (Table S1).

Data quality analysis results

Forty-nine input elements were included in the information system. In 2019, there were no mandatory elements to be submitted, and only the reporting elements available from the data collection of participating sites were submitted. Since 2020, the elements of ARs have been divided into mandatory and optional categories for hierarchical management. Among them, 16 were mandatory and 23 were optional. Elements with poor accessibility and low importance were selected as optional. General denominator data on the numbers of donations in broad categories of blood components (whole blood and apheresis platelets) were included in the data entry form.

The data quality analysis results are presented in Table 3. In 2019, 15 of the 36 reporting elements had a reporting rate above 50% and 21 had a reporting rate below 50%. The elements with reporting rates lower than 50% were mainly related to the follow-up of blood donors (10 items), vital signs of blood donors such as respiration, heart rate and blood pressure (6 items) before and after treatment of ARs of blood donation and three specific time points related to ARs of blood donation.

After the implementation of hierarchical management of reporting elements in 2020, 29 out of 39 reporting elements showed a reporting rate of more than 50%, while 10 had a reporting rate of less than 50%. The elements with reporting rates below 50% mainly

TABLE 3 Results of reporting elements of adverse reactions to blood donation.

Element			2019 Case	2019 Report rate (%)	2020 Case	2020 Report rate (%)	2021 Case	2021 Report rate (%)	Report type
Date			3529	100.0	6388	100.0	12,699	100.0	Mandatory
Gender			3494	99.0	6388	100.0	12,699	100.0	Mandatory
Age			N/A	N/A	6372	99.8	12,699	100.0	Mandatory
Weight			2764	78.3	5432	85.0	12,670	99.8	Mandatory
Height			1340	38.0	3960	62.0	10,748	84.6	Mandatory
Donation type			3529	100.0	6388	100.0	12,699	100.0	Mandatory
Volume			3288	93.2	6388	100.0	12,699	100.0	Mandatory
Donation history			3227	91.4	6388	100.0	12,699	100.0	Mandatory
Collection site			3495	99.0	6388	100.0	12,699	100.0	Mandatory
Sponsor group type			3471	98.4	6388	100.0	12,699	100.0	Mandatory
Inducement			2016	57.1	6384	99.9	11,497	90.5	Mandatory
Manifestation of adverse reaction	Link		2267	64.2	6388	100.0	12,293	96.8	Mandatory
	Start time		1611	45.7	3204	50.2	7847	61.8	Optional
	End time		1466	41.5	2850	44.6	5151	40.6	Optional
	Departure time		1131	32.1	2247	35.2	5064	39.9	Optional
	Symptom		2563	72.6	6388	100.0	10,119	79.7	Optional
Type of adverse reaction			1734	49.1	6388	100.0	12,699	100.0	Mandatory
Severity			1734	49.1	6388	100.0	12,699	100.0	Mandatory
Imputability			3295	93.4	6388	100.0	12,699	100.0	Mandatory
On-site treatment	Signs	Heart rate	1130	32.0	1868	29.2	4051	31.9	Optional
		Respiratory rate	842	23.9	1108	17.4	1428	11.2	Optional
		Blood pressure	1064	30.2	1971	30.9	4061	32.0	Optional
	Treatment measure		3055	86.6	512	80.0	9854	77.6	Optional
Treatment outcome	Recovery		2577	73.0	4546	71.2	8424	66.3	Optional
	Signs	Heart rate	1213	34.4	2791	43.7	5540	43.6	Optional
		Respiratory rate	846	24.0	1239	19.4	1428	11.2	Optional
		Blood pressure	1230	34.9	2619	41.0	5177	40.8	Optional
	Ways to leave		1791	50.8	2760	43.20	7513	59.2	Optional
Follow-up	Model		1812	51.4	3615	56.6	9340	73.6	Optional
	Subsequent processing		1707	48.4	3740	58.5	5853	46.1	Optional
	Diet		1034	29.3	3221	50.4	4956	39.0	Optional
	Drink		968	27.4	3178	49.8	5587	44.0	Optional
	Queue time		1044	29.6	3228	50.5	5591	44.0	Optional
	Puncture		1124	31.9	3274	51.3	4675	36.8	Optional
	AMT		955	27.1	3202	50.1	4596	36.2	Optional
	Conversation		1411	40.0	3394	53.1	4956	39.0	Optional
	Psychological status		1472	41.7	3439	53.8	4930	38.8	Optional
	Understanding of adverse reactions		1471	41.7	3444	53.9	4933	38.9	Optional
	Retention		1527	43.3	3457	54.1	5842	46.0	Optional

Note: Inducement: Incentives that cause adverse reactions during blood donation. Ways to leave: Blood donors leave alone or accompanied by others. Model: Method of follow-up, telephone or at home. Diet: Did you eat before donating blood? Drink: Did you drink water before donating blood? Conversation: Did you communicate with the staff during the donation process? Psychological status: Were you nervous or relaxed during the donation process? AMT: Did you have an AMT during the donation process? Understanding of adverse reactions: Are you aware of the potential for adverse reactions? Retention: Will you still participate in blood donation?
Abbreviation: AMT, applied muscle tension.

included the vital signs of blood donors before and after the treatment of ARs to blood donation and two specific time points related to ARs to blood donation. Among the 16 mandatory elements, there were 12 elements with 100% reporting rate, accounting for 75% of the total. The other four factors (inducement, age, weight and height) did not reach 100%, among which the height was reported at only 61.99%, while the other three factors exceeded 80%. Data from 2021 showed a similar distribution as 2020. The reported rates of height and weight increased to 84.6% and 99.8%, respectively.

DISCUSSION

Developed countries such as the United States, Britain and Italy have established relatively mature DHV systems [11–13], whereas a proper HV organization was not established in China until 2017 [14]. On the other hand, China's DHV was managed entirely at the local level before 2019.

Determination of monitoring criteria

The monitoring criteria were mainly based on the 2014 ISBT standard and China's health industry standards. The standard took the actual local situation of blood collection and supply into consideration, enabling the revised terms and classifications to be more applicable to China. For ARs that could not be diagnosed and clearly classified under our current conditions, such as the effect of blood donation on the heart of blood donors [15], the classification was summarized according to its subordinate level.

Electronic information system

The IHN data reporting system's hierarchical authorization model was adapted to our local needs [16]. The classification criteria of monitored ARs were designed into the information system, which provided a uniform method of data collection. In addition, our information system was embedded with auditing, summary and statistical analysis functions, which reduced the cost of sentinel unit data management. Additionally, a unified electronic information system was developed and provided to sentinels free of charge to improve information management and work efficiency. Besides, regular work reminders for timely data submission could be sent via the information system.

Pilot management

From 2019 to 2021, the number of sentinels increased from 61 to 81, and the covered area increased from 19 to 29 provinces. Recruitment was carried out on a confidential, non-punitive and voluntary basis by issuing recruitment letters to blood banks [17]. The aim of establishing a centralized data system for DHV in China was stated in the recruitment letter.

To ensure the data quality of sentinels and the foundation of the DHV, we developed a systematic pilot management scheme, and three training levels were established. The training included basic knowledge of DHV, the latest research results, international and domestic trends and so on, and was open to all blood bank practitioners. Good training can assist participating sites in better understanding the classification and definition of ARs to blood donation [18].

Reported incidence

From 2019 to 2021, sentinels submitted data reporting a total of 6,125,933 blood donations, including 5,615,679 whole-blood and 510,254 apheresis platelets donations. The reported incidence of ARs to whole-blood donation was 2.8‰–4.6‰. The incidence of ARs to apheresis platelets donation was lower than that of whole-blood donors (1.3%–2.9%). The overall reported incidence was lower than the 20.8‰–24.3‰ of the AABB Donor Haemovigilance Report for 2012–2017 [11] and the 17.9% of the Australian Haemovigilance Report for July 2017–June 2018 [19], while it was slightly higher than the 2.5‰ found in the 2016–2019 study conducted by Vanessa et al. on Italian donors [13]. This may be attributed to the data collection method on ARs to blood donation in China. The reported incidence of ARs varied greatly across different blood stations (from less than 1‰ to more than 5‰). This may be due to the differences in blood donation service mode, AR identification and the reporting mode of different blood stations. Three types of reporting methods were recommended in the guideline for monitoring ARs to blood donation: on-site recording; active reporting by blood donors after blood donation; and reporting during follow-up. However, most of the recorded ARs were acute that occurred on-site. Late-onset adverse events were rarely reported because of the fact that they require donors to report back to the blood bank actively [20, 21].

Data quality analysis

The quality of reported data was analysed to improve the monitoring quality [22]. Of the 38 reported elements in 2019, the top quartile of elements by completion rates included the donation type (100%), collection site (99.0%), gender (99.0%), sponsor group type (98.4%), imputability (93.4%), volume (93.2%), donation history (91.4%), on-site treatment (86.57%) and weight (78.3%). The elements in the top quartile were all part of the blood donation process (collection site, sponsor group type and volume) or information acquired during the health examination before donation (donation type, gender, donation history and weight). Therefore, these elements exhibited a high completion rate. In the AABB DHV report, a significant difference was observed in the reporting rate of different elements [23].

The analysis of the 2019 results suggested that there were too many elements for the collection of ARs, which were comprehensive but also complex and hindered the data collection. The contents of the Summary of Adverse Events of Blood Donation were readjusted based on the reporting rate and significance of the elements reported in 2019. The important reporting elements were retained, including some important factors with low reporting rates. Furthermore, essential elements such as donor age were added, and hierarchical management of the reporting elements was performed, dividing them into required and optional.

In the analysis of data quality, the presence or absence of information for each data element was only preliminarily analysed.

In the future, the data quality will be improved by further training the pilot blood bank staff, analysing the classification and severity of ARs and planning to incorporate the AR data into the IHN database. The severity of ARs is only divided into severe and non-severe, but severe reactions are rare in practice, and reporting standards remain unclear. AABB has been exploring the severity grading index [24, 25], but whether this index is applicable in China needs to be further verified and adjusted according to the actual situation.

In conclusion, the construction and continuous improvement of the blood donor safety monitoring system based on the current situation effectively promoted the establishment of the DHV system. A pilot project enabled the establishment of a DHV system at the national level. Hierarchical management according to the importance of AR elements is helpful in improving data quality. However, the number of blood banks participating in the pilot is still limited, and the coverage of blood banks and donors should be increased through advocacy. The pilot experience can be further popularized in China.

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J.Y. and D.F. performed the study and wrote the first draft of the manuscript; D.X. contributed to the definitions; X.G. and W.Z. performed the statistical analysis; T.H. and X.H. contributed to the study design and manuscript review.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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A novel quantitative method to evaluate the contribution of platelet products to white thrombus formation in reconstituted blood under flow conditions

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Abstract

Background and Objectives: Currently, the quality of platelet (PLT) products is evaluated using a series of in vitro tests, which only analyse PLTs as an inspection material. However, it would be ideal to assess the physiological functions of PLTs under conditions similar to the sequential blood haemostatic process. In this study, we attempted to establish an in vitro system where the thrombogenicity of PLT products was evaluated in the presence of red blood cells (RBCs) and plasma using a microchamber under constant shear stress (600/s).

Materials and Methods: Blood samples were reconstituted by mixing PLT products, standard human plasma (SHP) and standard RBCs. Each component was serially diluted keeping the other two components fixed. The samples were applied onto a flow chamber system (Total Thrombus-formation Analysis System [T-TAS]), and white thrombus formation (WTF) was assessed under large arterial shear conditions.

Results: We observed a good correlation between the PLT numbers in the test samples and WTF. The WTF of samples containing ≦10% SHP was significantly lower than those containing ≧40% SHP, and no difference was observed in WTF among samples containing 40%–100% SHP. WTF significantly declined in the absence of RBCs, whereas no change in WTF was observed in the presence of RBCs, over haematocrit range of 12.5%–50%.

Conclusion: The WTF assessed on the T-TAS using reconstituted blood may serve as a new physiological blood thrombus test to quantitatively determine the quality of PLT products.

Keywords

coagulation-related factors, haematocrit, platelet numbers, Total Thrombus-formation Analysis System, white thrombus formation

Highlights

- Platelet numbers in reconstituted blood correlate with white thrombus formation (WTF).

- WTF increases when incremental amounts of standard human plasma (SHP) are added to the mixture, reaching the maximum thrombus formation at SHP concentrations of 40%.
- WTF declines in the absence of red blood cells.

INTRODUCTION

Haemostasis, comprising multiple reactions, is an essential mechanism that prevents excessive bleeding from damaged vessels. Primary haemostasis is platelet (PLT) adhesion to the injured endothelium and aggregation by degranulation followed by secondary haemostasis, which includes fibrin clot formation by coagulation cascade [1–3]. The properties of the thrombus produced by blood flow shear stress differ: a PLT-dominant white thrombus forms under high shear conditions, whereas a fibrin-dominant white thrombus or red thrombus containing red blood cells (RBCs) develops under low shear conditions [4–6]. Changes in blood composition, such as increased coagulation factors associated with inflammation, increased fibrinolytic inhibitory factors or increased blood viscosity due to dehydration, play facilitating roles in thrombus formation [7]. Therefore, blood flow and composition are the blood-related factors of Virchow's triad affecting thrombus formation at the haemostasis site [8].

Blood banks evaluate the quality of a PLT product, which is transfused into patients with thrombocytopenia, based on the PLT product's morphology, metabolic features, surface antigens, hypotonic shock response and aggregation ability [9, 10]. Multiplate (Dynabyte Medical, Munich, Germany) and VerifyNOW (Accriva Diagnostics, San Diego, CA, USA) evaluate PLT reactivity to PLT-activating agents under non-flow conditions, while PFA-100 (Siemens Healthcare Diagnostics GmbH, Marburg, Germany) evaluates capillary occlusion due to PLT aggregate formation under flow conditions; however, these PLT function tests do not reflect secondary haemostasis [11]. ROTEM (Tem Innovations GmbH, Munich, Germany) can measure changes in blood viscoelasticity as a coagulation test using exogenous or endogenous coagulation-activating reagents under non-flow conditions; however, it is relatively insensitive for PLT function [11]. Therefore, it is difficult to predict the extent to which the PLT transfusion would restore the patient's whole thrombogenic ability using these tests. Recently, a new and simple thrombogenicity evaluation kit called Total Thrombus-formation Analysis System (T-TAS; Fujimori Kogyo, Tokyo, Japan) has emerged. It is an automated system that includes all the materials, utilizes the flow chamber system and allows feasible evaluation of whole blood thrombogenicity through monitoring of flow pressure associated with thrombus formation [12–14]. It enables the quantitative measurement of white thrombus formation (WTF), consisting of activated PLTs and fibrin, by adding a whole blood sample to a microchip coated with collagen and tissue thromboplastin, measuring the occlusion time (T_{80}: time to 80 kPa) when the pressure rise associated with thrombus formation reaches the near-complete occlusion of the capillary by WTF and analysing the integral value of time and pressure (area under the curve for 30 min [AUC_{30}]). Hence, the T-TAS can comprehensively evaluate primary and secondary haemostasis, providing more physiologically relevant conditions compared with existing evaluation systems.

In this study, we used artificial blood reconstituted with PLT products, standard human plasma (SHP) and standard RBCs to assess the effect of the blood component ratio and concentration on WTF abilities under shear conditions using the T-TAS and attempted to develop a more physiologically relevant system to evaluate the PLT product contribution to patient whole thrombogenic ability.

MATERIALS AND METHODS

Ethics statement

The institutional review board of the Ethics Committee of the Japanese Red Cross Society, Blood Service Headquarters, approved this study (ethical review number: 2019-016).

Sources of PLT, plasma and RBC samples

We used PLTs derived from PLT products as test samples. The PLT products were collected using apheresis systems (Trima Accel; Terumo BCT, Lakewood, CO, USA or CCS; Haemonetics Co., Boston, MA, USA). We used PLT products that were not supplied to a hospital because alanine aminotransferase (ALT) levels exceeded the safety limit (100 IU/L) 2 days after blood collection. SHPs (Siemens Healthcare Diagnostic Products GmbH, Marburg, Germany), containing 0.84–0.99 IU/mL of coagulation-related factors (CRFs) and 2.5 g/L of fibrinogen, were used as the plasma samples (Table 1). We used RBCs derived from 400 mL of leukocyte-reduced whole blood using the Terumo Automated Centrifuge & Separator Integration (Terumo BCT, Lakewood, CO, USA) suspended in 95 mL of mannitol–adenine–phosphate solution [15]. Briefly, RBC products were washed using equal volumes of phosphate-buffered saline followed by centrifugation at 500*g* for 15 min. The resultant RBC pellets were used as the RBC samples. We used RBC products that were not supplied to a hospital because ALT levels exceeded the safety limit 3 days after blood collection.

Preparation of reconstituted blood samples

We prepared reconstituted blood as test samples from each dilution series (Table 2) to investigate the effect of PLT numbers, CRF concentrations and RBC numbers on WTF abilities ($N = 10$). The reconstituted blood was prepared from different PLT samples to evaluate the effect of

TABLE 1 Coagulation-related factor concentrations in standard human plasma (SHP).

SHP (%)	FII (IU/mL)	FV (IU/mL)	FVII (IU/mL)	FVIII (IU/mL)	FIX (IU/mL)	FX (IU/mL)	FXI (IU/mL)	FXII (%)	FXIII (IU/mL)	VWF:Rco (IU/mL)	Fibrinogen (g/L)	AT (IU/mL)	Plasminogen (%)
100	0.93	0.87	0.84	0.92	0.86	0.88	0.89	94	0.99	0.89	2.5	0.91	93

Abbreviations: AT, antithrombin; F, factor; VWF RCo, von Willebrand factor ristocetin cofactor activity.

TABLE 2 Dilution series of reconstituted blood samples.

Evaluation components	Dilution series		
	PLT numbers (10^{10}/L)	CRF concentrations (%)	Hct value (%)
PLT numbers (Figure 2)	0, 5, 10, 20, 40	100	40
CRF concentrations (Figure 3)	30	0, 10, 20, 40, 60, 80, 100	40
Hct value substituting for RBC numbers (Figure 4)	15	100	0, 12.5, 25, 50

Abbreviations: CRF, coagulation-related factor; Hct, haematocrit; PLT, platelet; RBC, red blood cell.

each component. While evaluating any single factor, the other two factors were kept constant. The PLT numbers and haematocrit (Hct) value of the test samples were measured using an automated blood cell counter (XN-1000; Sysmex Co., Kobe, Japan) with no anticoagulant added to the test tubes (Eppendorf, Hamburg, Germany).

Within-run repeatability of reconstituted blood test using T-TAS

We prepared reconstituted blood with PLT numbers of 10×10^{10}/L and Hct value of 40% and verified the repeatability of the measurement when repeated five times.

Total Thrombus-formation Analysis System

We introduced the T-TAS, which enables the measurement of thrombus formation as a numerical value of change in flow pressure on a microchip (AR-chip; Fujimori Kogyo, Tokyo, Japan) with type I collagen plus tissue thromboplastin coated to the inside of a capillary channel (300 μm wide × 80 μm deep) (Figure 1a). Upon adding the test sample onto the microchip, adhesion of PLTs to collagen and the coagulation cascade with tissue thromboplastin as the initiation factor are promoted at 37°C under shear conditions (600/s), resulting in a WTF composed of PLTs and fibrin. The WTF gradually grows in size while repeating the collapse and reformation processes and subsequently occludes the flow path within the chip. The test time is no longer than 30 min. A typical analysis pattern and measured parameters are presented in Figure 1b.

Statistical analyses

The differences were analysed using the one-way analysis of variance for the post hoc analysis: Tukey–Kramer multiple comparison test, using the GraphPad Prism 8 (Graph Pad, Inc., La Jolla, CA, USA). We set the significance at $p < 0.01$. We calculated Spearman's rank correlation coefficient to assess the overall correlation between T-TAS parameters and the blood components.

RESULTS

Correlation between PLT numbers in reconstituted blood and WTF abilities

The correlations between the parameters including T_{10}, T_{80}, T_{10-80} or AUC_{30}, and PLT numbers in the test samples are presented in Figure 2. We observed no WTF in the test samples without PLTs. The onset time of WTF, T_{10} and the nearly complete occlusion time of the capillary, T_{80}, decreased as the PLT numbers in the sample increased and had a good correlation with the PLT numbers ($r = -0.91$, -0.91, respectively, Figure 2a,b). T_{10-80}, indicating the growth rate of WTF, also revealed a good correlation with the PLT numbers ($r = -0.74$, Figure 2c). AUC_{30}, the direct indicator for quantifying WTF, also revealed a high correlation with the PLT numbers in test samples ($r = 0.97$, Figure 2d). These results suggested that the WTF abilities of reconstituted blood may strongly depend on the PLT numbers.

Threshold values of CRF concentrations for WTF abilities

No white thrombus was formed in the test samples without CRFs, as expected. The correlation between the CRF concentrations and WTF abilities was weaker than that between the PLT numbers and WTF abilities (T_{10}: -0.54, T_{80}: -0.63, T_{10-80}: -0.70 and AUC_{30}: 0.76, respectively, Figure 3). The WTF abilities in the test samples containing ≦10% SHP were significantly reduced than in those containing ≧40% SHP. In addition, the T_{10-80} value did not reveal any noticeable change when SHP was used at 40%–100%, indicating a stable growth rate of thrombus in reconstituted blood with ≧40% SHP. The WTF abilities triggered by CRFs may depend on the threshold value of SHP (40%) rather than the

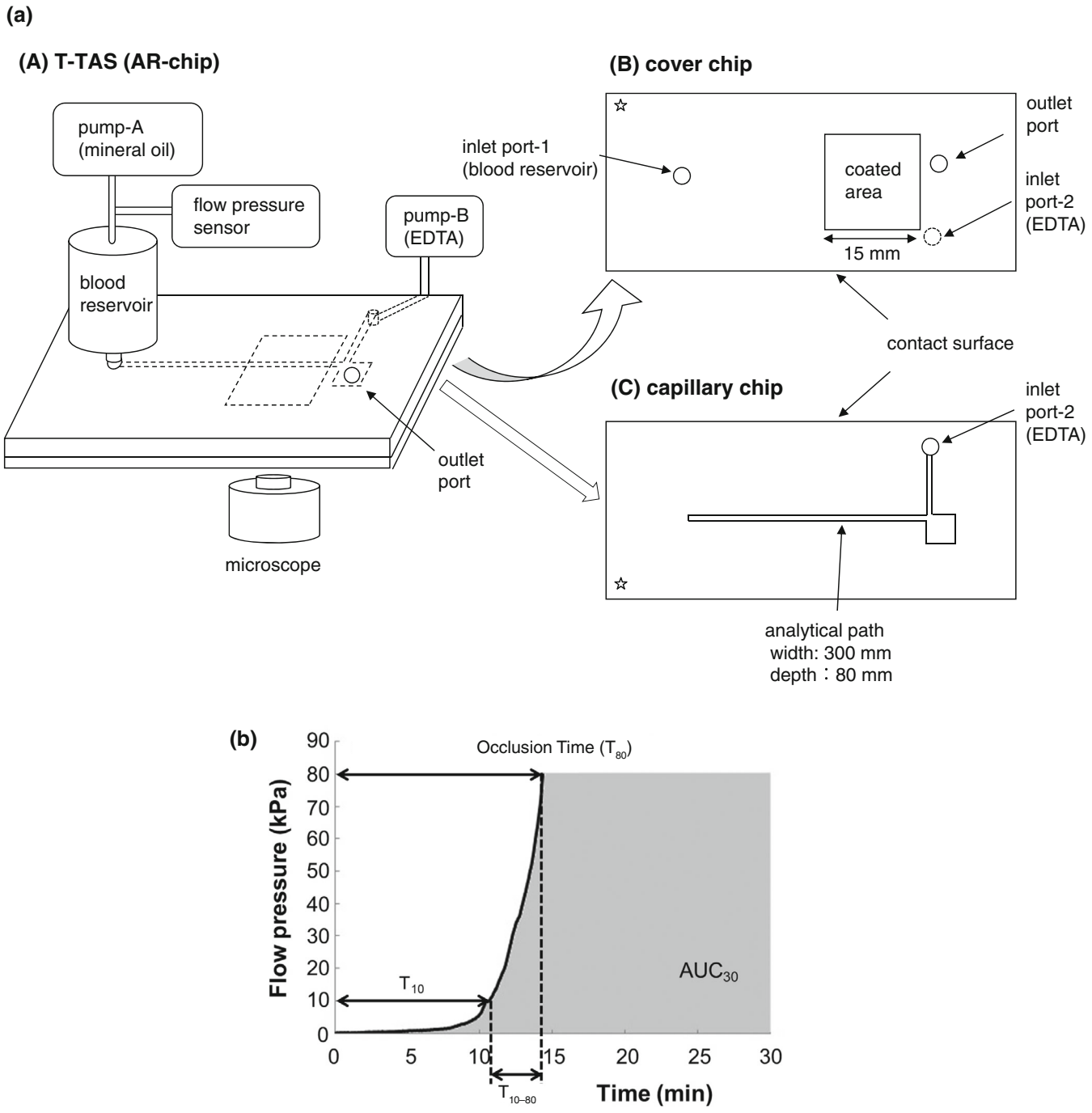

FIGURE 1 Total Thrombus-formation Analysis System (T-TAS) (AR-chip) and the analysis results. (a) Schematic diagrams of the T-TAS (AR-chip). (A) AR-chip consists of cover chip and capillary chip. (B) Type I collagen and tissue thromboplastin are coated on a portion of the cover chip and (C) The blood sample is perfused through the analytical path on the capillary chip. Pump-A (mineral oil) pushes the blood sample in the reservoir into the analytical path. Thrombus formation takes place over the area coated with collagen and tissue thromboplastin. Thrombus formation generates a back pressure that is monitored by the flow pressure sensor. White thrombus formation is also monitored by a microscope. Pump-B (EDTA) is used to prevent the outlet port from clotting. (b) A representative result of T-TAS presented as a flow pressure curve of reconstituted blood samples under flow condition. Analytical parameters, T_{10} (time in minutes for the flow pressure to reach 10 kPa), T_{10-80} (time in minutes for the flow pressure from 10 to 80 kPa), T_{80} (time to 80 kPa: occlusion time in minutes) and AUC_{30} (area under the curve for 30 min: shade of grey) are indicated in illustrated in the figure.

CRF concentrations since we did not observe significant differences in all the parameters obtained from the samples containing ≧40% SHP. These results indicate that CRFs are essential for WTF in reconstituted blood and that WTF abilities are not affected as long as the SHP contains 40% normal CRF concentrations or more.

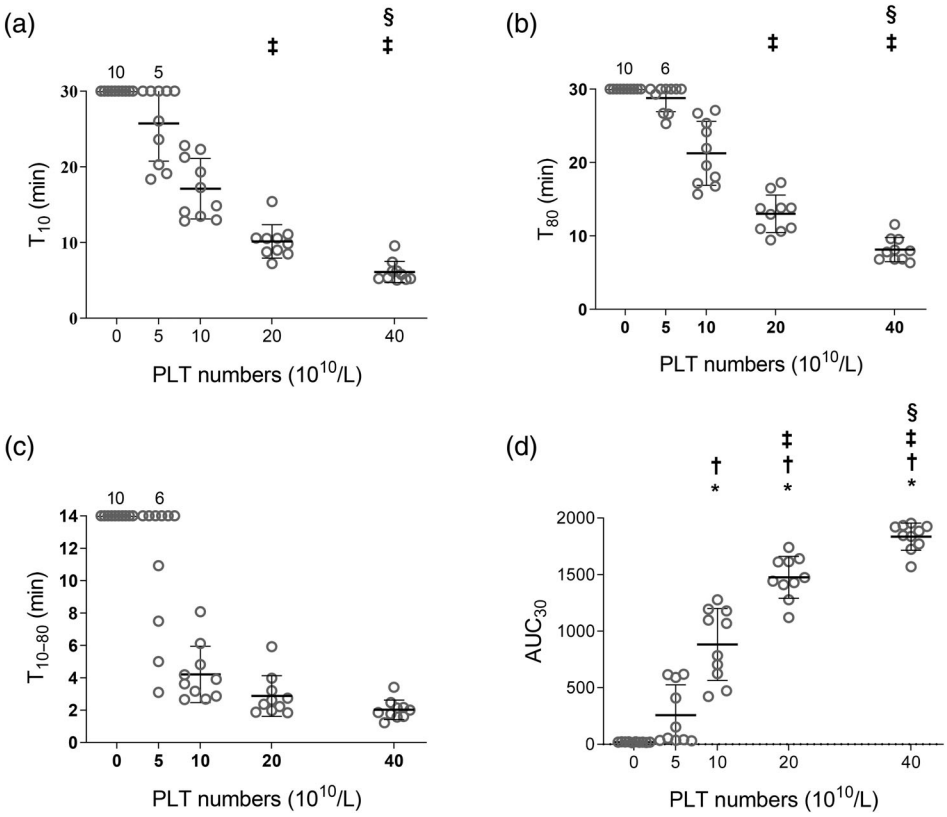

FIGURE 2 Effect of platelet (PLT) numbers on white thrombus formation abilities under blood flow conditions. Experiments were performed under the conditions described in 'Evaluation components of PLT numbers' in Table 2. The relationships between PLT numbers in reconstituted blood samples and each parameter, T_{10} (time to 10 kPa), T_{80} (time to 80 kPa), T_{10-80} (time from 10 to 80 kPa) and AUC_{30} (area under the curve for 30 min), are presented in (a), (b), (c) and (d), respectively ($N = 10$). The numbers above the dot plot indicate the frequency of the values for T_{10} or T_{80} exceeding 30 min. Significance was set at $^{*}p < 0.01$ (vs. PLTs 0), $^{\dagger}p < 0.01$ (vs. PLTs 5), $^{\ddagger}p < 0.01$ (vs. PLTs 10) and $^{\S}p < 0.01$ (vs. PLTs 20). Spearman R values against PLT numbers were T_{10}: −0.91, T_{80}: −0.91, T_{10-80}: −0.74 and AUC_{30}: 0.97, respectively.

WTF abilities declined in the absence of RBCs

No significant change in WTF abilities was observed in the presence of RBCs, with Hct value being 12.5%–50% (Figure 4). All parameters of WTF abilities excluding T_{10-80} were significantly reduced in the absence of RBCs. No parameter revealed a high correlation between WTF abilities and Hct value (T_{10}: −0.55, T_{80}: −0.67, T_{10-80}: −0.50 and AUC_{30}: 0.60, respectively). These results indicate that compared with PLT products with RBCs present, PLT products alone showed lower WTF abilities, while there was no difference in WTF abilities in the presence of RBCs in the range of 12.5%–50% Hct value.

Within-run repeatability of reconstituted blood test using T-TAS

The results of five experiments are summarized in Table 3. The coefficients of variation for T_{10}, T_{80}, T_{10-80} and AUC_{30} were 6.4%, 5.3%, 4.4% and 6.7%, respectively.

DISCUSSION

In this study, we attempted to establish an in vitro system, which can be used in blood banks, allowing to quantitatively evaluate how much the PLTs in PLT products contribute to thrombogenicity under more physiologically relevant conditions wherein PLTs, CRFs and RBCs are involved, by using reconstituted blood. We evaluated the effect of PLT numbers, CRF concentrations and the Hct value substituting for RBC numbers on the WTF abilities in the T-TAS microflow chamber system. Weiss et al. reported the essential role of PLTs in fibrin deposition and the good correlation between PLT numbers and WTF ($r = 0.8$) when blood samples from either healthy individuals or patients with PLT disorders were exposed to rabbit aortic segments under 650/s shear rate [16, 17]. Consistent with these reports, we observed that PLTs were essential for WTF, and PLT numbers in reconstituted blood samples revealed a high correlation with WTF abilities (Figure 2). Yamaguchi et al. tested whole blood samples collected from healthy volunteers (11 men and 20 women), between 25 and 62 (mean ± SD, 39 ± 11) years of age, by T-TAS, demonstrating normal values of individual T-TAS parameters (T_{10}: 11:39 ± 2:15,

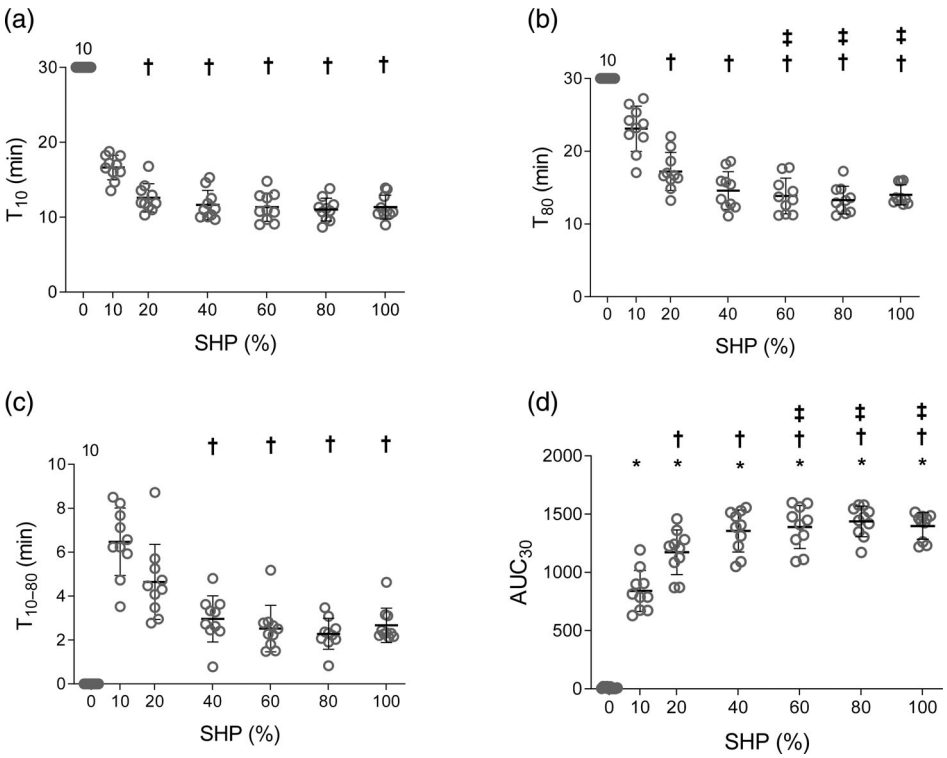

FIGURE 3 Effect of coagulation-related factor (CRF) concentrations in white thrombus formation abilities under blood flow conditions. Experiments were performed under the conditions described in 'Evaluation components of CRF concentrations' in Table 2. The relationships between CRF concentrations in reconstituted blood samples and each parameter, T_{10} (time to 10 kPa), T_{80} (time to 80 kPa), T_{10-80} (time from 10 to 80 kPa) and AUC_{30} (area under the curve for 30 min), are presented in (a), (b), (c) and (d), respectively ($N = 10$). The numbers above the dot plot indicate the frequency of the values for T_{10} or T_{80} exceeding 30 min. Significance was set at $^{*}p < 0.01$ (vs. standard human plasma [SHP] 0%), $^{\dagger}p < 0.01$ (vs. SHP 10%) and $^{\ddagger}p < 0.01$ (vs. SHP 20%). Spearman R values against CRF concentrations were T_{10}: −0.54, T_{80}: −0.63, T_{10-80}: −0.70 and AUC_{30}: 0.76, respectively.

T_{80}: 15:48 ± 2:56, T_{10-80}: 4:08 ± 1:11 and AUC_{30}: 1318.5 ± 203.2) [18]. These values are comparable to our results when PLT numbers and Hct were within normal range and supplemented SHP was sufficiently high. The European guideline on managing major bleeding and coagulopathy following trauma states that the clinical haemostatic threshold is 20%–25% for most coagulation factors, whereas fibrinogen is the highest at 40%–60% (1–1.5 g/L) [19, 20]. In our study as well, CRFs were essential for WTF and the respective thresholds for WTF abilities were comparable to the clinically required fibrinogen thresholds (Figure 3). The British Regional Heart Study reported a 30% increase in ischaemic heart disease in individuals with high Hct after adjusting for age, physical activity, cholesterol, body mass index and smoking. Other epidemiologic studies have also detected associations between increased Hct and arterial thrombosis [21, 22]. Some studies have claimed that increased blood viscosity is caused by increased Hct, which promotes thrombus formation as PLTs are extruded into the vascular endothelium by RBCs (margination) [23, 24]. However, we observed no difference in WTF abilities in the presence of RBCs as long as the Hct value was between 12.5% and 50%, although WTF abilities were reduced in the absence of RBCs (Figure 4). Blood flows under pulsative shear stress in vivo; however, our study provided a constant shear rate (600/s) to blood samples. Therefore, the difference in the effect of RBCs could be due to the pulsative versus constant shear stress. We assumed that pulsative shear stress, but not constant shear stress, could change blood viscosity and enhance RBCs to push PLTs to collagen depending on the Hct value, resulting in Hct affecting WTF abilities in vivo; however, we did not observe this in our system. A severe decrease in PLT numbers (below 5×10^{10}/L) and Hct value (10%) was reported to prolong closure times in PFA-100, which can evaluate primary haemostasis under blood flow conditions, showing a similar trend to our results [25, 26]. In contrast, it has been reported that closure times in PFA-100 are largely unaffected by the absence of coagulation factors (e.g., fibrinogen, factor V, factor VIII and factor IX) because it evaluates PLT plug formation, which consists of the relatively short time to thrombin generation and fibrin formation [27]. T-TAS was confirmed to be able to evaluate WTF including CRFs, which are central to secondary haemostasis, and therefore was considered to have a thrombogenic ability under more physiologically relevant conditions compared with instruments that evaluate only primary haemostasis. In addition, Atari et al. reported that AUC_{30} correlates well with the risk of bleeding due to thrombocytopenia and the therapeutic effect of PLT transfusion, suggesting that T-TAS relatively reflects the haemostatic process of transfused PLTs [28]. Based on these results, we conclude that our system allows us to analyse the degree of contribution of PLT products to WTF under the more physiologically relevant

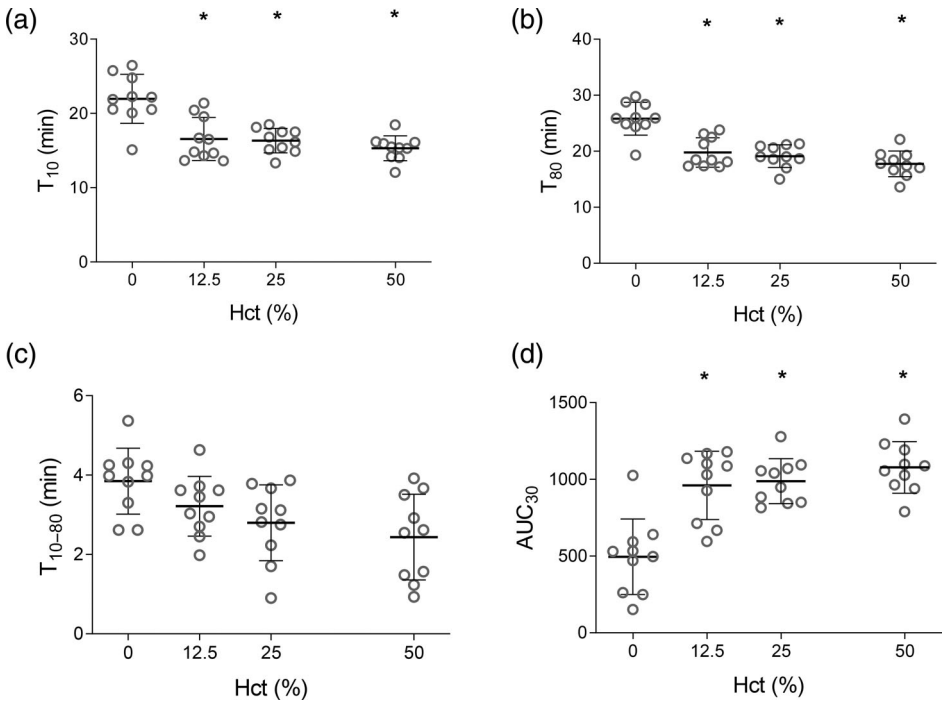

FIGURE 4 Effect of red blood cell (RBC) numbers on white thrombus formation abilities under blood flow conditions. Experiments were performed under the conditions described in 'Evaluation components of Hct (haematocrit) value' in Table 2. The relationships of RBC numbers and Hct value substituted for RBC numbers in reconstituted blood samples with each parameter, T_{10} (time to 10 kPa), T_{80} (time to 80 kPa), T_{10-80} (time from 10 to 80 kPa) and AUC_{30} (area under the curve for 30 min), are presented in (a), (b), (c) and (d), respectively ($N = 10$). Significance was set at $^*p < 0.01$ (vs. Hct 0%). Spearman R values against Hct levels were T_{10}: −0.55, T_{80}: −0.67, T_{10-80}: −0.50 and AUC_{30}: 0.60, respectively.

TABLE 3 Repeatability of reconstituted blood test using Total Thrombus-formation Analysis System.

No.	T_{10} (min)	T_{80} (min)	T_{10-80} (min)	AUC_{30}
1	14.4	17.0	2.6	1143
2	14.0	16.4	2.4	1195
3	15.6	18.0	2.4	1072
4	14.1	16.4	2.3	1206
5	16.1	18.4	2.3	1033
Mean	14.9	17.3	2.4	1130
Standard deviation	0.9	0.9	0.1	76
Coefficient of variation (%)	6.4	5.3	4.4	6.7

Note: The same reconstituted blood composed of the same concentrations of platelet product/red blood cells derived from a certain donor and standard human plasma was used for five repeated experiments.
Abbreviations: AUC_{30}, area under the curve for 30 min; T_{10}, time to 10 kPa; T_{10-80}, time from 10 to 80 kPa; T_{80}, time to 80 kPa.

conditions that plasma is mixed to achieve ≥40% of CRF concentrations and ≥1 g/L of fibrinogen, while RBCs are supplemented so that the Hct value ranges between 12.5% and 50%.

Current quality tests of PLT products focus on the in vitro characteristics of PLT before transfusion; however, they scarcely reflect the extent of transfused PLT products' contribution to the thrombus-forming ability in patients. For example, even the turbidimetric methods for qualifying PLT aggregating potential only use one or two activation agents at non-physiological concentrations. In contrast, haemostasis tests using experimental animals, although heterologous to humans, are extremely useful in evaluating the transfusion effect of PLT products; however, a routine daily inspection is challenging and impractical because of the time and labour required [29]. Hence, evaluating WTF in reconstituted blood using a microfluidic chamber may provide a semi-physiological condition and, therefore, is expected to be used as an intermediate quality test between the current in vitro quality tests and in vivo haemostasis tests.

Our study, which used reconstituted blood in a microchip flow chamber, had five limitations. First, blood components in the reconstituted blood might have been somewhat damaged during the preparation. Second, the reconstituted blood contained few leukocytes, including neutrophils, which have recently been recognized in a wide range of coagulation-promoting events, including the release of neutrophil extracellular traps or damage-associated molecular patterns, likely to occur in inflammatory conditions [30]. These leukocyte effects on blood coagulation were not reflected in our evaluation system. Third, the effect of PLT numbers and CRF concentrations on WTF in our system versus in patients or healthy individuals were comparable; however, that of the Hct value was not. To overcome these limitations, we may need to decrease the damage to RBCs during sample preparation, add a substantial number of leukocytes to the blood sample and alter shear stress over time similar to in vivo in the future. Fourth, the relationship between parameters of WTF abilities

and PLT quality parameters such as morphology, metabolic features, surface antigens, hypotonic shock response and aggregation ability is unclear. Lastly, our T-TAS platform is complicated and hence requires a lot of work. T-TAS can run with PLT products alone at fixed PLT numbers, enabling the analysis of primary and secondary haemostases under flow conditions. It indicates that this T-TAS application would provide more information about WTF abilities of PLT products in a shorter time compared with existing analysis methods. This method, however, does not reflect the effect of RBCs. We may need to carefully interpret the results when only PLT products are applied without RBCs. Thus, owing to some drawbacks, our current method may not yet adequately reflect the sequence of thrombus formation in vivo. However, if these challenges can be overcome with the establishment of a better system, it can be expected that blood reconstruction and the pressure-change-based sensitive flow chamber system would enable the quantitative evaluation of PLT quality as thrombus-forming ability.

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A.F. and T.H. performed the research; A.F. wrote the first draft of the manuscript; A.F., K.Y., M.T. and K.S. designed the research study; T.N., T.O.-W and K.H. participated in T-TAS analysis; K.Y., F.H., Y.T. and T.K. supervised the research and reviewed and edited the manuscript; all authors discussed the results and commented on the manuscript; all authors have approved the publication of the manuscript.

CONFLICT OF INTEREST STATEMENT

The Total Thrombus-formation Analysis System is a product of Fujimori Kogyo Co., Ltd., where T.N., T.O.-W. and K.H. are employed.

DATA AVAILABILITY STATEMENT

Data can be made available upon reasonable request from the corresponding author.

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

The estimated negative impacts on the red blood cell inventory of reducing shelf-life at two large health authorities in British Columbia, Canada, using a discrete-event simulation model

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Abstract

Background and Objectives: Reducing the maximum red blood cell (RBC) shelf-life is under consideration due to potential negative effects of older blood. An assessment of the impacts of this change on blood supply chain management is evaluated.

Materials and Methods: We performed a simulation study using data from 2017 to 2018 to estimate the outdate rate (ODR), STAT order and non-group-specific RBC transfusion at two Canadian health authorities (HAs).

Results: Shortening shelf-life from 42 to 35 and 28 days led to the following: ODRs (in percentage) in both HAs increased from 0.52% (95% confidence interval [CI] 0.50–0.54) to 1.32% (95% CI 1.26–1.38) and 5.47% (95% CI 5.34–5.60), respectively ($p < 0.05$). The estimated yearly median of outdated RBCs increased from 220 (interquartile range [IQR] 199–242) to 549 (IQR 530–576) and 2422 (IQR 2308–2470), respectively ($p < 0.05$). The median number of outdated redistributed units increased from 152 (IQR 136–168) to 356 (IQR 331–369) and 1644 (IQR 1591–1741), respectively ($p < 0.05$). The majority of outdated RBC units were from redistributed units rather than units ordered from the blood supplier.

The estimated weekly mean STAT orders increased from 11.4 (95% CI 11.2–11.5) to 14.1 (95% CI 13.1–14.3) and 20.9 (95% CI 20.6–21.1), respectively ($p < 0.001$). The non-group-specific RBC transfusion rate increased from 4.7% (95% CI 4.6–4.8) to 8.1% (95% CI 7.9–8.3) and 15.6% (95% CI 15.3–16.4), respectively ($p < 0.001$). Changes in ordering schedules, decreased inventory levels and fresher blood received simulated minimally mitigated these impacts.

Conclusion: Decreasing RBC shelf-life negatively impacted RBC inventory management, including increasing RBC outdating and STAT orders, which supply modifications minimally mitigate.

Keywords

blood components, quality management, red cell components

Highlights

- Based on simulation modelling across two health jurisdictions in Canada, shortening red blood cell (RBC) shelf-life from 42 to 35 and 28 days significantly increases their outdate and negatively impacts markers of appropriate RBC inventory management.
- This is despite a provincial redistribution programme that would transfer older RBCs from smaller sites to larger and higher utilization sites, where many of these redistributed RBCs would outdate. Inventory modifications also could not mitigate this effect.
- Extending the RBC shelf-life from 42 to 49 days decreases the number of units outdated by nearly half, based on simulation modelling across two health jurisdictions in Canada.

INTRODUCTION

Red blood cell (RBC) transfusion is a life-saving intervention with few alternatives. However, RBC components are perishable because of ongoing structural and metabolic cellular changes known as 'storage lesions', raising concerns about the safety and efficacy of older blood transfusion [1, 2]. Additive solutions (ASs) were developed to extend RBC storage by maintaining RBC metabolism, ATP levels and pH buffering. AS-1, AS-3 and SAGM (saline, adenine, glucose, mannitol) are widely used in North America because they provide a maximum RBC storage duration of up to 42 days post collection [3, 4]. However, little is known about the potential clinical harm of long-term storage of RBCs between 35 and 42 days because it has not been studied with significant numbers in randomized controlled trials [5, 6]. As a result, some jurisdictions, such as the United Kingdom, have taken a precautionary approach that decreases the maximum storage duration of RBCs from 42 to 35 days, although some European countries allow a maximum RBC storage duration of up to 49 days using more novel ASs [7, 8].

A major potential drawback to reducing the maximum RBC storage duration is increasing RBC wastage due to outdating. However, a previous study from Blake et al. in the province of Quebec, Canada, demonstrated that reducing RBC shelf-life to 21 or 28 days is feasible [9].

Moreover, there are other potential negative impacts, such as higher orders needed to be filled immediately without delay (STAT orders) or increased inappropriate use of group O, Rh D-negative RBC units to avoid expiry. However, it is unclear whether reducing RBCs' maximum storage duration significantly affects hospital blood bank inventories as a reasonable trade-off for putative safety.

Discrete-event simulation is an operational research tool used in unpredictable environments, and it has been repeatedly used in blood bank inventory planning [10]. This model can guide resource allocation by estimating adequate blood supply, thereby avoiding wastage due to expiry. Therefore, we sought to estimate the impacts of shortening maximum RBC storage duration on the outdate rate (ODR), STAT order and non-group-specific RBC transfusion rates, which are essential RBC inventory quality indicators, and possibly beneficial influences of lengthening the shelf-life. We carried this out in a regional setting encompassing our two major health authorities (HAs) in British Columbia, Canada, which also participate in an RBC redistribution programme that accepts older in-date RBC units from smaller and community hospitals.

MATERIALS AND METHODS

Study setting, inventory and redistribution management

The study was performed at Vancouver Coastal Health (VCH; 11 transfusing centres) and Providence Health Care (PHC; 2 transfusing centres), two large HAs in British Columbia, Canada. Combined, both HAs transfuse approximately 38,000 units annually, or 30% of the provincial RBC transfusion volume. Vancouver General Hospital and St. Paul's Hospital are the large tertiary care centres at the VCH and PHC HAs respectively. Canadian Blood Services (CBS), the single blood supplier, serves all of Canada (except Quebec).

Ordering practices differ between the two HAs. At VCH, RBC is ordered from the blood supplier under a demand-driven inventory planning method. During weekdays, the minimum value represents the number of available RBC units in general inventory (excluding allocated RBC units to patients) at 1:00 AM or re-order point. The difference between the minimum and maximum value represents the number of RBC units ordered to replenish the hospital's general inventory at 8:00 AM. These minimum and maximum inventories were determined using historical inventory modelling, previously validated to reduce ODRs [11]. PHC, however, requests RBC through a standing daily order delivery system, where the order sizes are modified in the morning at the PHC technologist's discretion.

VCH and PHC participate in a province-wide inter-hospital RBC redistribution programme where older in-date RBC units are sent from smaller hospitals to minimize outdating. Redistributed RBC accounts for 9% and 29% of VCH's and PHC's inventory, with a mean RBC age of 33 and 34 days on arrival, compared to 11 days if RBCs are delivered directly from the blood supplier (CBS). In our current redistribution protocol, smaller hospitals receive RBC directly from CBS and redistribute in-date 28–35-day-old RBC to VGH and SPH weekly or more often when they approach 35 days old.

Outcomes and study design

Discrete-event simulation was used to model operational RBC supply chain processes and to assess the potential impact of changes in RBC maximum storage duration on inventory management at both HAs. The methodology for modelling had been validated previously in its use to optimize inventory management to reduce ODRs at VCH [11]. Historical inventory data from 2017 to 2018 were used in the simulations to predict ODRs, the number of outdated RBC units and impacts on inventory management key quality indicators, including STAT order and non-group-specific RBC transfusion rates according to prospectively validated changes. Maximum storage duration of RBC units of 28, 35 and 49 days was assessed with a baseline set at 42 days used to predict their impact on inventory management outcomes, with 30 simulations performed per scenario. The historical ODRs at VCH and PHC were between 1.0% and 1.8% in 2017–2018.

The primary outcomes measured in each simulation are the ODRs and the number of outdated RBC units at VCH and PHC from ordered inventory or via redistribution programmes. The secondary outcomes included STAT orders and non-group-specific RBC transfusion rates due to changes in maximum storage duration. The rate of non-group-specific RBC transfusion indicates unnecessary group O RBC utilization, as these units being transfused to prevent expiry leads to an imbalance of RBC supply and demand.

Data collection

The RBC supply, inventory and disposition data were extracted retrospectively from the Laboratory Information System between 2017 and 2018. No ethical review was required, as only blood disposition data without patient information was utilized. RBC inventory comprises two different states: general and allocated. RBC units are moved from general to allocated inventory after transfusion is ordered and cross-matching is completed. Allocated RBC units will be issued for transfusion, segregated for a maximum of 3 days if not issued or returned to general inventory in the event of cancellation. The mean age of blood received (ABR) and the mean age of blood transfused (ABT) were calculated by subtracting the donor draw date printed on the RBC unit barcode label from the date the blood was received or transfused, also known as the analytical date. The RBC shelf-life in the hospital inventory was calculated by subtracting the ABR from the ABT of individual RBC units. An insignificantly small number of RBC units that underwent post-production modification, such as irradiation and leukoreduction, were included in the analysis.

Simulation modelling analysis

Our discrete-event simulation model was developed using the R software package [12]. The distributions of several processes were fit and/or empirically estimated from the data, including RBC request, ABR from both the blood supplier and inter-hospital redistribution and the cross-match release period. As the demand for blood varies between weekdays and weekends and the time of day, it has been modelled as a non-homogenous Poisson process. Each request represents a physician's order for an individual patient. Because each request is for a single blood group, the distribution of requests for each blood group is independent and was empirically estimated from the data. Similarly, the size of each request was modelled as a gamma distribution with parameters estimated from the data via maximum-likelihood estimation. A threshold-based allocation policy is also considered in this study. Approximately every hour, the stock is checked for RBC availability. Every Monday to Friday at 1:00 AM, routine orders are placed when the RBC inventory is less than the ideal threshold.

We also sought to determine through simulation whether specific inventory modifications can mitigate the adverse impacts of shortened maximum RBC storage duration. Scenarios tested included changes to daily ordering, twice-a-day ordering, daily ordering with a reduction of an inventory by 10% and twice-a-day ordering with a decrease of the age of blood received by 3 days from the blood supplier. There were minimal redistributed RBC units from PHC to VCH, and they were not applied to this simulation model.

RESULTS

Simulated changes to ODRs

Using a 42-day standard maximum RBC storage duration as a reference, the discrete-event simulation model demonstrated a significant increase in the ODR across both HAs from 0.52% (95% confidence interval [CI] 0.50%–0.54%) to 1.32% (95% CI 1.26%–1.38%) and 5.47% (95% CI 5.34%–5.60%) when reducing RBC shelf-life to 35 and 28 days, respectively ($p < 0.05$). The estimated median number of outdated RBC units per annum increased from 220 (interquartile range [IQR] 199–242) to 549 (IQR 530–576) and 2422 (IQR 2308–2470) units when reducing RBC shelf-life from 42 to 35 and 28 days, respectively. In contrast, extending the RBC shelf-life to 49 days reduced the ODR to 0.28% (95% CI 0.26%–0.29%) and the mean number of outdated RBC units to 115 (IQR 109–126) per annum ($p < 0.05$).

At PHC, reducing RBC shelf-life from 42 to 35 and 28 days led to significantly higher ODRs. The estimated ODRs for a shelf-life of 42, 35 and 28 days were 1.73% (95% CI 1.64%–1.82%), 4.34% (95% CI 4.21%–4.46%) and 16.85% (95% CI 16.46%–17.23%), respectively ($p < 0.05$; Table 1). At VCH, reducing RBC shelf-life from 42 to 35 and 28 days led to marginally higher ODRs. The estimated ODRs were 0.18% (95% CI 0.17–0.19), 0.46% (95% CI 0.38%–0.53%) and 1.75% (95% CI 1.66%–1.84%), respectively ($p < 0.05$; Table 1).

Changes in maximum RBC storage duration at both HAs to 35 and 28 days resulted in an approximate loss of $275,000 and $1.2 million, respectively, given a conservative estimated cost of $500 per unit of RBC.

For RBC units received via redistribution programmes, the estimated ODRs of both HAs combined significantly increased from 2.72% (95% CI 2.61%–2.83%) to 3.71% (95% CI 3.61%–3.81%) and 9.69% (95% CI 9.48%–9.89%) when reducing RBC shelf-life from 42 to 35 and 28 days ($p < 0.05$), respectively. The simulated median number of outdated RBC units per annum increased from 152 (IQR 136–168) to 356 (IQR 331–369) and 1644 (IQR 1591–1741) when reducing RBC shelf-life from 42 to 35 and 28 days (Table 2). Redistributed units

TABLE 1 Simulated outdate rates (ODRs) with 95% confidence intervals (CIs), median number of outdated units and interquartile range (IQR) for different red blood cell (RBC) shelf-lives (28, 35, 42, and 49 days).

RBC shelf-life (in days)	Both HAs		VCH		PHC	
	ODRs (95% CI)	Median number of outdated units (IQR)	ODR (95% CI)	Median number of outdated units (IQR)	ODR (95% CI)	Median number of outdated units (IQR)
28	5.47 (5.34–5.60)	2422 (2308–2470)	1.75 (1.66–1.84)	562 (523–629)	16.85 (16.46–17.23)	1816 (1754–1896)
35	1.32 (1.26–1.38)	549 (530–576)	0.46 (0.38–0.53)	140 (129–151)	4.34 (4.21–4.46)	406 (392–434)
42	0.52 (0.50–0.54)	220 (199–242)	0.18 (0.17–0.19)	59 (56–67)	1.73 (1.64–1.82)	155 (142–175)
49	0.28 (0.26–0.29)	115 (109–126)	0.09 (0.08–0.09)	28 (23–33)	0.96 (0.90, 1.02)	87 (78–97)

Note: Comparisons between the ODRs were statistically significant at the 5% level when using a 42-day shelf-life as the reference level.
Abbreviations: HAs, health authorities; PHC, Providence Health Care; VCH, Vancouver Coastal Health.

TABLE 2 Simulated outdate rates (ODRs) with 95% confidence intervals (CIs), median number of outdated units and interquartile range (IQR) for different red blood cell (RBC) shelf-lives (28, 35, 42 and 49 days) via redistribution programmes.

RBC shelf-life (in days)	Both HAs		VCH		PHC	
	ODRs (95% CI)	Median number of outdated units (IQR)	ODR (95% CI)	Median number of outdated units (IQR)	ODR (95% CI)	Median number of outdated units (IQR)
28	9.69 (9.48–9.89)	1664 (1591–1741)	1.91 (1.82–2.00)	170 (155–184)	18.22 (17.81–18.64)	1492 (1440 –1559)
35	3.71 (3.61–3.81)	356 (331–369)	0.99 (0.94–1.04)	47 (43–50)	6.38 (6.19–6.56)	307 (287–325)
42	2.72 (2.61–2.83)	152 (136–168)	0.96 (0.88–1.03)	26 (22–30)	4.41 (4.21–4.62)	122 (111–140)
49	2.03 (1.93–2.12)	85 (80–92)	0.57 (0.48–0.67)	11 (9–15)	3.43 (3.23–3.62)	68 (78–80)

Note: Comparisons between the ODRs were statistically significant at the 5% level when using 42-day shelf-life as the reference level.
Abbreviations: HAs, health authorities; PHC, Providence Health Care; VCH, Vancouver Coastal Health.

accounted for approximately two thirds of the total outdated units (68.7%, 64.8%, 69.0% and 73.9% for simulated RBC shelf-lives of 28, 35, 42 and 49 days, respectively). Therefore, reducing redistributed RBC shelf-life at both HAs to 35 and 28 days resulted in approximately $178,000 and $830,000 in losses, respectively.

Simulated changes to blood bank inventory metrics

STAT orders

In both HAs, a statistically significant increase in the estimated mean number of STAT orders per week was seen when reducing the RBC shelf-life. The estimated mean number of STAT orders placed per week increased from 11.4 (95% CI 11.2–11.5) to 14.1 (95% CI 13.9–14.3) and 20.9 (95% CI 20.6–21.1) when reducing RBC shelf-life from 42 to 35 and 28 days, respectively ($p < 0.001$). On the other hand, extending the RBC shelf-life to 49 days significantly reduced the mean number of STAT orders per week by 0.73 (95% CI −0.98 to −0.47) at VCH ($p < 0.001$).

The estimated mean number of STAT orders placed per week at VCH was consistently higher than at PHC for all RBC shelf-lives ($p < 0.001$), given the demand-driven inventory system. The STAT orders were negatively impacted by decreases in RBC shelf-life, increasing by 8.17 (95% CI 7.91–8.42) and 2.64 (95% CI 2.39–2.90) at VCH when reducing the RBC shelf-life from 42 to 28 and 35 days, respectively ($p < 0.001$) (Table 3).

Non-group-specific RBC transfusion

Reducing the RBC shelf-life increased the rate of non-group-specific RBC transfusion. The estimated non-group-specific RBC transfusion rate increased from 4.7% (IQR 4.6–4.8) to 8.1% (IQR 7.9–8.3) and 15.6% (IQR 15.3–16.4) when reducing RBC shelf-life from 42 to 35 and 28 days, respectively. Conversely, extending the shelf-life to 49 days decreased the non-group-specific RBC transfusion rate to 3.0% (IQR 2.9–3.1) at both HAs. Similar effects were seen in the estimated odds of non-group-specific RBC transfusions and the median number of group-compatible RBC units transfused (Table 4).

Simulating mitigation of ODRs

To determine whether modifying inventory and/or supplier logistics would mitigate the increased ODRs observed by shortening RBC shelf-life, we simulated the effects of the following modifications: a daily ordering system including weekends (DO), a twice-per-day ordering system (2DO), daily ordering with a decrease in inventory by 10% (DO-10% inventory) and a twice-a-day ordering with a decrease in the ABR by 3 days (2DO ABR-3).

With an unchanged ordering system at both HAs, the ODRs were estimated at 5.47 (95% CI 5.34–5.60), 1.32 (95% CI 1.26–1.38) and 0.52 (95% CI 0.50–0.54), for a RBC shelf-life of 28, 35 and 42 days respectively. DO results in ODRs of 1.20 (95% CI 1.15–1.24) and 4.67 (95% CI 4.56–4.77) and 2DO in ODRs of 1.30 (95% CI 1.21–1.39) and

TABLE 3 Estimated mean number of STAT orders placed per week and corresponding 95% confidence intervals (CIs) for different red blood cell (RBC) shelf-lives (28, 35, 42 and 49 days).

	Both HAs			VCH			PHC		
RBC shelf-life (in days)	Mean number of STAT orders (95% CI)	Difference in mean (95% CI)	*p*-value	Mean number of STAT orders (95% CI)	Difference in mean (95% CI)	*p*-value	Mean number of STAT orders (95% CI)	Difference in mean (95% CI)	*p*-value
28	20.9 (20.6–21.1)	9.51 (9.24–9.78)	<0.001	17.9 (17.7–18.2)	8.17 (7.91–8.42)	<0.001	2.9 (2.8–3.0)	1.34 (1.24–1.44)	<0.001
35	14.1 (13.9–14.3)	2.69 (2.42–2.96)	<0.001	12.4 (12.2–12.6)	2.64 (2.39–2.90)	<0.001	1.6 (1.5–1.7)	0.04 (−0.06 to 0.14)	0.400
42	11.4 (11.2–11.5)	Reference		9.8 (9.6–9.9)	Reference		1.6 (1.5–1.7)	Reference	
49	10.8 (10.7–11.0)	−0.55 (−0.82 to −0.28)	<0.001	9.1 (8.9–9.2)	−0.73 (−0.98 to −0.47)	<0.001	1.8 (1.7–1.8)	0.18 (0.08–0.28)	<0.001

Note: The *p*-values obtained from *t*-tests comparing the mean number of STAT orders for 28-, 35- and 49-day shelf-lives to that of 42 days are provided. STAT orders denote orders that are required to be filled immediately without delay.
Abbreviations: HAs, health authorities; PHC, Providence Health Care; VCH, Vancouver Coastal Health.

TABLE 4 Median non-group-specific red blood cell (RBC) transfusion rate and corresponding interquartile range (IQR) for different RBC shelf-lives (28, 35, 42 and 49 days).

	Both HAs				VCH		PHC	
RBC shelf-life (in days)	Median RBC units (IQR)	Non-group-specific RBC transfusion rate (%, IQR)	Odds ratio (95% CI)	*p*-value	Median RBC units (IQR)	Non-group-specific RBC transfusion rate (%, IQR)	Median RBC units (IQR)	Non-group-specific RBC transfusion rate (%, IQR)
28	6520 (6371–6846)	15.6 (15.3–16.4)	3.80 (3.77–3.84)	<0.001	3452 (3269–3709)	10.6 (9.9–11.3)	3109 (3045–3150)	34.1 (33.7–34.7)
35	3392 (3335–3472)	8.1 (7.9–8.3)	1.80 (1.78–1.82)	<0.001	1395 (1346–1444)	4.3 (4.1–4.5)	1994 (1952–2030)	22.0 (21.5–22.6)
42	1987 (1906–2026)	4.7 (4.6–4.8)	Reference		825 (793–896)	2.5 (2.4–2.7)	1145 (1119–1169)	12.6 (12.4–13.0)
49	1282 (1221–1311)	3.0 (2.9–3.1)	0.63 (0.62–0.64)	<0.001	507 (502–533)	1.5 (1–1.6)	758 (720–789)	8.4 (8.0–8.8)

Note: The estimated odds of a non-group-specific RBC transfusion using 42-day shelf-life as the reference are provided with corresponding 95% confidence intervals (CIs) and *p*-values.
Abbreviations: HAs, health authorities; PHC, Providence Health Care; VCH, Vancouver Coastal Health.

TABLE 5 Estimated outdate rates (ODR) and corresponding 95% confidence intervals (CIs) under different mitigation strategies for an red blood cell shelf-life of 28 and 35 days.

Mitigation strategies	ODR (95% CI) of 28-day shelf-life	ODR (95% CI) of 35-day shelf-life
Unchanged	5.47 (5.3–5.60)	1.32 (1.26–1.38)
DO[a]	4.67 (4.56–4.77)	1.20 (1.15–1.24)
2DO[b]	4.95 (4.83–5.07)	1.30 (1.21–1.39)
DO-10% inventory[c]	4.30 (4.15–4.44)	1.05 (1.02–1.08)
2DO ABR–3[d]	4.03 (3.94–4.11)	0.99 (0.96–1.02)

[a]A daily ordering system including weekends (DO).
[b]A twice-per-day ordering system alone (2DO).
[c]A daily ordering with decreasing inventory by 10% (DO-10% inventory).
[d]A twice-a-day ordering with decreasing the ABR by 3 days (2DO ABR-3).

4.95 (95% CI 4.83–5.07) for 35- and 28-day shelf-life. With DO-10% inventory and 2DO ABR-3, the estimated ODRs were 1.05 (95% CI 1.02–1.08) and 0.99 (95% CI 0.96–1.02) for 35-day shelf-life and 4.30 (95% CI 4.15–4.44) and 4.03 (95% CI 3.94–4.11) for 28-day shelf-life and 1.05 (95% CI 1.02–1.08; Table 5).

Age of transfused RBC

The mean age of transfused RBC was 21 days. Using the first, second and third quartiles of the transfused age of RBC units, 25%, 50% and 75% of the transfused units were under 15, 18 and 25 days old, respectively.

DISCUSSION

RBCs have limited storage life due to ongoing metabolic, morphologic and functional changes over time, known as 'storage lesions', which may lead to deleterious effects when transfused into patients. Although randomized controlled trials have not demonstrated the link between older blood transfusion and higher mortality, they did not focus on the oldest RBC units [6, 13–17]. Multiple observational studies, in contrast, suggested that older RBC units were associated with increased mortality rates, infections and multi-organ failure in hospitalized patients, which led to the consideration of reducing RBC shelf-life to avoid potential adverse outcomes of older RBCs [18–20]. Still, this approach requires careful evaluation of the potential critical impacts on blood inventory.

Our study applied historical inventory data for simulations to predict the consequences of shortened RBC shelf-life on the RBC ODRs internally at both HAs and via the redistribution programme, which accounts for 9% and 29% of VCH's and PHC's inventory, respectively. Our simulation study confirmed that decreasing RBC shelf-life will cause excessive outdates at both HAs. Our historical ODRs at both HAs were 1.0% in 2017 and 1.8% in 2018, with a 42-day RBC shelf-life, which is lower than the set target of <2.0% determined by the Provincial Blood Coordinating Office of British Columbia. So, if the maximum shelf-life is reduced to 35 days, the ODRs will increase significantly, particularly for PHC, with the ODR reaching 4.3%.

The previous study performed by Fontaine et al. at a university-based tertiary hospital using a trace-driven simulation method also found that a reduction in the maximum storage duration of RBCs significantly increased RBC wastage [21]. Our study demonstrates concordant findings through multiple health jurisdictions involved in an RBC redistribution programme involving the province of British Columbia.

The discrete-event simulation model also offers computational advantages with dynamic variables because RBC shelf-life changes consequentially influence the number of in-date and outdated RBC units in the available inventory. Another study using this approach by Blake et al. simulated the ordering behaviours involving both suppliers and broad geographically distributed consumers. Both studies involved jurisdictions that had RBC redistribution programmes between smaller hospitals and larger regional sites to accept units with less than 10 days of remaining shelf-life. In contrast to our results, theirs found that reducing the shelf-life from 42 to 28 or 21 days is feasible in the province of Quebec with no excessive increases to system-wide outdate, shortage or emergency ordering rates [9]. The different findings can be explained as due to the study settings and scopes of blood-supplying services between the two studies, where the modelling assumptions of our study are more applicable to large hospitals involved in a provincially led RBC redistribution programme and in an effort to minimize the impact to the blood supplier.

Grasas et al. also used a trace simulation model to demonstrate that the maximum RBC shelf-life could be reduced to 28–35 days without major increases in the outdate and shortage. With a 35-day shelf-life, their ODR was only 1.3%–1.4% and a shortage rate of 0.05%, relying on the strategies to limit the length of inventory held at the blood supplier headquarters to 8–10 days. This approach could not be adopted at our HAs because the median age of blood received from the blood supplier was higher at 11 days [22].

Referring to the data from the Joint UKBTS Professional Advisory Committee Report from the United Kingdom released in 2020, the UK RBC ODR with a maximum RBC shelf-life of 35 days was 2.7%. Though appropriate for a jurisdiction with a significant geographical expanse, this would be slightly above our historical provincial target and should be put into account for our future consideration to shorten the shelf-life [23].

British Columbia also has further geographic and logistical challenges, including blood production being in a different province and provincial hub transport over an ocean. Thus, in British Columbia, a mean redistributed RBC age usually approaching 35 days makes shortening the shelf-life impractical. The costs and supply chain disruptions associated with these changes would also be high, particularly in a Canadian jurisdiction with a single blood supplier covering many large provinces. Another challenge in an RBC redistribution programme management is a higher proportion of RBC inventory transshipped from small hospitals with low transfusion rates. Our results demonstrate this contribution to the high ODR in receiver hospitals, although thoughtful assessment is needed given lowering the

inventory in these rural and remote areas may lead to patient harm from local blood shortages.

We demonstrated that inventory depletion due to shorter RBC maximum storage duration would increase STAT orders, which will increase the cost of RBC transportation from blood supplier and possible delay in receiving blood at the hospitals.

Routine orders from the smaller hospitals could have been made more frequently to overcome this issue. However, this was not simulated in the current study because, in practice, this would be impractical to implement at smaller, more remote hospitals, which have both staffing constraints and transportation challenges.

Increasing non-group-specific but compatible RBC transfusions produces further strain on group-O donors and has led to many clinical proposals to decrease the demand [24, 25]. Our study showed that lowering the maximum storage duration will lead to increased non-group-specific RBC transfusion rates.

Both large and rural hospitals in our HAs stocked a significant proportion of group O, both Rh D-positive and Rh D-negative, to serve patients requiring emergent transfusion such as trauma patients. The redistribution programme is a key strategy to reduce ODRs through sending these units and increasing non-group-specific RBC transfusion rates at larger centres which can accommodate the volume of units redistributed through expiry. This is observed most at VGH, the largest hospital of VCH, which is a Level 1 Trauma Centre, where emergent transfusion when the blood group is unknown uses group O Rh D-positive RBCs for males and females older than 45 years and group O Rh D-negative RBCs for females younger than 45 years similar to other jurisdictions.

At VGH, the emergency stock of group O Rh D-negative RBCs has its set target without heavily relying upon redistribution to avoid a shortage and an increase in STAT orders. Non-group-specific RBC transfusion rates at VGH are variable depending on RBC consumption during trauma resuscitation and redistribution volume. Like the increased ODR, non-group-specific RBC transfusion rates would be exceptionally high at PHC, which is not a Level 1 Trauma Centre and would burden the non-trauma patients to receive more group O blood.

Our study also explored whether changing hospital practices, such as targeting lower inventory, more frequent ordering and lowering the age of blood received, could mitigate the adverse effects of decreased RBC maximum storage duration. Our results show that these strategies alone are relatively ineffective. Reducing inventory and requiring fresher RBC units from the blood supplier appears to have the best effect of mitigating outdates, but these changes would have negative impacts for both hospitals and blood suppliers, respectively.

A limitation of our study is that it does not specifically address unusual circumstances, such as blood demand spikes during natural disasters and mass casualties. However, we recognize that the purpose of our study was not to assess inventory management in these heavily labile scenarios, and the variety of scenarios created for simulations cannot capture all possible challenges affecting inventory management. Additionally, we did not simulate the effect of more frequent rotations within the HAs at the earlier days because our provincial redistribution programme policy is fixed. Also, our model did not consider changes in clinical practice, such as an expansion of haematology/oncology services, seasonality or RBC discards that are not due to outdating. Our simulated outcomes may not be applicable to other centres with different RBC consumption dynamics.

Given the marked impact of shortening RBC maximum storage duration being balanced with the putative concerns of harm from various observational studies with increased storage duration, we advocate that, instead, efforts be made to mitigate the negative impacts of the storage lesions. Currently, ASs, such as AS-1 and AS-3, and SAGM in most North American jurisdictions have allowed the maximum RBC storage duration to 42 days thanks to their abilities to buffer against pH changes, maintain redox homeostasis and minimize oxidative lesions that occur during storage [4]. Other blood suppliers, such as the German Red Cross, have used PAGGSM (phosphate, adenine, glucose, guanosin, saline, mannitol) to store RBCs for up to 49 days [26]. As well, AS-7 (SOLX) has a higher buffering capacity against pH changes and offers a higher degree of protection against oxidative lesions compared to other additive solutions, where it has demonstrated improved quality measures for a broad range of RBC parameters, such as reduced haemolysis, reduced micro-vesicles, improved morphology and in vivo recovery [27].

It might be impossible to establish a causal association between transfusions of RBC storage lesions and observed adverse outcomes in recipients with diverse conditions. Therefore, improving RBC processing and storage methods should be pursued to ensure the safety of RBC transfusion while sustaining the RBC supply chain at a reasonable cost.

Currently, more studies are evaluating two new approaches to reduce oxidative damage during storage: the inclusion of antioxidants in the additive solutions and the reduction of pro-oxidants in stored RBCs by hypoxic storage. However, the former appeared insufficient in maintaining RBC metabolism, and the latter required further examination of its metabolic modulation [28, 29].

It is worth mentioning that CBSs implemented leukoreduction by filtration for the production of RBCs and platelets in the late 1990s, which could potentially minimize reactive oxygen species and hydroxyl radical generation and better maintain stored RBC quality [30, 31].

In conclusion, our study found that decreasing the maximum storage duration of RBCs can lead to an increase in RBC outdates and other negative RBC inventory impacts, such as increased STAT orders and non-group-specific RBC transfusions, when simulated with a discrete-event simulation model in two large HAs. Changing supplier logistics, such as frequency of orders, lowering inventory and age of blood received, yielded only a marginal decrease in outdates caused by reduced RBC maximum storage duration. The patient effects of RBC storage lesions are still controversial, and an alternative method of mitigating these adverse effects would be to use a metabolomics approach to minimize the storage lesions. However, further studies, particularly in large jurisdictions and health institutions involved in the RBC redistribution programme, are needed to determine the benefit from a longer RBC shelf-life.

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CONFLICT OF INTEREST STATEMENT

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ORIGINAL ARTICLE

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Digital polymerase chain reaction to monitor platelet transfusions in cardiac surgery patients

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Abstract

Background and Objectives: Corrected count increment (CCI) measurements monitor the effectiveness of platelet transfusions in haemato-oncology, but they usually fail in patients undergoing cardiac surgery. We investigated whether polymerase chain reaction (PCR) of mitochondrial single-nucleotide polymorphisms (SNPs) is able to monitor the survival of transfused platelets in these patients.

Materials and Methods: Leukocyte-free, platelet-rich plasma was prepared from patients' blood to measure platelet counts based on patient-/donor-specific SNPs by digital PCR after DNA extraction. Platelet counts in samples from patients with severe thrombocytopenia were analysed by both PCR and flow cytometry. Ten patients undergoing cardiac surgery with the use of heart lung machine and without overt bleeding received a single apheresis platelet concentrate because of either dual platelet inhibition during a non-elective intervention or a complex procedure. Blood samples were collected at nine defined intervals (0–120 h) post transfusion.

Results: The digital PCR of the seven SNPs reliably quantified levels ≥0.6 G/L platelets, in good agreement with flow cytometry and without interference by other SNPs or by platelet activation. A mean 24-h CCI of 11.8 (range: 5.6–19.8) and a mean 120-h area under the curve (AUC) of 1386 (915–1821) hxG/L were observed for the transfused platelets. The mean AUC of 14,103 (3415–27,305) hxG/L for the patients' endogenous platelets indicates that transfused platelets represented only 11% (5–25) of the total platelet counts during 120 h post transfusion.

Conclusion: PCR of mitochondrial SNPs offers a tool to assess the survival of platelets from apheresis concentrates in cardiac surgery patients to facilitate the implementation of improved transfusion strategies.

Keywords

cardiovascular surgery, mitochondrial DNA, platelet increment, platelet transfusion, quantitative PCR

Highlights

- Digital polymerase chain reaction of seven mitochondrial single-nucleotide polymorphisms (SNPs) reliably measuring platelet counts of >0.6 × 10^9/L was validated by flow cytometry.
- The quantification was not affected by the presence of an excess of different SNPs nor by platelet activation.

- Monitoring the survival of apheresis platelets for 120 h after their transfusion in 10 patients with complex cardiosurgical interventions demonstrated the feasibility of this approach to evaluate platelet transfusions.

INTRODUCTION

Patients with severe hypoproliferative thrombocytopenia often receive platelet transfusions to prevent or treat bleeding. Pivotal clinical trials in this patient group [1] have been instrumental in elaborating the benefit–risk relation of prophylactic platelet transfusions. They have also helped in assessing the platelet count thresholds. International consensus has evolved for the platelet count threshold for platelet transfusions [2, 3]. Evidence suggests that haemato-oncological patients may need platelet support only if their platelet count declines to 10 G/L or less and they do not suffer from additional risk factors for bleeding [4].

Patients undergoing surgery, especially neuro- or cardiovascular surgery, usually require substantially higher platelet counts than haemato-oncologic patients to prevent bleeding [3, 5]. In this setting, platelet transfusions may be indicated even in individuals with normal or high platelet counts if their platelet function has been impaired by treatment with platelet inhibitors or if bleeding occurs after prolonged heart lung machine exposure [6].

In haemato-oncological patients, the profound and often prolonged thrombocytopenia in response to chemotherapy provides the setting for the 'corrected count increment (CCI)' to assess the effect of platelet support against the background of a very low invariable platelet count [7]. In cardiosurgical patients, however, this parameter usually may be limited because of normal platelet counts. Moreover, anti-platelet therapies with the combination of aspirin and clopidogrel and related agents induce an irreversible platelet dysfunction, which can be associated with relevant bleeding despite a normal platelet count. An enhanced platelet production triggered by the surgical intervention may further limit the validity of platelet count increments to investigate the effect of platelet transfusion.

In the present study, we assessed the analytical qualities of droplet digital PCR (ddPCR) measurements of platelet counts for seven different mitochondrial markers in vitro to investigate in a pilot study their value for monitoring platelet transfusions in cardiac surgery patients.

MATERIALS AND METHODS

Samples

Samples collected from blood donors as well as haemato-oncological and cardiosurgery patients with their written informed consent were investigated as approved by the institutional review boards (ethics committee, Medical University Oldenburg, reference 109/2016; ethics committee, Technical University Braunschweig, reference FV-2020-16).

ddPCR

Internal control

The *SPEF1* gene (Gene ID: 100195749; product: sperm flagellar protein 1) of the Atlantic salmon (*Salmo salar*) was used as an internal control for sample preparation, DNA extraction and amplification. Thus, 200 pg DNA from fish sperm (UltraPure Salmon Sperm DNA Solution 10 mg/mL, Thermo Fisher-Scientific Inc., Waltham, USA) was added to 1 mL EDTA anticoagulated blood.

Preparation of platelet rich plasma and extraction of mtDNA

Samples of 1 mL EDTA anticoagulated blood were centrifuged at 100*g* for 10 min without brake (Hettich Rotina 46S, Hettich, Tuttlingen, Germany). Mitochondrial DNA (mtDNA) was extracted from platelet suspensions (500 µL) with the MagnaPure Large Volume protocol (MagnaPure compact, Roche Diagnostics GmbH, Mannheim, Germany) into an elution volume of 50 µL. Alternatively, the MagCore Plus II device (Radim Germany, Freiburg, Germany) with the RBC-Bioscience plasma kit (RBC Bioscience Corp. New Taipei City, Taiwan) was used.

Primer and conditions of the ddPCR assay

Polymerase chain reaction (PCR) amplified a mitochondrial sequence to detect single-nucleotide polymorphism (SNP) by allele-specific probes (Table 1) fluorescently labelled with either 6-carboxyfluorescein (FAM) or 2′-chloro-7′-phenyl-1,4-dichloro-6-carboxy-fluorescein (VIC). Droplet generation, amplification and detection of amplificates followed the manufacturer's instructions (QX200 ddPCR System, Bio-Rad Laboratories GmbH, München, Germany).

SPEF1 sequences and controls for 'contaminating' genomic DNA were quantified in each blood sample [8].

Calculation of platelet count

The platelet count was calculated from the readout of the ddPCR system (copies/µL) as quotient of

$$\frac{(\text{Concentration [copies/µL]} \times \text{dilution factor [PRP preparation; PCR assay]})}{\text{Genome mass (pg/platelet)}}$$

and normalized to the internal control.

TABLE 1 Primer and probes for the droplet digital PCR.

SNP	Variation	Primer and probe sequences (5′ ⇒ 3′)	Final concentration (nmol/L)	Annealing temperature (°C)
HVR1–16069	T/C			
Forward		ATGGGGAAGCAGATTTGGGTAC	900	58
Reverse		GCAGTAATGTACGAAATACATA GCGGT	900	
Probe-T		FAM-AAGTATTGACTTACCCATCA	300	
Probe-C		VIC-AGTATTGACTCACCCATC	300	
HVR1–16399	A/G			
Forward		TTCTCGTCCCCATGGATGAC	900	61
Reverse		AGCACTCTTGAGCGGGATATTG	900	
Probe-A		FAM-ATAGGGGTCCCTTGAC	300	
Probe-G		VIC-GTCCCTTGGCCACC	300	
HVR1–16519	T/C			
Forward		CACTTGGGGGTAGCTAAAGTGAAC	900	61
Reverse		GATGTCTTATTTAAGGGAACGTGTGG	900	
Probe-T		FAM-TTCCTACTTCAGGGTCATA	300	
Probe-C		VIC-TCCTACTTCAGGGCCATA	300	
HVR2–73	A/G			
Forward		CTATCACCCTATTAACCACTCACGG	800	61
Reverse		AGATACTGCGACATAGGGTGCTC	800	
Probe-A		FAM-TGGGGGGTATGCAC	600	
Probe-G		VIC-TCTGGGGGGTGTGCAC	400	
HVR2–195	C/T			
Forward		TCGCACCTACGTTCAATATTATCG	800	61
Reverse		CTGTGCAGACATTCAATTGTTATTATTATG	800	
Probe-T		FAM-CGAACATACTTACTAAAGTGTGTT	250	
Probe-C		VIC-CGAACATACCTACTAAAGT	250	
HVR2–295	C/T			
Forward		TGTCTGCACAGCCACTTTCC	800	56
Reverse		TGTTTAAGTGCTGTGGCCAGA	800	
Probe-C		FAM-TCATAACAAAAAATTTCCACC	250	
Probe-T		VIC-TCATAACAAAAAATTTTCACC	250	
HVR2–310	C/CC			
Forward		TGTCTGCACAGCCACTTTCC 600 nM	600	58
Reverse		TGTTTAAGTGCTGTGGCCAGA	600	
Probe-C		FAM-CAAACCCCCCCTCC	100	
Probe-CC		VIC-CACAAACCCCCCCCTCC	100	

Abbreviation: SNP, single-nucleotide polymorphism.

Investigations to evaluate the ddPCR assay performance

Limit of blank, limit of detection and limit of quantification

Each parameter was determined by 10 different PCR runs with duplicate samples [9]. Limit of blank (LoB) characterizes the apparent platelet count observed when platelet-free samples are analysed. Limit of detection (LoD) represents the lowest platelet count that can be reliably distinguished from the LoB. Limit of quantification (LoQ) is the lowest platelet count detectable with a coefficient of variation ≤25% in inter-assay imprecision tests.

Assay linearity

Samples were diluted for platelet counts in the range 0.07–10 G/L and tested in quadruples per marker. The concentration after each dilution step was checked in parallel by flow cytometry.

Accuracy

Samples from patients were analysed with the ddPCR and compared with results of a flow cytometric method (modified from [10]) and haematology analysers (Sysmex XS 800i, Sysmex GmbH Norderstedt, Germany and ADVIA 2120i, Siemens Healthcare GmbH, Germany).

Specificity

Samples from donors differing in their biallelic SNPs were combined in vitro to test the effect of a substantial excess of platelets on the quantitation of platelet counts for a marker near its LoD.

Effect of strong platelet activation

Platelet samples with a count of 210–350 G/L were incubated and agitated for 10 min at 37°C in the presence or absence (control) of 20 µmol/L of the thrombin-receptor activating peptide (TRAP)-6 (Bachem Inc., Bubendorf, Switzerland) for ddPCR.

Reproducibility of internal SPEF1 control

Amplification of *SPEF1* sequences was performed with triplicates for intra-assay variability and in 15 independent PCR runs for determination of inter-assay variability. Recovered DNA of *S. salar* was calculated by using a genome size of 3×10^9 base pairs and a haploid *C* value of 3.27 pg.

Stability of samples

EDTA-anticoagulated samples were stored for 5 days at 4°C, and DNA was extracted on a daily basis. In addition, DNA samples were stored at −30°C to repeat the ddPCR assay after 1, 3, 6 and 12 months.

Investigations in cardiosurgery patients

Blood samples

Patients receiving a single platelet transfusion in the operating theatre were included. Venous blood samples were collected into 5-ml EDTA tubes just before 0 h and at 6 (5.5–6.5), 12 (11–13), 24 (22–26), 36 (34–38), 48 (46–50), 72 (70–74), 96 (94–98) and 120 (118–122) h after completion of the transfusion of a single platelet concentrate. The demographics and additional characteristics of these patients are summarized in Table 2.

Platelet concentrates

Single-donor apheresis platelet concentrates were prepared according to the requirements of the German National Guidelines. The minimum platelet number per concentrate was 2.0×10^{11} with a minimum platelet count of 0.8 T/L.

24-h count increment and 24-h CCI measurements in cardiosurgery patients

The post-transfusion count increment (CI) was calculated as the difference of the transfused platelet counts (G/L) between the samples collected 24 h after the end of transfusion and before starting the transfusion. CCI was calculated as quotient of the CI value and the platelet dose (G) transfused divided by the body surface area (in square metres).

Statistical analysis

Parameters for assay performance and method comparison were calculated with Analyze-it (Analyze-it for Microsoft Excel 5.66, Analyze-it Software, Ltd., Leeds, UK).

The individual AUC values for the "area under the curve" of the platelet counts from 0 to 120 h post transfusion; Figure 1 were calculated in R (version 4.2.2).

RESULTS

Analytical qualities of the ddPCR

DNA from *S. salar* was added to each freshly drawn blood sample to control for losses during sample preparation and DNA extraction. A mean recovery of 79.9% ± 7.9% (±SD) was observed for triplicates in 15 independent series. Platelet counts of each sample were normalized to this internal control. The storage stability allowed us to maintain the blood samples at 4°C for up to 5 days before DNA extraction and DNA samples at −30°C for at least 12 months without significant losses.

Tests of no-template controls generated always less than 10 positive droplets per sample and served to assess the LoB. Samples from patients with profound thrombocytopenia were analysed to determine the LoD and LoQ. The results are summarized for each of the seven mitochondrial markers as well as the incorporated fluorescence label in Table 3. The mean imprecision above the LoQ depending on the mitochondrial marker and the platelet concentration varied from 3.3% to 10.5%.

Platelet suspensions diluted to counts between 0.07 and 10 G/L (confirmed by flow cytometry) showed a linear relation between the expected and the observed ddPCR counts with R^2-values above 0.99, a slope close to 1.0 for the different markers (0.738–1.382) and an intercept of approximately 0.0 (−0.03 to −0.16).

The accuracy of the ddPCR assay was further evaluated by comparing flow cytometric and ddPCR results of 225 venous blood samples from patients with severe thrombocytopenia. The LoQ for the flow cytometric method was 0.25 G/L platelets. The results from flow cytometric and ddPCR assays agreed very well (correlation coefficient

TABLE 2 Demographics and characteristics of the cardiac surgery patients.

Patient	Sex	Age (years)	Weight (kg)	Height (m)	LoSICU (days)	EF (%)	CAD	Diagnosis	Procedure	Pre-treatment	Clamp time (min)	Bypass time (min)	RBC (units)	FFP (units)
1	M	73	73	1.73	2	53	1	AS	AVR	dualPI	41	54	0	0
2	M	74	70	1.68	2	35	3	Three-vessel disease	CABG		72	115	1	0
3	M	71	74	1.76	1	60	1	Three-vessel disease	CABG	dualPI	64	93	0	0
4	F	61	59	1.81	2	55	0	AR, TAA, AAA, DTAA	Valve-sparing root replacement (David), total arch replacement with frozen elephant trunk		169	191	3	6
5	F	75	62	1.62	1	75	0	AR, TAA, AAA	AVR, ascending aortic and partial arch replacement		64	122	2	1
6	M	37	81	1.89	2	65	0	TAA, AAA, DTAA	Ascending aortic and total arch replacement with frozen elephant trunk		147	188	4	2
7	F	74	57	1.67	6	65	0	AR, MR, TR, AF	Mitral valve replacement, tricuspid valve repair and MAZE ablation		101	142	2	0
8	M	64	83	1.80	1	65	3	3-vessel disease	CABG	dualPI	49	63	3	0
9	M	54	71	1.71	6	8	1	LV aneurysm, MR	LV reconstruction		102	138	2	8
10	M	52	90	1.90	7	65	0	TAA, AAA, DTAA	Ascending aortic and total arch replacement		74	117	6	8

Abbreviations: AAA, aortic arch aneurysm; AF, atrial fibrillation; AVR, aortic valve regurgitation; AS, aortic valve stenosis; ASA, acetyl salicylic acid; AV, aortic valve replacement; CABG, coronary artery bypass graft; CAD, number of diseased coronary arteries; DTAA, descending thoracic aortic aneurysm; EF, ejection fraction; FFP, fresh frozen plasma; LoSICU, length of stay in the intensive care unit; LV, left ventricle; MR, mitral valve regurgitation; dualPI, dual platelet inhibition by ASA and clopidogrel; RBC, red blood cell concentrate; TAA, thoracic ascending aorta aneurysm; TR, tricuspid valve regurgitation.

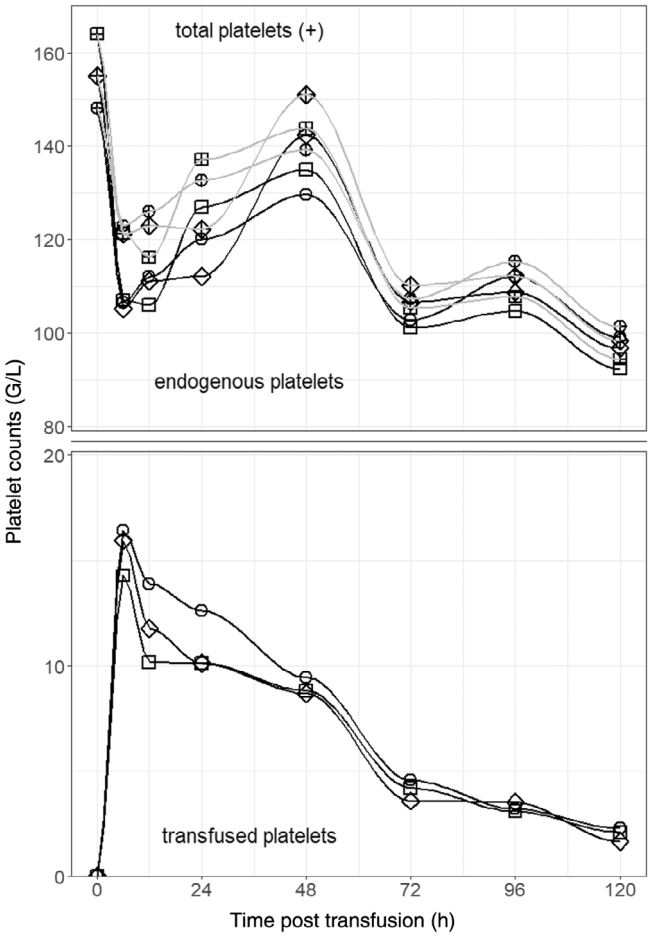

FIGURE 1 Time course of counts for endogenous and transfused (connected by black lines) or total (+, connected by grey lines) platelets (representing results from three different single-nucleotide polymorphisms) after administration of a single apheresis platelet concentrate in a cardiac surgery patient.

TABLE 3 Levels of blank (LoB), limits of detection (LoD) and limits of quantitation (LoQ) presented as platelet counts (in G/L) for the droplet digital PCR assays detecting single-nucleotide polymorphisms (SNPs) with FAM- or VIC-labelled probes (Table 1).

	LoB (G/L)		LoD (G/L)		LoQ (G/L)	
SNP	FAM	VIC	FAM	VIC	FAM	VIC
73	0.025	0.036	0.070	0.126	0.280	0.446
195	0.025	0.022	0.067	0.064	0.237	0.429
295	0.010	0.013	0.027	0.031	0.238	0.202
310	0.025	0.088	0.079	0.251	0.444	0.376
16,069	0.023	0.016	0.059	0.070	0.294	0.579
16,399	0.040	0.054	0.057	0.071	0.301	0.197
16,519	0.051	0.033	0.087	0.069	0.176	0.314

of 0.92). Only at higher platelet counts (>10 G/L), we observed consistent results from identical samples measured with the haematology analysers ADVIA2120i or Sysmex XS 800i with flow cytometry (correlation coefficients of 0.93 for ADVIA and of 0.94 for Sysmex) as well as ddPCR (correlation coefficients of 0.89 for ADVIA and of 0.88 for Sysmex).

We also assessed the specificity of the determinations of relatively low platelet counts (0.5–1.0 G/L) of one platelet population in the presence of an excess of another platelet population (110–180 G/L) to mimic, in vitro, the conditions for platelet transfusions in cardiosurgery patients.

Moreover, we compared ddPCR results from samples stimulated with the potent platelet activator TRAP (20 μmol/L) to control samples from identical donors to maximally activate platelets. Such platelet activation is expected to occur in heart surgery patients because of several factors including mechanical stress exerted by the heart lung machine, systemic inflammatory reactions post surgery or in response to vascular injuries or exposure to artificial surfaces. The results of these in vitro experiments showed that platelet activation did not affect the ddPCR platelet count measurements.

Monitoring platelet transfusions in cardiac surgery patients by ddPCR

Details of patients and their surgery are summarized in Table 2. After weaning from cardiopulmonary bypass and protamine administration, one unit of platelets was transfused intra-operatively to improve haemostasis.

The platelet counts observed for an individual patient (patient 10, Table 4) after transfusion of a platelet concentrate after cardiopulmonary bypass surgery are presented in Figure 1. The endogenous platelet counts declined from their initial level above 150 G/L to a nadir of approximately 105 G/L 6 h post transfusion. Thereafter, the endogenous platelet counts increased to a transient peak of about 130 G/L 48 h post transfusion to return to levels between 90 and 115 G/L from 72 to 120 h post transfusion.

The counts of endogenous and transfused platelets measured by ddPCR in each blood sample were added to the total platelet count and compared with the counts detected in the same sample by a haematology analyser. The line fitted by the least squares method showed good agreement between the ddPCR results and the conventional measurements (correlation coefficient of 0.94).

PCR detected a mean count of 17.2 (range: 10–26) G/L of transfused platelets in the peripheral blood collected at 24 h post transfusion for the 10 patients. This increment corresponded to a 24-h CCI of 11.8 (range: 5.6–19.8). Based on the widely accepted definition that a successful platelet transfusion requires a CCI above 4.5, the platelet support appeared to be effective during surgery in all patients.

The integral of the counts of transfused platelets over the 120-h period after the end of transfusion was approximated as the AUC of endogenous platelet counts for each patient. The AUC values of transfused platelets for individual patients varied between 915 and 2032 hxG/L with a mean of 1416 hxG/L and a relative variation coefficient of 25%.

Furthermore, we calculated the integral of endogenous platelets in the patient's blood during the 120-h period after the end of transfusion. The AUC value for endogenous platelets varied between 3415 and 27,305 hxG/L with a mean of 14,103 hxG/L. It indicates that, on

TABLE 4 Platelet (plt) count increment (CI in G/L), corrected count increment (CCI) at 24 h post transfusion and area under the curve (AUC) values of platelet counts (AUC: 0–120 h post transfusion in hxG/L; as AUC values of transfused platelets [AUCtr] without and as AUCcorr with correction for both platelet dose and body surface area [BSA]) for 10 patients receiving platelet concentrates during cardiac surgery (Table 2).

Patient	plt dose (10^{11})	BSA (m^2)	Dose/BSA	24-h CI	24-h CCI	AUCtr	AUCcorr	ABO-BG: Recipient	ABO-BG: Donor
1	2.9	1.87	1.55	19.8	12.80	1482	957	A	A
2	2.6	1.81	1.44	14.6	10.2	1306	908	O	O
3	2.3	1.90	1.21	23.9	19.8	1768	1462	O	O
4	3.0	1.72	1.74	9.7	5.6	1171	672	O	O
5	3.6	2.03	1.80	25.3	14.1	1821	1014	O	O
6	2.5	1.67	1.48	26.5	17.9	2032	1374	A	A
7	2.5	2.06	1.21	14.5	11.9	1144	944	O	A
8	3.3	2.04	1.61	12.6	7.8	1339	829	B	A
9	2.9	1.84	1.58	12.8	8.1	1183	749	A	A
10	2.5	2.18	1.16	11.5	9.9	915	791	O	A

Abbreviations: 24-h CCI, corrected count increment at 24 h post transfusion; 24-h CI, count increment at 24 h post transfusion; BG, blood group.

average, transfused platelets represented about 11% (range: 5%–25%) of all platelets in the patient's blood during the 120-h observation period.

DISCUSSION

In this pilot study, we investigated whether monitoring the survival of transfused platelets in the circulating blood by quantitative PCR of mitochondrial SNPs is feasible in cardiosurgery patients. As a first step, we evaluated the analytical qualities of the ddPCR for platelet counting. For all seven SNPs, the LoQ was below 0.6 G/L. This sensitivity exceeded that of conventional haematology analysers by at least 10-fold [11, 12]. The strong correlation between platelet counts detected in samples from thrombocytopenic patients by flow cytometry and ddPCR corroborated the validity of counting very low platelet numbers by ddPCR. The specificity of the ddPCR assay ensured that low platelet counts with one marker were reliably detectable in the presence of a high excess of another marker. The inter-assay reproducibility of this platelet counting method depends on the platelet levels. Mean variation coefficients of approximately 5% for platelet counts 10-fold higher than the LoQ appear to be sufficient to reliably monitor platelet survival. Profound platelet activation in vitro did not affect the quantification. It remains to be demonstrated that this method reliably quantifies platelets in patients suffering from disease conditions such as renal or hepatic failure with their impact on platelet function.

Routine clinical application of this approach to follow platelet transfusions in surgical patients may be limited by the time of approximately 5 h required from sampling to the final results. However, approaches to shorten the PCR (120 min) as the time-limiting step or to eliminate the DNA extraction (90 min) are under development and should help overcome this limitation in the future.

Based on these analytical profiles for the ddPCR assays, we performed a feasibility study in 10 cardiac surgery patients without overt bleeding to differentiate between the levels of endogenous and transfused platelet counts at pre-defined intervals for 120 h after the transfusion of an apheresis platelet concentrate. Assays for seven mitochondrial SNPs were sufficient to eliminate the need to preselect platelet donors for each individual recipient.

Our pilot study was limited to 10 patients treated with a single platelet concentrate, which is insufficient to detect differences with respect to the platelet product (e.g., storage time or blood group difference) or recipients (e.g., gender, extent of platelet inhibition, duration of the extracorporeal circulation).

The conventional monitoring of the CI after a platelet transfusion was not applicable because of the background of endogenous platelets in these patients (mean platelet count before the surgery 209 [range: 108–338] G/L) and their inter-individual temporal variation in response to the surgery. The approach to identify and count the transfused platelets by a mitochondrial SNP surmounted this limitation. The inter-individual variation of the AUC values for the transfused platelets during the 120-h period was 25%, which is lower than that of the 24-h CCI values with 36%. In multi-centre trials, inter-individual variations of the 24-h CCI even >50% have been observed. This could reflect a less precise timing of the sampling at 24 h post transfusion than the range of 22–26 h of our pilot study. A potential decline or a recovery of endogenous platelet counts within the 24-h interval depending on the individual state of thrombopoiesis may contribute to this high variability. Integrating the counts of endogenous platelets over 120 h post transfusion, moreover, demonstrated that the transfused platelets, on average, represented only 11% of the total platelet mass in these cardiac surgery patients.

Previous studies had established the strategy to detect platelets of different origin in patients [13], including platelet support in haemato-oncology [8] by mitochondrial SNPs.

The PCR of mitochondrial DNA to detect the levels of transfused platelets circulating in the blood of the recipient allowed, for the first time, calculation of the CCI values for transfused platelets despite the high and varying background of endogenous platelets in cardiac surgery patients. Studies in haemato-oncological patients [7, 14–17] have evaluated the value of 1-h or 24-h CCI determinations as an efficacy parameter for prophylactic platelet support. Although the CCIs fail to predict bleeding, they do correlate with the time to the next transfusion [17]. In the face of difficulties to accumulate a sufficient number of the relatively rare and clinically relevant bleeding events even in

very large controlled trials in hundreds of patients, CCI studies have been debated as a surrogate marker in haemato-oncology to improve platelet transfusion therapy [18]. The pure presence of circulating platelets may not suffice, and adequate function of the platelets and additional blood cells may also be essential for the complex interplay between plasmatic and cellular activities to ensure haemostasis.

Nevertheless, CCI data could help improve the platelet transfusion support of cardiac surgery patients. These patients receive platelet concentrates as the second largest group following haemato-oncological patients [19]. Among surgery and trauma patients, cardiosurgical procedures may offer a relatively high degree of both standardization and monitoring. They may be ideally suited to differentially profile in haemato-oncology and surgery patients the effects of novel approaches including pathogen reduction [20], major changes of storage conditions (e.g., in the cold [21] or for more than 5 days after preparation [22]) as well as products harvested from megakaryocyte culture [23].

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data available from the corresponding author.

ORCID

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

DSLK and Kg: Antithetical antigens in the RHAG blood group system, and characterization of anti-DSLK antibody

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Abstract

Background and Objectives: The RHAG blood group system contains five antigens: Duclos (RHAG001), Ol^a (RHAG002), DSLK (RHAG003), Kg (RHAG005) and SHER (RHAG006). Individuals who are DSLK-negative and Kg-positive have the same allele *RHAG*01.–3*, with a single-nucleotide variation (rs144305805), c.490A>C (p.Lys164Gln), in exon 3 of the *RHAG* gene. We aimed to confirm whether DSLK and Kg are antithetical antigens.

Materials and Methods: Blood samples of the original DSLK-negative proband with anti-DSLK, her son and another DSLK-negative individual were examined. The *RHAG* gene was analysed by polymerase chain reaction and Sanger sequencing. Immunocomplex capture fluorescence assays (ICFAs) and monocyte phagocytosis assays were performed to characterize the anti-DSLK antibody. Cross-testing of alloanti-DSLK and monoclonal anti-Kg (OSK46) was performed using transduced HEK293 cells by inducing the construct of expression vectors encoding wild-type *RHAG*01* or the variant *RHAG*01.–3*.

Results: ICFA using monoclonal anti-RHAG (LA18.18) revealed that the anti-DSLK and anti-Kg antibodies reacted with the wild-type and variant RhAG (Rh-associated glycoprotein), respectively. The proband and a DSLK-negative individual appeared to be homozygous for variant *RHAG*01.–3*, and the proband's son was typed as *RHAG*01/RHAG*01.–3* heterozygote. HEK293 cells with wild-type RhAG reacted with the anti-DSLK but not anti-Kg antibody, whereas HEK293 cells expressing the variant RhAG reacted with the anti-Kg but not anti-DSLK antibody. Monocyte phagocytosis assays indicated that 64% of red cells sensitized with anti-DSLK were phagocytosed by monocytes.

Conclusion: Our results demonstrate that DSLK and Kg are antithetical antigens in the RHAG blood group system. Anti-DSLK may be a clinically significant antibody.

Keywords

antithetical antigens, DSLK antigen, Kg antigen, RHAG blood group, *RHAG* gene

Highlights

- DSLK (RHAG003) and Kg (RHAG005) are two antigens of the RHAG blood group system.
- Our results show that the common DSLK and the rare Kg are antithetical antigens.
- Anti-DSLK may be a clinically significant antibody.

INTRODUCTION

In 2010, three new antigens, namely Duclos (RHAG001), Ol^a (RHAG002) and DSLK (RHAG003), were reported [1], and RHAG was recognized as the 30th blood group system by the International Society of Blood Transfusion (ISBT). Duclos and DSLK are high-frequency antigens (HFAs) and Ol^a is a low-frequency antigen (LFA). Recently, an LFA of the 700 series, Kg (RHAG005) [2], was identified as belonging to the RHAG blood group system [3]. In addition, a new LFA in the RHAG blood group system encoded by the novel *RHAG* gene with the variation c.1063A>C (p.Asn355His) was registered as SHER (RHAG006) [4, 5].

Individuals who are DSLK-negative and Kg-positive have the same single-nucleotide variation (SNV, rs144305805), c.490A>C (p.Lys164Gln), in exon 3 of the *RHAG* gene, and this SNV was designated allele *RHAG*01.–3* by the ISBT. However, DSLK has been awarded only provisional status, awaiting further examples of the DSLK−phenotype to confirm the polymorphism [5], and whether DSLK (i.e., HFA) and Kg (i.e., LFA) are antithetical antigens has not been confirmed by serological-based tests [1, 3].

In this study, we analysed blood samples from a DSLK-negative proband with anti-DSLK [1], her son and another DSLK-negative individual using an immunocomplex capture fluorescence assay (ICFA) and Sanger sequencing. In addition, we investigated cross-reactivity between the anti-DSLK (eluate of proband plasma) and monoclonal anti-Kg (OSK46) antibodies using HEK293 cells expressing RhAG (Rh-associated glycoprotein) with the deduced amino acids lysine or glutamine at position 164.

Two cases of haemolytic disease of the newborn (HDN) caused by anti-Kg antibody and requiring exchange transfusion therapy have been reported, demonstrating the clinical importance of anti-Kg antibodies [2, 6]. In addition, a study of anti-Kg antibody-mediated phagocytosis of red blood cells (RBCs) by monocytes suggested that these antibodies can cause haemolytic disease of the fetus and newborn [7]. Because anti-DSLK and anti-Kg antibodies are predicted to recognize the amino acid at the same RhAG position, lysine 164 and glutamine 164, respectively, we also examined alloanti-DSLK antibodies using a monocyte phagocytosis assay to estimate the antibody's clinical significance.

MATERIALS AND METHODS

Blood samples

This study was approved by the ethics committee of the Japanese Red Cross Society (#2018-033). In this study, we used archived blood samples re-donated from the original case of a DSLK-negative proband carrying anti-DSLK antibody with titre of 1:128 [1] and a blood sample from her son. In addition, we used Kg-positive and Kg-negative blood samples obtained from donors screened by serology using human monoclonal anti-Kg (OSK46, Kinki Block Blood Center, Osaka, Japan) and an automated blood grouping system (PK7300; Beckman Coulter, Koto-ku, Tokyo, Japan). Another DSLK-negative blood sample was found at the Chu-Shikoku Block Blood Center and analysed by Sanger sequencing and serological tests using the proband's plasma containing anti-DSLK antibody. Anti-DSLK eluate was prepared from Kg-negative RBCs sensitized with anti-DSLK according to a method described previously [8] using in-house acid elution reagents: 100 mM glycine-HCl (pH 2.5; Sigma-Aldrich Japan, Tokyo, Japan) containing 250 mM ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid, disodium salt, dihydrate (EDTA-2Na; Dojindo Laboratories, Kumamoto, Japan) for elution and 1 M Tris (hydroxymethyl) aminomethane (Sigma-Aldrich Japan) for neutralization.

Flow cytometry for RBCs

RBCs were washed with phosphate-buffered saline (PBS) (pH 7.4; Sigma-Aldrich Japan), and a 4% RBC suspension was prepared with PBS. An aliquot of 2 μL of the 4% RBC suspension was incubated with 10 μL of the proband's plasma or anti-Kg antibody at room temperature (RT) for 30 min. The sensitized RBCs were washed three times with PBS and incubated with 20 μL of 5 μg/mL R-phycoerythrin (R-PE)-conjugated anti-human IgG (Jackson ImmunoResearch, West Grove, PA, USA) at RT for 15 min. After washing with PBS, antibodies bound to the RBCs were detected by flow cytometry (FCM; BD FACS Lyric, BD, Franklin Lakes, NJ, USA), and the data were analysed using the BD FACSuite software, v1.3.

Immunocomplex capture fluorescence assays

To confirm the molecules carrying DSLK and Kg antigens, ICFA was performed according to a method described previously [9]. In brief, several different colours of Luminex beads (MicroPlex™ Microspheres, Luminex Japan Co. Ltd., Minato-ku, Tokyo, Japan) were coupled with one of the following mouse monoclonal antibodies: LA18.18 (Sanquin, Amsterdam, The Netherlands) for RhAG; CBC-450 (in-house) for the cytoplasmic domain of GPA; BIII 136 (Santa Cruz Biotechnology, Dallas, TX, USA) for DI; CBC-458 (in-house) for LW; CBC-374 (in-house) for GE and CBC-371 (in-house) for KEL. The obtained blood group capture (BGC) beads were mixed just before use.

DSLK+/Kg− and DSLK+/Kg+ RBCs were mixed with the proband's plasma and anti-Kg antibody, respectively, and these antibody-sensitized RBCs were lysed with Triton X-100 (Nacalai Tesque, Inc., Kyoto, Japan) buffer containing 1 μg/mL R-PE-conjugated anti-human IgG (Jackson ImmunoResearch Laboratories Inc., PA, USA). The immune complex in the lysate was incubated with the BGC beads mixture, and then these mixtures were analysed using Luminex200 (Luminex Japan Co. Ltd., Tokyo, Japan). The fluorescence intensity of R-PE was calculated by the software xPONENT, version. 4.3 (Luminex Japan Co. Ltd.).

RHAG gene analysis

Genomic DNA was extracted from whole blood using a DNA blood mini kit (QIAamp, Qiagen, Chuo-ku, Tokyo, Japan). All primers and polymerase chain reaction (PCR) conditions are shown in Tables S1

and S2, respectively. The constituents of the PCR mixture were the same as for amplification of individual *RHD* exons described previously [10]. Exons 1–10 of the *RHAG* gene were amplified by PCR 1–9, and then Sanger sequencing was performed using a cycle sequencing kit (BigDye Terminator, version 1.1, Applied Biosystems, Carlsbad, CA, USA) and genetic analyser (Model 3500xL, Applied Biosystems).

Lentiviral transduction of HEK293 cells

Wild-type *RHAG*01* and variant *RHAG*01.-3* with plasmid vector (pENTR/SD/D-TOPO; Thermo Fisher Scientific, Waltham, MA, USA) were constructed as described previously [3]. The constructs were recombined with CSII-EF-RfA (RIKEN BioResource Research Center, Tsukuba, Japan) using the Gateway LR clonase II enzyme mix (Thermo Fisher Scientific). Lentiviral particles were prepared as described previously [9]. HEK293 cells were maintained in Dulbecco's Modified Eagle Medium containing 10% foetal bovine serum. Each *RHAG* gene was introduced into 1–2 $\times$ 10^5 HEK293 cells and incubated overnight by co-culturing the cells with lentiviral particles in the presence of 8 μg/mL polybrene. The next day, the transduced HEK293 cells were washed twice with 10 mL of fresh medium and re-cultured for 6 days. Then RHAG-positive cells were stained with anti-RHAG antibody (LA18.18) followed by Alexa Fluor 488-conjugated goat anti-mouse IgG (Thermo Fisher Scientific) and then purified using a SH800Z Cell Sorter (SONY Biotechnology Inc., Tokyo, Japan).

The established transduced HEK293 cells were washed with PBS and suspended 1×10^6 cells/mL with PBS containing 0.2% BSA (Sigma-Aldrich Japan) and 10 mM EDTA-2Na. An aliquot of 20 μL of the cell suspension was stained with 10 μL of anti-DSLK eluate or anti-Kg monoclonal antibody, and then FCM analysis was performed in the same manner as RBCs using R-PE-conjugated anti-human IgG (Jackson ImmunoResearch).

Monocyte phagocytosis assays using FCM

Monocyte phagocytosis assays were performed according to a method similar to a previously described method used for analysing platelet phagocytosis by monocytes [11]. Ten volumes of 10% RBC suspension with PBS were mixed with one volume of 10 μg/mL pH-sensitive pHrodo dye (pHrodo™ Red, SE, Thermo Fisher Scientific) and incubated at RT for 30 min in the dark. The labelled RBCs were washed thrice with PBS and suspended to 3% with PBS. Then the same volume of serially diluted (1:1) antibodies was added to the labelled RBC suspension and incubated at 37°C for 1 h. After washing with PBS, the antibody-sensitized RBCs were resuspended to 1% with RPMI 1640 medium (Sigma-Aldrich Japan) containing 10% foetal calf serum (Nichirei Biosciences Inc., Tokyo, Japan). An aliquot of 50 μL of the sensitized RBC suspension was added to 100 μL of mononuclear cells (1×10^6 cells) prepared using density separation medium (Lympholyte; Cedarlana Laboratories, Burlington, Canada) in a 96-well culture plate (Corning Inc., NY, USA) and then incubated at 37°C for 2 h under 5% CO_2. The monocytes were stained with FITC-CD14 (BD), and then 200 μL of RBC lysis buffer (155 mM ammonium chloride [Fujifilm Wako Chemicals, Hiratsuka, Japan] containing 10 mM potassium hydrogen carbonate [Fujifilm Wako Chemicals] and 1 mM EDTA-2Na [Dojindo Laboratories]) was added to the wells to lyse the un-phagocytosed RBCs. The monocytes were counted by FCM (FACSLyric), and the ratio of red-coloured cells (i.e., monocytes that phagocytosed RBCs) to the total number of monocytes was calculated as the percent phagocytosis. Human monoclonal anti-Kg (OSK46) and anti-D (HIRO-3, in-house) antibodies were used as positive controls, and PBS was used as the negative control. To estimate the correlation between the phagocytosis percentage and the amount of IgG on the RBCs, the amount of IgG bound to the RBCs was measured by FCM using R-PE-conjugated anti-human IgG, and the mean fluorescence intensity was evaluated.

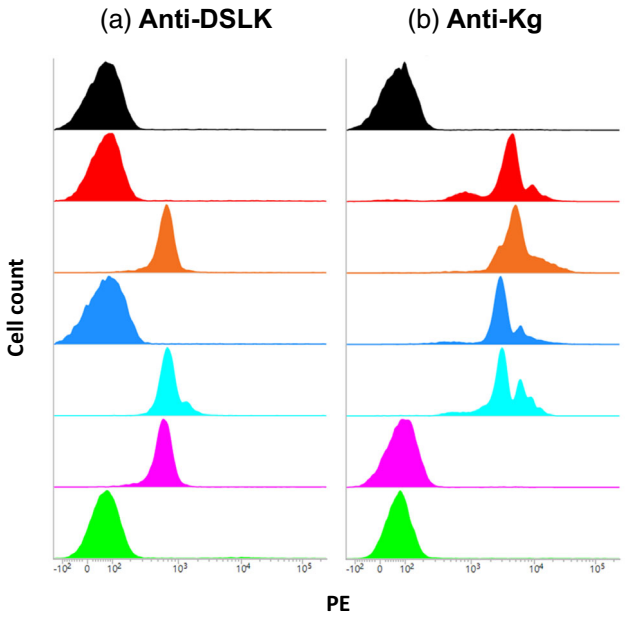

FIGURE 1 Reactivity of red blood cells to anti-DSLK and anti-Kg antibodies. Histograms show the intensity of antigen expression (phycoerythrin [PE]; *X*-axis) versus the number of events (cell count; *Y*-axis). Black: negative control (phosphate-buffered saline was used instead of primary antibody), red: proband; orange: proband's son; blue: DSLK-negative individual; sky blue: DSLK+/Kg+ individual; purple: Kg-negative individual; green: Rh_{null} individual. (a) Reactivity with anti-DSLK antibody (proband's plasma). (b) Reactivity with anti-Kg antibody (OSK46).

RESULTS

DSLK-negative individuals express the Kg antigen on the RBCs

The results of FCM analysis of DSLK and Kg antigens on RBCs are shown in Figure 1. RBCs of the proband and the DSLK-negative

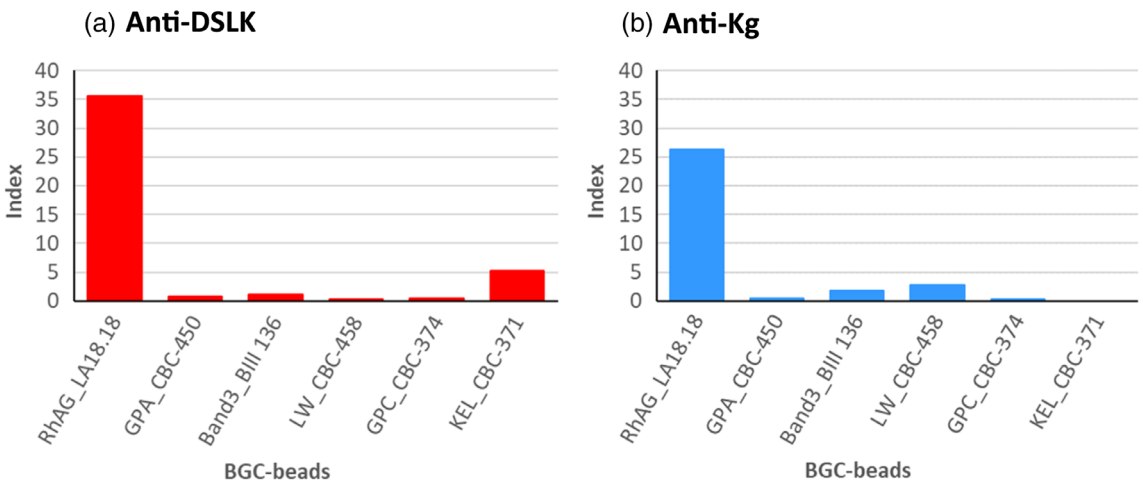

FIGURE 2 Immunocomplex capture fluorescence assay identification of the red blood cell (RBC) membrane molecule that binds to anti-DSLK and anti-Kg antibodies. Immune complex of RBC membrane molecule was incubated with the blood group capture (BGC)-beads mixture (*X*-axis) and the intensity of the captured molecule was shown as index (*Y*-axis). (a) Kg-negative RBCs reacted with anti-DSLK antibody. (b) Kg-positive RBCs reacted with anti-Kg antibody.

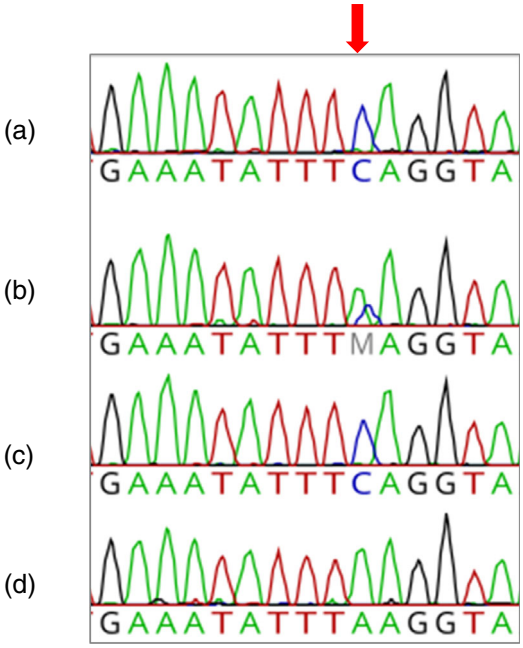

FIGURE 3 Sanger sequencing of the *RHAG* gene. Regions near the exon 3/intron 3 boundaries are shown: (a) proband; (b) proband's son; (c) DSLK-negative individual and (d) Kg-negative individual. Red arrow indicates nucleotide position 490 in the *RHAG* gene.

individual did not react with the anti-DSLK antibody (proband's plasma), exhibiting reactivity similar to that of Rh_{null} RBCs (Figure 1a). In contrast, RBCs of the proband and the DSLK-negative individual exhibited positive reactivity with anti-Kg antibody (Figure 1b). Furthermore, the RBCs of the proband's son reacted with both the anti-DSLK and anti-Kg antibodies, indicating that he is a DSLK+/Kg+ heterozygote.

ICFA results confirm DSLK and Kg antigens are carried on RhAG

To confirm the molecule that carries the DSLK and Kg antigens, we performed ICFAs targeting the RHAG, MNS, DI, LW, GE and KEL blood group systems. The anti-DSLK immunocomplex and anti-Kg immunocomplex reacted only with the anti-RHAG-conjugated beads as shown in Figure 2a,b, respectively, indicating that the DSLK and Kg antigens are carried by RhAG.

Analysis of the *RHAG* gene

The whole exons of the *RHAG* gene of the proband, her son and the DSLK-negative individual were analysed using Sanger sequencing. The proband and DSLK-negative individual were apparently homozygous for *RHAG*01.–3* with a missense SNV, c.490A>C (p.Lys164Gln), in exon 3 of the *RHAG* gene (Figure 3a,c), whereas the proband's son was a *RHAG*01/RHAG*01.–3* heterozygote (Figure 3b).

Cross-reactivity testing of anti-DSLK and anti-Kg antibodies by FCM

We established HEK293 cells expressing RhAG with the deduced amino acids p.Lys164 (wild-type) and p.Gln164 (variant) by inducing the *RHAG*01* and *RHAG*01.–3* constructs, respectively. Cells expressing wild-type RhAG and cells expressing variant RhAG reacted with anti-RhAG antibody (LA18.18) and exhibited similar histogram profiles (Figure 4a). The wild-type RhAG-expressing cells reacted with anti-DSLK eluate but not with anti-Kg, whereas variant RhAG-expressing cells reacted with anti-Kg but not with

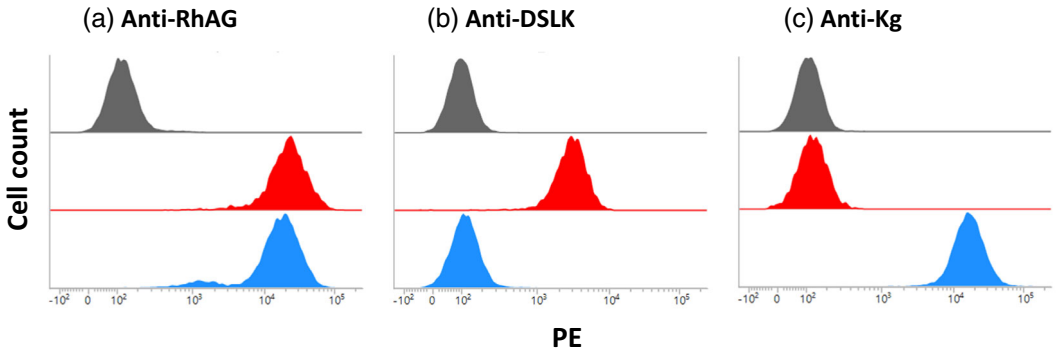

FIGURE 4 Cross-reactivity testing of the anti-DSLK and anti-Kg antibodies using HEK293 cells expressing wild-type or variant RhAG. Histograms show the intensity of antigen expression (phycoerythrin [PE]; *X*-axis) versus the number of events (cell count; *Y*-axis). HEK293 cells induced by wild-type *RHAG* (red) and variant *RHAG* with c.490A>C (blue) are shown. Mock cells are indicated as black. (a) Reactivity of the cells with anti-RhAG antibody (LA18.18), (b) anti-DSLK antibody (eluate) and (c) Anti-Kg antibody (OSK46).

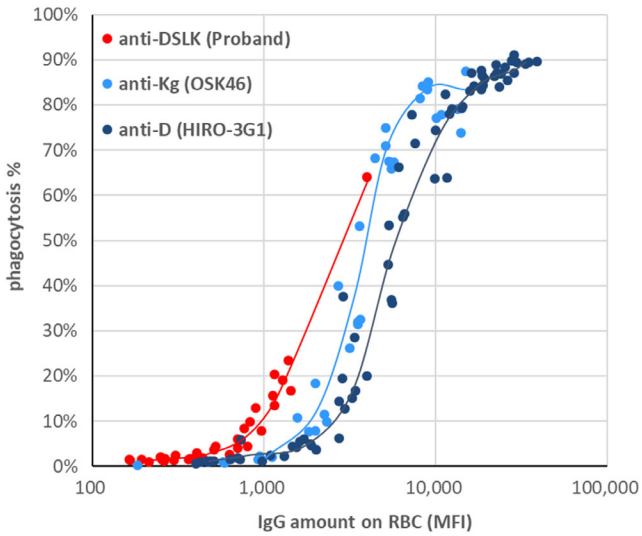

FIGURE 5 Flow cytometric assays of monocyte phagocytosis of red blood cells (RBCs) bound with anti-DSLK, anti-Kg and anti-D antibodies. Correlations between the percent phagocytosis (*Y*-axis) and amount of IgG on RBCs (*X*-axis) with anti-DSLK (red), anti-Kg (blue) and anti-D (dark blue) antibodies.

anti-DSLK (Figure 4b,c). These results clearly demonstrate that DSLK and Kg are antithetical antigens.

Monocyte phagocytosis assays using anti-DSLK-sensitized RBCs

The results of monocyte phagocytosis assays using RBCs sensitized with anti-D, anti-Kg or anti-DSLK antibodies are shown in Figure 5. In many cases, the percent phagocytosis increased dramatically as the amount of IgG bound to the RBCs increased within a particular range. The titres of the anti-DSLK eluate, anti-Kg and anti-D antibodies were 1:256, 1:512 and 1:4096, respectively. The maximum percent phagocytosis of RBCs sensitized with anti-DSLK, anti-Kg or anti-D antibodies was 64%, 83%, and 89%, respectively. The percent phagocytosis of RBCs with the negative control (PBS) was 1%. These results suggest that anti-DSLK may potentially cause haemolytic disease.

DISCUSSION

Since RHAG was recognized as the 30th blood group system, consisting of three antigens (Duclos [RHAG001], OI^a [RHAG002] and DSLK [RHAG003], as designated by the ISBT) [1], no additional antigens have been identified in this system in the last 10 years. Recently, an LFA of the 700 series, Kg (RHAG005) [2], was identified as belonging to the RHAG blood group system. Furthermore, a novel LFA encoded by *RHAG* with a missense SNV, c.1063A>C (p.Asn355His) [4], has been recognized as SHER (RHAG006) by the ISBT.

Interestingly, the missense SNV (rs144305805) c.490A>C (p.Lys164Gln) in the *RHAG* gene is responsible for both the DSLK-negative and Kg-positive phenotypes. In this study, we confirmed that the DSLK and Kg antigens are carried on RhAG by ICFA and that they are antithetical antigens by cross-reactivity testing using anti-DSLK and anti-Kg with HEK293 cells expressing wild-type RhAG and variant RhAG.

The frequency of the missense SNV rs144305805 is 0.001498 in the Japanese population [12] but extremely rare in other populations [13]. Takahashi et al. [14] screened 68,395 Japanese blood donors using a monoclonal anti-Kg antibody and reported a prevalence of Kg-positive donors of 0.19%. Tanaka et al. [3] reported that the frequency of Kg-positive individuals in the Japanese population is 0.22%. Taken together, these data suggest that the frequency of Kg-positive Japanese is approximately 0.2%. Based on these data, the calculated frequency of DSLK-negative (Kg homozygous) individuals in the Japanese population is 0.0001%.

Although cases of individuals harbouring anti-DSLK or anti-Kg antibodies are very rare, two cases of HDN caused by anti-Kg antibodies have been reported [2, 6] to date. Another study suggested that anti-Kg antibodies can cause haemolytic transfusion reaction

(HTR) [7]. Because there is currently no information available regarding the clinical significance of anti-DSLK antibodies, we performed monocyte phagocytosis assays to determine whether anti-DSLK antibodies can cause HTR or HDN. The maximum percent phagocytosis of RBCs sensitized with anti-D and anti-Kg antibodies was >80%, consistent with the results of a previous study [7], whereas the maximum percent phagocytosis of RBCs sensitized with anti-DSLK was 64%. In addition, the percent phagocytosis increased as the amount of anti-DSLK bound on the RBCs increased. These results suggest that anti-DSLK antibodies also have the potential to induce haemolytic reactions. Screening of Kg-positive RBCs using monoclonal anti-Kg antibody followed by anti-DSLK antibody or molecular testing of the *RHAG* gene will be useful in efforts to obtain extremely rare DSLK-negative blood.

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W.O. and Y.T. provided the proband samples; T.M. analysed serological and ICFA data; K.I. analysed the *RHAG* gene; R.K. established the RhAG-expressing cells and K.O. and T.M. wrote the paper, which was edited by S.M. and M.S.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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SUPPORTING INFORMATION

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

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SHORT REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

Use of selective phenotyping and genotyping to identify rare blood donors in Canada

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Abstract

Background and Objectives: The distribution of rare and specific red cell phenotypes varies between races and ethnicities. Therefore, the most compatible red cell units for patients with haemoglobinopathies and other rare blood requirements are most likely to be found in donors from similar genetic backgrounds. Our blood service introduced a voluntary question asking donors to provide their racial background/ethnicity. Results triggered additional phenotyping and/or genotyping.

Materials and Methods: We analysed the results of additional testing performed between January 2021 and June 2022, and rare donors were added to the Rare Blood Donor database. We determined the incidence of various rare phenotypes and blood group alleles based on donor race/ethnicity.

Results: Over 95% of donors answered the voluntary question; 715 samples were tested, and 25 donors were added to the Rare Blood Donor database, including five k−, four U−, two Jk(a−b−) and two D− − phenotypes.

Conclusion: Asking donors about their race/ethnicity was well received by donors, and the resulting selective testing enabled us to identify individuals with a higher likelihood of being rare blood donors, support patients with rare blood requirements and better understand the incidence of common and rare alleles and red blood cell phenotypes in the Canadian donor population.

Keywords

blood donors, donor race and ethnicity, genotyping

Highlights

- The vast majority of donors answered a voluntary question about race/ethnicity.
- Extended phenotyping and genotyping were selectively performed in specific groups to identify rare donors.
- We identified 3.5% of donors undergoing additional testing as rare and added them to our Rare Blood Donor database.

INTRODUCTION

Red blood cell (RBC) antigens are encoded by the human genome [1]. Variations within the alleles encoding RBC antigens can lead to differences in antigen expression and are inherited across generations [2]. Distribution of various genetic and associated RBC phenotypes is known to vary across different races and ethnicities because of their underlying genetic diversity [3, 4].

Blood donors with rare phenotypes are found in less than 0.1% of the population and include individuals with specific phenotypic combinations or the absence of high-prevalence antigens. Because of genetic inheritance, the odds of finding the most compatible RBCs for individuals with particular red cell requirements increases considerably when looking at donors within the same racial or ethnic background. Therefore, the ability to identify these donors is of great value to the blood supplier.

Different methods can be used to determine a donor's RBC phenotype, including the use of serological phenotyping antisera or genotyping. RBC antigen genotyping is used to accurately predict phenotypes by inferring expression from the donor's genetic material and can also identify novel or variant alleles that lead to altered expression [5]. With the knowledge that antigen expression varies by race and ethnicity, donor testing laboratories can perform targeted investigations on specific donor samples to increase the likelihood of finding rare donors.

Canadian Blood Services (CBS) supplies blood components for all Canadian provinces and territories apart from the province of Quebec and has recently made advances towards expanding the identification of rare blood donors. CBS chooses to identify donors as rare based on the presence of a rare blood group or extended phenotypic combination that is useful in transfusion, who can then be recruited into the national Rare Blood Program [6].

This program ensures the availability of various blood components for patients who have unique requirements and whose needs are not easily met by the general donor population. Donors enrolled in the program receive extra information about the rarity of their blood type and how important their donations are for patients with particular transfusion needs, and are coded in the computer system. There are currently approximately 1100 donors with rare donor status. While providing service to the Canadian population, CBS's Rare Blood Program also interfaces with the transfusion community at an international level through the ISBT Rare Donor Working Party and the International Rare Donor Panel to meet the needs of transfusion recipients outside of Canada.

We describe the results of a recently implemented protocol where selected serological testing and genotyping are applied to whole-blood donor samples following the implementation of an optional donor question asking donors about their race/ethnicity.

MATERIALS AND METHODS

Donor questionnaire

An electronic donor questionnaire was first introduced at CBS in 2016. After reviewing their questionnaire answers with staff, eligible donors are verbally asked an optional question: 'This is an optional question about your ethnic background that will be used to help us identify rare blood groups. Are you willing to provide the information?'

If the donor answered affirmatively, they are then asked if their background is Aboriginal, Arabic, Asian, Black, Latin American, South Asian, White and other ethnicity. Choices were previously based on the contemporary Statistics Canada census and have since been expanded upon after the study period. Implementation of this question was initially limited to permanent donation sites because of technical limitations, with expansion to include all mobile blood donation centres completed by September 2020. The question is asked only on one occasion, and the donor can select only one race/ethnicity.

TABLE 1 Distribution of self-reported donor/ethnicity race during study period in all donors and in donors undergoing additional testing.

Self-reported donor race/ethnicity	Total blood donors ($N = 437,542$)	Donors with extended testing ($N = 715$)
Indigenous	6283 (1.43%)	13 (1.8%)
Arabic	6275 (1.43%)	1 (0.1%)
Asian	27,820 (6.36%)	28 (3.9%)
Black	3285 (0.75%)	255 (35.7%)
Latin	7480 (1.71%)	7 (1.0%)
South Asian	23,528 (5.37%)	47 (6.6%)
White	315,691 (72.1%)	306 (42.8%)
Other ethnicity	20,358 (4.65%)	21 (2.9%)
No answer	26,822 (6.13%)	37 (5.2%)

Donor testing

CBS performs routine testing on all donor samples for ABO, RhD and K(KEL1) phenotype. A subset of donors are also routinely tested for extended Rh phenotypes (C/c/E/e) Kidd (Jk^a/Jk^b), Duffy (Fy^a/Fy^b) and MNS (S/s) using automated solid-phase testing regardless of race or ethnicity.

When donors answer the optional question on donor race/ethnicity, specific codes are applied to their donation, which can be used by donor testing laboratories to flag these samples for further testing, including extended serological phenotyping by automated solid-phase methods, manual phenotyping using rare antisera and PCR-based red cell antigen genotyping performed via probe-hybridization coupled to bead-based flow cytometry (Progenika ID CORE XT platform, Progenika Biopharma-Grifols, Barcelona, Spain) [7]. The donors' geographic location, donation frequency and results of initial Rh testing are also part of the algorithmic approach to further testing.

For example, Arabic donors were serologically phenotyped for AnWj, indigenous donors for Diego and Black donors for Rh status; all serological results were confirmed via RBC genotyping. Samples were also occasionally sent to a reference laboratory for next-generation sequencing to identify additional rare or novel variant alleles or to resolve phenotype and genotype discrepancies encountered with the probe hybridization targeted genotyping method.

Results of testing and Rare Blood Program recruitment

We analysed the results of extended serological donor testing and genotyping performed between January 2021

and June 2022 based on the optional donor question by donor race/ethnicity. We reviewed donor records to identify donors recruited into CBS's Rare Blood Program due to rare blood groups or phenotypes identified through this policy.

Donors routinely provide consent for extended blood group typing. According to procedures developed by our Research and Ethics Board, this study did not require additional ethics approval since no additional information was collected from donors and no donor identifiers are present in the manuscript.

TABLE 2 Rare blood donor phenotypes confirmed by targeted genotyping.

Rare donor phenotype	Total ($N = 25$)
k−	5
Jo(a)	4
U−	4
Co(a−)	3
Yt(a−)	2
dCe	2
Jk(a−b−)	2
D−−	2
Lu(b−)	1

RESULTS

Donor response to optional question

Over 95% of donors agreed to answer the optional question on race/ethnicity; 715 donors were selected for extended testing based on their response. Table 1 shows the breakdown of total donor percentage by race/ethnicity across all donors, and among those selected for extended testing. Although donors who self-identified as Black only accounted for 0.75% of all donors, they constituted 35.7% of donors undergoing additional testing.

Donors identified as rare by testing protocol

In total, 25 out of 715 (3.5%) of donors flagged for extended testing were identified as rare and referred for inclusion in the Rare Blood Program. Table 2 shows the reason for rare donor status. Among the rare donors identified, there were five k− donors, four U− Black donors, two Asian Jk(a−b−) donors and two donors with D− − phenotype (one Asian, one other race/ethnicity).

Extended phenotypes were also available either by serological or genotyping methods for all 715 donors flagged for additional testing. Table 3 shows the distribution of predicted Rh and extended phenotypes among the four most tested donor groups. Black donors showed the highest proportion of specific Rh and extended phenotypes

TABLE 3 Distribution of selected Rh and extended phenotypes by self-reported donor race/ethnicity.

	Self-reported donor race/ethnicity			
Red blood cell phenotype	Asian ($N = 28$)	Black ($N = 255$)	South Asian ($N = 47$)	White ($N = 306$)
RhD-positive phenotypes				
R_1R_1	19 (67.8%)	10 (3.92%)	27 (57.4%)	82 (26.8%)
R_1R_2	3 (10.7%)	10 (3.92%)	6 (12.7%)	30 (9.80%)
R_2r	1 (3.57%)	19 (7.45%)	2 (4.25%)	30 (9.80%)
R_2R_2	1 (3.57%)	0	0	16 (5.22%)
R_0r	0	138 (54.1%)	0	8 (2.61%)
R_1r	1 (3.57%)	53 (20.7%)	9 (19.1%)	71 (23.2%)
R_1R_z	1 (3.57%)	0	0	0
D− −	1 (3.57%)	0	0	0
rr	0	24 (9.41%)	1 (2.1%)	63 (20.6%)
RhD-negative phenotypes				
r′r	0	1 (0.39%)	1 (2.1%)	1 (0.32%)
r″r	1 (3.57%)	0	0	3 (0.98%)
r′r″	0	0	1 (2.1%)	0
r′r′	0	0	0	1 (0.32%)
r″r″	0	0	0	1 (0.32%)
Extended phenotypes				
D+C-E-K− Jk^b−Fy^a−	0	73 (28.6%)	0	0
D+C-E-K− Jk^b−Fy^a−S−	0	50 (19.6%)	0	0

Note: Percentages are calculated from all donors genotyped within a specific race/ethnicity.

commonly requested for transfusion in recipients with sickle cell disease, who often receive extended-matched RBCs [8, 9].

DISCUSSION

Expansion of donor testing based on self-reported donor race/ethnicity enabled the efficient discovery of more donors with rare red cell phenotypes. Within our timeframe of analysis, 3.5% of donors whose samples underwent additional testing were added to the Rare Blood Program. While not all donors of a particular racial/ethnic background could be tested using the study protocol, emphasis was placed on selecting donors from minority populations. This is illustrated by the observation that Black donors represented 35.7% of all donors tested, whereas they make up only 0.75% of the total donor population. Without this protocol, rare donors in the Black population may have otherwise been undiscovered. Such policies provide CBS with the best ability to support what is a growing and increasingly diverse patient population.

Approximately 95% of Canadian donors are comfortable answering this question on race and ethnicity. We acknowledge the inherent limitations in the classification categories used for this analysis, which include a lack of specificity and depth to describe various backgrounds. This issue was recently addressed through changes to the donor questionnaire to harmonize categories with those utilized by the Canadian Stem Cell Registry.

We were able to provide an overview on the genetic differences in our donor population by race/ethnicity. However, many populations are under-represented in our analysis, which is due to a combination of limited availability of widespread RBC genotyping for all donors and decreased overall representation in the donor pool. We plan to continue increasing the degree to which donor genotyping is performed as our expansion of donor identification continues, particularly for particular phenotype combinations in the Rh system. Genotyping remains the only readily available means of identifying RBC variant alleles in these populations.

This study represents the first description of Canadian whole-blood donors by their red cell genotypes and racial/ethnic background. In addition to supporting our rare blood donor program, this data is also helpful for monitoring the recruitment, retention and deferral patterns of donors based on their racial/ethnic background. In the 2016 census of the general Canadian population, 2.5% of respondents self-identified as Black, compared to 0.75% of our donors [10]. Minority blood donors may face many barriers to donation [11]. CBS remains committed to developing community partnerships across the country to further strengthen and diversify our donor population to meet the needs of all blood recipients.

In conclusion, implementation of a voluntary question on donor race/ethnicity and associated additional serological testing and genotyping has resulted in the discovery of multiple donors with rare blood groups and phenotypes commonly requested in the transfusion of patients with sickle cell disease and other haemoglobinopathies.

Identification of a donor's race/ethnicity remains a limited but useful means for identifying specific and rare blood donors. These results highlight the ongoing efforts to maintain a diverse donor population in order to meet recipient needs.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflicts of interest.

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SHORT REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

Secretor status of blood group O mothers is associated with development of ABO haemolytic disease in the newborn

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Abstract

Background and Objectives: Identification of antibody characteristics and genetics underlying the development of maternal anti-A/B linked to inducing haemolytic disease of the foetus and newborn could contribute to the development of screening methods predicting pregnancies at risk with high diagnostic accuracy.

Materials and Methods: We examined 73 samples from mothers to 37 newborns with haemolysis (cases) and 36 without (controls). The secretor status was determined by genotyping a single nucleotide polymorphism in *FUT2*, rs601338 (c.428G>A).

Results: We found a significant association between secretor mothers and newborns developing haemolysis ($p = 0.028$). However, stratifying by the newborn's blood group, the association was found only in secretor mothers to blood group B newborns ($p = 0.032$). In fact, only secretor mothers were found in this group. By including antibody data from a previous study, we found higher median semi-quantitative levels of IgG1 and IgG3 among secretor mothers than non-secretor mothers to newborns with and without haemolysis.

Conclusion: We found that the maternal secretor status is associated with the production of anti-A/B, pathogenic to ABO-incompatible newborns. We suggest that secretors experience hyper-immunizing events more frequently than non-secretors, leading to the production of pathogenic ABO antibodies, especially anti-B.

Keywords

ABO, ABO-antibodies, *FUT2*, haemolysis, HFDN, secretor

Highlights

- A significant association was found between mothers with a secretor (*FUT2*) genotype and newborns with haemolysis.
- There were differences in maternal anti-A and anti-B regarding maternal secretor status and the development of haemolysis in the newborn.
- We also found that the levels of maternal IgG1 and IgG3 of both anti-A and anti-B were higher in secretor mothers than in non-secretor mothers to newborns with and without haemolysis.

INTRODUCTION

Previous studies on ABO-incompatible pregnancies of blood group O women have shown a significant association between the level of maternal anti-A/B IgG and hyperbilirubinaemia requiring treatment of the newborn [1, 2]. In addition, studies have investigated the possible characteristics of the maternal antibodies causing ABO haemolytic disease of the newborn [3–5].

We have previously reported an association between the frequency of the foetal secretor allele and newborn haemolysis [6]. Since this association was found only at the allele level and not with the phenotype, we hypothesized that the association might reflect an effect exerted by the maternal secretor status [6]. Furthermore, a previous study found an effect of secretor status on the level of anti-B, where the level was found considerably lower in non-secretors than in secretors [5]. The incidence of newborns with blood group B developing haemolytic disease of the foetus and newborn (HDFN) is disproportionately high when considering the frequency of blood group B in the general population [1, 2, 7], suggesting a more pathogenic potential of anti-B.

In the present study, we therefore analysed the maternal secretor status and aimed to determine (i) if maternal secretor status is associated with the development of ABO haemolytic disease of the newborn and (ii) if such potential association might be particularly pathogenic for blood group B newborns.

MATERIALS AND METHODS

Subjects

This study is an extension of a previously published case–control study of ABO HDFN causative and protective factors in the newborn and mother [2, 6]. Details of inclusion criteria of the subjects are given in that study [2, 6]. In short, a group of 38 blood group O mothers and their incompatible A−/B− newborns with haemolysis (cases) and a group of 38 blood group O mothers and their incompatible A−/B− newborns without haemolysis (controls) were included in the study [2].

Materials

Maternal EDTA-stabilized blood samples were separated into plasma and cell-rich suspensions and stored below −20°C. DNA was extracted from the cell-rich suspension by the QIAamp DNA Blood Mini Kit (ID: 51104, Qiagen Inc.) according to the manufacturer's protocol.

Determination of secretor status

The maternal secretor status was determined by genotyping a single nucleotide polymorphism (SNP) in *FUT2*, rs601338 (c.428G>A), using KASP chemistry as previously described [6].

Determination of the semi-quantitative level of maternal anti-A/B IgG1, 2, 3 subclasses and total IgG

In a previous study, we determined semi-quantitatively the level of maternal IgG1, 2, 3 anti-A and anti-B using a flow-cytometric method and included the data in the present study [6].

Also, we included data of total IgG anti-A/B determined in a previous study by testing serial plasma dilutions against A_1/B red blood cells, using the Galileo NEO automated ABO titration assay employing Immucor Capture-R technology [2].

Ethics statement

The study was approved by Danish Data Protection Agency (RH-2017-295) and the Regional Scientific Ethical Committee of the Capital Region of Denmark (H-17029655). Verbal and written consent was obtained from the women and parents of the newborns.

Statistical analyses

Descriptive statistics and differences between groups assessed using Fisher's exact test were performed using IBM SPSS statistics 25. Violin plots were drawn using GraphPad Prism 9.4.1.

RESULTS

The secretor status was determined in samples from 73 blood group O mothers to 37 newborns with haemolysis (with 22 blood group A and 15 blood group B) and 36 newborns without haemolysis (with 28 blood group A and 8 blood group B). DNA from three samples was not available. We determined the secretor status by the c.428G>A SNP in *FUT2*, where secretors were defined by G/G and G/A and non-secretors were defined by A/A.

First, we found a significant association between mothers with secretor genotypes and newborns with haemolysis ($p = 0.028$; Table 1). Next, we stratified by newborn blood group and found a significant association only between mothers with secretor genotypes and blood group B newborns with haemolysis ($p = 0.032$). Additionally, no non-secretors were found in this group (Table 2).

The median semi-quantitative levels of maternal IgG1 and IgG3 of anti-A were higher in secretor mothers than in non-secretor mothers in cases (newborns with haemolysis) as well as in controls (newborns without haemolysis), whereas the median levels of IgG2 and total IgG were similar or slightly lower (Figure 1). For maternal anti-B, the median semi-quantitative levels could be compared only between secretor mothers and non-

TABLE 1 Comparison of the secretor status of blood group O mothers with newborns with and without haemolysis.

Newborn blood group		Secretor[a] frequency (*n*)	Non-secretor[a] frequency (*n*)	*p*-value[b]
A/B	Mothers to newborns with haemolysis ($n = 37$)[c]	0.92 (34)	0.08 (3)	**0.028**
	Mothers to newborns without haemolysis ($n = 36$)[c]	0.72 (26)	0.27 (10)	

[a]As determined by single nucleotide polymorphism rs601338; the frequency of non-secretors in Europeans and Africans = 0.2; South Asians = 0.1 (11).
[b]Fisher's exact test, bold value indicates *p*-value <0.05.
[c]Results are missing in one case (A-newborn) and two controls (A-newborns).

secretor mothers in controls, where levels of total IgG were similar and levels of IgG1, 2 and 3 were found higher in secretors (Figure 1). Descriptive data are shown in Table S1.

TABLE 2 Comparison of the secretor status of blood group O mothers with newborns with and without haemolysis, separated by newborn blood group.

Newborn blood group		Secretor[a] frequency (*n*)	Non-secretor[a] frequency (*n*)	*p*-value[b]
A	Mothers to newborns with haemolysis ($n = 22$)[c]	0.86 (19)	0.14 (3)	0.263
	Mothers to newborns without haemolysis ($n = 28$)[c]	0.75 (21)	0.25 (7)	
B	Mothers to newborns with haemolysis ($n = 15$)	1.00 (15)	0 (0)	**0.032**
	Mothers to newborns without haemolysis ($n = 8$)	0.63 (5)	0.38 (3)	

[a]As determined by single nucleotide polymorphism rs601338. The frequency of non-secretors in Europeans and Africans = 0.2; in South Asians = 0.1 (11).
[b]Fisher's exact test, bold value indicates *p*-value <0.05.
[c]Results are missing in one case (A-newborn) and two controls (A-newborns).

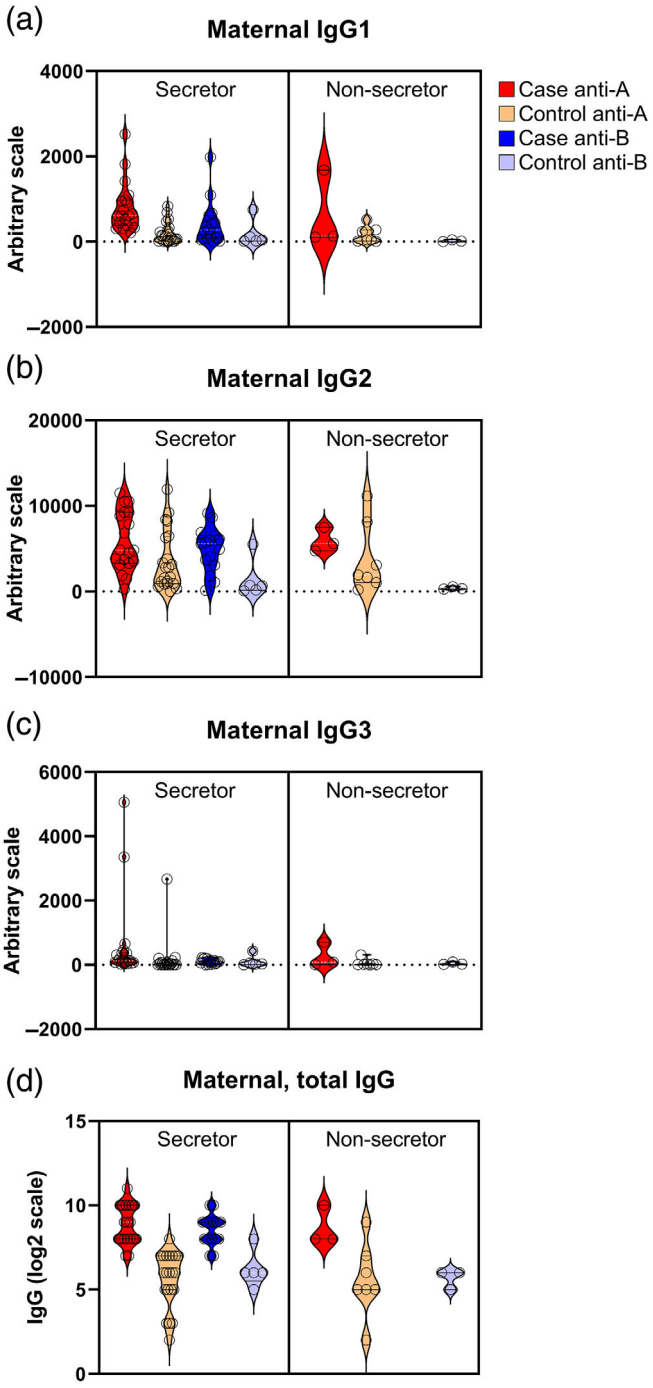

FIGURE 1 Maternal level of IgG1 (a), IgG2 (b), IgG3 (c) and total IgG (d) stratified by maternal secretor status and in relation to newborns with hemolysis (cases) and newborns without hemolysis (controls); median (white lines) and first/third quartiles (black lines). In the IgG3 assay, three extreme outliers with values 5059, 3356 and 2668 were all found from three secretor mothers to two newborns with blood group A^1 and one newborn with the protective A^2 blood group. Secretor mothers: anti-A cases $n = 19$, anti-A controls $n = 21$, anti-B cases $n = 15$ and anti-B controls $n = 5$. Non-secretor mothers: anti-A cases $n = 3$, anti-A controls $n = 7$, anti-B cases $n = 0$ and anti-B controls $n = 3$. Descriptive data is shown in Table S1.

DISCUSSION

The maternal secretor status was examined to investigate a potential influence on the level of ABO IgG antibodies causing ABO HDFN. The secretor genotype (*FUT2*) was determined for blood group O mothers to newborns with or without haemolysis. The median semi-quantitative level of anti-A and anti-B IgG1, 2, 3 subclasses and the total IgG titre, determined in earlier studies, were included for comparison [2, 6]. We found evidence that the maternal secretor status was significantly associated with haemolysis in the newborn induced by ABO IgG antibodies. However, the significant association was apparent only in cases with secretor mothers to blood group B newborns. The fact that non-secretor mothers to blood group B newborns were found only among newborns without haemolysis emphasizes the observed impact of secretor status on predominantly maternal anti-B. The link between maternal secretor status and anti-B is not clear. However, our findings are consistent with a previous report showing a considerable higher level of anti-B in secretor individuals [5].

Secretor mothers displayed a trend towards elevated levels of both anti-A and anti-B IgG1 and IgG3 antibodies compared to non-secretors. A noteworthy finding is three extreme outliers of IgG3 anti-A, all from secretor mothers.

The clinical impact of the *FUT2* gene on the formation of higher levels of more pathogenic IgG1 and IgG3 anti-A/B is hidden behind the higher quantity of IgG2. The fact that IgG2 comprises a larger fraction of the total IgG weakens the predictive effect of total IgG anti-A/B on the development of ABO HDFN.

Anti-A and anti-B are naturally occurring antibodies produced in response to particularly Gram-negative gut microbiota [8]. Anti-A/B are predominantly of the IgM isotype, and class-switching to IgG requires an event of hyper-immunization, such as an ABO-incompatible pregnancy or a probiotic intake [8, 9]. We speculate that the class-switch to IgG1 and IgG3 may be related to an impact of secretor status on the selection and abundance of intestinal bacteria. One study found that secretors have both higher overall counts of intestinal bacteria and an increased abundance of bifidobacteria species [10].

We hypothesize that a higher quantity of anti-A/B, especially anti-B, is a response to a higher amount of and differences in certain species in the gut microbiota, which in turn is influenced by diet and other environmental factors [8]. As blood group B [9] and the secretor genotype [11] are more common in Asian ethnic groups, we also hypothesize that a more potent anti-B could be a part of an explanation of the difference in incidence of ABO HDFN among ethnic populations [9, 12].

As our dataset included a relatively small number of individuals in each group—especially among blood group B newborns—statistics were limited to descriptive in relation to the semi-quantitative level of IgG1, 2, 3 and total IgG of anti-A/B. Neither the number of multigravida individuals nor the consumption of probiotics was recorded for this study, which may confound data interpretation. Consequently, the results should be interpreted with caution.

To the best of our knowledge, this is the first study to highlight a significant association between the maternal secretor status and newborns with haemolysis. The results may provide the incitement for further study in a larger cohort.

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G.R.K. designed and performed the research; G.R.K. and H.L. wrote the first draft of the manuscript and edited the manuscript; F.B.C. and M.H.D. supervised, reviewed and edited the manuscript.

CONFLICT OF INTEREST STATEMENT

All authors have declared no conflict of interest.

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SUPPORTING INFORMATION

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

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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

Low neutralization capacity against SARS-CoV-2 Omicron BQ.1.1 of convalescent plasma collected during circulation of Omicron BA.1

The emerging Omicron BQ.1.1 sublineage has become dominant in France (69% of circulating strains, Week 1, 2023). It shows a worrying overall level of resistance to marketed therapeutic monoclonal antibodies (mAbs) [1, 2]. This may increase, for the treatment of immunocompromised patients, the interest in using hyperimmune human plasma such as those collected in France from COVID-19 convalescent vaccinated (CCP-V) blood donors.

Between 26 January 2022 and 9 February 2022, we collected samples from 54 French plasma donors (voluntary and non-remunerated) who had received a complete vaccination regimen (i.e., at least two doses of the Pfizer/BioNTech mRNA vaccine), and who were recently infected with SARS-CoV-2 (tested PCR- or antigenic-positive after 30 December 2021). Most of them (96%, 52/54) were qualified as convalescent plasma donors (>3500 BAU/mL at that time in France). During this period, the Omicron BA.1 variant was largely predominant in France.

Samples were tested for SARS-CoV-2 IgG anti-S1 (Euroimmun Quantivac) and by virus neutralization test (VNT) against different strains: ancestral (B.1) and Omicron variants (BA.1, BA.5 and BQ.1.1), as previously reported [3].

VNT was performed in an NSB3 laboratory and consisted of (i) bringing into contact (1 h at 37°C) a sample dilution of plasma (1/10–1/20,480) with a fixed amount of a given variant of SARS-CoV-2 corresponding to 0.5 TCID50 per µL of final plasma dilution, (ii) inoculating 100 µL of this mixture onto Vero E6 TMPRSS2+ cells (for 5 days at 37°C under 5% CO_2) and (iii) reading cytophatic effect at Day 5. The test was used in a duplicate format. Neutralization titres ≥40 were considered positive.

The mean anti-S1 IgG level was extremely high: 9877 BAU/mL (3046–20,727 BAU/mL). The highest neutralizing titres were found against B.1 (geometric mean titre [GMT] = 2006 [320–20,480], see Figure 1), suggesting an increase in antibodies against the vaccine strain upon infection with BA.1. The neutralizing capacities against the other strains were significantly lower ($p < 0.0001$, Friedman's non-parametric test) with GMT = 419 [40–2560], 156 [20–640] and 21 [10–80], for BA.1, BA.5 and BQ.1.1, respectively.

Overall, this work provides a rare picture of the reduction in neutralizing capacity against new emerging variants in BA.1-infected vaccinees. This loss is concordant with what was observed with therapeutic

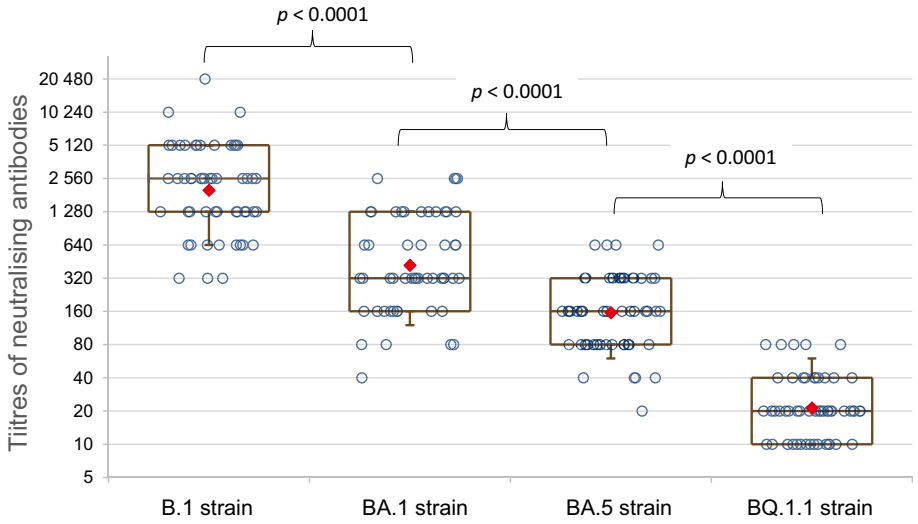

FIGURE 1 Distribution of neutralizing titres of vaccinated COVID-19 convalescent plasma antibodies against B.1, BA.12, BA.5 and BQ.1.1 strains. Titres are expressed in $\log_2$ scale. The boxes represent the geometric mean titre (red diamonds) and the interquartile range. The whiskers represent the 10th and 90th percentiles. Positive cut-off value for neutralizing titres is ≥40.

mAbs [1, 2, 4] or in triply vaccinated individuals [4, 5]. This is understandable since the receptor binding domain of the spike protein is a common preferred target. However, the neutralizing capacity against BQ.1.1, the latest variant circulating in France, is particularly low.

With regard to CCP-V plasma, these data confirm the need to constantly adjust plasma donor selection criteria to emerging strains, in particular by favouring the collection of donors infected recently, ideally after a variant has become the majority strain.

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CONFLICT OF INTEREST STATEMENT

C.I., P.G., N.B., S.L.C., S.L., P.M., P.T. and P.R. are employed by the French transfusion public service (Etablissement Français du Sang) in charge of the manufacturing and issuing of blood components in France.

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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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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

Comment on White paper on pandemic preparedness in the blood supply

We read with interest the article titled 'White paper on pandemic preparedness in the blood supply' [1]. Whilst we strongly support the majority of the recommendations, we question the emphasis placed on pathogen inactivation. A more accurate term is 'pathogen reduction', given current technologies do not completely eliminate transmission risk for all pathogens and all products and additional testing is required in some scenarios.

Pathogen reduction needs to be considered in terms of local context. In Australia, pathogen reduction has not been introduced as it cannot be cost-justified. This is supported by recent research demonstrating implementation of the INTERCEPT™ Blood System (Cerus Corporation, Concord, CA) would have an incremental cost-effectiveness ratio of approximately 8.1 million dollars per quality-adjusted life-year gained in the Canadian province of Quebec [2], although the estimated incremental cost-effectiveness ratio fell to $123,063 in the presence of a new highly contagious human immunodeficiency virus (HIV)-like pathogen without diagnostic testing. Whilst this may appear to support pathogen reduction as a key pandemic response strategy, it is important to consider the context of the origins of the HIV pandemic when the ability to rapidly identify a virus and develop screening assays was not available. Therefore, for future pandemics, the reliance on pathogen reduction because a test would not be rapidly developed cannot be assummed. Testing by individual donation nucleic acid testing for a high-consequence virus may be more efficient than pathogen reduction.

Since 2007, there have been seven public health emergencies of international concern including the H1N1 influenza pandemic, poliomyelitis, Ebola, Zika, COVID-19 [3] and mpox. Zika virus is the only known transfusion-transmitted agent listed. The other pathogens are transmitted primarily through person-to-person contact, and although their transient detection in blood has been confirmed, it has not been confirmed that they can be transmitted through transfusion. A primary concern during the COVID-19 pandemic was not blood safety, but minimizing transmission to staff and donors during donation [4]. Whilst pathogen reduction is effective for a number of these viruses, such as Ebola virus [5], transfusion transmission of these agents is either rare or remains theoretical. Therefore, it is not an effective use of resources to spend large sums on preventing rare transfusion-transmission cases, if any. These resources could be better directed towards decreasing community spread, which is a far larger public health problem for all of these diseases. This could involve vector control, vaccination or other public health interventions. In addition, if a donor is infectious, dependent on the virus, person-to-person transmission is generally more significant in the context of exposure during blood donation. We therefore agree it is imperative that blood operators' decision-making is integrated with a broad public health response that considers the relative contribution of all modes of pathogen transmission, the cost-effectiveness of potential intervention strategies and resource limitations of each jurisdiction.

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CONFLICT OF INTEREST STATEMENT

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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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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

Reply to Hoad et al. Comment on White paper on pandemic preparedness in the blood supply

We appreciate and read with interest the Letter to the Editor by Hoad et al. [1]. While the authors strongly support the majority of the recommendations in our paper 'White paper on pandemic preparedness in the blood supply' [2], they suggest that the term 'pathogen reduction' may be more appropriate than 'pathogen inactivation' and assert that pathogen reduction needs to be considered in terms of local context.

Regarding the term, we acknowledge the potential for confusion with nomenclature related to the treatments developed to decrease transfusion-transmitted infection risk. The terms 'pathogen reduction' and 'pathogen inactivation' are both commonly used. Lozano et al. have addressed these differences in terminology and proposed standardization by referring to the technologies used as 'pathogen inactivation' methods and the treated products as 'pathogen-reduced' blood components [3]. In other words, pathogen-reduced platelets and plasma are described as blood components that are treated with pathogen inactivation technology. These definitions are also used by US Food and Drug Administration (FDA) and the European Directorate for the Quality of Medicines & Healthcare (EDQM) [4, 5]. In our paper, we refer to the technologies used.

Regarding the need for local context, we agree that all countries will evaluate adoption of pathogen inactivation methods against the background of perceived infectious risks, available resources and other considerations. However, in addition to the public health emergencies mentioned in the letter, countries may eventually face serious risks from other globally emerging and re-emerging pathogens that put the blood donor population and patients at risk [6]. Future dangers to the blood supply may derive from a return to robust air travel, climate change and attendant effects on the range of disease vectors or the effects of animal husbandry methods, among other factors. We view platelet and plasma pathogen-reduced components as the precursor to a future where all products can effectively be pathogen-reduced. It is not inconceivable that a new pathogen might emerge that can be transmitted by person-to-person contact and by blood; if the sequencing of the pathogen and the production of test materials are delayed, the safety of blood products would be compromised. In such a case, pathogen inactivation would be essential.

The cost effectiveness remains to be seen and is necessarily influenced by local context, such as how much infectious disease testing or donor deferrals might be reduced and other operational issues. From our perspective, the central issue is whether pathogen-reduced products are likely to be safer than the standard paradigm of testing and donor selection if a new pathogen emerges. Our proposal, along with the other six recommendations on pandemic preparedness in the blood supply, is to support the implementation and continuing development of pathogen inactivation technologies for all blood components because this will help guarantee blood supply during a future pandemic.

CONFLICT OF INTEREST STATEMENT

None declared.

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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DIARY OF EVENTS

See also https://www.isbtweb.org/events/hvwebinars.html	
17–21 June 2023	ISBT Gothenburg 2023
18–21 November 2023	ISBT Cape Town 2023

