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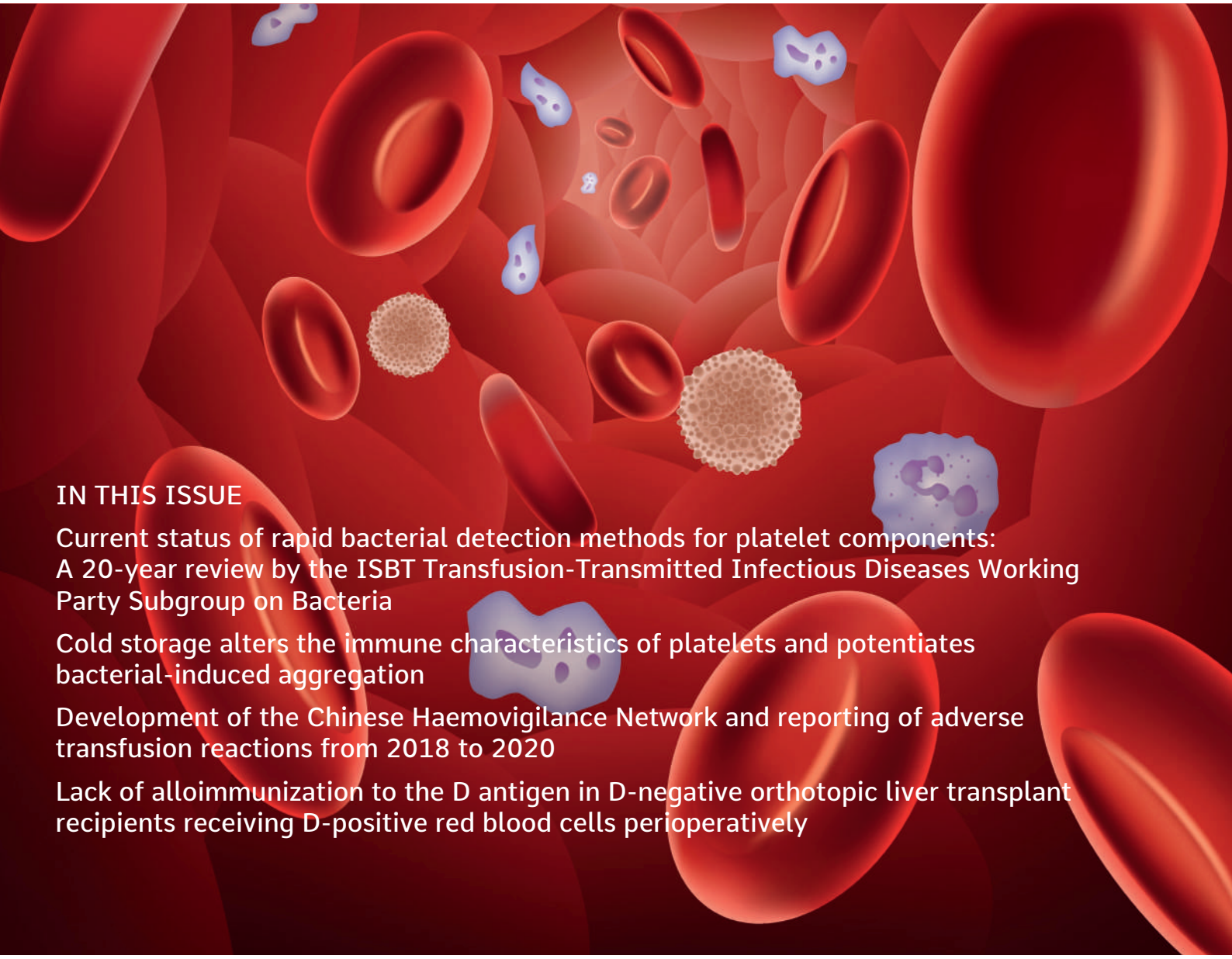

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Aims and Scope

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

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Current status of rapid bacterial detection methods for platelet components: A 20-year review by the ISBT Transfusion-Transmitted Infectious Diseases Working Party Subgroup on Bacteria

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Abstract

Background and Objectives: Bacterial contamination of platelet components (PCs) poses a safety challenge for transfusion patients. Despite mitigation interventions, the residual risk of transfusion-transmitted bacterial infections remains predominant. PC safety can be improved either by pathogen reduction or by implementation of bacterial detection methods. Detection methodologies include culture methods and rapid detection methods. The current review focuses on currently available rapid detection methods.

Materials and Methods: We reviewed published manuscripts since 2000 on rapid bacterial detection methods used for PC screening with result determination within 4 h. Methods meeting this criterion included Verax PGD*prime*, BacTx and nucleic amplification testing. The analytical and diagnostic sensitivity and specificity of these systems were assessed.

Results: The analytical sensitivity between the different detection methods ranged between 50 and 100,000 CFU/ml. The sample volume used by these testing systems varies between 0.5 and 1.0 ml of PCs. A delay of at least 48 h before sampling enhances detectability. All rapid detection methods generate results in a timely manner, allowing testing to be performed before transfusion with optimal sensitivity.

Conclusion: Rapid detection methods improve PC safety regarding bacterial contamination. The assays are optimal for rapidly growing bacteria, which are more likely to cause septic transfusion reactions in patients. Because of the reduced diagnostic sensitivity, the sample collection should be late in shelf-life and ideally just before transfusion. The major benefit of these methods is that the test result can be obtained before releasing PCs for transfusion or to be used in combination with other screening methods applied early during PC storage.

KEYWORDS
bacterial contamination, platelet components, rapid detection method

Highlights

Rapid bacterial detection methods can identify bacterial contaminated platelet products.

INTRODUCTION

The introduction of nucleic amplification testing (NAT) [1] for transfusion-transmitted viral infections (e.g., HBV, HCV and HIV-1) into blood donor screening has led to the current residual risk of such infections to less than 1:1 million [2, 3]. In contrast, the residual transfusion-transmitted risk for bacterial infections is calculated to be in the range between 1:2000 and 1:100,000 [4–6]. Although the introduction of first aliquot diversion of donated blood by most blood services was able to reduce the residual bacterial infection risk by 50%, there are fundamental differences to viral infections [7]. Bacteraemia usually causes fever in humans, and it is unlikely that a donor will be asymptomatic and able to donate. However, there could be donors with silent transient bacteraemia; for example, intensive tooth cleaning can induce short-term bacteraemia [8]. A major risk of bacterial contamination during donation comes from the donor's skin at venipuncture. Improved donor arm disinfection procedures have been implemented by most blood donor services in the developed world [9–13]. Bacteria differ from viruses in the fact that they are competent microorganisms able to multiply during blood component storage. Platelet component (PC) storage at 20–24°C, which is optimal for platelet functionality, presents an ideal environment for a broad range of bacterial species to proliferate. In addition to donor selection programmes (e.g., temporary exclusion of donors who have undergone endoscopy), first aliquot diversion and improved donor arm disinfection, blood safety can be improved by the implementation of bacterial testing or by the introduction of pathogen reduction (PR) systems [14, 15]. In contrast to culture methods with a maximum incubation time of 7 days, rapid detection methods are defined with a total test time of less than 4 h. The Food and Drug Administration (FDA) has recently issued guidelines to enhance PC safety in the United States [16]. The FDA recommendations include rapid testing methods for screening of whole-blood-derived PCs stored for a maximum of 5 days or in combination with primary culture for extension of the PC storage shelf-life up to 7 days [16]. This review focuses on the current status of rapid bacterial detection methods. All currently available methods are briefly described and compared regarding their analytical sensitivity, diagnostic specificity and implementation into routine blood donor screening. Figure 1 describes the timeline of development and implementation of rapid testing methods.

Rapid detection methods

Most bacterial detection assays focus on PCs, because the storage temperature of these blood components poses the greatest risk with regard to bacterial proliferation. The analytical sensitivity of the rapid detection methods ranges between 50 and 100,000 colony forming units (CFU)/ml. The testing time of rapid methods varies from 0.5 to 4.0 h. There are two methods that use the bacterial cell wall as a target for detection: Verax Pan Genera Detection (PGD and PDG*prime*) and BacTx assays. Rapid systems also include nucleic acid detection methods (NAT) and PC screening using flow cytometry, which can be used as stand-alone tests. A literature review was performed in

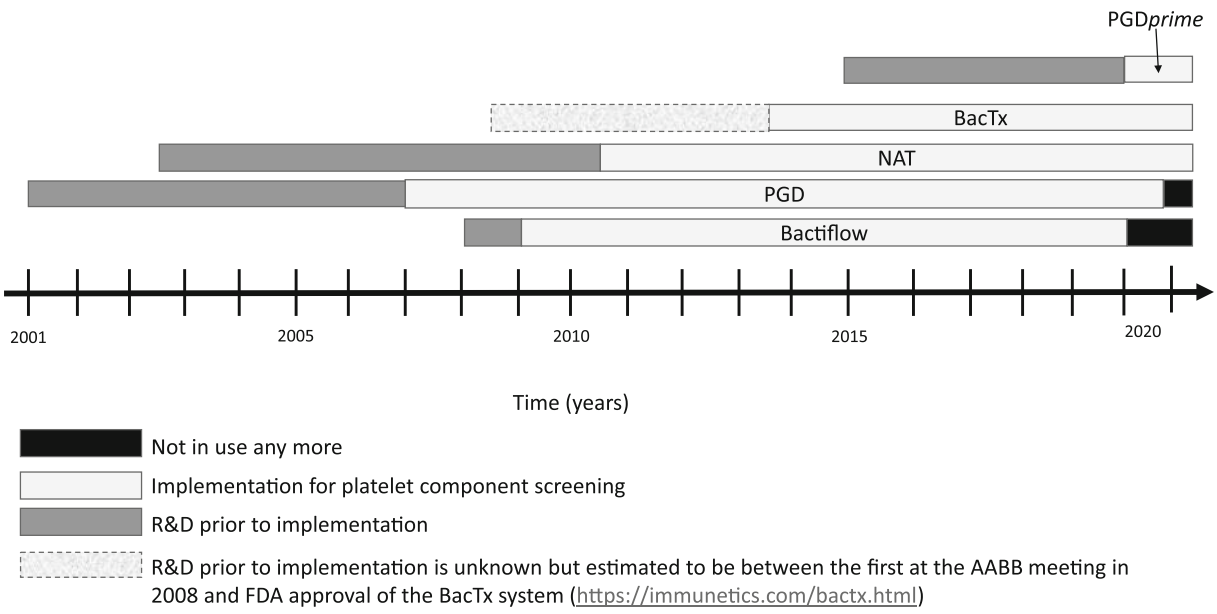

FIGURE 1 Timeline of development of rapid detection systems

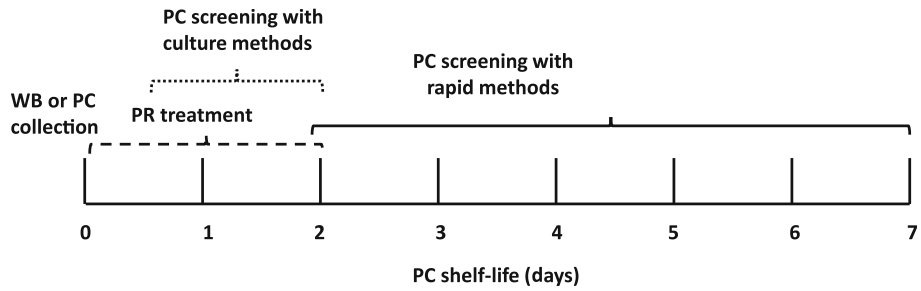

FIGURE 2 Timeline for application of platelet component (PC) safety strategies. PR, pathogen reduction; WB, whole blood

PubMed (https://pubmed.ncbi.nlm.nih.gov/) using the key words 'rapid method' + 'platelet screening' + 'bacteria contamination'. This review article focuses on methods that are currently licensed and in use in different jurisdictions, which include the PGD test, the BacTx bacterial detection kit for platelets and NAT tests. The flow-cytometry-based method BactiFlow [17] was used in Germany for several years but has been discontinued and therefore will not be discussed in this review. Figure 2 shows timeline of applicability of rapid testing methods in the PC shelf-life in comparison with PC culture methods and PR treatment.

Verax Pan Genera Detection (PGD and PGD*prime*) assays

These immunoassays detect the lipopolysaccharide component of Gram-negative bacteria and lipoteichoic acids of Gram-positive bacteria [18]. A negative PGD result requires PCs to be transfused within 4–24 h depending on the PC type and with certain products after prior screening by a culture method or in combination with primary PC culture as per the FDA recommendations to extend the PC shelf-life from 5 to 7 days in the United States [16]. PDG*prime* is the next-generation test system, which superseded the precedent PDG assay in mid-2021. To perform PC screening with the PGD*prime* test, 0.2 ml of the PC sample is mixed by inversion with six drops of Reagent 1A, as per manufacturer's instructions. The resulting yellow solution is then mixed by inversion with six drops of Reagent 1B. The solution should turn from pink to red to proceed with the test. The mix is then added to well 1 of the PDG*prime* device. Once the sample has flowed from one-fourth to three-fourths of the way across the GP/GN test result window, six drops of reagent 2 are added to well 2. After 25 min, the reaction can be read to confirm the following criteria: well 1 should be red but empty, well 2 should be white or light grey, and a strong pink/red line should be present in the procedural control line window. If these criteria are not met, the device should be checked every 5–10 min for a maximum of 45 min. Once the criteria have been met, testing results are read in the GP/GN test result window. Any pink/red line in this window is considered to be a positive result (i.e., reactive). If a reactive result is obtained, the test should be repeated for confirmation [19].

The platelet PDG*prime* assay has a simplified workflow, and shows increased sensitivity and specificity compared to those of the original test [18]. Enhanced limit of detection with the PGD*prime* test compared to the original PGD assay has been reported for *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis* [18]. The specificity of the PGD*prime* test was determined in different PC components and was found to be 99.9%–100% (lower one-sided 95% confidence limit [CI]) [18, 19]. More recently, an enhanced PGD*prime* test was developed to include recognition of *Acinetobacter* spp. because this bacterium has been implicated in several transfusion reactions in the United States in recent years, including one that was missed with the PGD test [20, 21]. A comprehensive review of the PGD assays has been recently published by Mintz and Vallejo [18].

BacTx bacterial detection kit for PCs

BacTx is a qualitative colorimetric method for bacterial detection in PCs, which targets detection of cell wall peptidoglycan of Gram-positive and Gram-negative bacteria [22, 23]. This rapid method can also be used alone or in combination with primary PC culture, according to the recommendations by the FDA [16]. To test PCs for the presence of bacterial peptidoglycan, a small volume (0.5 ml) is sampled from the PC bag and added to a microfuge tube containing 1 ml of a lysis reagent (aqueous solution containing sodium hydroxide, surfactant and *n*-butanol) and mixed by inversion. The microfuge tube is briefly centrifuged to pelletize insoluble PC debris and bacterial cell wall fragments, if present. The pellet is then homogenized in 0.5 ml of an extraction reagent (aqueous sodium hydroxide solution) by pipetting up and down 10 times. The alkaline extraction reagent effectively releases peptidoglycan from bacterial cell walls for optimal detection. Lastly, 0.5 ml of the suspension is added to a clean microfuge tube containing 0.5 ml of a neutralization reagent (4-morpholinopropanesulfonate [MOPS] solution) and the tube is mixed by inversion. From the resulting sample, 0.3 ml is added to a tube containing lyophilized detection reagents, the tube is vortexed for 3 s, and then placed in the BacTx analyser. The BacTx analyser is a photometer that automatically monitors the detection reaction and interprets the result using the software installed in the provided laptop. If bacteria are detected within the 30-min reading time, a 'Fail' result accompanied by an audible alarm is generated; otherwise, a 'Pass' result will be recorded. This test has a specificity of

99.8% (with a lower one-sided 95% CI of 99.0%) and an analytical sensitivity that ranges from approximately 10^3 CFU/ml for *Escherichia coli*, *Klebsiella oxytoca*, *S. epidermidis* and *Bacillus cereus* to 10^5 CFU/ml for *Streptococcus agalactiae*, *P. aeruginosa* and *Serratia marcescens* [24]. The BacTx test was evaluated in spiking studies, showing high specificity and reproducibility with limits of detection ranging from 10^2 to 10^4 CFU/ml for clinically relevant PC contaminants, including slow-growing and biofilm-forming species [25, 26]. There are no published reports on the routine use of the BacTx assay for PC screening.

Nucleic amplification testing

NAT is a universal screening method for the detection of bacterial DNA or ribosomal RNA. NAT testing of PC is based on real-time polymerase chain reaction (PCR). Important aspects need to be considered with implementing molecular methods, including the target gene(s), the type of assay, technical consideration for nucleic acid extraction method, and validation of results [27]. In general, low PC volumes (approximately 0.5 ml) are used for these assays, and nucleic acids are extracted on the basis of the test used and manufacturer's instructions (manually or using automated platforms). Universal bacterial detection can be performed targeting the 16s [25, 26] or 23s [28] ribosomal genes or other genes such as *rpoB* or *groEL* [28]. Quality control of these tests is very important and is usually performed using culture-negative PC. Dreier et al. reported the development of two RT-PCR assays for bacterial detection in PCs [28]. They introduced reagent treatment with 8-methoxypsoralen and UV irradiation to reduce contamination of the DNA. The authors noted that the assay sensitivity depended on the amplification enzyme used. r*Tth* DNA polymerase allowed the detection of 500 CFU/ml of *E. coli* or *S. epidermidis*, and the sensitivity improved to the detection of 16 CFU/ml of these organisms when a combination of two enzymes (Moloney murine leukaemia virus RT and *Taq* DNA polymerase) was used. In the same study, the *groEL* gene allowed the detection of 125 CFU/ml of *E. coli*, and no cross-contamination issues with human DNA were found when using this target gene. Another important aspect of RT-PCR assays is to demonstrate bacterial viability, which was addressed in this study by the amplification of mRNA. A NAT method was approved by the Paul Ehrlich Institute and accepted as a release test for individual donations, mini-pools of 5, or mini-pools of 10 samples in Germany [29]. During the development of this method, 1 ml of PC spiked with bacteria was processed for real-time PCR amplification of the 16 s ribosomal gene. The analytical sensitivity was evaluated with the bacteria repository standards *Klebsiella pneumoniae* PEI-B-08-09, *S. epidermidis* PEI-B-13-03, *S. aureus* PEI-B-23-07, *Streptococcus pyogenes* PEI-B-20-05, *B. cereus* PEI-B-07-23 [30] and *S. marcescens* ATCC 43862. Each bacterial strain was tested in seven different concentrations (0.1–64 CFU/ml). The 95% level of detection (LOD) was calculated using probit analysis with SPSS version 32. The mean 95% LOD was 13 $\pm$ 4.94 CFU/ml. This method was first used from 2010 to 2015, and has been in use again since 2021 in routine bases (i.e., for daily release of PCs) by the German Red Cross Baden-Wuerttemberg—Hesse. Dreier et al. have given a summary of the application of PCR or RT-PCR to detect bacterial contamination in different blood components including whole blood, red blood cell concentrates and PCs [27]. They concluded that NAT assays have great potential to screen blood components but require stringent method standardization and comparability to other PC screening systems. More recently, a Brazilian team reported the optimization of an RT-PCR method to detect bacterial contamination in PCs [26]. In this protocol, a bacterial ribosomal 16s gene sequence representing common PC contaminants, including *Cutibacterium acnes*, was targeted, and the method was optimized to avoid non-specific background noise by treating the PCR master mix with ethidium monoazide [31]. The whole assay takes 4 h to completion with a limit of detection of 10 genome equivalents. The method was shown to be specific when used to test 50 PC pools, which tested negative for bacterial contamination.

Although NAT methods have been mainly developed for universal bacterial detection, assays for specific organisms have also been developed. Mastronardi and Ramirez-Arcos optimized an RT-PCR assay targeting the cell division *divIVA* gene for detection of the predominant PC contaminant *S. epidermidis* [32]. Limitations of NAT assays for PC screening are related to potential contamination of assay reagents, which affects specificity and sensitivity, feasibility for routine implementation and cost of the assays. At present, there are no commercial generic bacterial NAT assays available to the blood community.

DISCUSSION

PC screening methods can be subdivided into culture methods and rapid detection methods. A rapid bacterial detection method is expected to achieve the final test result within 4 h. An ideal rapid bacterial detection system should have high diagnostic sensitivity and high diagnostic specificity. With bacterial culture, to reduce the risk of non-detection, a holding period of 24–48 h can be provided to allow bacteria to proliferate to a sufficient number to be captured in the test sample. Countries using culture methods with the 'negative-to-date' concept have been able to reduce the risk for false-negative results and detect most extremely 'pathogenic' bacteria before transfusion [33–37]. Rapid detection methods such as NAT, PGD and BacTx recommend performing the tests later in the PC shelf-life to enable bacteria in a contaminated PC to grow up to concentrations in the order of the detection sensitivity for rapid assays, which is lower than in culture methods. For example, NAT methods implemented in Germany are used after a PC holding period of 48–72 h [32, 38–40]. NAT can be used for individual donation testing as well as in small mini-pools of up to 10 PC units per pool and is appropriate for buffy-coat-derived pooled PCs as well as for apheresis PCs.

The 'negative-to-date' concept for bacterial culture screening is utilized with automated microbial detection systems and implemented in many countries throughout the world [35–38, 41]. Samples are taken from PCs, inoculated into both an aerobic and an anaerobic culture bottle, and incubated for 7 days. Re-calls are made if a reactive

culture result occurs. The American Red Cross reported a reduction in septic transfusion reactions by 50% when implementing bacterial culture screening of PCs at 24 h after donation and using only an aerobic culture bottle and low fill volume of 4 ml. However, a negative aspect of the 'negative-to-date' release criterion is that units may be transfused before positive detection with an automated system [36, 38]. The FDA guidance for industry has recommendations to enhance detection with culture methods [16]. Large-volume culture of 8 ml per culture bottle, the use of aerobic and anaerobic culture, and a delay in sampling to 36–48 h from donation have shown to increase detection and significantly reduce the number of septic transfusion cases reported by passive surveillance to fewer than 1 in a million [36, 38].

The PGD*prime* and BacTx methods can be used as point-of-care tests before transfusion. This may shift the responsibility and implementation in many countries from the blood collection facility to the hospital. Trained technicians will be required to perform these tests. The advantage of a point-of-care test is that it can be used close to the time of transfusion, which is the most appropriate time to test for bacterial contamination. This late test time can then partially compensate for the reduced sensitivity. NAT assays are highly specific and sensitive; however, these methods require robust validation and pose difficulties for routine implementation due to requirements of special equipment.

In addition to general methods for blood safety regarding bacterial transmissions, such as donor selection programmes, efficient donor arm disinfections and the introduction of diversion, rapid bacterial detection methods are now available and able to improve blood component safety. This literature review shows that not all experimental approaches, that is, BacTx, have been implemented into routine diagnostics. The variety of methodological approaches shows once again the complexity of bacterial screening in which each method has advantages but also disadvantages. Rapid screening methods have a potential to be used as point-of-care tests, to extend PC shelf-life, or to supplement culture methods.

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CONFLICT OF INTEREST

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

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REVIEW

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Comparative evaluation of efficacy and safety of automated versus manual red cell exchange in sickle cell disease: A systematic review and meta-analysis

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Abstract

Background and Objectives: Exchange transfusion is a valuable treatment option in sickle cell disease (SCD) and is preferred over simple transfusion as it removes abnormal haemoglobin S (HbS) levels and reduces complications. This meta-analysis aims to evaluate the efficacy and safety profile of automated red cell exchange (aRBX) procedure over manual red cell exchange transfusion (MET) in SCD patients.

Materials and Methods: A standard meta-analysis protocol was developed, and after performing a comprehensive literature search in PubMed/MEDLINE, Cochrane and International Clinical Trial Registry Platform (ICTRP), reviewers assessed eligibility and extracted data from nine relevant studies. A random effects model was used to estimate the pooled effect size calculated from the mean difference in HbS percentage, serum ferritin level and risk ratio for the adverse events. Quality assessment was done using the risk-of-bias assessment tool, and a summary of observations was prepared using standard Cochrane methodology with GradePro GDT.

Results: The random-model analysis revealed a mean difference of 4.10 (95% CI: −3.29–11.49; $Z = 1.09$; $p = 0.28$) for HbS percentage, mean difference of 435.29 (95% CI: −73.74–944.32; $Z = 1.68$; $p = 0.09$) for serum ferritin and pooled risk ratio of 1.35 (95% CI: 0.63–2.87; $Z = 0.77$; $p = 0.44$) for adverse events.

Conclusion: This meta-analysis did not reveal any significant benefit of aRBX in reducing HbS percentage and attenuating the serum ferritin level when compared with MET. There was also no significant increased risk of adverse events detected in association with aRBX.

KEYWORDS
apheresis: therapeutic, patient blood management, transfusion reactions, transfusion strategy: red cell components, transfusion therapy

Highlights

- Sickle cell disease (SCD) is a haematological disorder with autosomal recessive inheritance.
- Exchange transfusion is a life-saving treatment modality in vaso-occlusive crisis due to SCD.
- The efficacy and safety profile of automated versus manual exchange procedures have been hardly recognized in the literature.

INTRODUCTION

Sickle cell disease (SCD) is one of the most common inherited genetic disorders caused by a single point mutation of the β-globin gene that produces haemoglobin S (HbS) [1]. This abnormal HbS leads to many acute complications such as stroke, acute chest syndrome (ACS), vaso-occlusive crisis (VOC), splenic sequestration and severe infection. Patients with SCD also have some chronic complications such as pulmonary hypertension, retinopathy, nephropathy and sometimes osteonecrosis [1, 2].

Presently, three major therapeutic modalities are available: blood transfusion, administration of hydroxyurea and bone marrow transplantation [3]. Although hydroxyurea has shown clinically proven efficacy with significant improvement of VOC and ACS, many breakthrough trials have shown that chronic transfusion therapy can prevent stroke, reducing the frequency of vaso-occlusive pain crisis and acute chest syndrome–related hospitalizations as well as other chronic complications related to SCD [4–6]. Blood transfusion in SCD mainly aims to correct anaemia and improve the oxygen-carrying capacity, as transfusion of normal red blood cells (RBCs) carrying normal haemoglobin A (HbA) dilutes the circulating level of sickled HbS, thus diminishing impaired erythropoiesis, haemolysis and vaso-occlusive events, which are major contributors to the complications of SCD [7, 8].

There are three different modalities of RBC transfusion in SCD: simple transfusion (ST), manual exchange transfusion (MET) and automated red cell exchange (aRBX) transfusion, also termed erythrocytapheresis [9]. In exchange transfusion, the sickled RBCs in SCD are removed and replaced with normal donor RBCs. Exchange transfusion is preferred over ST, as it maintains the HbS level within target ranges or even rapidly decreases the level of HbS, prevents iron overload and volume overload, and also minimizes the rise in blood viscosity [8, 10]. In MET, whole blood is phlebotomized immediately before transfusion, and the net iron load is reduced by more than 15% when compared to ST. MET is advocated if there is a lack of high-flow venous access, patients have low body weight (less than 25 kg) and the setting is resource-constrained [7]. However, unlike MET, aRBX requires specialized equipment called apheresis, as well as expertise. Automated exchange is a faster procedure with continuous fluid balance but requires good venous access and the use of anticoagulation. In aRBX, the net iron load can be reduced further by modulating the post-exchange target haematocrit (Hct) [9, 11, 12]. It also increases RBC exposure, as the number of RBCs needed for each procedure is higher than in MET and possibly increases the risk of alloimmunization [10].

Presently, evidence from observational studies and clinical trials exploring the efficacy of aRBX over MET in attenuating the post-exchange sickled HbS as well as reducing the iron overload is limited. Moreover, the safety profile of aRBX over MET is also contradictory [8, 10, 13]. Hence, it is important to generate evidence regarding the magnitude of the efficacy and safety of aRBX in SCD patients for framing therapeutic guidelines on red cell exchange procedures. So, the present meta-analysis has been planned to evaluate the efficacy, safety and tolerability profile of aRBX as one of the treatment modalities in SCD.

MATERIALS AND METHODS

Development and registration of protocol

A standard meta-analysis protocol for systematic reviews and meta-analysis was registered in the International Prospective Register of Ongoing Systematic Reviews (PROSPERO Registration Number: CRD42021268069) [14–16]. The protocol of the meta-analysis was exempted from full review and approved by the Institutional Ethics Committee, All India Institute of Medical Sciences (AIIMS), Bhubaneswar, as per the Indian Council of Medical Research (ICMR) 2017 guideline, on 21 April 2018.

Literature search

A systematic literature search was performed independently by four review authors (S.M., A.S., S.P. and G.K.R.) using PubMed/MEDLINE, Cochrane and the WHO International Clinical Trials Registry Platform (ICTRP) databases for prospective clinical trials and observational studies on the efficacy and safety of aRBX procedure in reducing HbS percentage and attenuation of iron overload in terms of serum ferritin in comparison to MET procedure till May 2021. Only studies published in English language were included for the meta-analysis; however, the search strategy was not restricted by the date of publication. PICO scheme was followed for reporting the inclusion criteria. Key elements that we used in our search using MESH terms were the 'P' (anaemia, sickle cell/sickle cell disease/disease, haemoglobin S/cell disorder, sickle/HBS disease); the 'I' (Exchange transfusion, whole blood/Cytapheresis, Instrumentation/Cytapheresis, methods/Erythrocyte transfusion, methods/Erythrocyte transfusion, therapy/Erythrocyte transfusion, automation); the 'C' (Manual exchange, Erythrocyte transfusion/Erythrocyte transfusion/whole blood/Exchange transfusion, whole blood) and the 'O' (Efficacy/percentage decrease in Sickled Haemoglobin [HBS]/decrease in serum ferritin/tolerability and safety profile in terms of any complications or adverse events associated with aRBX procedure [e.g., hypocalcemia, hypotension, vasovagal attack]), and complications related to venous access (e.g., catheter flow obstruction, venous port infection, or thrombosis), alloimmunization, or any other technical concerns.

Study selection criteria

Types of studies

Prospective clinical trials and observational studies that evaluated the effect of red cell exchange in decreasing the HbS percentage in SCD as a primary outcome were included in this meta-analysis. Review articles, letters to the editor, comments, case series, case reports and

studies in which it was impossible to retrieve or calculate data of interest were excluded from this review.

Types of participants

Both paediatric and adult patients of the age group range 4–66 years of both sexes with a history of acute SCD complications such as stroke, ACS and VOC; history of chronic SCD complications such as primary and secondary stroke; recurrent acute complications such as VOC, ACS, or priapism, fulfilling as criteria for indication of chronic RBC exchange transfusion in preventing these episodes as per the American Society for Apheresis (ASFA) guideline were included in this review [17]. SCD patients who required exchange transfusion as a pre-operative measure of a major surgery to reduce the HbS percentage were also included in the study.

The exclusion criteria included SCD patients who were only on an ST regime and not indicated for red cell exchange transfusion as per the ASFA guideline.

Types of interventions

Experimental intervention: aRBX procedure performed on SCD patients in different clinical conditions as indicated by the ASFA guideline.

Control intervention: MET performed on SCD patients as per the same indication mentioned in the ASFA guideline.

Types of outcome measures

1. Primary outcome
   a. Change in the percentage of reduction of HBS
2. Secondary outcomes
   a. Change in serum ferritin level
   b. Complications related to the procedure, especially during aRBX such as vasovagal syncope/attack, hypocalcaemia, hypotension and hypothermia and catheter-related adverse events
3. Rate of RBC alloimmunization

Data extraction and management

We contacted authors of the studies to resolve any uncertainties and asked for additional unpublished data for this meta-analysis. Extracted data included the following:

1. Publication type and source
2. Trial design including timing, follow-up, sequence generation and allocation concealment
3. Setting, including the country, and the level of care
4. Participants satisfying the selection criteria and number of dropouts
5. Interventions including the number of procedures/sessions performed for each type of red cell exchange (i.e., MET vs. aRBX), time between the procedures, route of administration and duration of treatment
6. Outcome measures as specified above

Data analysis

This meta-analysis was conducted using the Cochrane Program Review Manager Version 5.3 and the 'meta' package of R programming (Version 4.1.0) [18].

The mean difference and risk ratio were calculated to estimate the effect size for continuous and categorical variables, respectively. The random effects model was used for between-group analyses, irrespective of heterogeneity between individual sample sizes. The outcome was depicted as a point estimate with 95% confidence interval. Chi-squared test was used to assess whether the observed differences in results were compatible with chance alone. I^2 statistics, which describes the percentage of the variability in effect that is due to heterogeneity, was performed for quantifying inconsistency.

In case of significant heterogeneity, the result was further investigated by performing sensitivity analysis to test the robustness of the results obtained in the present meta-analysis. We also performed a subgroup analysis between the studies, as the effect size may be potentially modified because of inclusion of both observational studies and clinical trials. We constructed funnel plots and performed Egger regression test and Begg and Mazumdar rank correlation as a quantitative test for publication bias. Standard Cochrane methodology and the GRADE Working Group guidance were followed to create a 'Summary of findings' table, and five grade considerations (risk of bias, consistency, imprecision, indirectness and publication bias) of the included methods and results of the included studies were considered to conclude the certainty of the evidence for each outcome [19]. We described the risk of bias for observational studies and for randomized controlled trials and judged them as low moderate, serious, critical and low, some concerns and high.

RESULTS

Description of included studies

The database searches identified 483 publications, which were reduced to 460 after duplicates were removed. These publications were screened by title and abstract for eligibility, and 444 studies were excluded from the study. The reasons for exclusion were as follows: review articles, case reports, letters to the editor, practice guidelines, literature in Spanish, French, German and Dutch, and studies unrelated to the subject of the present meta-analysis. The results of the searching and screening process are shown in the PRISMA flow chart (Figure 1). After screening, 16 studies were retrieved for full-text assessment. Finally, nine full-text articles were included in the meta-

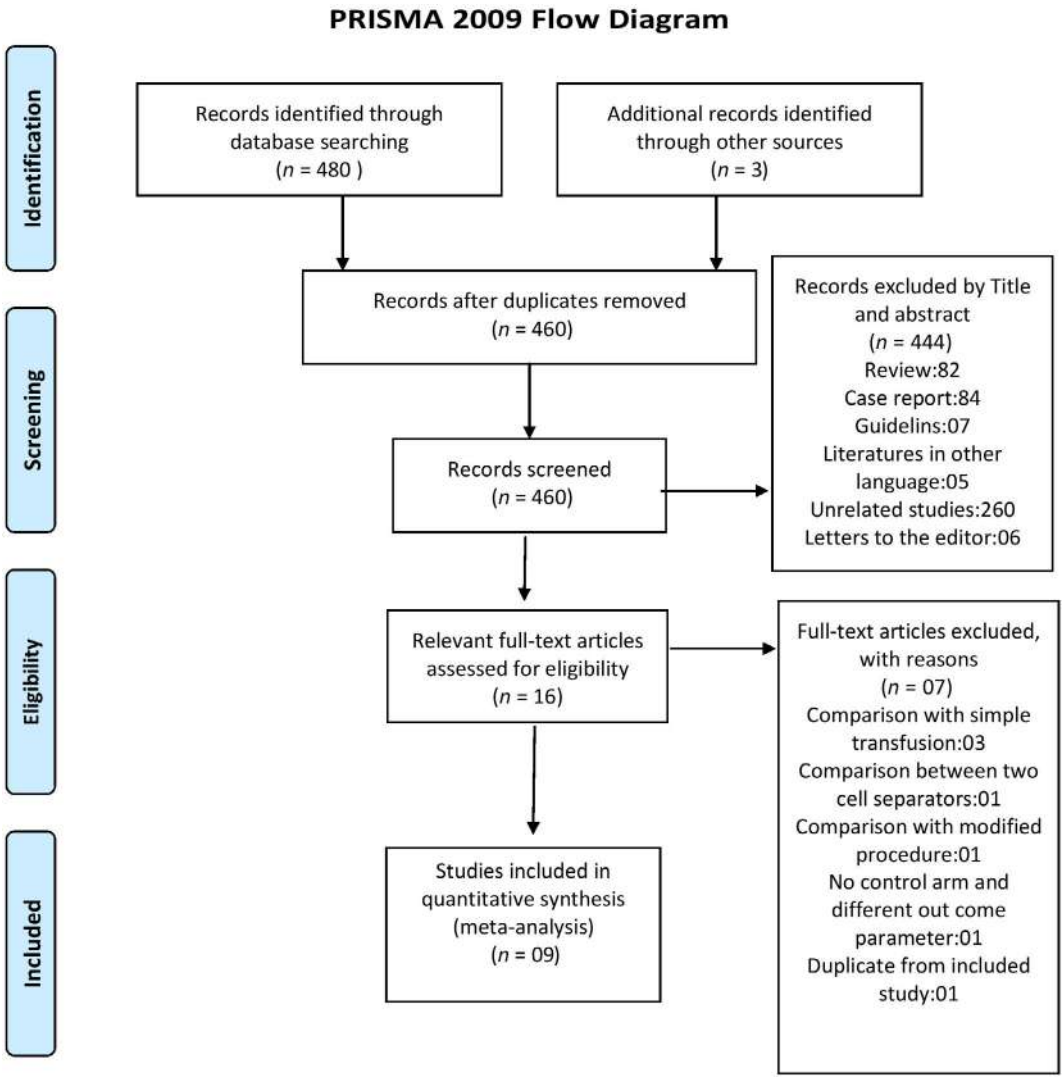

FIGURE 1 Study identification and selection process as per PRISMA guideline

analysis [3, 7–10, 13, 20–22]. Out of these included studies, eight studies were either prospective or retrospective observational studies [3, 7–10, 13, 21, 22] and one study was a randomized controlled trial [20]. The details of the included studies are described in Table S1. The remaining seven full-text articles [23–29] were excluded; three of them [23, 28, 29] compared aRBX with ST; one study [26] compared the effect of aRBX in SCD with two different cell separators (Spectra OPTIA vs. COBE Spectra); another study [27] compared conventional RBC exchange with the modified procedure of isovolumic haemodilution; another one [25] had no control arm as a comparator and a different outcome parameter; and one study [24] was duplicate of an already included study (Table S2). Assessment of the risk of bias of all included studies was separately performed for data of randomized controlled trials and observational studies with the help of the R programming in the 'robvis' package, that is, ROB2 and ROBINS-I, respectively. The risk-of-bias plot was produced by the 'robvis' package via the 'rob_traffic_light' function for better understanding, and is shown in Figure 2a,b.

Effect of intervention

The effect of aRBX compared with MET in SCD patients on the reduction of HbS level, decrease of serum ferritin level, and other complications associated with the procedures, such as catheter-related complications, vasovagal attack, hypotension, hypocalcaemia and any other complications observed in the included literature, was evaluated in this meta-analysis. The effect sizes of the included studies were compared using the Cochrane Program Review Manager, version 5.4 using a random effects model.

HBS level

Six studies [7, 8, 10, 13, 20, 22] from the included literature described the primary outcome, that is, change in the reduction of HbS percentage. The mean and standard deviation from median and inter-quartile range were determined with the on-line calculator

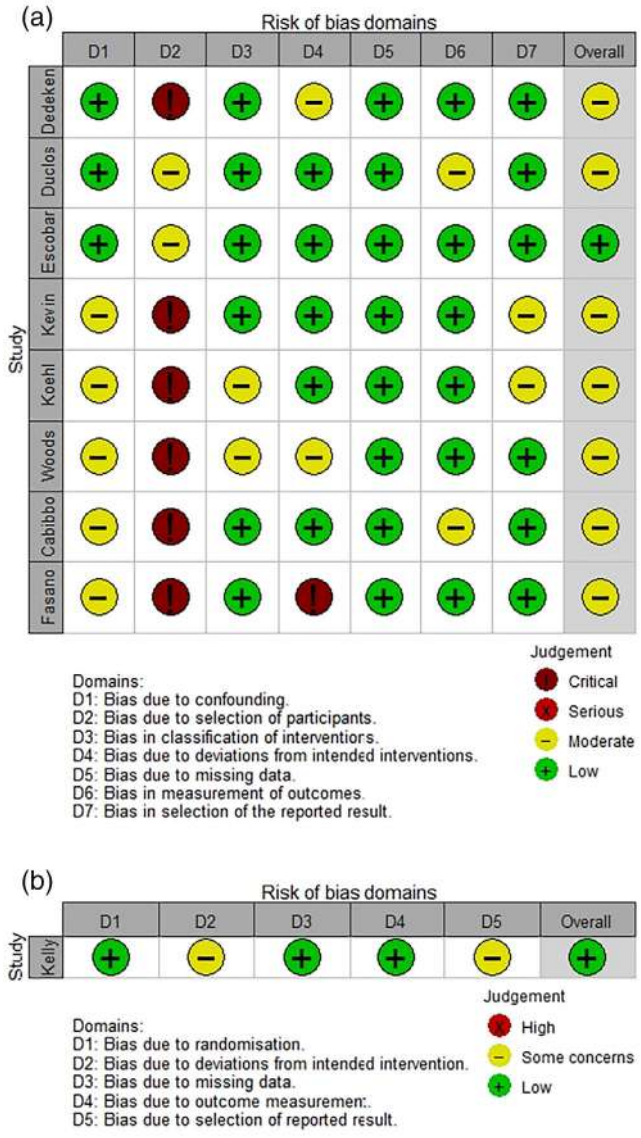

FIGURE 2 Risk-of-bias graph: (a) observational study, (b) clinical trial

formulated and described by Luo and Wan et al. [30, 31]. The test of heterogeneity was 45% and the *p*-value was not significant. The random model analysis of the studies did not find any significant reduction of the HbS value in the aRBX group (Figure 3a). Despite low heterogeneity, we excluded the study by Kelly et al. [20], as their study included data retrospectively from a randomized trial [20]. We observed a significant mean difference of HbS of 5.72 (95% CI: 0.48–10.95; $Z = 2.14$; $p = 0.03$), favouring the higher reduction of HbS percentage in the aRBX group (Figure 3b). Moreover, the heterogeneity of the studies was also reduced considerably to 11%. The study by Escobar et al. [8] had shown a significant reduction of HbS percentage in the aRBX arm. After excluding this study, the heterogeneity had dropped significantly to 9%, but, overall, the effect size remained non-significant (Figure 3c). Subgroup analysis comparing observational study with the clinical trial to detect the impact of clinical trial over observational study was also significant, as shown in Figure 3d.

Serum ferritin level

Six studies from the included records described the change in serum ferritin level [3, 7, 9, 10, 20, 21]. The test of heterogeneity was significant (heterogeneity: $\chi^2 = 75.91$, $df = 5$ [$p < 0.00001$]; $I^2 = 93\%$; $n = 175$). The random effects model analysis of these studies indicated a trend of serum ferritin reduction towards the aRBX group as compared to those treated with MET despite no statistical significance in the pooled effect size (Figure 4a). Sensitivity analysis was performed in view of significant heterogeneity. The heterogeneity was nullified, and the pooled mean difference became significant after removing the study by Kelly et al. [20], indicating a significant reduction of serum ferritin in the aRBX group (Figure 4b). The heterogeneity and the pooled effect size were not significant following exclusion of the other studies. Further, subgroup analysis by comparing the observational studies with the clinical trial by Kelly et al. [20] was significant (Figure 4c).

Adverse events

We included seven studies from the records and calculated the risk ratio of the adverse events in our meta-analysis [3, 7, 8, 10, 13, 21, 22]. The test of heterogeneity was not significant and random effects model analysis revealed a pooled risk ratio of 1.35 (95% CI: 0.63–2.87; $Z = 0.77$; $p = 0.44$), indicating that adverse events were not significantly associated with aRBX. We did not perform sensitivity analysis because there was no significant heterogeneity observed among the studies (Figure 5a).

Procedure-related adverse events

We analysed the adverse events that were specifically related to the red cell exchange procedure, and the test of heterogeneity showed its almost absence (heterogeneity: $\chi^2 = 5.13$, $df = 5$ ($p = 0.40$); $I^2 = 3\%$; $n = 33$). The pooled risk ratio of this random analysis was 2.27 (95% CI: 0.99–5.19; $Z = 1.94$; $p = 0.05$), showing a trend towards aRBX, as we observed a higher number of procedural complications with aRBX (Figure 5b). Although the heterogeneity among the studies was very small, we still performed sensitivity analysis after excluding the study by Escobar et al. [8], as this study did not mention any procedural complications with aRBX. We noted nil heterogeneity (heterogeneity: $\chi^2 = 1.83$, $df = 4$ ($p = 0.77$); $I^2 = 0\%$; $n = 29$) following sensitivity analysis and with the significant pooled risk ratio of 2.8 (95% CI: 1.22–6.51; $Z = 2.42$; $p = 0.02$), indicating that a significantly high number of procedure-related adverse events are encountered with aRBX compared to MET (Figure 5c).

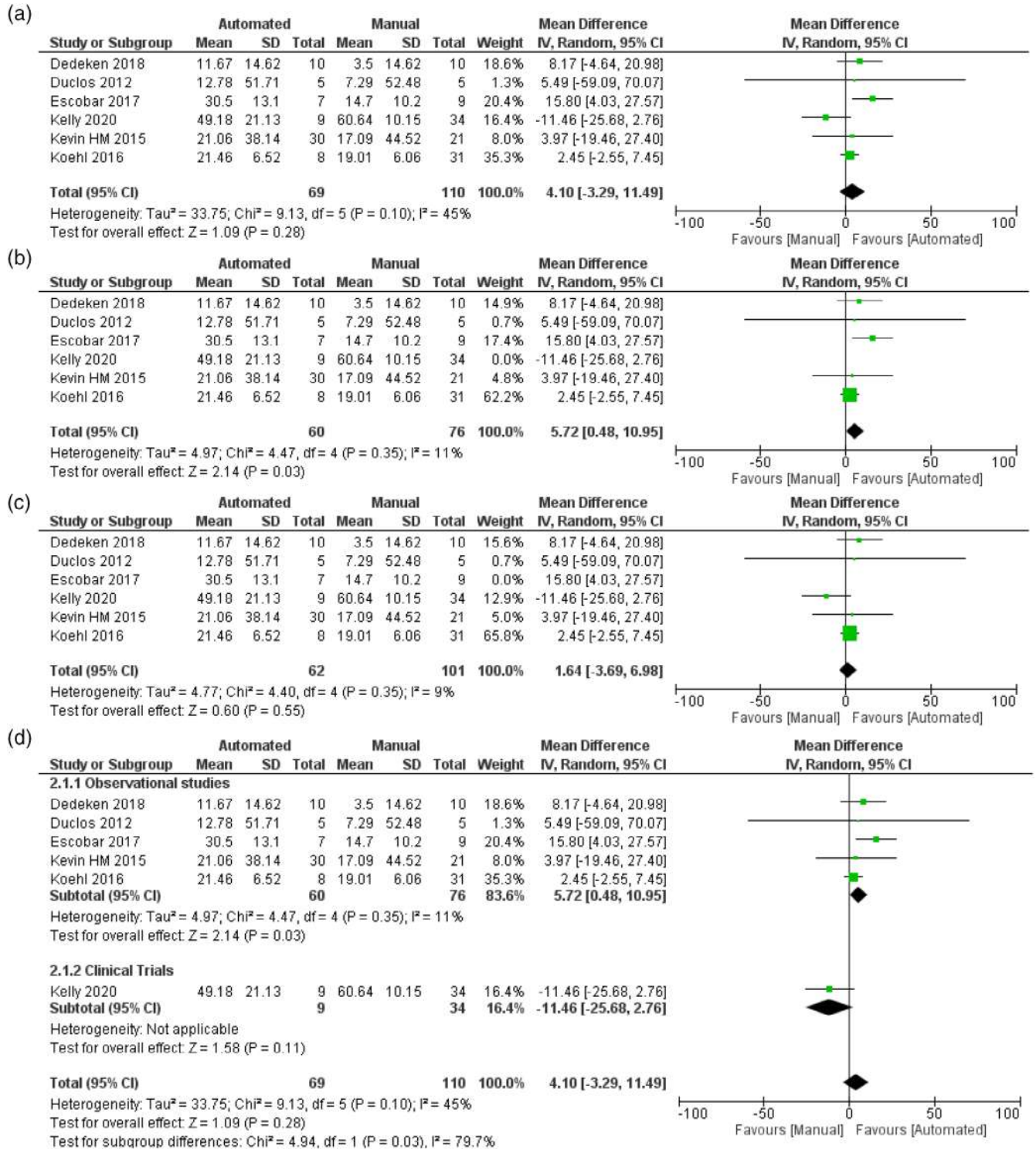

FIGURE 3 Forest plot illustrating the mean difference of change in HbS level in automated versus manual red cell exchange procedure (a), after sensitivity analysis excluding Kelly et al. (b), after sensitivity analysis excluding Escobar et al. (c), and subgroup analysis for observational studies and clinical trial (d)

Catheter-related adverse events

We separately analysed catheter-related adverse events, especially infection, blockage, or thrombosis, due to the intervention with aRBX or MET. We identified only three studies that described catheter- or venous access-related complications [8, 21, 22]. Out of these three studies, the data from Woods et al. [21] were not suitable for meta-analysis because there were no catheter-related adverse events in the MET group. The random effects model revealed a high heterogeneity among the studies (heterogeneity: $\chi^2 = 2.77$, $df = 1$ ($p = 0.10$);

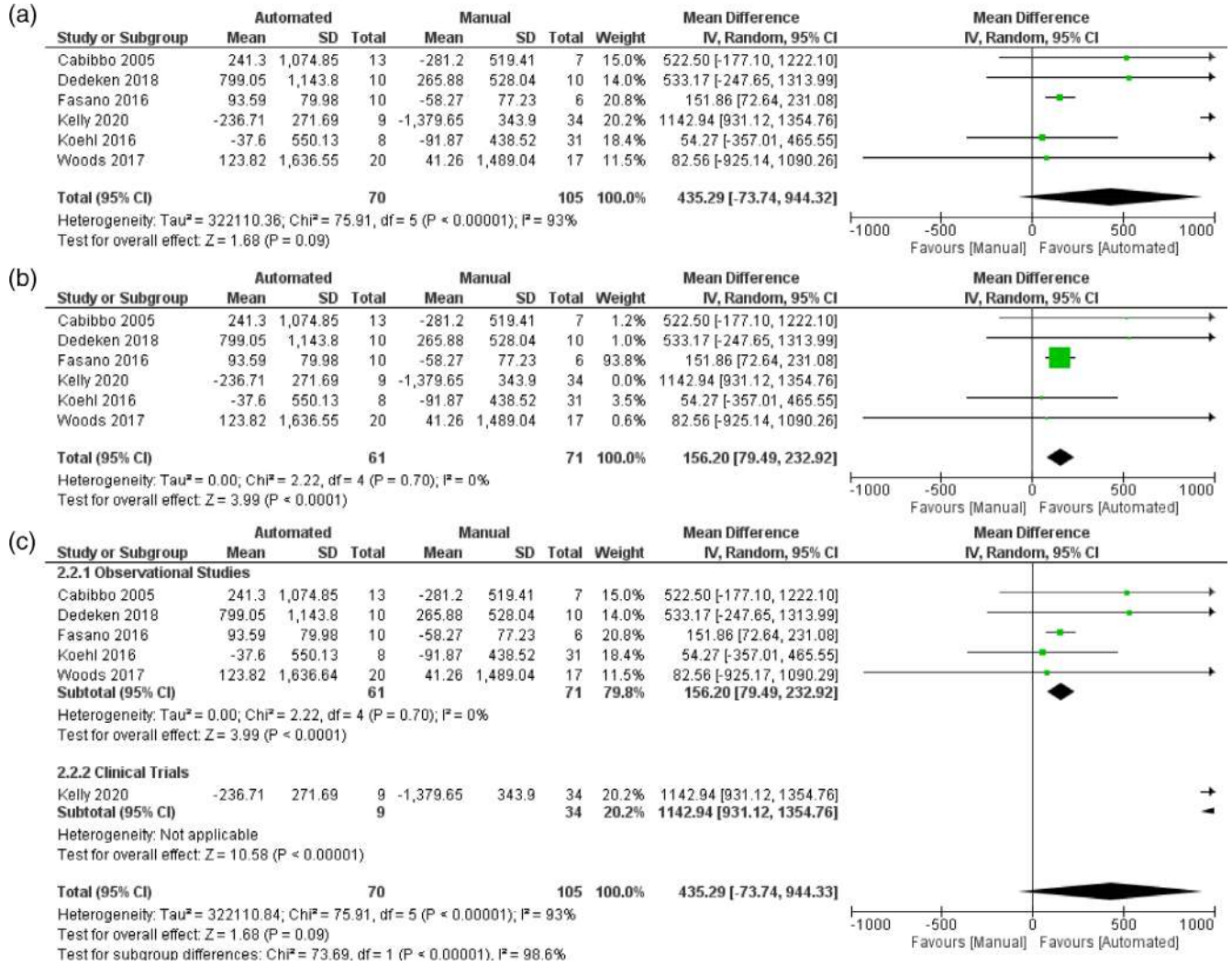

FIGURE 4 Forest plot comparing mean difference in serum ferritin level in automated versus manual red cell exchange procedure (a), after sensitivity analysis excluding Kelly et al. (b), and subgroup analysis for observational studies and clinical trial (c)

$I^2 = 64\%$; $n = 58$) but it was not significant. The pooled risk ratio of these studies was 0.61 (95% CI: 0.18–2.14; $Z = 0.77$; $p = 0.44$), showing no significant increase in the risk of these complications with the erythrocytapheresis procedure (Figure 5d).

Publication bias in included studies

There was no obvious publication bias within the review process as seen from the funnel plot (Figure 6), which was almost symmetrical. The assessment of publication bias using the Begg and Mazumdar rank correlation test showed Kendall's tau value of 0.164 (with continuity correction) with a two-tailed *p*-value of 0.87, which is not significant.

Certainty of evidence

Details of the effect estimates and GRADE ratings are summarized in Table S3. Compared with the control, the certainty of evidence was found to be high for HbS percentage reduction. Therefore, we are very confident that the estimated effect and the true effect lie very close. For serum ferritin level attenuation, the certainty of evidence was found to be low, suggesting that our confidence in the effect estimate was limited and that the true effect might be substantially different from the estimate of the effect.

DISCUSSION

Blood transfusion is the mainstay of therapy in preventing complications, especially primary and secondary strokes, in SCD patients [32]. However, the optimal transfusion remains to be determined. More importantly, the comparison between manual and aRBX and their benefit in SCD in preventing complications have been hardly recognized in the literature. In this meta-analysis, we tried to perform a comparative evaluation of the efficacy of aRBX over MET in attenuating the post-exchange sickled HbS and reducing the iron overload, as well as the safety profile in SCD.

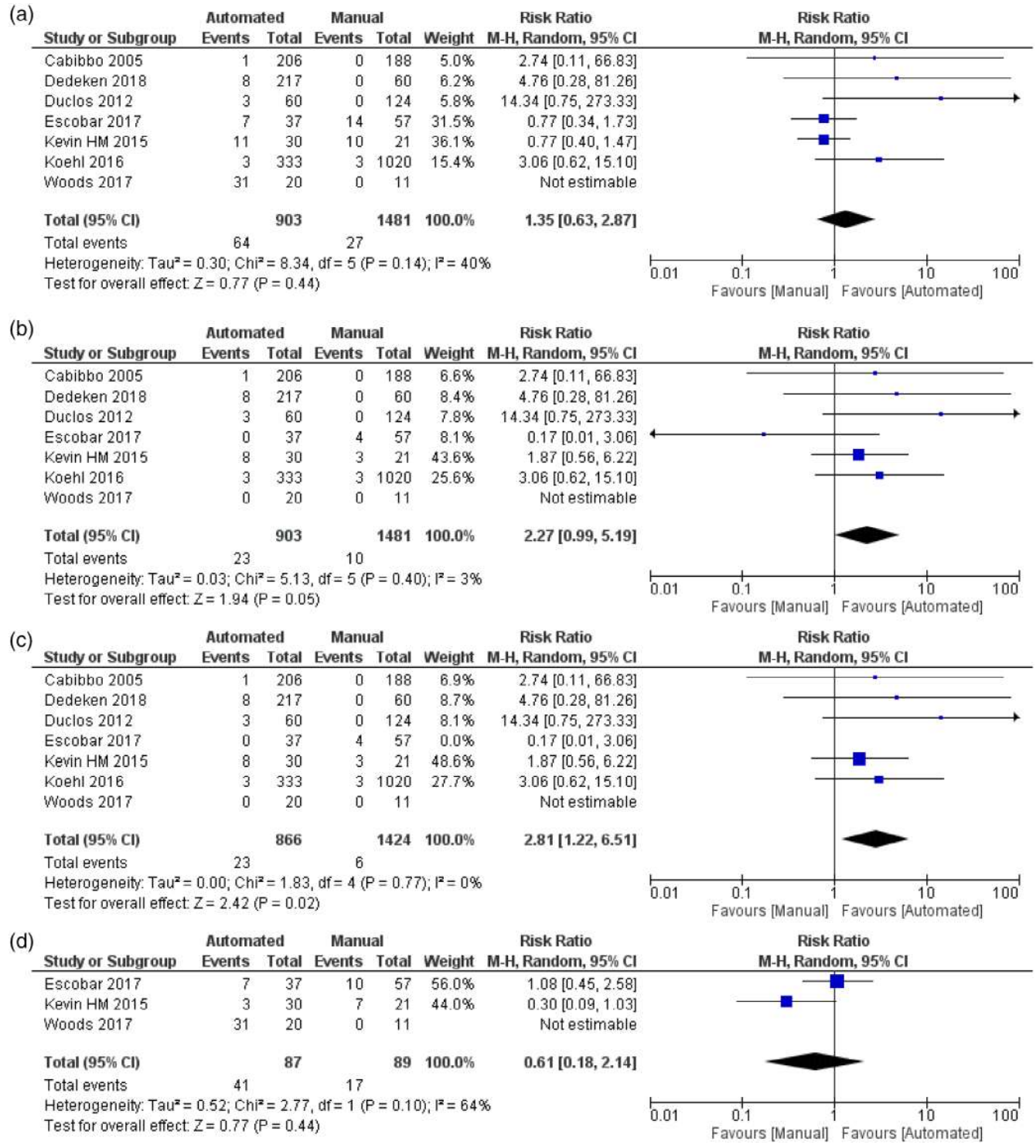

FIGURE 5 Forest plot comparing risk ratio of total adverse events in automated versus manual red cell exchange procedure (a), comparison of the risk ratio of procedure-related adverse events in automated versus manual red cell exchange procedure (b), after sensitivity analysis excluding Escobar et al. (c), and comparison of the risk ratio of catheter-related adverse events in automated versus manual red cell exchange procedure (d)

Percentage HbS level

The result of the present meta-analysis showed no significant reduction in HbS level in the aRBX group [7, 8, 10, 13, 20, 22]. When the result was compared with those of individual studies in the meta-analysis, the trend of the individual study showed HbS reduction towards the aRBX arm, except in the study by Kelly et al. [20], which showed less reduction of HbS in the aRBX arm compared to MET. However,

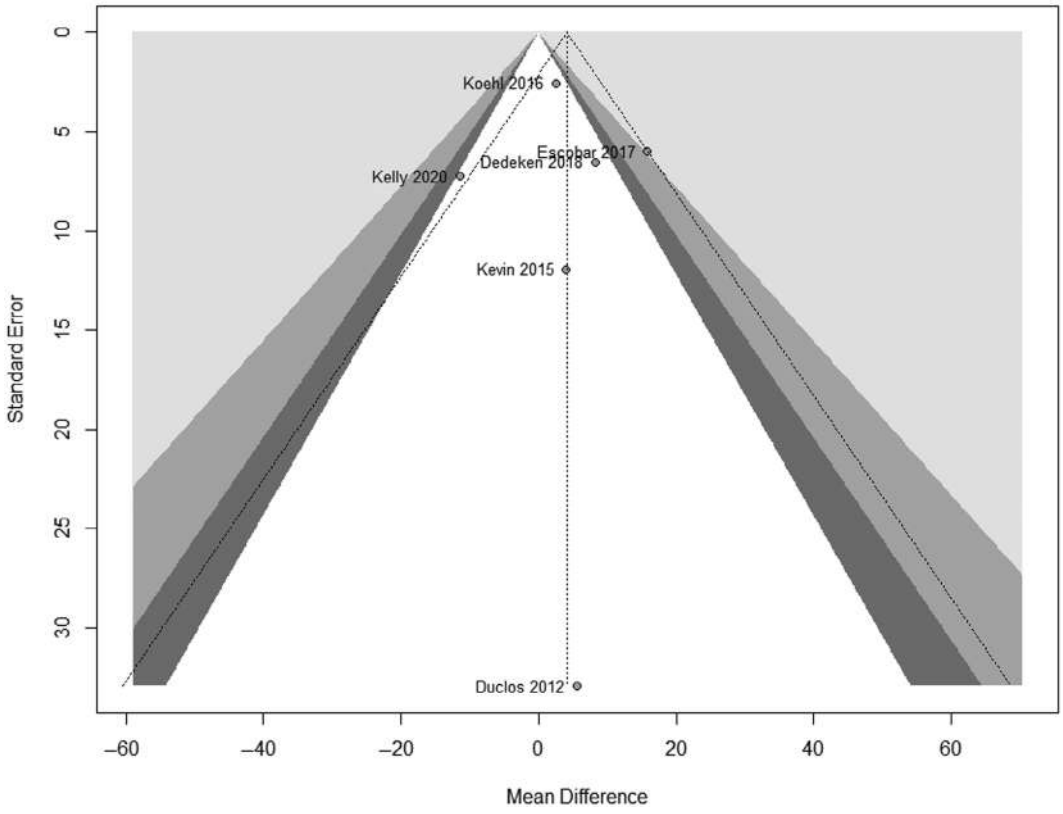

FIGURE 6 Contour-enhanced funnel plot of effect estimate (mean difference of change in HbS level) against standard error

sensitivity analysis by excluding the study of Kelly et al. showed significant reduction of the HbS level in the aRBX group. Subgroup analysis indicated a significant difference between the observational studies and clinical trials. The higher reduction of HbS level in the MET arm observed in the study by Kelly et al. could be due to fewer patients recruited into the aRBX group than the MET group ($n = 9$ in aRBX vs. $n = 34$ in MET). Accordingly, the number of aRBX procedures might be much less than that of MET.

The recommendation to maintain an HbS level below 30% immediately before the next transfusion was largely derived from STOP (Stroke Prevention in Sickle Cell Anaemia) and STOP2 trials and the SWiTCH (Stroke With Transfusions Changing to Hydroxyurea) trial [33–35]. But, it is difficult to comply with the recommendations because the literature frequently reported higher pre-exchange HbS levels [13, 22]. Improving the pre-exchange HbS level by MET may be a formidable task, as the duration of the manual exchange session would be very long, which might increase the risk of hypotension and syncope [22]. aRBX has the advantage to circumvent all these issues, as it could achieve the target pre-exchange HbS level in cases with low baseline haematocrit or in those who were not able to achieve the target HbS consistently by MET [22]. However, Dedeken et al. experienced a significant increase of median HbS (33.5% on MET compared to 45% on aRBX; $p < 0.001$) in 10 patients who were switched from MET to aRBX and had shorter intervals between the procedures while on MET [10]. Woods et al. found that the achievement of HbS goal was not significantly associated with the transfusion mode. Although their study showed a trend towards meeting more frequently the pre-transfusion HBS target with aRBX, the adherence to scheduled transfusion appointments was an important factor contributing to achieving the pre-transfusion HbS goal [21].

Serum ferritin level

In terms of attenuating the serum ferritin level following the intervention of exchange transfusion, we observed that patients who had undergone aRBX had a higher level of serum ferritin reduction compared to those treated with MET. However, there was significant heterogeneity among the studies as revealed in the meta-analysis. The study by Kelly et al. contributed maximum heterogeneity as observed from the sensitivity analysis. Even subgroup analysis showed a significant difference between observational studies and the study by Kelly et al. [20]. Fasano et al. and Kelly et al. reported a significant decline in serum ferritin in patients who underwent aRBX, which was also obvious in our meta-analysis [9, 20]. The possible explanation may be related to chelation adherence: it was observed that patients on MET had been on chelation for a longer duration, and hence had a greater likelihood of chelation mis-adherence [9]. Woods et al. observed that children who received transfusion therapy by aRBX were more adherent to appointments of chelation therapy as well [21]. Therefore, chelation therapy could be a potential confounder, which deserves consideration while analysing the benefit of exchange transfusion [9].

Interestingly, the studies of Woods et al. and Koehl et al. did not find any significant difference in the decline of serum ferritin [7, 21]. Savage et al. and Aloni et al. reported relatively stable serum ferritin levels in children receiving MET [12, 36]. The result of Kelly et al. was distinctly different from those of other studies [20]. Although this study indicated a significant decrease in the risk of excess iron store using aRBX, it did not require adjustment for treatment with or adherence to chelation therapy and any differences in absorption of chelation within the individual. Another strength of this study was that none of the participants was heavily transfused before participating in the study. None of them had a history of regular blood transfusions and had high initial serum ferritin levels. The authors described that the difference between the pre-automatic exchange transfusion and post-automatic exchange transfusion haematocrit is an essential parameter in assessing the benefit of aRBX for lowering the rate of rising serum ferritin. Patients with increased post-transfusion haematocrit will have a positive iron balance. However, there is a lack of guidelines or standards on the post-automatic exchange transfusion haematocrit target value. Hence, patients with low baseline haematocrit are likely to have a positive iron balance following each procedure irrespective of MET or aRBX. The authors observed an increase in ferritin level in children who had lower baseline haemoglobin compared to those who had higher baseline haemoglobin. However, the result was not significant. The comparison of cost effectiveness and duration between red cell exchange by manual and automated methods is shown in Table S1. A meta-analysis of cost effectiveness could not be performed because of inadequate data. Only two studies assessed the cost effectiveness [8, 10] and four studies assessed the duration [8–10, 22], and the results of these studies are contradictory. We searched the literature related to cost effectiveness from SCD-prevalent developing countries as well, but there were no articles comparing the cost effectiveness of manual and automated exchange procedures. The cost effectiveness varies depending on the region and structure of the healthcare system.

Adverse events

Regarding the adverse outcome events due to either red cell exchange procedures, a random effects model analysis from seven included studies [3, 7, 8, 10, 13, 21, 22] did not reveal any significant increase in the risk; however, there was a trend of increased risk of adverse events in the aRBX group. We segregated these adverse events into procedure-related and catheter-related events and performed a separate meta-analysis.

Procedure-related adverse events

The random effects model analysis of procedure-related adverse events showed a trend of increased risk in the aRBCX group. Sensitivity analysis was performed after removing the study by Escobar et al., as only this study reported four adverse events (three hypotension and one transitory hypothermia) during the manual exchange procedure [8]. We found a significant increase in risk related to the aRBX procedure. Cabibbo et al. reported one episode of haemolytic transfusion reaction following exchange transfusion with compatible unit by automated apheresis procedure, but no clinically significant antibody was found. The authors predicted that it could be HLA-antibody-mediated or due to bystander haemolysis (a mechanism of hyperhaemolysis in SCD patients) [3]. Alloimmunization has been frequently encountered in SCD patients with multiple transfusions [37]. However, recent literature shows that the aRBX procedure does not increase the risk of alloimmunization in SCD patients even when donor exposure and blood requirement are higher [38, 39]. Similarly, none of the included studies, except the one by Koehl et al., in our meta-analysis had reported increased incidence of alloimmunization following erythrocytapheresis [7]. Koehl et al. reported three patients who developed alloantibodies out of 39 patients but with no clinically significant antibodies. Furthermore, it was not clear under which group these patients developed alloantibodies [7].

Catheter-related adverse events

Catheter-related complications are the major deterrents of RBX procedures, especially in children. Woods et al. reported 31 events of catheter-related complications, especially catheter-associated thrombosis, infection and mechanical malfunctions in the recipients of aRBX group but not a single incidence of catheter-related complications in the MET group [21]. Catheter-related complications are not uncommon as the Cooperative Study of Sickle Cell Disease found that, by the age of 40, 11.3% of the participants developed catheter-related venous thromboembolism [40]. Catheter stenosis and thrombosis are directly related to the duration of catheter use and the diameter of the catheter [41]. Woods et al. observed that patients in the aRBX group had the catheters inserted for a longer period than MET [21]. In addition, in the aRBX group, double-lumen infusion ports were used, having a larger catheter diameter than the single-lumen infusion ports used for most MET patients, thus possibly increasing the risk of thrombosis. Furthermore, aRBCX needs larger diameter needles to access the port in aRBX than in MET. This might induce a higher risk of catheter malfunction and greater stress of the port's reservoir [42]. In the meta-analysis, we included only three studies for analysis, as only these studies described catheter-related adverse events [8, 21, 22]. The random-effect model revealed high heterogeneity, but the pooled risk ratio showed no significant increased risk of adverse events in the aRBX group. This is possible because in the meta-analysis, the study of Woods et al. could not be analysed as there were no adverse events reported in the manual exchange group [21]. Therefore, data from more such studies are required to arrive at any conclusion regarding adverse events due to RBX procedures. aRBX is preferred over manual exchange, as the latter is less precise and not standardized. Moreover, the manual procedure is prolonged and labour intensive, causing greater discomfort for the patient and therefore poor patient compliance. The automated procedure is more

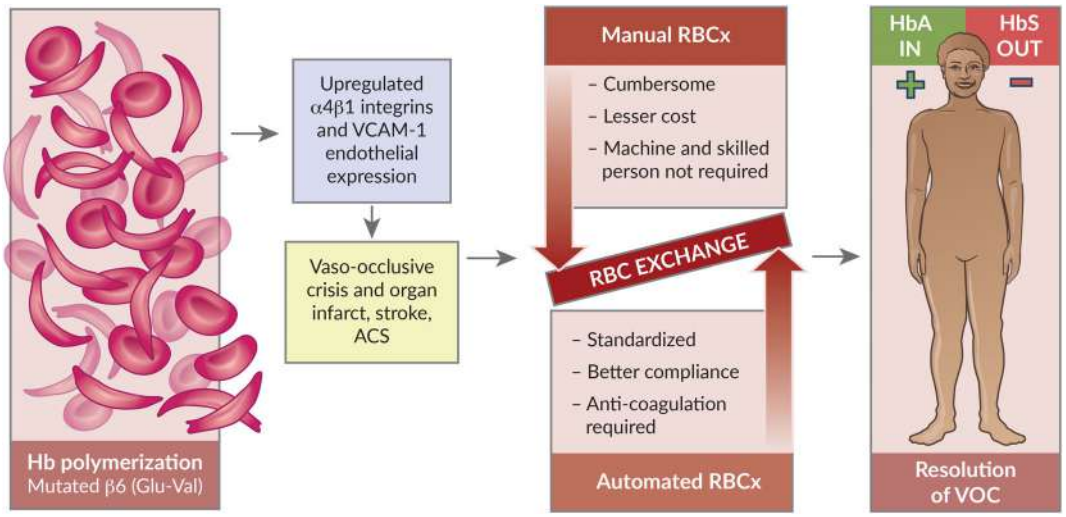

FIGURE 7 Pros and cons of manual versus automated red cell exchange (aRBCx)

efficient in reducing the HbS level (Figure 7). However, it needs an apheresis machine along with costly consumables and skilled personnel [13, 17].

There were a few limitations in our meta-analysis. We could include only nine studies, and most of them were observational and retrospective. Therefore, selection bias and some unclear risk of bias due to improper reporting of the methodology were major drawbacks, resulting in difficulty in suitable comparison between the control and experimental groups. We also could not perform meta-regression analysis from these studies. Also, we recognized that the interval between or frequency of RBX procedures, volume of blood phlebotomized, volume of blood transfused, haematocrit of the transfused unit, pre-exchange and post-exchange haematocrit or haemoglobin of the patient, and pre-transfusion target HbS could potentially impact the outcome of the RBX procedures. However, the data available from the included studies are inconsistent, especially the target HbS values, which vary widely for different clinical conditions in SCD. Nevertheless, to the best of our knowledge, this is the first meta-analysis that compared the efficacy and safety profile of erythrocytapheresis versus manual RBX.

CONCLUSION

In conclusion, this meta-analysis revealed that aRBX did not significantly reduce the HbS and serum ferritin levels when compared with the MET procedure in SCD patients. In terms of adverse events, aRBX also did not show any significant association with the increased risk related to this procedure. Therefore, more high-quality evidence from large randomized controlled trials designed and powered are desirable to reach any conclusion on the efficacy of aRBX over MET as well as on the safety profile of aRBX in both children and adult patients with SCD.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Haemoglobin S testing using HEA BeadChip™ technology: Lifeblood comparison with clinical diagnosis

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Abstract

Background and Objectives: Red cell antigen genotyping is commonly performed on patients requiring chronic transfusion support, such as sickle cell disease and thalassaemia. The Immucor HEA BeadChip™ test, in addition to assessing red cell antigen expression, can also detect the haemoglobin S (HbS) mutation. Our aim was to compare HbS results using HEA BeadChip™ performed at the Australian Red Cross Lifeblood with conventional haemoglobin studies.

Materials and Methods: Patients with thalassaemia and sickle cell trait (SCT) or disease (SCD) referred for red cell genotyping between 2017 and 2019 were assessed. The HbS result obtained from HEA BeadChip™ was compared with that obtained from high-performance liquid chromatography (HPLC) performed by the referring pathology provider.

Results: One-hundred and nineteen cases had comparable HPLC and HEA BeadChip™ results. On HEA BeadChip™ testing, 40 cases showed a negative HbS result, 31 cases showed HbS+ and 47 cases showed HbS++. There was one case with 'low signal' result. Of the negative HbS cases, there was none with SCT. The HbS+ group comprised a mixture of SCT and SCD due to compound heterozygosity for HbS and β-thalassaemia mutations. The HbS++ group comprised predominantly SCD due to homozygosity for HbS.

Conclusion: HEA BeadChip™ is an accurate screening test for the detection of HbS. There were no false positives or false negatives. The identification of donors with the HbS mutation through the targeted genotyping programme would enable early intervention, improved donor management and reduced wastage.

KEYWORDS
donor screening, haemoglobin studies, red cell genotyping, sickle cell disease

Highlights

- The HEA BeadChip™ test is comparable to high-performance liquid chromatography in detecting HbS mutation.
- Identifying donors with sickle cell trait or disease through targeted red cell genotyping will allow early intervention, improved donor management and reduced wastage.

INTRODUCTION

Sickle cell disease (SCD) is an inherited red cell disorder that causes red cells to 'sickle' due to valine for glutamic acid substitution at position 6 of the β-globin chain (β6 Glu→Val) [1]. Haemoglobin S (HbS) polymerizes when deoxygenated, reducing red cell deformability and increases adhesion to endothelial cells, resulting in haemolysis and vaso-occlusion. Complications from SCD result in major morbidity and mortality.

Red cell transfusions are used in acute complications or as prophylaxis [2–4]. However, alloimmunization, acute and delayed haemolytic transfusion reactions and iron overload are critical challenges in the management of patients with SCD due to the need for lifelong transfusion support [5].

In patients with SCD, the prevalence of red cell alloimmunization with ABO and RhD matching alone ranges from 18% to 76% [3]. Patients once alloimmunized are likely to develop additional alloantibodies through further transfusions. Alloimmunization poses considerable challenge to transfusion services, limiting the number of compatible red cell units available, potentially delaying timely transfusion support. A contributing factor is the red cell antigenic mismatch between Caucasian donors and African recipients [2, 6, 7].

Current international guidelines recommend the provision of Rh and K matched red cell units for all patients with SCD [7, 8]. These units should also be HbS negative. In addition, extended red cell phenotyping or genotyping should be considered at baseline [2, 8]. Red cell genotyping can help determine red cell antigen expression, detect Rh variants and resolve potential phenotype–genotype discrepancies to help reduce the incidence of alloimmunization [6].

In Australia, red cell genotyping is performed exclusively at the Red Cell Reference Laboratory, Australian Red Cross Lifeblood (formerly known as the Australian Red Cross Blood Service, hereinafter Lifeblood) in Queensland since 2015 using the Immucor PreciseType™ HEA Molecular BeadChip™ test. The major advantage of genotyping is that testing is performed on DNA. Unlike serological phenotyping, genotyping is not influenced by immunoglobulin coating of red cells, the presence of recently transfused red cells or polyagglutination [9, 10]. The HEA BeadChip™ kit detects 24 polymorphisms associated with 38 red blood cell antigens and phenotypic variants. In addition, it also detects the HbS mutation.

People with sickle cell trait (SCT) do not have any symptoms of SCD, have normal red cell indices and no abnormal red cell morphology. Because of this, these people are often not aware of their carrier state. Donors with SCT have a higher risk of causing filter issues when their blood is leuco-depleted, often leading to discard of the donation [11].

HbS results obtained from HEA BeadChip™ are currently not issued on the red cell genotype report issued by Lifeblood. As such, we sought to externally validate this method of HbS detection by comparing it with conventional haemoglobin studies to assess its potential use in donor screening in Australia to improve donor management and reduce wastage. Haemoglobin studies currently used in the diagnosis of haemoglobinopathies or thalassaemia include high-performance liquid chromatography (HPLC), capillary electrophoresis, gel electrophoresis and multiplex ligation-dependent probe amplification.

MATERIALS AND METHODS

Patients with SCT, SCD or thalassaemia who were referred for red cell genotyping at Lifeblood were assessed. Red cell genotyping reports from January 2017 to May 2019 were manually extracted and assessed. Both paediatric and adult cases were included. Genotyping results for blood donors were excluded owing to the unavailability of diagnostic haemoglobin studies. Ethics for this study was sought and approved by the Australian Red Cross Lifeblood Ethics Committee in May 2019.

The Immucor PreciseType™ HEA Molecular BeadChip™ test uses proprietary Elongation-mediated Multiplexed Analysis of Polymorphisms (eMAP®) technology. This in vitro test identifies single nucleotide polymorphisms affecting red cell antigen expression. The technology identifies the presence or absence of these polymorphisms via multiplex polymerase chain reaction amplification. Genomic DNA is extracted from EDTA whole-blood samples. The DNA is then isolated, amplified, captured and fluorescently labelled. The elongation product is imaged and read with the BioArray Array Imaging System (AIS™ 400). Data are then imported and analysed by the BioArray Solutions Imaging System™ (BASIS®), which interprets the intensity of each reaction to determine a genotype and predicted phenotype for each polymorphism [10, 12].

HbS results using HEA BeadChip™ are reported as negative (0), heterozygous (HbS+) or homozygous (HbS++). Historical haemoglobin studies were obtained from the referring pathology provider. The HbS results from HEA BeadChip™ were compared with each patient's haemoglobin studies for concordance.

RESULTS

A total of 133 patients were referred for red cell genotyping because of underlying haemoglobinopathy. Haemoglobin studies were available for 119 cases. All the haemoglobin studies were pivoted on HPLC testing. Of these, 106 had clinical information provided on the referral sheet. There were 45 cases with 'thalassaemia' and 61 with 'SCT' or 'SCD'.

In the cases where HbS was negative using HEA BeadChip™, all cases were proven to be thalassaemia with no detection of HbS by HPLC (Table 1). There were 31 cases with HbS+ on genotyping. Six cases were reported as SCT, including one case with coexisting α-thalassaemia 3.7 kb deletion. The other 25 cases were compound heterozygotes for the HbS mutation, with 21 being compound heterozygous for HbS and β-thalassaemia (including 3 cases with coexisting α-thalassaemia 3.7 kb deletion). The remaining four cases included two compound heterozygous HbS and HbE, and two compound heterozygous HbS and haemoglobin D-Punjab (Table 2).

TABLE 1 Haemoglobin study results grouped by HbS strength on HEA BeadChip™ testing

HbS strength	No. of cases (%)	Haemoglobin study result (no.)
0	40 (34)	Thalassaemia (40)
+	31 (26)	Sickle trait (6) Compound sickle (25)
++	47 (39)	Compound sickle (7) Homozygous sickle (40)
LS	1 (1)	Homozygous δ-β (1)

TABLE 2 Compound heterozygotes for HbS with other mutations grouped by strength of HbS positivity on HEA BeadChip™ testing

HbS strength	Compound sickle state (no.)
+	HbS/β-thalassaemia (21)
	HbS/E (2)
	HbS/D-Punjab (2)
++	HbS/β-thalassaemia (4)
	HbS/C (3)

There were 47 cases with HbS++ on genotyping. Forty cases (85%) were homozygous HbS, with two of these cases reported as having a coexisting α-thalassaemia 3.7 kb deletion, and one with coexisting α-chain variant haemoglobin G-Philadelphia plus an α-thalassaemia 3.7 kb deletion. Of the seven cases that were reported as compound heterozygotes, four were compound heterozygous for HbS and β-thalassaemia, and three were compound heterozygous for HbS and HbC (Table 2).

A 'low signal' (LS) was detected for one case with homozygous δ-β-thalassaemia. The LS was due to the deletion of the target sequence within the *HBB* gene at chromosome 11p15.4, resulting in insufficient amplification.

Thirteen cases did not have information provided on the referral sheet. There were eight cases with HbS+ on genotyping. Of these, there was one case of SCT, one case of compound heterozygous for HbS and haemoglobin D-Punjab, and eight cases of compound heterozygous for HbS and β-thalassaemia as demonstrated by HPLC. Of the five cases with HbS++ on genotyping, all cases were homozygous HbS.

There were four cases with notes on the referral form stating 'thalassaemia', which showed positive HbS results on genotyping. Corresponding haemoglobin studies showed two cases of homozygous HbS demonstrating HbS++ on genotyping. The other two cases were compound heterozygous for HbS and β-thalassaemia, both recording HbS+ on genotyping. In these instances, the clinical information provided was considered incorrect and there was concordance between the HEA BeadChip™ results and haemoglobin studies.

DISCUSSION

In our study, HEA BeadChip™ was able to detect HbS mutation in every case of known SCT or SCD. While the original intention of performing this external validation was to see whether HEA BeadChip™ was able to accurately differentiate between SCT and homozygous HbS, the number of compound heterozygous sickle cases encountered provided useful insight into the ability of HEA BeadChip™ to correctly predict the HbS phenotype.

There was one case in which haemoglobin studies were reported as consistent with homozygous HbS but demonstrated only HbS+ by genotyping. Clinical correspondence obtained supported a diagnosis of compound heterozygous for HbS and β-null thalassaemia based on family history. Although HPLC remains the gold standard, this highlights the issue of interpreting haemoglobin studies in isolation and the importance of family history in determining potential SCD genotypes. Hydroxyurea and transfusion therapies can affect haemoglobin variant levels on haemoglobin studies. As such, there can be difficulty in differentiating SCD genotypes by haemoglobin studies alone: in particular, homozygous HbS, homozygous HbS with coexisting α-thalassaemia and compound heterozygous for HbS and β-null thalassaemia. In this case, HEA BeadChip™ correctly identified the SCD genotype.

Of the 32 cases of compound heterozygous sickle disorder, 25 (78%) demonstrated HbS+. The HbS+ result is to be expected in sickle compound heterozygous states, as only one β-globin allele carries the sickle mutation. However, there were seven cases of compound heterozygous sickle disorder that showed HbS++. Of these, three were compound heterozygous for HbS and HbC and four were compound heterozygous for HbS and β-thalassaemia.

Compound heterozygosity for the HbS and HbC mutations has been documented by HEA BeadChip™ to interfere with HbS detection. HbC is a variant haemoglobin resulting from the substitution of lysine for glutamic acid at position 6 of the β-globin chain (β6 Glu→Lys), which is the same mutation site as HbS (β6 Glu→Val) [1]. As such, this may result in invalid or inaccurate HbS phenotype call by HEA BeadChip™ (HbS++ instead of HbS+).

Similarly, there were four cases of compound heterozygous for HbS and β-thalassaemia showing HbS++. β-Globin gene sequencing would be useful in these cases to determine whether other β-globin chain variants could result in inaccurate HbS phenotype call similar to HbC.

One of the limitations of this study is the low number of cases with SCT (5%). Nevertheless, there was 100% concordance between HEA BeadChip™ and haemoglobin studies for all cases of SCT. Overall, data from this study have shown that HEA BeadChip™ is a sensitive screening tool for the detection of the HbS mutation. However, its ability to correctly call HbS zygosity in certain compound heterozygous sickle disorders requires further investigation.

In Australia, all blood products come from voluntary, non-remunerated donors. Donors with SCT are eligible for plasma donations at Lifeblood. Plasma collection is done through whole-blood donation rather than apheresis [13, 14]. As whole-blood donation for plasma only is not encouraged, donors with known SCT are effectively deferred. Conversely, in the United Kingdom, red cell donations are acceptable from donors with SCT, but these red cell units are not to be used for neonatal and intrauterine transfusions or for patients with SCD [15].

Lifeblood does not routinely screen all donors for SCT. Sickle solubility testing is a screening method used to detect the presence of HbS. A previous study comparing HEA BeadChip™ with sickle solubility testing (Pacific Hemostasis® SickleScreen) demonstrated that HEA BeadChip™ was superior to SickleScreen in detecting SCT [16]. False positives can occur with sickle solubility testing in cases of polycythaemia, multiple myeloma, and cryoglobulinaemia. False negatives may occur in neonates and cases of severe anaemia or recent transfusion [17].

Leucodepletion is universally performed on red cells and platelet components manufactured by Lifeblood [18]. It is reported that blood from donors with SCT can obstruct the filters used in leucodepletion. Hypothesized triggers of haemoglobin polymerization in these donated red cells include pH, temperature, oxygen saturation of the blood unit, storage time, type of anticoagulant and thrombin generation caused by exposure of phosphatidylserine on red cell membranes [11, 19, 20]. As we do not routinely screen donors for SCT, donors can make multiple donations, which may repeatedly cause blockage of the leucodepletion filter, leading to repeated discard of their donations.

At present, in cases where a donor has repeated donations resulting in filter blockage, the donor is deferred and a referral is made to the donor's general practitioner for further investigation. Tests such as haemoglobin studies are requested to confirm or exclude SCT as a potential cause of recurrent filter blockage. At Lifeblood, we have found SCT to account for 41.5% of cases of recurrent leucodepletion filter blockages [21].

A recent study supported the potential utility of HEA BeadChip™ testing for donor screening [22]. Although HEA BeadChip™ reliably identifies the HbS mutation, it is not an economical alternative to HPLC for this select group of donors. However, Lifeblood currently performs red cell genotyping on a proportion of donors to assist with the provision of compatible red cell units. Donors incidentally identified as carrying the HbS mutation through this targeted donor genotyping programme can now be appropriately managed to avoid potential recurrent wastage due to filter blockage.

In conclusion, the Immucor PreciseType™ HEA Molecular BeadChip™ test is an accurate screening test for the detection of HbS. We have provided external validation at Lifeblood by comparing the HbS results from HEA BeadChip™ with haemoglobin studies pivoted on HPLC. We did not find any false-positive or false-negative cases. We now intend to include the HbS result as part of our red cell genotype report.

The identification of donors with SCT or SCD through the targeted genotyping programme will enable early intervention, improved donor management and reduced wastage. The utility of HEA BeadChip™ in the diagnosis of haemoglobin disorders remains a screening tool for the HbS mutation. Although HEA BeadChip™ can determine the presence of HbS, haemoglobin studies are still required for definitive characterization of haemoglobin genotypes.

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P.W. performed the research, collected the data and wrote the paper; S.B. and J.D. supervised the research and edited the manuscript; T.P. designed the research study and provided the red cell genotyping data. We thank Stephen Miller for assistance with the preparation of the manuscript. The Australian government funds Australian Red Cross Lifeblood for the provision of blood, blood products, and services to the Australian community.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

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Cold storage alters the immune characteristics of platelets and potentiates bacterial-induced aggregation

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Abstract

Background and Objectives: Cold-stored platelets are currently under clinical evaluation and have been approved for limited clinical use in the United States. Most studies have focused on the haemostatic functionality of cold-stored platelets; however, limited information is available examining changes to their immune function.

Materials and Methods: Two buffy-coat-derived platelet components were combined and split into two treatment arms: room temperature (RT)-stored (20–24°C) or refrigerated (cold-stored, 2–6°C). The concentration of select soluble factors was measured in the supernatant using commercial ELISA kits. The abundance of surface receptors associated with immunological function was assessed by flow cytometry. Platelet aggregation was assessed in response to *Escherichia coli* and *Staphylococcus aureus*, in the presence and absence of RGDS (blocks active conformation of integrin $\alpha_2\beta_3$).

Results: Cold-stored platelet components contained a lower supernatant concentration of C3a, RANTES and PF4. The abundance of surface-bound P-selectin and integrin $\alpha_2\beta_3$ in the activated conformation increased during cold storage. In comparison, the abundance of CD86, CD44, ICAM-2, CD40, TLR1, TLR2, TLR4, TLR3, TLR7 and TLR9 was lower on the surface membrane of cold-stored platelets compared to RT-stored components. Cold-stored platelets exhibited an increased responsiveness to *E. coli*- and *S. aureus*-induced aggregation compared to RT-stored platelets. Inhibition of the active conformation of integrin $\alpha_2\beta_3$ using RGDS reduced the potentiation of bacterial-induced aggregation in cold-stored platelets.

Conclusion: Our data highlight that cold storage changes the in vitro immune characteristics of platelets, including their sensitivity to bacterial-induced aggregation. Changes in these immune characteristics may have clinical implications post transfusion.

KEYWORDS
bacteria, cold storage, immunology, platelets

Highlights

- Cold storage alters the immune phenotype of platelets.
- Cold storage reduces the abundance of pathogen recognition receptors on the surface membrane.
- Cold-stored platelets exhibit increased aggregation in response to stimulation by *E. coli* and *S. aureus*.

INTRODUCTION

Cold storage has re-emerged as an alternative platelet storage methodology. Several clinical trials are ongoing to determine the appropriate shelf-life and clinical indications, including 4CPLT (NCT02495506), CHIPS (NCT04834414) and CriSP-TBI (NCT04726410) trials. Additionally, cold-stored platelets have been approved for limited use by the US Food and Drug Administration (FDA) [1,2], and delayed cold storage has been implemented in some blood centres to counter pandemic-induced shortages [3]. Overall, cold storage better preserves the in vitro haemostatic function of platelets and lowers the risk of bacterial proliferation [4,5], compared to conventional room-temperature (RT) storage [6–11].

While traditionally considered haemostatic cells, platelets are also key mediators of the immune response [12,13]. The platelet surface membrane houses a range of co-stimulatory (CD40, CD40L, HCAM, B7-2, MHC-I, siglec-7) and adhesion (P-selectin, ICAM-2, DC-SIGN, CLEC2, integrin $\alpha_2\beta_3$) receptors, which enable leukocyte activation and facilitate the formation of platelet–leukocyte aggregates [12,14–16]. Further, activation by haemostatic or immunological agonists can induce degranulation and release of biological response modifiers (BRMs) [7,12,17]. BRMs can have numerous effects, facilitating inflammation and leukocyte activation (CD40L, endocan, IL-1β, IL-13, IL-27, HMGB1, OX40L and RANTES), and anti-pathogenic activity (PF4, C3a, C4a and C5a) [7,12,13,18–23]. Notably, the accumulation of BRMs in platelet components has been associated with an increased incidence of adverse events following transfusion [18].

Platelet surface receptors also mediate the detection and elimination of infiltrating pathogens. Toll-like receptors (TLRs; TLR1, TLR2,

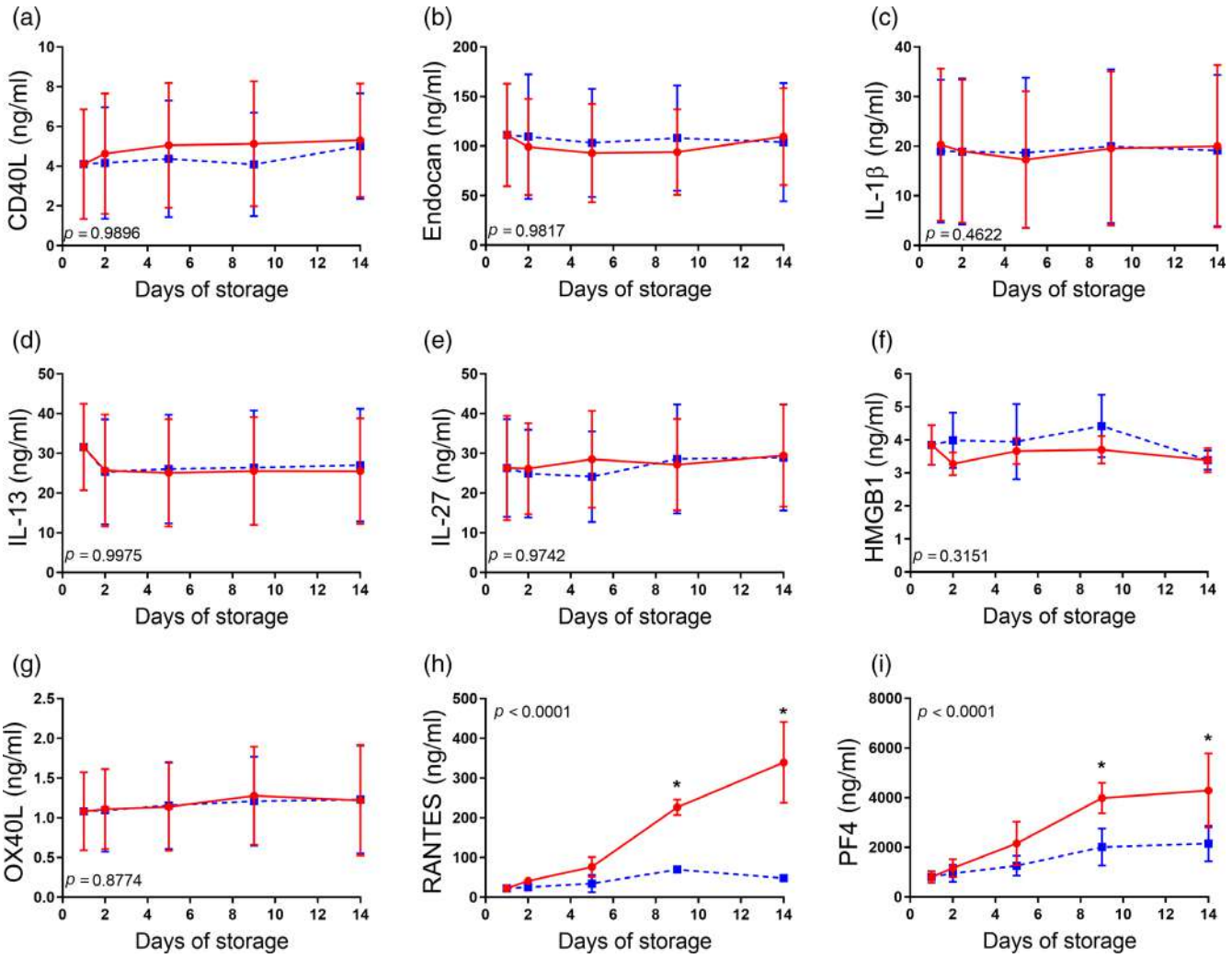

FIGURE 1 Refrigeration alters the concentration of cytokines in the supernatant. Platelet supernatants were collected from RT-stored (red lines) and cold-stored (broken blue lines) platelets stored at the indicated days post collection. The concentration of (a) CD40L, (b) endocan, (c) IL-1β, (d) IL-13, (e) IL-27, (f) HMGB-1, (g) OX40L, (h) RANTES and (i) PF4 was measured by ELISA. Data represent mean ± standard deviation (error bars, $n = 8$ in each group). Significance was determined by two-way ANOVA comparing the effects of each treatment over the storage period. $^*p < 0.05$ compared to RT-stored platelets.

TLR4 and TLR6) and adhesion molecules (P-selectin) are capable of recognizing and binding pathogens in vivo [24,25]. Additionally, platelets are unique in that TLR3, TLR7 and TLR9, which are generally expressed in intracellular compartments in other cells, are present on the surface membrane, where they are believed to assist in pathogen clearance from the circulation [24,25]. Platelets can also adhere to pathogens indirectly through FcγRIIa and the activated form of $\alpha_2\beta_3$ integrin and their bridging molecules IgG and fibrin/fibrinogen, respectively [26–29]. Platelet–pathogen binding can be beneficial, facilitating pathogen immobilization and destruction [26,30,31], or detrimental, allowing pathogens to evade aspects of the immune system [16].

Therefore, this study aimed to examine the effect of cold storage on the immune characteristics of platelets, with a particular focus on functions associated with pathogen interaction.

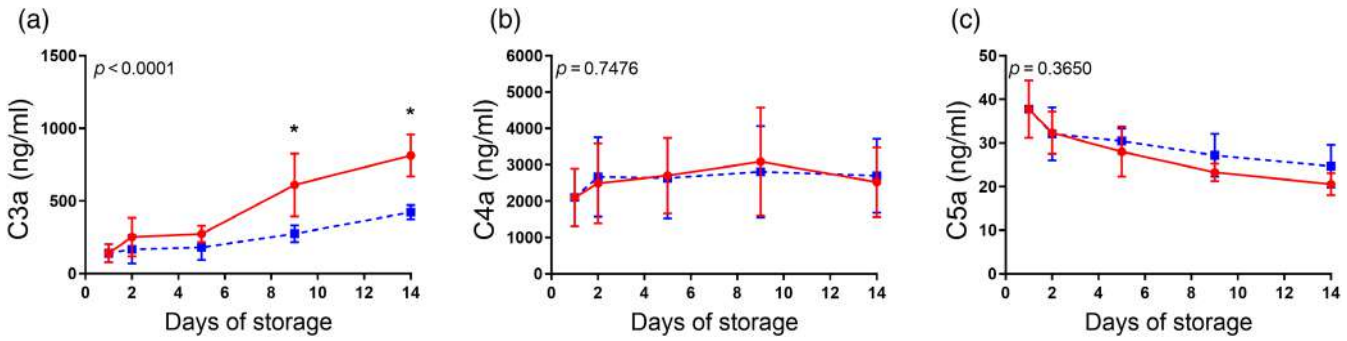

FIGURE 2 Refrigeration alters the concentration of complement factors in the supernatant. Platelet supernatants were collected from RT-stored (solid red lines) and cold-stored (broken blue lines) platelets stored at the indicated days post collection. The concentration of (a) C3a, (b) C4a and (c) C5a was measured by ELISA. Data represent mean ± standard deviation (error bars, $n = 8$ in each group). Significance was determined by two-way ANOVA comparing the effects of each treatment over the storage period. *$p < 0.05$ compared to RT-stored platelets.

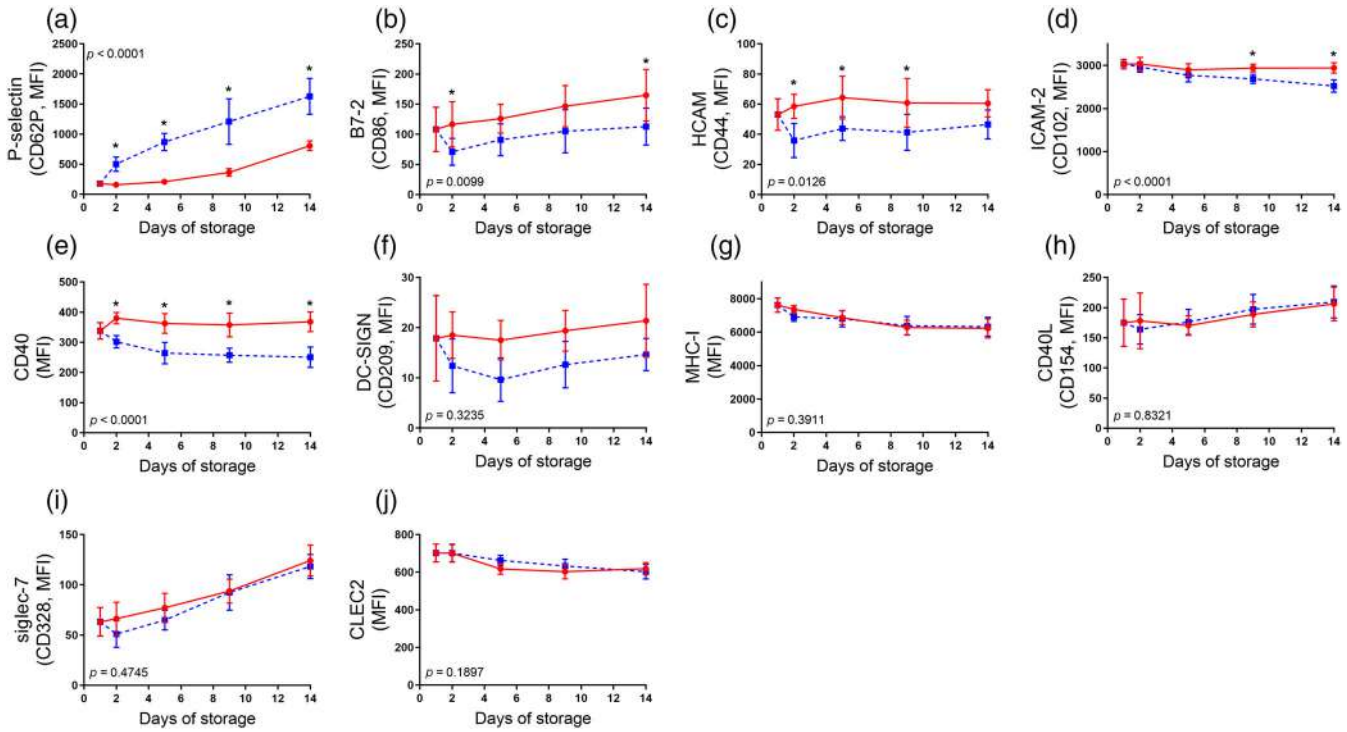

FIGURE 3 Refrigeration alters the abundance of leukocyte interaction receptors on the platelet surface membrane. The surface abundance of receptors was measured in RT-stored (solid red lines) and cold-stored (broken blue lines) platelets at the indicated days post collection. Platelets were stained with (a) CD62P-PE, (b) CD86-PE, (c) CD44-APC, (d) CD102-APC, (e) CD40-PE, (f) CD209-PE, (g) MHC-I-PE, (h) CD154-APC, (i) CD328-APC and (j) CLEC2-APC. The median fluorescent intensity (MFI) was determined by flow cytometry. Data represent mean ± standard deviation (error bars, $n = 8$ in each group). Significance was determined by two-way ANOVA comparing the effects of each treatment over the storage period. *$p < 0.05$ compared to RT-stored platelets.

MATERIALS AND METHODS

Experimental design

Ethics approval was obtained from the Australian Red Cross Lifeblood Ethics Committee (Johnson 10052019). Blood donations were obtained from voluntary blood donors. Pooled platelet components were prepared by combining buffy coats from four whole-blood donations. Platelet components were re-suspended in 30% plasma/70% PAS-E (SSP+; MacoPharma, Mouvaux, France) and leuko-reduced by filtration (AutoStop BC filters; Haemonetics Corp., Boston, MA) before storage in 1300-ml PVC bags (ELX; Haemonetics Corp.). Each replicate consisted of two pooled platelet concentrates, which were combined and split into two components of equal weight at Day 1 post collection. Components ($n = 8$ in each group) were randomly assigned to be either RT-stored (20–24°C with agitation) or cold-stored (2–6°C with no agitation). The combined component was sampled on Day 1 post collection (10–15 ml) to establish baseline parameters, and components in each treatment arm were sampled on Day 2, 5, 7, 9 and 14 post collection. Platelet counts were obtained using an automated haematology analyser (CellDYN Emerald, Abbott Core Laboratory, Abbott Park, IL).

Flow cytometry

The surface membrane abundance of platelet receptors was examined using flow cytometry (FACS Canto II, Becton Dickinson, Franklin Lakes, NJ). Approximately 500,000 platelets were individually stained with the following antibodies diluted in Tyrode's buffer for 30 min at 20–24°C in the dark: CD40-PE (5C3), CD32-APC (FUN-2), CD154-APC (24–31), CD286-PE (TLR6.127), CD86-PE (IT2.2),

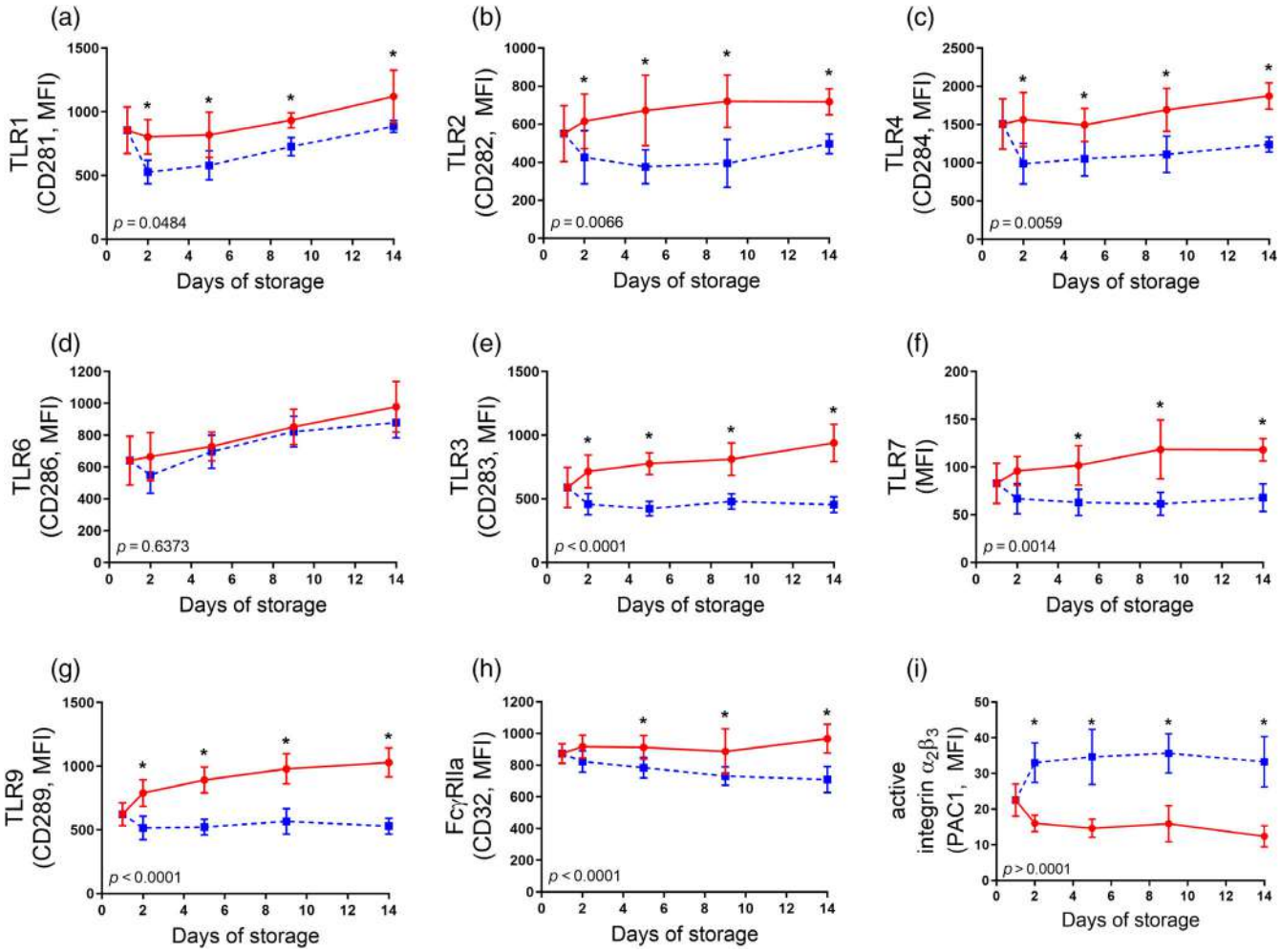

FIGURE 4 Refrigeration alters the abundance of pathogen recognition receptors on the platelet surface membrane. The surface abundance of receptors was measured in RT-stored (solid red lines) and cold-stored (broken blue lines) platelets at the indicated days post collection. Platelets were stained with (a) CD281-PE, (b) CD282-PE, (c) CD284-PE, (d) CD286-PE, (e) CD283-PE, (f) TLR7-PE, (g) CD289-PE, (h) CD32-PE and (i) PAC1-FITC. The median fluorescent intensity (MFI) was determined by flow cytometry. Data represents mean ± standard deviation (error bars, $n = 8$ in each group). Significance was determined by two-way ANOVA comparing the effects of each treatment over the storage period. *$p < 0.05$ compared to RT-stored platelets.

CD44-APC (VI A034, BioLegend, San Diego, CA), PAC1-FITC (PAC1), CD62P-PE (AC1.2, BD Biosciences, Franklin Lakes, NJ), CD328-PE (194211), CD209-APC (120507), MHC-I-PE (W6/32), CLEC2-APC (219133), TLR7-PE (533707, R&D Systems, Minneapolis, MN), CD281-PE (GD2.F4), CD282-PE (TL2.1), CD283-PE (TLR-104), CD284-PE (HTA125), CD289-PE (eB72-1665), CD102-PE (V BP363, eBioscience, San Diego, CA). Following incubation, each tube was diluted 1:20 with Tyrode's buffer, and 10,000 gated platelet events were collected.

Soluble factor analysis

Platelet supernatant was collected at each time point by double centrifugation, as previously described [7]. Briefly, the platelet concentrate was centrifuged at 1600*g* for 20 min and again at 12000*g* for 5 min, and the supernatant was stored at −80°C for later analysis. The concentration of soluble factors in the supernatant was examined using commercially available enzyme-linked immunosorbent assay (ELISA) kits: endocan (limit of detection: 10 pg/ml; Abcam, Cambridge, UK), HMGB-1 (limit of detection: 19 pg/ml; Novus Biologicals, Littleton, CO), C3a, C4a (limit of detection: 7 and 6 pg/ml respectively; BD Bioscience), C5a, RANTES, PF4, IL-13, IL-27, IL-1β, OX40L and CD40L (limit of detection: 31, 16, 16, 94, 156, 4, 47 and 16 pg/ml, respectively; R&D Systems). Each sample was tested in duplicate or triplicate and compared with a standard curve, as per the manufacturer's instructions.

Platelet–bacteria aggregometry

Platelet–bacteria aggregation was measured by light transmission aggregometry (Helena Laboratories, Beaumont, TX). A common gram-positive (*Staphylococcus aureus*; NCTC 10788) and a gram-negative (*Escherichia coli*; NCTC 12923; Bioball, Biomerieux, Marcy-l'Etoile, France) bacterial strain were included in this study because of their association with post-surgical infection risk [32,33]. Bacterial vials were rehydrated to a concentration of 1.1×10^9 in rehydration fluid for 30 min at 22°C. Platelets were diluted to 300×10^6 cells/ml in fresh frozen plasma to obtain platelet-rich plasma (PRP). All reagents, including PRP, were pre-heated to 37°C before use. Platelet aggregation was monitored for 40 min following stimulation with either 20 μM adenosine diphosphate (ADP; Sigma, MO) or bacteria at a ratio of 1:2 (bacteria/platelets, $n = 6$). Further, at Day 14 of storage, four of the replicates were randomly selected for incubation with RGDS peptide (400 μM, Cayman Chemical Company, MI)

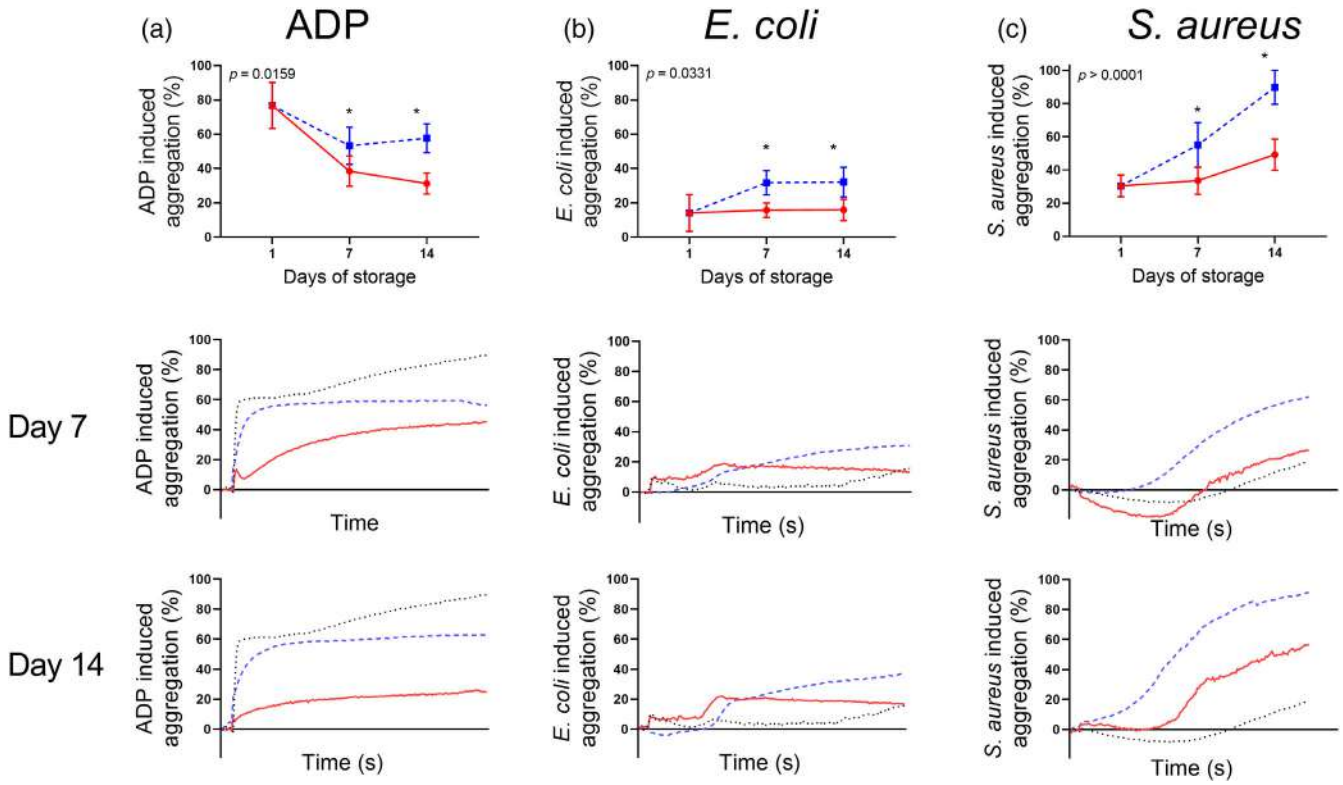

FIGURE 5 *Escherichia coli* and *Staphylococcus aureus* stimulation induces a higher aggregation response in refrigeration platelets. Light transmission aggregation was measured in RT-stored (solid red lines) and cold-stored (broken blue lines) platelets at the indicated days post collection. Aggregation was induced by the addition of (a) 20 μM ADP or bacteria, (b) *Escherichia coli* (NTCC 12923) and (c) *Staphylococcus aureus* (NTCC 10788) at a ratio of 1:2 (bacteria/platelets). Data represents mean ± standard deviation (error bars, $n = 6$ in each group). Significance was determined by two-way ANOVA comparing the effects of each treatment over the storage period. *$p < 0.05$ compared to RT-stored platelets. Representative aggregometry traces are presented below the corresponding graphs for Days 1 (dotted black lines), 7 and 14 of storage.

before bacterial-induced activation. RGDS binds to the active site of integrin $\alpha_2\beta_3$, acting as a competitive inhibitor of fibrin/fibrinogen binding [34].

Statistical analysis

Data are presented as mean ± SD. Statistical analysis was conducted using GraphPad Prism 8.4.3 (GraphPad Software, Inc.). A two-way repeated-measures analysis of variance (ANOVA) was used to compare RT-stored and cold-stored platelets over the storage period. RGDS data was analysed by a one-way ANOVA comparing all treatment groups. Post hoc Bonferroni multiple comparisons were performed to determine differences at each time point or treatment. In all cases a p-value < 0.05 was considered to be statistically significant.

RESULTS

The platelet components had a starting count of $380 \pm 24 \times 10^9$/unit, which decreased by 13% over storage, but to the same degree in both arms. Platelets contain significant internal stores of soluble factors, which can be released upon activation, promoting inflammation and anti-pathogenic functions [12,26,31]. In general, cold storage did not significantly alter the release of most immunomodulatory factors

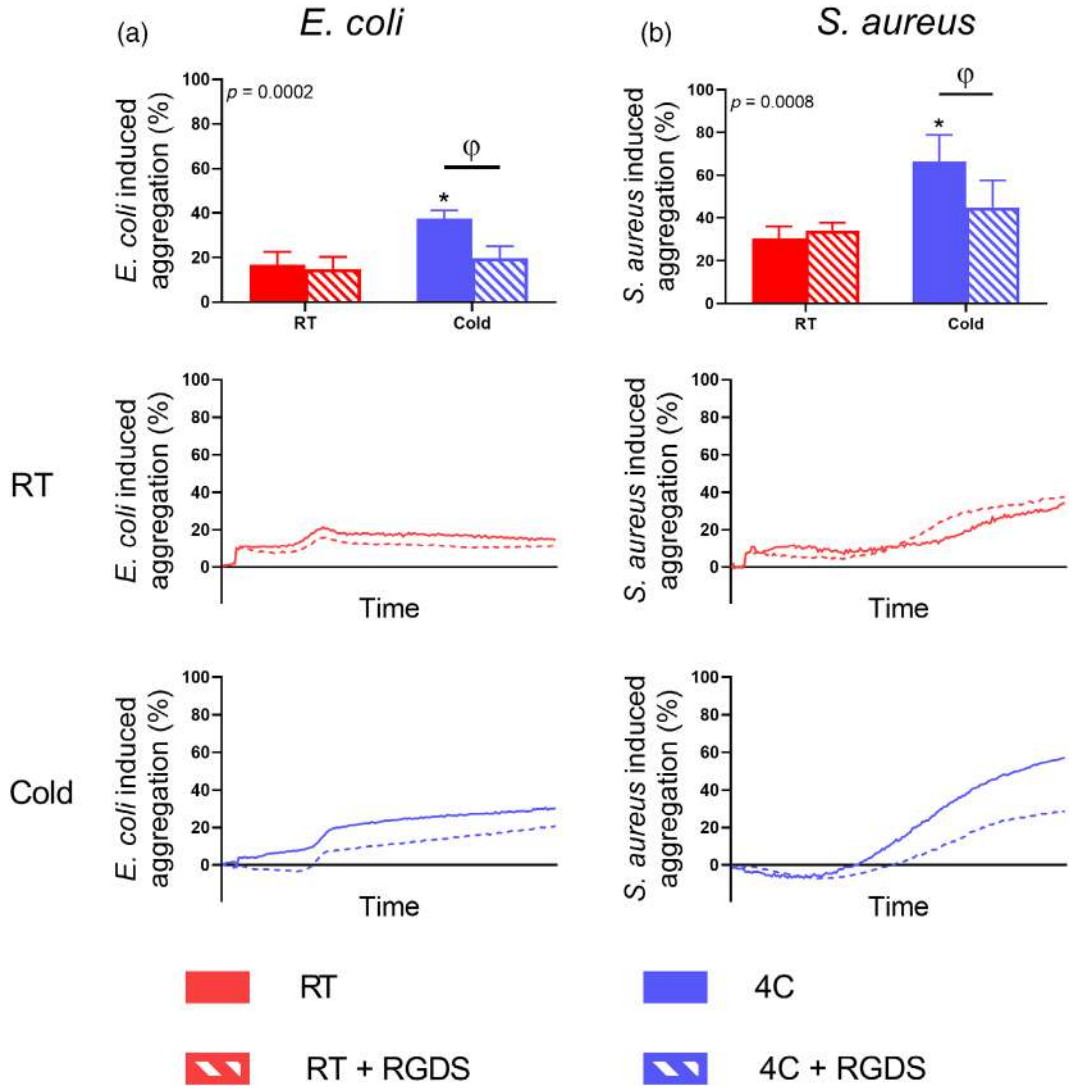

FIGURE 6 The activated conformation of integrin $\alpha_2\beta_3$ mediates the cold-induced potentiation of *Escherichia coli* and *Staphylococcus aureus* aggregation response. Platelet aggregation was measured in the presence or absence of RGDS (400 μM) and (a) *Escherichia coli* (NTCC 12923) and (b) *Staphylococcus aureus* (NTCC 10788) at a ratio of 1:2 to RT-stored or cold-stored platelets at Day 14 post collection. Data represents mean ± standard deviation (error bars, $n = 4$). Significance was determined by one-way ANOVA comparing means between treatments. *p < 0.05 compared to RT-stored platelets without RGDS. φ indicates p < 0.05 compared to cold-stored platelets without RGDS. Representative aggregometry traces for untreated platelets (solid lines) and RGDS-treated platelets (dashed lines) are presented below the corresponding bar graph.

examined. The concentration of CD40L, endocan, IL-1β, IL-13, IL-27, HMGB1 and OX40L did not vary significantly compared to RT-stored platelets at any time point (Figure 1a–g). However, the concentration of RANTES and PF4 increased two- and three-fold, respectively, in RT-stored platelets from Day 9 onwards compared to cold-stored components (Figure 1h,i).

Complement factors are stored in α-granules and can be released following platelet activation and degranulation [17]. The concentration of C3a gradually increased in RT-stored components, becoming two-fold higher than cold-stored platelets at Days 9 and 14 (Figure 2a). In contrast, no significant difference was observed in the supernatant concentration of C4a or C5a between RT-stored and cold-stored platelets (Figure 2b,c).

The surface membrane of the platelet contains a range of adhesion and co-stimulatory receptors, which allow binding and activation of leukocytes [12]. Cold storage significantly increased the abundance of P-selectin on the surface membrane from Day 2, compared to RT-stored components (Figure 3a). In contrast, the abundance of B7-2, HCAM, ICAM-2 and CD40 was reduced by Day 2 on the surface membrane of cold-stored platelets, compared to those stored at RT (Figure 3b–e). The abundance of DC-SIGN appeared lower on the surface membrane of cold-stored platelets; however, the reduction was not statistically significant (Figure 3f). In contrast, there was no difference in the membrane abundance of MHC-I, CD40L, siglec-7 or CLEC2 between the treatment arms (Figure 3g–j). Overall, cold storage differentially altered the abundance of certain platelet receptors associated with the immunological function.

Platelets can respond and adhere to pathogens directly through Toll-like receptors and indirectly through binding FcγRIIa or the activated conformation of integrin $\alpha_2\beta_3$ [27,29]. In general, RT-stored platelets exhibited a higher abundance of most TLRs and FcγRIIa on the surface membrane over storage (Figure 4a–h). The exception was TLR6, which was comparable between the treatment arms over storage (Figure 4d). Notably, only the abundance of the active conformation of integrin $\alpha_2\beta_3$ was significantly increased by cold storage, compared to RT-stored components (Figure 4i).

As cold storage reduced the abundance of pathogen receptors on the surface membrane of platelets, we used aggregometry to determine whether this impacted the ability of platelets to aggregate in the presence of common bacterial strains (*E. coli* and *S. aureus*). Platelet aggregation in response to ADP decreased in both treatment arms but was better maintained in cold-stored platelets at Days 7 and 14 (Figure 5a). *E. coli* induced a relatively low level of aggregation in RT-stored platelets, which remained stable over storage (Figure 5b). In contrast, a two-fold higher aggregation response to *E. coli* was observed in cold-stored platelets at Days 7 and 14 (Figure 5b). Similarly, *S. aureus* induced aggregation in RT-stored platelets, which increased slightly after 14 days of storage (Figure 5c). Notably, cold-stored platelets exhibited a significantly higher aggregation response to *S. aureus* at Day 7, which further increased at Day 14, compared to RT-stored components.

The activated conformation of integrin $\alpha_2\beta_3$ is a key mediator of *E. coli*- and *S. aureus*-induced aggregation [28,35]. Inhibition of the active site of the integrin with RGDS did not significantly affect *E. coli*-induced platelet aggregation in RT-stored platelets (Figure 6a). However, RGDS treatment significantly reduced *E. coli*-induced aggregation after cold storage to levels comparable to those of RT-stored components (Figure 6a). Likewise, RGDS addition had no effect on *S. aureus*-induced aggregation of RT-stored platelets (Figure 6b) but inhibited the cold-stored potentiation of aggregation (Figure 6b).

DISCUSSION

Cold storage alters the haemostatic phenotype and function of platelets [8–10]. In this study, we found that cold storage also induces changes in the immune characteristics of platelets. Specifically, cold storage reduced the release of a range BRMs into the supernatant and altered the surface abundance of receptors associated with immunological function. Cold storage also potentiated the aggregation responses induced by *E. coli* and *S. aureus*, in an activated integrin $\alpha_2\beta_2$-dependent manner.

Platelet surface receptors facilitate haemostasis, pathogen recognition and leukocyte interaction [12,14,15]. Previous studies have shown that cold storage results in reduced abundance of specific haemostatic receptors (GPIb, GPVI, GPIIb and GPIX), which is believed to be related to changes in the morphology of the platelet surface membrane [10,36,37]. In this study, we have seen a cold-associated decrease in the abundance of a range of TLRs and leukocyte-associated receptors, which may occur through a similar mechanism. Additionally, we report that RT storage results in a significant increase in surface abundance of TLRs during extended storage, which may be indicative of the mobilization of internal stores.

Platelets are often the first cells to reach the sites of tissue damage and thus have a high chance of encountering infiltrating pathogens [12]. Despite a decrease in the abundance of TLRs and FcγRIIa, which are typically associated with pathogen binding, an increase in bacterial-induced aggregation was observed in cold-stored platelets. While still under investigation, current research suggests that cold storage better maintains metabolic parameters and potentially primes the internal signal transduction pathways [9,38,39]. Previous work has identified that cold storage increases the phosphorylation of the Src and Syk family of kinases [38,39]. Notably, bacterial-induced platelet activation of TLRs, integrin $\alpha_2\beta_3$ and FcγRIIa is reliant on signal transduction through Src and Syk [40,41]. Consequently, cold-stored platelets may still be able to respond to activation by bacteria through TLRs and FcγRIIa, despite the observed reduction in pathogen adhesion receptors on the surface membrane.

Prior research has highlighted the importance of $\alpha_2\beta_3$-mediated fibrinogen binding in facilitating agonist-mediated (ADP, collagen) and bacterial-induced (*E. coli* and *S. aureus*) platelet aggregation [28,34,35,42]. Notably, cold-stored platelets exhibited increased activation of the integrin $\alpha_2\beta_3$ (higher PAC-1 binding), which was associated with increased aggregation in response to *E. coli* and *S. aureus*. Further, the addition of RGDS inhibited the cold-induced potentiation of bacteria-induced aggregation. As such, the activated conformation

of integrin $\alpha_2\beta_3$ may be responsible for the observed increase in bacterial-induced aggregation. Interestingly, RGDS inhibition reduced but did not eliminate *E. coli*- and *S. aureus*-induced aggregation. Additionally, platelets in both treatment arms exhibited increased aggregation in response to *S. aureus* at Day 14 compared to Day 7, despite no further change in integrin $\alpha_2\beta_3$ activation. A potential explanation may be found in the increased abundance of P-selectin on the surface membrane of both RT- and cold-stored platelets over storage. Surface P-selectin is known to mediate *S. aureus*-induced platelet activation and adhesion [16], but is not the primary mechanism for *E. coli*-induced aggregation [28].

Alterations in platelet immune function ex vivo can cause post-transfusion immunomodulation, contributing to adverse events [18,43]. The likelihood of adverse events has been linked to changes in the platelet activation state and release of immunomodulatory BRMs into the supernatant of components [18,43]. The concentration of most BRMs was comparable between treatments. However, C3a, RANTES and PF4 concentrations were found to increase in RT-stored components, which aligns with previous reports [7,21,44]. PF4 and C3a are involved in anti-pathogen responses [26,45], while RANTES has been linked to a higher risk of adverse transfusion events [43]. Further, cold-stored platelets exhibited a lower abundance of several key leukocyte adhesion (HCAM, ICAM-2) and co-stimulatory (B7-2, DC-SIGN, CD40) receptors. Taken together, these changes suggest a decreased capacity to bind and activate leukocytes. In contrast, cold storage results in an activated haemostatic phenotype, with phosphatidylserine and P-selectin exposure and activation of the integrin $\alpha_2\beta_3$. To date, one study has reported a slight but not statistically significant increase in platelet leukocyte aggregates following the transfusion of refrigerated whole blood in coagulopathic rats [46]. Although increased activation in RT-stored components is associated with a higher risk of adverse events [18,43], the evidence is less clear for cold-stored platelets, warranting further investigation.

In this study, we have shown that cold storage potentiates bacterial-induced aggregation using single strains of *E. coli* and *S. aureus*. However, bacteria-induced platelet aggregation varies significantly between species and strains [29,31]. As such, further examination of a diverse range of bacteria, including other species associated with post-operative infection, such as *Pseudomonas*, *Klebsiella* and *Enterobacter*, would be informative [47]. Further, bacteria-induced platelet aggregation is associated with both bacterial destruction and immune evasion [16,26,28,30,31]. Consequently, it is unclear what impact increased platelet–bacteria aggregation would have post transfusion. Notably, most cold-stored components will be used for the treatment of acute bleeding resulting from trauma or surgery, which is associated with an elevated risk of bacterial infection post surgery [32,33], highlighting the need for further work in this area.

This study presented a general overview of the effects of cold storage on a range of characteristics associated with the immune function of platelets. The impact of these in vitro changes on clinical outcomes following transfusion of cold-stored platelets should be a major consideration as the implementation of cold-stored platelets progresses.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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ORIGINAL ARTICLE

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Risk of variant Creutzfeldt–Jakob disease transmission by blood transfusion in Australia

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Abstract

Background and Objectives: Most of the 233 worldwide cases of variant Creutzfeldt–Jakob disease (vCJD) have been reported in the United Kingdom and 3 have been associated with transfusion-transmission. To mitigate the potential vCJD risk to blood safety, Australian Red Cross Lifeblood imposes restrictions on blood donation from people with prior residency in, or extended travel to, the United Kingdom during the risk period 1980–1996. We have modified a previously published methodology to estimate the transfusion-transmission risk of vCJD associated with fresh component transfusion in Australia if the UK residence deferral was removed.

Materials and Methods: The prevalence of current pre-symptomatic vCJD infection in the United Kingdom by age at infection and genotype was estimated based on risk of exposure to the bovine spongiform encephalopathy agent for the period 1980–1996. These results were used to estimate the age-specific prevalence of undiagnosed, pre-symptomatic vCJD in the Australian population in the current year due to prior UK residency or travel. The primary model outputs were the 2020 vCJD risks/unit of vCJD contamination, transfusion-transmission (infections) and clinical cases.

Results: The overall (prior UK residency in and travel to United Kingdom, 1980–1996) mean risk of contamination per unit was 1 in 29,900,000. The risks of resulting vCJD transmission (infection) and clinical case were 1 in 389,000,000 and 1 in 1,450,000,000, respectively.

Conclusion: Our modelling suggests that removing the Lifeblood donation deferral for travel to, or UK residence, would result in virtually no increased risk of vCJD transfusion-transmission and would be a safe and effective strategy for increasing the donor base.

This work does not represent any official conclusion, policy, or decision of the US Food and Drug Administration.

KEYWORDS
blood transfusion, risk, transfusion-transmission, variant Creutzfeldt–Jakob disease, vCJD

Highlights

- Adapting a published vCJD transfusion risk model, we predict a donation from an Australian person with vCJD to occur once every 65 years but this decreases over time.
- The current estimated risk of a clinical case in an Australian recipient (vCJD transfusion-transmission) was approximately 1 in 1.4 billion.
- Our modelling concludes removing the Lifeblood donation deferral for travel to, or UK residence would result in virtually no increased risk of vCJD transfusion-transmission and would result in a sufficiency gain of at least 57,000 donations annually.

INTRODUCTION

Variant Creutzfeldt–Jakob disease (vCJD) is a human transmissible spongiform encephalopathy first reported in 1996 [1]. Pathogenesis is associated with an abnormal infectious isoform of the naturally occurring cellular prion sialoglycoprotein PrP^{c}. The abnormal isoform, which does not contain nucleic acid, is designated as PrP^{res}, PrP^{sc} or PrP^{TSE}. The origin of the prion strain associated with vCJD is the strain associated with bovine spongiform encephalopathy (BSE) in cattle [2, 3]. Infection with vCJD is universally fatal, and there is no effective treatment to control or cure vCJD [1]. Following infection, there is an extended pre-symptomatic period, which, although not well defined, is estimated at 10–16 years for primary vCJD for UK cases (modelled range: 8–55 years depending on the genotype) [4–6].

vCJD has primarily occurred in the United Kingdom (178 of 233 cases worldwide to March 2021) with only 55 cases in 11 countries recorded outside that country (Figure 1). The peak of the UK epidemic occurred in 2000 (28 deaths), and none of the 178 cases is alive [8]. The primary route of human infection is through dietary consumption of beef from cattle infected with BSE over the period 1980–1996 associated with contaminated stock feed [1, 9].

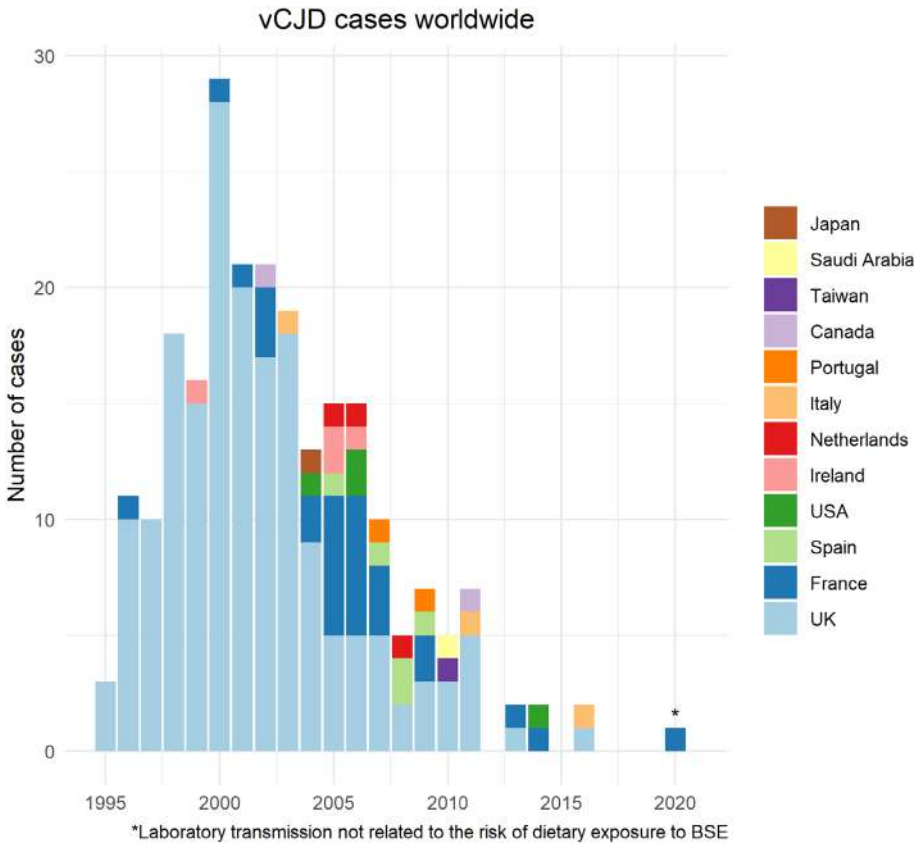

FIGURE 1 Reported cases of variant Creutzfeldt–Jakob disease worldwide. *Source*: https://www.eurocjd.ed.ac.uk/data and 2020 case report [7].

The primary route of infection via beef consumption in the United Kingdom (and other European countries) was reportedly eliminated by industry regulation from 1996. However, three human cases of vCJD transmission associated with transfusion have been reported [10–12], as well as a potential sub-clinical case diagnosed at post-mortem following death from an unrelated condition [13], all in the United Kingdom, involving non-leucodepleted red blood cells transfused between 1996 and 1999. Additionally, there is a single report of possible transmission of vCJD by plasma-derived products [14].

Animal experiments support that vCJD infectivity in blood is highly associated with leucocytes [15, 16], and therefore leucodepletion is an effective measure in reducing the risk of vCJD. Accordingly, leucodepletion was an early risk minimization strategy, introduced in the United Kingdom in 1999 [17] and in Australia in 2008 (Australian Red Cross Lifeblood, hereafter Lifeblood, unpublished). The proportion of vCJD infectivity removed by leucodepletion of human blood is not well defined but data from animal model suggest it as 42%–71% [15, 16].

Because of the potential risk of secondary infection via transfusion, many countries have imposed restrictions on blood donation from people with potential exposure risk through prior residency in, or extended travel to, the United Kingdom and/or other countries recording vCJD cases [18]. Under regulations current in 2022, Lifeblood defers from donation individuals who have resided in/visited the United Kingdom for a cumulative period exceeding 6 months within the period 1980–1996. The current predicted impact is the loss of 3.5% (approx. 57,000) donations annually (see Data S1 and S2). This policy remains in effect due to continuing uncertainty about disease pathogenesis and transmission, despite the absence of local cases [19], and reports of international transfusion-transmission after 1999 [20]. Although the primary epidemic peak appears to have passed, there remains concern about potential further cases associated with *PRNP* genotypes MV and VV at codon 129. These cases may have longer disease incubation periods compared to the MM genotype, which was present in 42% of a sample of UK blood donors [21] and represented all genotyped vCJD cases reported before 2016. MV heterozygous and VV homozygous individuals with accumulation of prion protein have been identified by retrospective tonsil/appendix tissues [22–25], as well as in a recipient of non-leucodepleted red blood cells (RBCs) who died 5 years later from unrelated causes [13]. In addition, the last case of vCJD identified in the United Kingdom (2016) was MV heterozygous [26], adding to concerns of a potential second wave of vCJD [18].

Blood services use risk tolerability principles to balance risk minimization to blood recipients with sufficiency of blood supply, which requires ongoing assessment of donor eligibility criteria [27, 28]. In this context, deferral of donors who present negligible transfusion-transmission risk is not consistent with a risk-based approach to blood safety and may present a threat to the blood supply. Lifeblood uses an internal framework for determining the tolerable transfusion risk level based on the Alliance of Blood Operators Risk Based Decision Making Framework, which acknowledges that risk cannot be reduced to zero but should be as low as reasonably achievable while maintaining sufficiency of supply [29]. Lifeblood has determined that the tolerability threshold for an agent classified as posing a threat of 'catastrophic severity', such as vCJD, is 'tolerable' if kept below 1 in 5 million per unit transfused.

Concerns over the potential for a second wave of vCJD and the lack of an effective treatment are potential reasons for the relative lack of widespread policy change around vCJD transfusion risk [18, 20]. However, recently the US Food and Drug Administration (FDA) amended and removed some of its vCJD-related geographical deferrals [30], and Ireland became the first country to lift a vCJD deferral for past UK residents [31]. Notably, the United Kingdom has also rescinded its precautionary vCJD risk reduction measure requiring the use of imported plasma and apheresis platelets for individuals born on or after 1 January 1996 and/or with thrombotic thrombocytopenia [31], and, most recently, announced it would lift its 1998 ban on fractionating locally sourced plasma [17, 32]. Review of deferral policy in the United States by the FDA has been guided by stochastic risk assessments modelling a range of risk scenarios, including those with extended incubation periods [5].

We adopted the FDA methodology for red cell transfusion risk [5] to develop a vCJD risk model for transfusion of fresh components including red cells, platelets, and clinical plasma in Australia. Our aim was to estimate the risk of vCJD transmission in Australia should the UK residence deferral be discontinued.

METHODS

Australian exposure assessment model

An overview of the simulation model process used to estimate vCJD transmission risk is shown in Figure 2, with specific input parameters listed in Table 1 and technical detail provided in Data S1.

We applied a very similar methodology to that used by the FDA to estimate the prevalence of undiagnosed pre-symptomatic vCJD in the Australian population (Figure 2: Stage 1a). We based our modelling on published stochastically modelled UK prevalence estimates (100 asymptomatic undiagnosed cases in 2011, equivalent prevalence of 1.7/million) [6], which are broadly consistent with outbreak numbers [8]. We assumed no change in prevalent undiagnosed, pre-symptomatic vCJD cases. Relative risk according to year and duration of exposure during 1980–1996 was based on relative risk of UK BSE cases per given year (Table S2) [37]. These data were used to develop estimates of the current age-specific prevalence of undiagnosed pre-symptomatic vCJD in the United Kingdom by calendar year and duration of exposure. The age distribution of prevalent undiagnosed pre-symptomatic vCJD for MM genotypes was based on observed cases to 2003 (Table S1) [23] and adjustment was made to incorporate a longer estimated incubation time for non-MM genotypes (35 years) (Table 1) [5, 6, 21, 33–35].

Outputs were generated through Monte Carlo simulation using at least 10,000 replications, or convergence to 1% level of deviance for all output parameters (Figure S1) using Stata statistical software

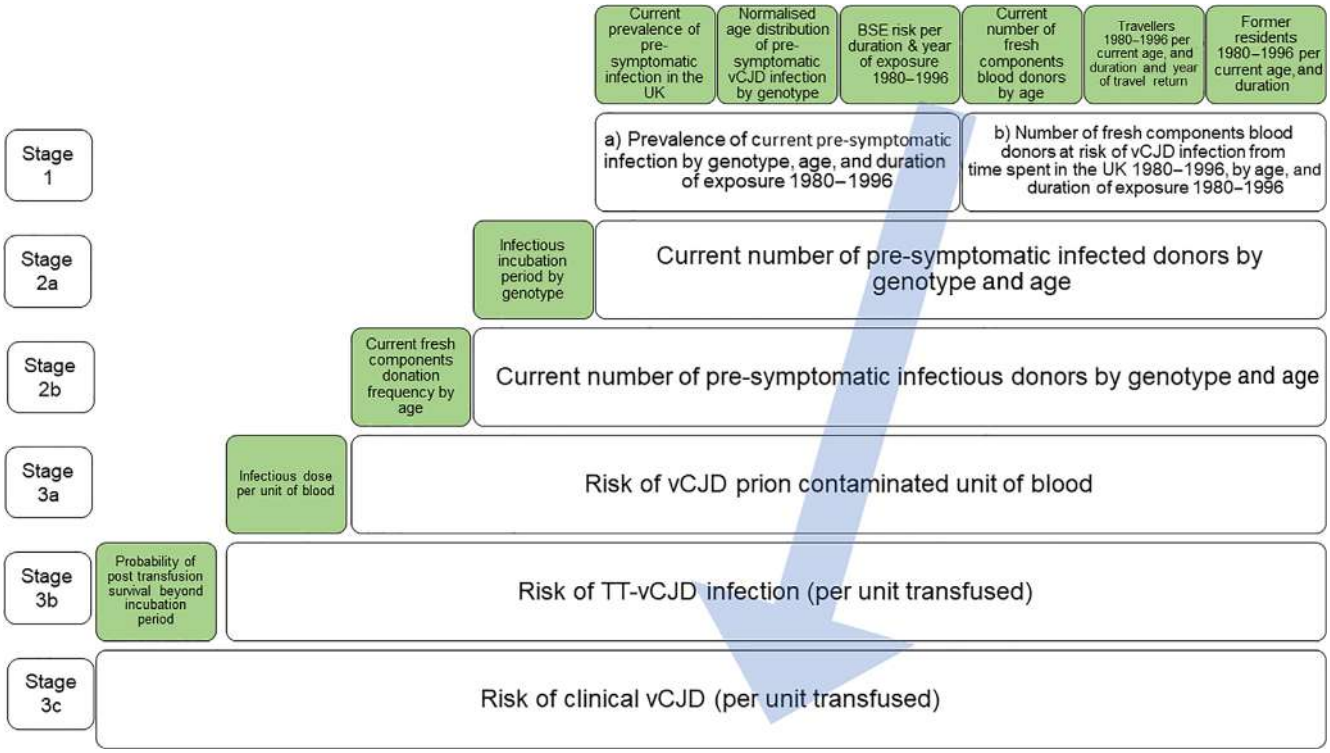

FIGURE 2 Model overview. Stage of model process represented by rows. Boxes in upper rows contributing to estimation of boxes immediately below. Green boxes represent external inputs, white boxes represent calculated outputs.

(Stata 15.1, StataCorp, College Station, TX). Where sufficient data was available, and unless otherwise specified, parameter inputs were drawn from assumed triangular distributions, which concentrate simulated outputs about the mean and which have restricted ranges of risk and are therefore not strongly affected by extreme simulated values.

Outputs

Primary outputs were the 2020 risks of a (1) vCJD contaminated unit, (2) vCJD transfusion-transmission, and (3) case of clinical vCJD (infection resulting in disease).

We estimated exposure risk associated with time spent in the United Kingdom but not in the rest of Europe since Lifeblood restricted deferral to UK residence only. Because no donor-specific exposure data were available, exposure risk in the general population was used based on Australian Bureau of Statistics (ABS) population data, while blood donor numbers were based on Lifeblood internal data (Figure 2, Stage 1b). This approach assumes that the annual age-specific vCJD prevalence in donors is equal to that of the Australian general population. We estimated prevalence separately for both travellers to the United Kingdom and for former UK residents currently living in Australia. An at-risk traveller to the United Kingdom was defined as a resident who spent time in that country during the risk period 1980–1996 and who has returned to Australia from the United Kingdom at some time before 2007 (up to 10 years after the end of the risk period). A former resident was defined as a person counted as living in Australia by usual place of residence who was born in the United Kingdom.

Blood donor population

Overall donor numbers were based on Lifeblood data 2014–2018 (Table S3) as well as Lifeblood projections for 2021–2025 (Table S4). We limited consideration of risk to donors of fresh blood components, given prion infectivity is substantially decreased during the fractionation process [42]. The age distribution of fresh component donors for each year was based on observed age-specific fresh component data for 2018 (Table S5). Annual age-specific frequencies of donation per donor were based on 2014–2018 donation frequency in all donors, which were extrapolated to later years 2019–2025 using linear regression. Annual age-specific donation rate per capita was then estimated based on ABS annual estimated resident population and projected population data [43, 44].

Prior UK resident population

Age-specific ABS census data on number of citizens with the United Kingdom as the country of birth by year of arrival were used to estimate the age-specific at-risk population of former residents for each calendar year by duration (6–12, 12–36, 36–60, and

TABLE 1 Major input distributions used in Australian exposure assessment model

Parameter	Selected value	Simulation distribution	Source
Primary exposed vCJD cases remaining at 2012+	Mean = 100 (95% CI [11–220])	Triangular	Garske [6]
(Sensitivity analysis) Appendix survey abnormal prion protein prevalence	Mean = 493 cases/million (95% CI [282–801] cases/million)	Triangular	Gill [24]
Prion protein genotype distribution MM:MV:VV	42%:47%:11%	Point estimates	Nurmi [21]
vCJD incubation (MM genotype)	Mean = 15 years (90% CI [9–35])	Log-normal	Yang [5], Garske [6]
vCJD incubation (MV and VV genotypes)	Mean = 35 years, (90% CI [23–55])	Log-normal	Yang [5], Garske [6], Collinge [33, 34], Kaski [35]
Age distribution of vCJD cases (MM)	Table S1	Point estimates	Hilton [23]
UK population by age group 2003	Table S1	Point estimates	UK National Statistics [36]
Annual UK BSE cases 1980–1996	Table S2	Point estimates	World Organization for Animal Health [37]
Age- and year-specific frequency of blood donors 2014–2018	Table S3	Triangular using linear regression prediction 2020	Lifeblood
Projected fresh components donor numbers 2018–2025	Table S4	Triangular using linear regression prediction 2020	Lifeblood
Age distribution of fresh components donors 2018	Table S5	Point estimates	Lifeblood
Year-specific duration of travel to UK (1980–1996)	File S2	Point estimates	ABS (custom report)
Age specific distribution of travel	Data table: ABS Cat. 3401	Point estimates	ABS
Age-specific Australian population (1980–2019)	Data table: ABS Cat. 3101059	Point estimates	ABS
Age-specific Australian population (2020–2060)	Data table: ABS Cat. 32220	Point estimates	ABS
Age-specific survival	Data table: ABS Cat. 3302055001	Point estimates	ABS Life tables 2016–2018
Repeat travel to the UK 1980–1996	61%	Point estimate	Seeteram [38]
Age, year of arrival specific Australian residents born in the United Kingdom	Data table 2006 Census: Persons by age, year of arrival and birthplace; 2011 Census: Persons by age, year of arrival and birthplace; 2016 Census: Persons by age, year of arrival and birthplace	Point estimate	ABS
Proportion of late incubation period infectious blood	Mode = 0.75 (min = 50% max = 0.90)	Triangular	Yang [5], Houston [39]
Infectious doses per unit of blood	Mode = 0.09 (min = 0.01 max 0.14)	Triangular	Salamat [16]
Incubation for transfusion-transmitted case (MM)	Mean = 10 years (min = 6 max = 20)	Triangular	Bennet and Darachtchiev [40]
Incubation for transfusion-transmitted case (MV and VV)	Mean = 20 years (min = 16 max = 30)	Triangular	Bennet and Darachtchiev [40]
Post-transfusion survival rate	Data S1, Function (genotype, incubation period)	Function	Borkent-Raven [41]; ABS Lifetables

>60 months) and period at risk 1980–1996 [45–47]. Because there was no detailed data on durations of exposure of less than a year, any exposure during 1980–1996 of less than a year was considered deferrable with duration '6–12 month'. Age-specific former resident proportions of census population totals by year and duration of exposure were scaled by ABS-estimated residential population numbers and projected population numbers to estimate final numbers at risk [43, 44]. Intercensal periods were interpolated, while projected populations were extrapolated and then adjusted for mortality using ABS life tables [48].

Prior UK traveller population

To estimate numbers of travellers at risk, we used an ABS custom-generated report of travellers returning from the United Kingdom by the duration of stay (<1, 2–3, 3–6, 6–12, 12–36, 36–60, and >60 months) and year of return to Australia (1980–2006) [49]. For returning travellers whose stay commenced prior to the start of the risk period (1980) or whose stay finished after the end of the risk period (1996), travel duration was reduced to reflect only the interval of travel at-risk (1980–1996). We developed an age distribution for these travellers based on ABS-published national travel data, which assumed constant age distribution by duration and year of travel [50]. We adjusted estimates by an index based on probability of repeat travel to the same destination by Australian travellers [38]. We assumed an average of two trips over the at-risk period for the proportion of travellers who repeatedly travelled where that travel was less than 1 year total duration, and an average of one trip if over 1 year duration.

Prevalence of vCJD in blood donors

Age- and genotype-specific prevalence estimates were applied to estimated Australian at-risk donor populations described above to estimate current age-specific prevalence of asymptomatic, undiagnosed infection in the Australian blood donor population (Figure 2, Stage 2a). Simulated genotype-specific vCJD incubation periods were drawn from log-normal distributions (Table 1) and adjusted by the proportion of the incubation period where blood was infectious to estimate the current number of asymptomatic donors who were infectious (Table 1; Figure 2, Stage 2b).

vCJD transmission risk

Donor prevalence data were used to estimate risks per unit of blood transfused for primary outputs:

- Risk of vCJD contamination ($P_{\text{TT-vCJD\_U}}$), by estimating prevalence of vCJD−associated prion contamination per unit transfused based on age-specific donation frequencies and quantities in infected donors relative to the general donor population (Table 1; Figure 2, Stage 3a). This was calculated as the complement of the cumulative binomial probability of receiving no vCJD-infected blood ($B = 0$) during transfusion per unit transfused ($N_u = 1$) and given prevalence of infected units (P_u):

$$P_{\text{TT-vCJD\_U}} = 1 - \text{Binomial distribution}\,(B = 0, N_u = 1, P_u)$$

- Risk of vCJD transfusion-transmitted infection, by scaling the risk of vCJD prions by simulated probabilities of infectious dose per unit transfused (Table 1; Figure 2, Stage 3b) [16].
- Risk of clinical vCJD, by scaling risk of vCJD infection by simulated probabilities of post-transfusion survival beyond the genotype-specific TT-vCJD incubation period (Table 1; Figure 2, Stage 3c) [40, 41].

Sensitivity analysis

The sensitivity of estimates to changes in assumptions about distributions of model parameters was evaluated. Specifically, low (2.5th percentile) and high (97.5th percentile) bounds on parameter distributions were sequentially used as fixed parameter values and model estimates generated as for the primary analysis using Monte Carlo simulation.

Detailed assessment of the sensitivity of estimates to changes in assumed prevalence of undiagnosed vCJD infections was conducted. We plotted the distribution of vCJD risk using the range of prevalences from the lower half of the distribution generated by Garske and Ghani (11–100 future cases) [6].

Because of theoretical concerns of potentially higher UK vCJD infectivity prevalence suggested by the UK Appendix surveys, we conducted additional analyses using the higher prevalence estimate of 493 cases per million (95% CI [282–801]) derived from the Appendix II survey results and as used in the FDA 'high-prevalence' analysis [5, 24, 25, 51].

To validate model findings, we estimated the cumulative total number of transfusion-transmitted cases expected to have arisen in Australia over the period 1980–2020 using both the Garske-modelled prevalence, as well as the Appendix II-based prevalence used in sensitivity analyses. We adjusted model parameters to account for the impact of the implementation of the donation deferral in December 2000 and the national implementation of leucodepletion for RBC and platelets in October 2008. Model outputs were also used to estimate the total number of clinical cases in Australia associated with primary exposure for the period 1980–2020 under both prevalence assumptions (see Data S1 for methodological details).

RESULTS

Donation

Donor numbers exposed to risk

The predicted number of donors providing fresh blood components for 2020 was 395,625. Of these donors, 14,016 (3.5%) met UK deferral criteria, comprising 9145 (2.3%) deferrable due to prior UK residency (2.0% donation rate from 448,372 individuals in general population with deferrable prior UK residency) and 4871 (1.2%) due to prior travel to the United Kingdom (1.7% donation rate from 288,740 individuals in general population with deferrable UK travel). The potential eligible donor population increase associated with ending the deferral was 737,112 (Table S6).

Predicted vCJD risk in donors

Mean predicted number of donors with vCJD in 2020 was 0.015 (2.5th–97.5th percentile: 0–0). A donation from a person with vCJD was predicted to occur once every 65 years (i.e. based on the same conditions as 2020). This risk was higher in donors with prior residency (1 in 70 years of donation) than for donors with travel-related exposure (1 in 900 years).

Transfusion

The risk of vCJD-contaminated donations, transfusion-transmissions and clinical cases of vCJD are presented in Table 2. Mean risk of contamination per unit was 1 in 30,000,000, the risk of vCJD transmission (infection) was 1 in 389,000,000 and the risk of a clinical case in a recipient (vCJD transfusion-transmission) was 1 in 1,450,000,000.

Sensitivity analysis

The results of the importance analysis indicated that the infectious dose per transfused unit and prevalence of vCJD infections in the United Kingdom had the most impact on the risk estimates for vCJD cases per unit of blood transfused in 2020 (Figure 3).

Sensitivity of estimated vCJD infection risk to change in assumed prevalence

Change in assumed prevalence of pre-symptomatic vCJD infection from 11 to 100 cases was associated with an increase in vCJD transmissions from less than 1 in 1.5×10^{10} to 1 in 1.6×10^{9} for prior resident donation exposure; and from less than 1 in 2.1×10^{11} to 1 in 1.4×10^{10} for travel-related donation exposure (Figure 4). Risk of a clinical case in a recipient (vCJD transfusion-transmission) based on the Appendix II-based prevalence estimate was 1 in 5,240,000 (Table S7). The model predicted 0.04 cases of TT-vCJD (2.5th–97.5th percentile, 0–0) for the period 1980–2020, with 1.1 cases (2.5th–97.5th percentile, 0–0) in the Australian population for the same period associated with primary exposure. Sensitivity analysis based on the Appendix II-based prevalence assumption predicted 8.0 cases of TT-vCJD (2.5th–97.5th percentile, 0–46), with 368 cases in the Australian population (2.5th–97.5th percentile, 0–1408) (Table S8).

DISCUSSION

Our modelling suggests that removing the UK residence deferral in Australia would result in virtually no increased risk of vCJD transfusion-transmission to recipients. In sensitivity analyses based on lower assumed prevalence, consistent with observed global case numbers, we found even lower risk. Risk will also reduce further as donors meeting current exclusion criteria exceed age thresholds for blood donation.

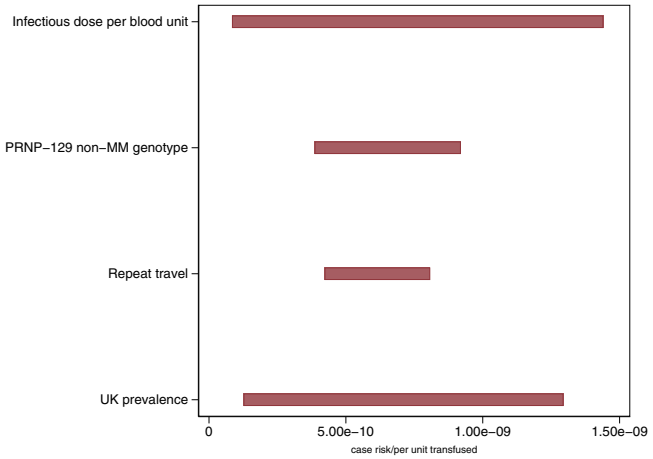

FIGURE 3 Importance analysis of input parameters to estimated annual number of future cases arising from infected Australian donors in 2020, for all blood fractions and combined travel and residency exposure groups. Analysis based on Monte Carlo models using 10,000 simulations, with assumptions regarding all other parameter distributions unchanged.

TABLE 2 Mean vCJD risk in 2020 by risk exposure group

Risk	Exposure group	Mean (2.5th–97.5th percentile)[a]	Point estimate (1 in *x*)
vCJD contamination/unit	Prior UK resident 1980–1996	3.00×10^{-8} (0–0)	33,000,000
	Travel to UK 1980–1996	3.34×10^{-9} (0–0)	299,000,000
	Total	3.34×10^{-8} (0–0)	29,900,000
vCJD transfusion-transmission/unit	Prior UK resident 1980–1996	2.30×10^{-9} (0–0)	435,000,000
	Travel to UK 1980–1996	2.69×10^{-10} (0–0)	3,720,000,000
	Total	2.57×10^{-9} (0–0)	389,000,000
Clinical vCJD/unit	Prior UK resident 1980–1996	6.16×10^{-10} (0–0)	1,620,000,000
	Travel to UK 1980–1996	7.20×10^{-11} (0–0)	13,900,000,000
	Total	6.88×10^{-10} (0–0)	1,450,000,000

[a]Distribution is positively skewed with mean greater than 97.5th percentile, associated with rare simulation instances of the occurrence of infected potential donors.

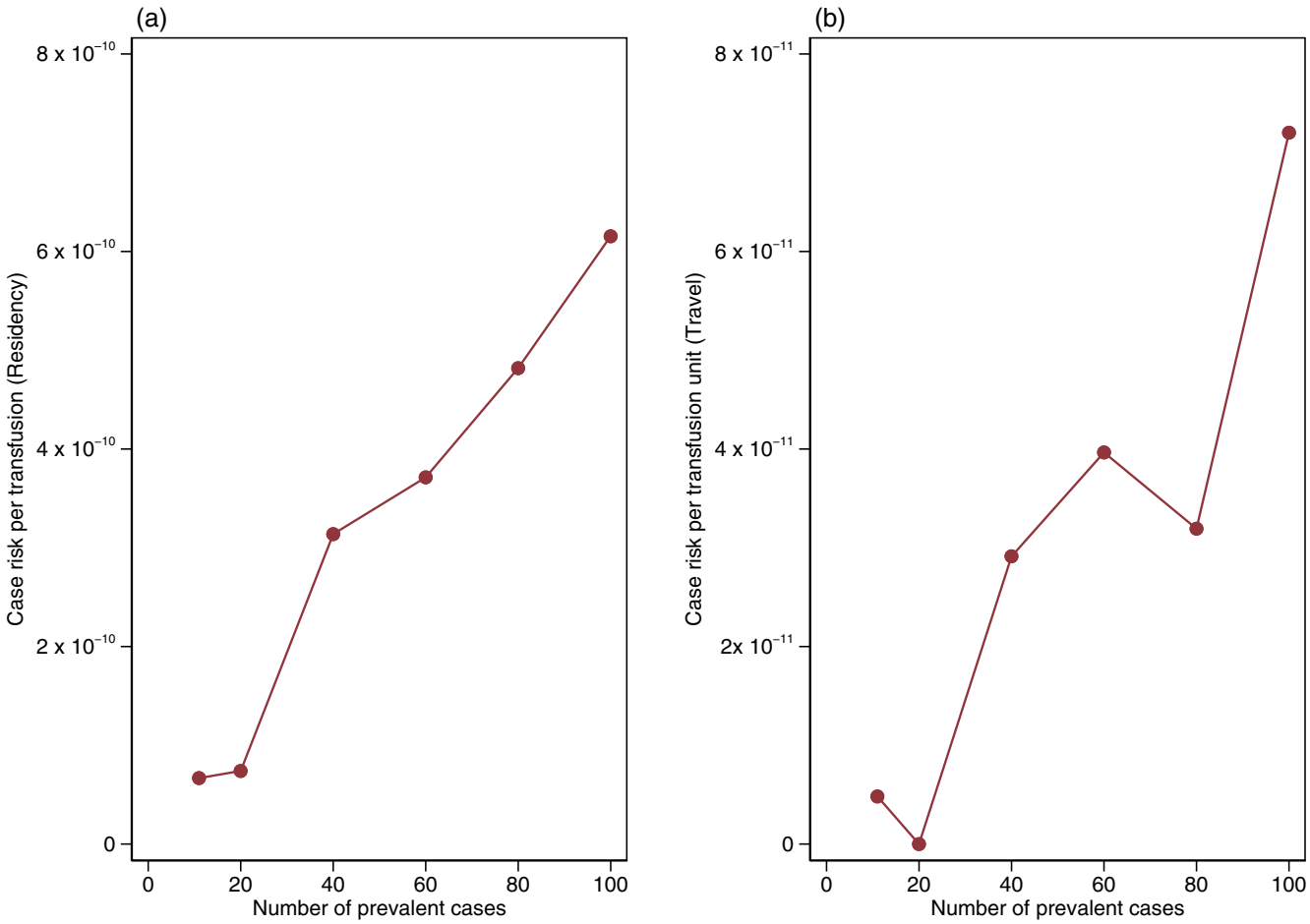

FIGURE 4 Estimated risk of transfusion-transmitted infection 2020 by change in assumed number of remaining prevalent cases and blood fraction by donor exposure group: (a) former residents 1980–1996; (b) travellers 1980–1996.

The mean risk of a clinical vCJD case per unit transfused for 2020 was estimated to be 1 in 1.5 billion if no UK residence deferral applied. This estimate was influenced primarily by risk from donors with prior residency in the United Kingdom (1 in 1.6 billion) compared to donors with travel-related history. No infections from transfusion were predicted for 2020 with or without a deferral. Over 99% of simulations estimated zero vCJD transmission risk (97.5th percentile equals zero), with the mean risk estimate driven by less than 1% of simulations generating rare cases.

Our risk estimates were sensitive to assumed current prevalence of UK primary exposure vCJD cases. We based analyses on an estimated 100 primary exposure cases (95% CI [11–220]) in the United Kingdom during or after 2020 according to Garske and Ghani, who predicted this number from 2010 onwards [6]. The Garske model estimated an annual case load increase from around 5 cases per year in 2011 to a peak in the period 2020–2030 at around 10 cases per year [6]. Given that there has been less than one case per year on average in the United Kingdom over that interval, and one case since 2014, this projection has substantially overestimated current risk [8].

In their vCJD transfusion risk modelling of transfused RBCs in the United States in 2011, Yang et al. [5] presented risk estimates using both a 'low' UK vCJD prevalence estimate based on the Garske model (100 asymptomatic undiagnosed cases in 2011, equivalent prevalence of 1.7/million) and a 'high' estimate derived from the rate of abnormal prion detection (493 cases per million) in surgically removed appendices in the UK [6, 24]. Yang et al. concluded that while the 'low' prevalence estimate provided reasonably accurate predictions for clinical cases of primary food-borne vCJD in the United States and transfusion-transmitted cases of vCJD in the United Kingdom and France, the use of the 'high' prevalence estimate led to much higher numbers than recognized at the time (2011) [5]. They concluded 'predictions based on the low prevalence estimate are more consistent with clinical cases actually observed to date, implying that the risk, while highly uncertain, is likely very small' [5]. In the intervening decade, no further cases of TT-vCJD have been recorded worldwide, and the results of a follow-up study (Appendix III) of the rate of abnormal prion protein in surgically removed appendices in the United Kingdom from before and after exposure to the BSE epizootic have been published [8, 25]. The latter study was intended as the 'control' for the two prior UK appendix studies (Appendices I and II) [23, 24], but the detection of positive appendices removed in both the pre-1980

and post-1996 cohorts (where no positive appendices were predicted because individuals were not 'exposed' to BSE contaminated products) complicated the interpretation of Appendices I and II results and, arguably, casts doubt on the original assumption that detection of abnormal prion protein in appendices correlates with pre-symptomatic vCJD [23–25] or a plausible infectivity estimate (refer to Data S2 for comprehensive discussion and analysis). The Appendix III investigators suggested two possible interpretations for their findings: (1) a low background prevalence of abnormal PrP in human lymphoid tissues that may not progress to vCJD or (2) all positive specimens are attributable to BSE exposure, a finding that would necessitate human exposure having begun in the late 1970s and continuing through the late 1990s [25]. Considering the conclusions of Yang et al. on the predictive accuracy of observed cases of vCJD and TT-vCJD using the 'low' and 'high' prevalence estimates, combined with the subsequently published results of the Appendix III study and the lack of additional cases of TT-vCJD despite more than 50 million transfusions in the United Kingdom since 2000, we consider it inappropriate to use the Appendix II data as a valid model parameter estimate for UK vCJD infectivity prevalence, and therefore use the 'low' (Garske-modelled) estimate as the basis for our risk estimates [5]. However, in the context of a sensitivity analysis, we did use the Appendix II-based estimate and confirmed the conclusions of Yang et al. that the predictions for vCJD cases and TT-vCJD cases in Australia to 2020 were incompatible with the (zero) observed cases (Tables S7 and S8) [5].

Our analysis took account of the potential for new peaks in vCJD risk associated with differing genotype-specific incubation periods, applying a mean incubation period for non-MM genotypes of 35 years as per Yang et al. [5]. Our resulting projections over the period 2020–2025 found continued declining transfusion risk in Australia under this assumption (Figure S2). In an importance analysis, non-MM incubation period was found to be an influential parameter in the model, after assumed prevalence levels and infectious dose per unit transfused. While it is possible that mean incubation periods for non-MM genotypes might exceed 35 years [33–35], the importance analysis showed only moderate change in potential future peak risk under this assumption. This predicted risk is reduced over time by decreases in numbers of at-risk donors as they reach the upper age of eligibility. Observed low case numbers may simply reflect that the susceptibility to vCJD in people with non-MM genotypes is well below the level assumed by Garske and Ghani [6].

In respect of infectivity per unit transfused, our modelling is based on the recent publication of updated ovine data by Salamat et al. [16]. This sheep transfusion model included sheep at all stages of the illness and therefore increased the risk, and suggests a transmission rate of 0.31 per unit in non-leucodepleted blood, with a 71% reduction to 0.09 with leucodepletion (0.01–0.14). This is also consistent with the updated UK-modelled risk assessment that used an infectivity per unit of 0%–20% [17].

There are some limitations to our analyses. We were not able to take account of the possible overlap between travel and prior-residency status in donors and therefore may have overestimated number of potential donors at risk who were categorized as both travellers and prior residents. However, the much stronger influence of prior residency as compared to travel on exposure risk means that any such adjustment is likely to have limited effect on estimates. A second issue is that projections of future vCJD transmission risk rely strongly on predicted future population growth, and predicted numbers of donors with prior UK residency, which in light of the ongoing COVID-19 pandemic are particularly uncertain in the post-2020 Australian environment.

Our modelled projections suggest that removal of the UK residence deferral would be a safe and effective strategy for increasing the donor base, as the potential risk is so small it can be considered essentially equivalent to the vCJD risk under the current deferral policy, while providing a substantial sufficiency benefit. The 2019 UK SHOT report reports a risk of death of 1 in 135,705 from overall transfusion-related adverse reactions [52]. Our estimate of 1 in 1.5 billion vCJD risk adds 0.01% to the baseline risk, which does not materially change the overall risk (1 in 135,690). However, there is a significant sufficiency gain, and we conservatively predict that the total donor number would increase by over 17,000 (57,000 donations annually) under this scenario, from the current population base of over 700,000 who are not eligible to donate in Australia because of the current deferral. [43].

Concluding, we predict a miniscule additional risk of vCJD transmission by transfusion in Australia if the current donor deferral for UK residence were to be ceased. Given that the predicted transmission risk without the deferral is magnitudes below that considered tolerable for blood safety in Australia and does not materially contribute to the total risk inherent in a blood transfusion, the Australian authors submitted to the Australian regulator a proposal to end this deferral and safely expand the donor base, which has now been approved. Our method may be useful to other blood operators wishing to reassess their vCJD transfusion risk exposure.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Development of the Chinese Haemovigilance Network and reporting of adverse transfusion reactions from 2018 to 2020

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Abstract

Background and Objectives: To advance blood transfusion safety, the Chinese Haemovigilance Network (CHN) was put into operation in 2018. This report describes the development of the CHN and evaluates its role by analysing reported adverse transfusion reactions (ATRs) from 2018 to 2020.

Materials and Methods: All data in this study were obtained from the CHN online reporting platform. A timeline of CHN development is presented, and the activities of CHN-enrolled facilities are analysed by year. The reported ATRs were analysed in detail for ATR types, blood components involved and adherence to case definition, severity and imputability criteria. Incidence rates were calculated and compared with international examples.

Results: During 2018–2020, a total of 3061 ATRs were reported through the CHN online reporting system. The rate of reported ATRs in all facilities and the 10 highest reporting facilities was 0.7‰ and 1.8‰, respectively. When analysed by year, the incidence rate showed an increasing trend from 2018 to 2020. Allergic (68.2%) and febrile non-haemolytic transfusion reaction (27.1%) were the most common. The vast majority of ATRs (92.0%) were not serious, but serious cases of transfusion-associated circulatory overload, transfusion-associated dyspnoea and hypotensive reaction were common. Most (86.0%) of reported cases were definitely or probably associated with transfusion.

Conclusion: Under-reporting of ATRs occurs in many Chinese hospitals, but the establishment of CHN has increased ATR recognition and management. More effort will be needed in the future to detect transfusion problems and improve transfusion practice in China.

KEYWORDS
adverse transfusion reactions, healthcare quality improvement, haemovigilance, national development, patient safety

[†]The Collaborators of the CHN Main Alliance Team are given in Appendix A.

Ling Li and Zhong Liu contributed equally as joint corresponding authors.

Highlights

- This report describes the development of the Chinese Haemovigilance Network (CHN) over a recent 3-year period and highlights the importance of haemovigilance implementation in China.
- Although CHN had a number of limitations at the early stages, progress has been made in the management of adverse transfusion reaction reporting.
- More effort is needed to develop CHN in the future, and it is hoped that the development of the CHN can provide a useful reference for other countries.

INTRODUCTION

Blood transfusion is a critical therapeutic modality that carries both benefits and risks. The establishment of a haemovigilance system is one of the most important strategies to reduce transfusion risks and ensure transfusion safety [1–3]. Many countries have established haemovigilance systems and some achievements have been made in blood transfusion management [4–7].

China uses more than 20 million units of blood components every year and this large number creates great challenges in transfusion safety management [8–10]. In the absence of a national haemovigilance system, adverse transfusion reactions (ATRs) in China have not been well studied, and its incidence, types and severity remain unclear. Therefore, the Chinese Haemovigilance Network (CHN) was established to meet the transfusion challenges and achieve better investigation of ATRs [11]. In this report, we describe the development of the CHN since its establishment in 2018, along with an analysis of reported ATRs from 2018 to 2020, aiming to obtain a better understanding of ATR reporting and evaluate the effectiveness of the work of CHN.

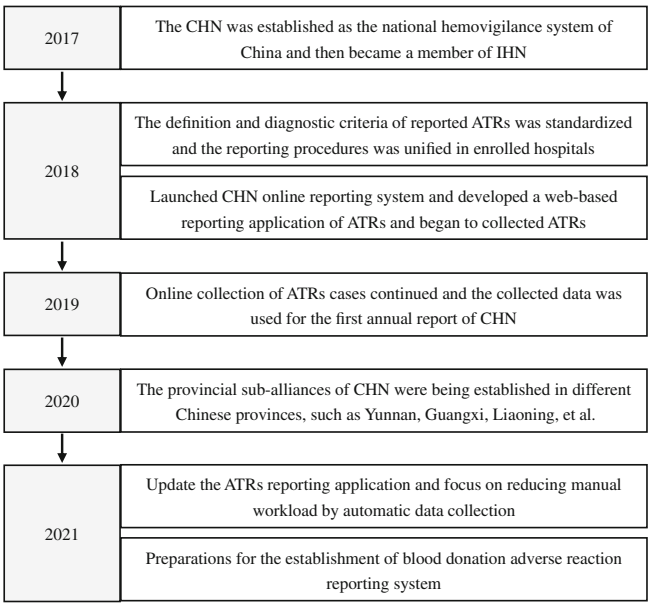

FIGURE 1 The timeline of CHN development. ATR, adverse transfusion reaction; CHN, Chinese Haemovigilance Network; IHN, International Hemovigilance Network.

MATERIALS AND METHODS

Data collection

In order to ensure the quality of reported data, the enrolment of CHN facilities was limited to grade III and class A hospitals, which are the highest quality hospitals with top-tier designation in China. The hospitals were requested to report the cases as soon as possible after the ATR occurrence through the online reporting platform of the CHN. Any abnormal conditions considered to be associated with blood transfusion were requested to be reported, regardless of the severity of the symptoms. In addition, the number of patients transfused was to be reported monthly.

The reported ATRs were to be classified into the reaction types listed in Table 2 and then graded for 'case definition', 'severity' and 'imputability' as shown in Table 3. All reported cases were evaluated twice according to the CHN procedure. The first evaluation was made by the hospital staff immediately after the ATR

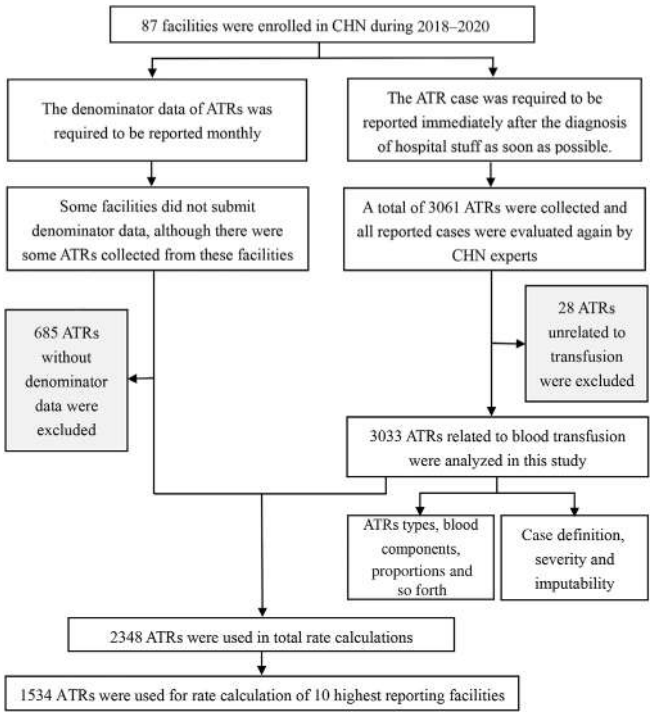

FIGURE 2 Flow diagram of reported data inclusion and adverse transfusion reactions analysis steps, 2018–2020

occurrence. Then, the reported case was evaluated again, and the final diagnosis was made by CHN staff based on the information in the CHN system.

Statistical analysis

The development of CHN is represented by a timeline from 2017 to 2021 (Figure 1). The reported ATRs were analysed in aggregate for incidence rate, reaction types and transfused components and graded for case definition, severity and imputability. For the calculation of ATR incidence rate, some cases were excluded because of the lack of corresponding denominator information. The incidence rate was calculated based on the number of single-unit transfusion episodes regardless of the actual volume transfused. The total incidence rate was calculated according to the data from all reported hospitals with the denominator information. A second incidence rate was calculated on the basis of data from the 10 highest reporting hospitals. The observed incidence rates were not only analysed from the different facilities but also compared between the years 2018 and 2020.

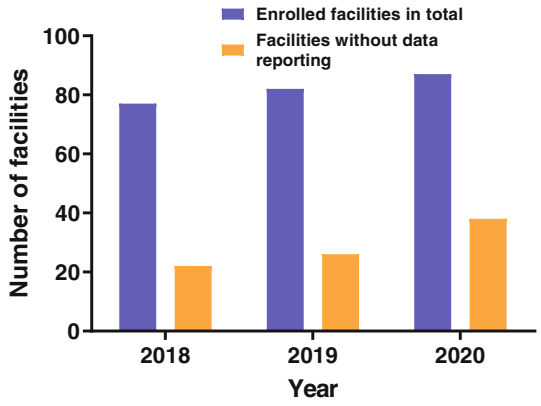

FIGURE 3 Reporting condition of facilities enrolled in the network, 2018–2020

RESULTS

Through the study period, 87 facilities were enrolled in the CHN and registered in the ATR online reporting system (Figure 2). All facilities were viewed as highest quality hospitals, having been rated as grade III and class A healthcare institutions. Many facilities did not submit the data as requested; the annual non-response rate was 28.6%, 31.7% and 43.7%, respectively, in 2018, 2019 and 2020 (Figure 3).

A total of 3061 cases were collected during the study period; 28 cases were judged as not associated with the transfusion process. Another 685 cases were excluded because the corresponding denominator data were missing. The remaining 2348 ATRs were included for incidence rate calculations (Figure 2). The total incidence rate of ATRs observed in this study was 0.7‰ (2348/3,375,301); annual rates during 2018–2020 were

TABLE 1 Incidence rate of reported adverse transfusion reactions (ATRs), 2018–2020

	All facilities			Ten highest reporting facilities		
Year	ATRs	Transfusions	Rate (‰)	ATRs	Transfusions	Rate (‰)
2018	732	1,248,952	0.6	422	398,639	1.1
2019	885	1,369,107	0.7	543	270,990	2.0
2020	731	757,242	1.0	569	166,629	3.4
Total	2348	3,375,301	0.7	1534	836,257	1.8

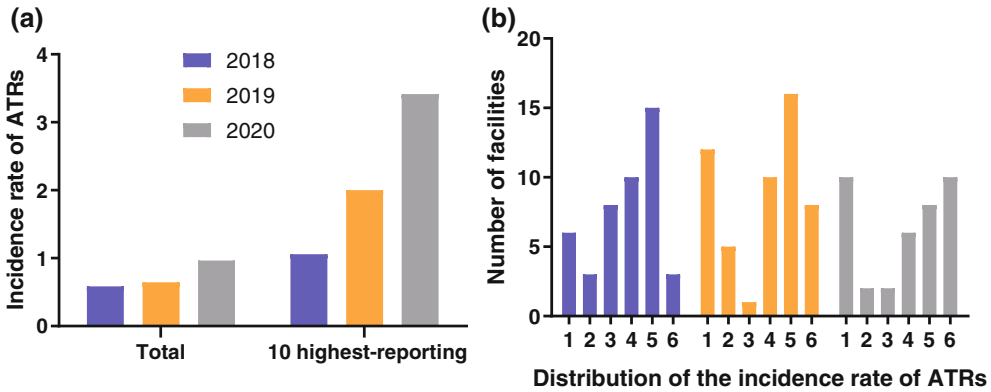

FIGURE 4 Distribution of the incidence rate of reported adverse transfusion reactions (ATRs) (a) by year and (b) by facility with the groupings as 1: Incidence rate of ATRs ≥2.0‰; 2: 1.5‰ ≤ incidence rate of ATRs <2.0‰; 3: 1.0‰ ≤ incidence rate of ATRs <1.5‰; 4: 0.5‰ ≤ incidence rate of ATRs <1.0‰; 5: 0.0‰ < incidence rate of ATRs <0.5‰; and 6: Incidence rate of ATRs = 0.0‰.

TABLE 2 Types of reported adverse transfusion reactions (ATRs) and the corresponding blood components transfused, 2018–2020

Components	ATRs	Allergic	FNHTR	TACO	TAD	Hypo	DHTR	AHTR	DSTR	Other/unknown
All	3033	2067 (68.2%)	822 (27.1%)	19 (0.6%)	37 (1.2%)	11 (0.4%)	14 (0.5%)	12 (0.4%)	2 (0.1%)	49 (1.6%)
RBCs	939 (31.0%)	320	524	14	18	6	12	10	2	33
Plasma	1014 (33.4%)	845	142	4	11	4	0	0	0	8
Platelets	1008 (33.2%)	842	146	0	8	1	2	2	0	7
Cryo	62 (2.0%)	54	6	1	0	0	0	0	0	1
Other	10 (0.3%)	6	4	0	0	0	0	0	0	0

Abbreviations: AHTR, acute haemolytic transfusion reaction; Cryo, cryoprecipitate; DHTR, delayed haemolytic transfusion reaction; DSTR, delayed serological transfusion reaction; FNHTR, febrile non-haemolytic transfusion reaction; Hypo, hypotensive transfusion reaction; Hypo, hypotensive; Other, autoblood, whole blood and granulocytes; RBC, red blood cell; TACO, transfusion-associated circulatory overload; TAD, transfusion-associated dyspnoea.

TABLE 3 Grade assessment of ATRs by case definition, severity and imputability

	Case definition			Severity				Imputability				
ATRs	Def	Prob	Poss	Non-S	Sev	LT	Death	Def	Prob	Poss	Dou.	ND
All (3033)	2085 (68.7%)	811 (26.7%)	137 (4.5%)	2790 (92.0%)	236 (8.5%)	5 (0.2%)	2 (0.1%)	1327 (43.8%)	1281 (42.2%)	362 (11.9%)	52 (1.7%)	11 (0.4%)
Allergic (2067)	1294 (62.6%)	773 (37.4%)	0 (0.0%)	1945 (94.1%)	118 (5.7%)	4 (0.2%)	0 (0.0%)	1185 (57.3%)	802 (38.8%)	75 (3.6%)	5 (0.2%)	0 (0.0%)
FNHTRs (822)	687 (83.6%)	0 (0.0%)	135 (16.4%)	760 (92.5%)	62 (7.5%)	0 (0.0%)	0 (0.0%)	137 (16.7%)	430 (52.3%)	225 (27.4%)	28 (3.4%)	2 (0.2%)
TACO (19)	7 (36.8%)	12 (63.2%)	0 (0.0%)	8 (42.1%)	10 (52.6%)	0 (0.0%)	1 (5.3%)	0 (0.0%)	11 (57.9%)	8 (42.1%)	0 (0.0%)	0 (0.0%)
TAD (37)	37 (100.0%)	0 (0.0%)	0 (0.0%)	11 (29.7%)	25 (67.6%)	1 (2.7%)	0 (0.0%)	2 (5.4%)	14 (37.8%)	13 (35.1%)	7 (18.9%)	1 (2.7%)
Hypo (11)	9 (81.8%)	0 (0.0%)	2 (18.2%)	2 (18.2%)	9 (81.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (36.4%)	6 (54.5%)	1 (9.1%)	0 (0.0%)
DHTR (14)	0 (0.0%)	14 (100.0%)	0 (0.0%)	11 (78.6%)	2 (14.3%)	0 (0.0%)	1 (7.1%)	1 (7.1%)	3 (21.4%)	8 (57.1%)	2 (14.3%)	0 (0.0%)
AHTR (12)	0 (0.0%)	12 (100.0%)	0 (0.0%)	8 (66.7%)	4 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	7 (58.3%)	4 (33.3%)	1 (8.3%)	0 (0.0%)
DSTR (2)	2 (100.0%)	0 (0.0%)	0 (0.0%)	2 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Other/unknown (49)	49 (100.0%)	0 (0.00%)	0 (0.00%)	43 (87.8%)	6 (12.2%)	0 (0.0%)	0 (0.0%)	1 (2.0%)	9 (18.4%)	23 (46.9%)	8 (16.3%)	8 (16.3%)

Abbreviations: AHTR, acute haemolytic transfusion reaction; ATR, adverse transfusion reaction; Def, definitive; DHTR, delayed haemolytic transfusion reaction; Doub, doubtful; DSTR, delayed serological transfusion reaction; FNHTR, febrile non-haemolytic transfusion reaction; LT, life-threatening; ND, not determined; Non-S, non-severe; Poss, possible; Prob, probable; Sev, severe; TACO, transfusion-associated circulatory overload; TAD, transfusion-associated dyspnoea.

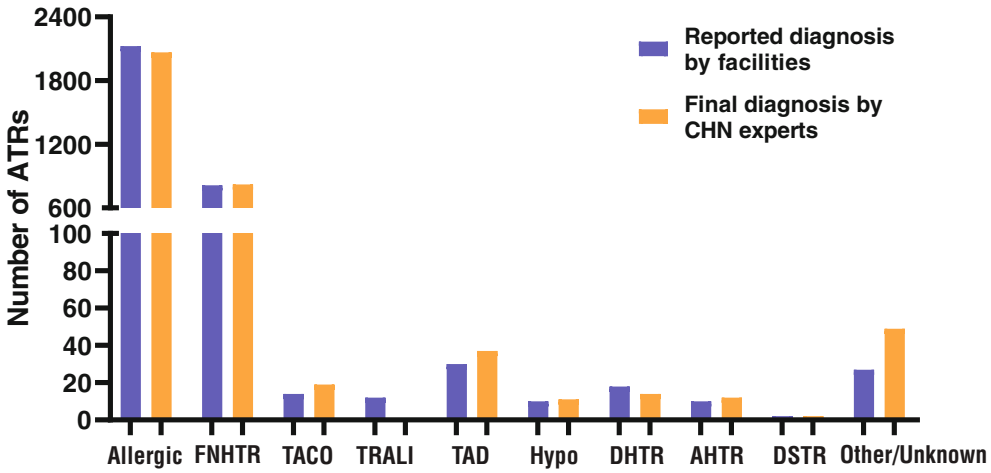

FIGURE 5 Comparison of adverse transfusion reaction diagnosis between facilities and Chinese Haemovigilance Network experts

0.6‰ (732/1,248,952), 0.7‰ (885/1,369,107) and 1.0‰ (731/757,242), respectively. More than half of the ATRs (65.3%, 1534/2348) were reported from the 10 highest reporting hospitals; the overall incidence rate based on these facilities was 1.8‰ (1534/836,257). Annual rates during 2018–2020 were, chronologically, 1.1‰ (422/398,639), 2.0‰ (543/270,990) and 3.4‰ (569/166,629) (Table 1). The incidence of ATRs in most individual hospitals was less than 1.5‰ (Figure 4).

When we analysed the associated blood components of the remaining 3033 ATRs, those attributed to red blood cells (RBCs), plasma and platelets were roughly the same: 31.0% (939/3033), 33.4% (1014/3033) and 33.2% (1008/3033), respectively. Allergic reaction (2067/3033; 68.2%) and febrile non-haemolytic transfusion reaction (FNHTR) (822/3033; 27.1%) were the most common reaction types. The majority of allergic reactions (1687/2067; 81.6%) were associated with either plasma or platelets, with almost equal proportions between them. Unlike allergic reactions, nearly two-thirds (524/822; 63.6%) of the FNHTRs were associated with RBC transfusion, while those attributable to plasma and platelets were about 17% each. There were 56 non-allergy cases with pulmonary symptoms, including 19 transfusion-associated circulatory overloads (TACO) and 37 transfusion-associated dyspnoeas (TAD). Small numbers of hypotensive transfusion reactions (11/3033, 0.4%), acute haemolytic transfusion reactions (AHTR) (12/3033, 0.4%), delayed haemolytic transfusion reactions (DHTR) (14/3033, 0.5%), delayed serological transfusion reactions (DSTR) (2/3033, 0.1%) and other/unknown reactions (49/3033, 1.6%) were also reported. No transfusion-related acute lung injury (TRALI), transfusion-associated graft versus host disease (TAGVHD), post-transfusion purpura (PTP) or transfusion-transmitted infection (TTI) was reported during the study period (Table 2).

All reported cases were graded in terms of meeting case definitions, severity and causal imputability. Based on case definition assessment, 2085 cases (68.7%) met definitive criteria, 811 (26.7%) met probable criteria and 137 (4.5%) met possible criteria. (Table 3) Based on severity assessment, most of the reported ATRs were non-severe (2790/3033; 92.0%). Of the 243 cases judged severe, 236 (8.5% of 2790; 97.1% of 243) were serious, 5 (0.2% of 2790, 2.1% of 243) were life-threatening and 2 (0.1% of 2790, 0.8% of 243) were fatal. The most frequently reported reactions were allergic reactions and FNHTRs; however, most of these were not considered serious (94.1% [1945/2067] and 92.5% [760/822], respectively). In contrast, some ATRs with relatively low reporting frequency were more likely to be serious, especially those involving pulmonary complications and the circulatory system. Well over half of all TACO, TAD and hypotensive reactions were judged to be serious (TACO: 11/19 [57.9%]; TAD: 26/37 [70.3%]; hypotensive: 9/11 [81.8%]). Based on imputability assessment, 43.8% (1327/3033) were meeting definite criteria, 42.2% (1281/3033) were probable, 11.9% (362/3033) were possible, 1.7% (52/3033) were doubtful and 0.4% (11/3033) were non-determinable.

DISCUSSION

CHN was established as the national haemovigilance system to improve blood donation and transfusion safety. The responsibilities of CHN include the monitoring of adverse effects for donors and recipients, providing transfusion medicine education for medical staff, identifying systematic problems in the transfusion field in China, and providing evidence as a basis for future transfusion practice improvements. The monitoring of ATRs was the key work of CHN in its early days. With the help of CHN data, we published the first Chinese Haemovigilance Report and provided an analysis of ATRs during 2019. In the current study, we give an overall view of CHN development and analyse the reported ATRs in detail to show the current reporting status and evaluate the work of CHN in its first 3 years (Figure 5).

The total incidence rate of reported ATRs observed in this study was 0.7‰ based on the overall reported data, which is low compared to other studies [12–15]. The incidence of ATRs reported in this study is probably lower than the real situation, but the data from the 10 highest reporting hospitals are approaching internationally reported rates. Owing to difficulties in data collection, staff shortage, poor compatibility of hospital software and other reasons, ATR

reporting in some hospitals could not be carried out smoothly. Some have not reported any ATR data after registration, and some were not able to report data persistently. In addition, the outbreak of the corona virus disease 2019 (COVID-19) pandemic had a serious impact on Chinese hospitals, resulting in the stoppage of ATR reporting in 2020. However, there are still some facilities that insist on reporting ATR data, even in the face of many difficulties. Through the investigation of these hospitals, we found that the leader's attention to ATR and the number of staff have considerable influence on data reporting quality. In the 10 highest reporting facilities, the leaders of hospital and the transfusion department are willing to pay more attention to ATRs and are always able to ensure sufficient number of staff for reporting work. In addition, staffs in these hospitals generally have a better understanding of ATRs because of more transfusion training.

In many enrolled hospitals, the ATR reporting process means additional workload, so the complexity and volume of data on ATR reporting have a huge impact on the initiative of hospital participation. Therefore, we have simplified the whole reporting process and minimized the amount of data required, so as to reduce the reporting burden of hospital as much as possible. Despite these strategies, some hospitals are still unable to handle the reporting workload, which poses an obstacle to ATR data collection. Therefore, the automatic capture of patient information may be the ultimate way to comprehensive data acquisition and manual workload reduction. When analysing the ATRs incidence rate by year, we did observe that the rate of reported ATRs showed an upward trend in over the 3 years of our study period, not only among the 10 highest reporting units but also in hospitals overall. This suggests that although under-reporting of ATRs exits, ATR awareness and the diagnostic ability of medical staff have improved gradually, which may be partially attributed to the education, guidance and persistence of CHN.

Allergic reactions and FNHTRs were the most common transfusion reaction types in this study, as reported in previous studies also by others [16,17]. The clinical symptoms of these two ATRs are relatively easy to identify, making them less likely to be under-reported. As can be seen from the grade assessment results of case definition, all allergic reactions and the vast majority of FNHTRs met definitive or probable level, and similar results were found in imputability assessment also, which suggests that the reactions were relatively easy to diagnose (Table 3). Thirty-seven cases were reported as TAD because the definitions of TACO, TRALI and allergic reaction were not applicable. No TRALI was reported, which may be related to missing diagnosis and to fewer female blood donors in China. Most of the reported ATRs were not considered serious; symptomatic treatment was sufficient to handle them. However, those involving pulmonary complications often proved serious, also consistent with other studies [18–20]. More than half of the reported TACO were suspected cases because the available evidence was not sufficient to meet the definite level of case definition criteria, limiting the reliability of the diagnosis [21,22].

Hypotensive reactions were reported in 11 cases; most of these patients experienced a sharp drop in blood pressure shortly after the transfusion was started. Some studies show that hypotensive transfusion reactions are more common among patients on angiotensin-converting enzyme inhibitors (ACEI) [23,24]. However, no correlation between ACEI and the occurrence of acute hypotensive reactions could be identified in our study, as only one patient received an ACEI among the 11 reported cases. Some other possible mechanism than ACEI may have contributed to the hypotension during transfusion [25,26].

Only two cases of DSTR were reported in our study, which is evidence of significant under-reporting. DSTRs and DHTRs share similar serological findings, but the DSTR patients do not have clinical signs or laboratory evidence of haemolysis [27,28]. Many Chinese hospitals do not screen patient antibody status after transfusion, especially when no abnormal symptoms are observed in the patients. A centralized, multicentre system based on CHN that keeps a record of patient's red cell antibody histories may be a good option to reduce the risk of DSTR and DHTR.

In the early days of CHN, the total number of participating facilities was limited and the quality of the enrolled hospitals could be screened. All participating hospital haemovigilance units were managed centrally by the CHN Main Alliance Team. In this mode, lead hospitals developed skills to identify many ATRs. However, many recruited hospitals had difficulty meeting their responsibilities, and some failed to report them altogether. The centralized mode contributed to better management of early collected data, but it was not conducive to understanding the overall situation in the thousands of hospitals throughout China. Accordingly, CHN is being restructured based on provincial-level units being established in the different Chinese provinces, such as the already running sub-alliances in Yunnan, Guangxi, Neimenggu and Liaoning. Enrolment in the sub-alliance will be open to all hospitals in the province, which will help expand CHN.

In conclusion, this report described the development of CHN over a recent 3-year period and highlighted the importance of haemovigilance implementation in China. Although many limitations existed in the early stage of CHN implementation, progress has been made in the management of ATR reporting. The analysis of ATRs in this study is useful and provides valuable information about the need for further training in ATR recognition. In addition, the outbreak of COVID-19 pandemic has made us more aware of the importance of an all-sided haemovigilance system, not only ATR but also the donor side, cell therapy and so on, for the comprehensive monitoring of the whole transfusion chain [29]. The donor side of CHN was established at the end of 2021, but more efforts are needed to develop CHN in the future. We hope the development of CHN can provide much valuable data and experiences to share in the near future and thus provide a useful reference for other countries.

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to the study design and manuscript revision. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

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

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APPENDIX A

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ORIGINAL ARTICLE

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Autologous red blood cell transfusion does not result in a more profound increase in pulmonary capillary wedge pressure compared to saline in critically ill patients: A randomized crossover trial

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Abstract

Background and Objectives: Transfusion-associated circulatory overload (TACO) is a major cause of severe transfusion-related morbidity. Transfusion of red blood cells (RBCs) has been shown to induce hydrostatic pressure overload. It is unclear which product-specific factors contribute. We set out to determine the effect of autologous RBC transfusion versus saline on pulmonary capillary wedge pressure (PCWP) change.

Materials and Methods: In a randomized crossover trial, patients who had undergone coronary bypass surgery were allocated to treatment post-operatively in the intensive care unit with either an initial 300 ml autologous RBC transfusion (salvaged during surgery) or 300 ml saline infusion first, followed by the other. Primary outcome was the difference in PCWP change. Secondary outcome measures were the difference in extra-vascular lung water index (EVLWI) and pulmonary vascular permeability index (PVPI).

Results: Change in PCWP was not higher after autologous RBC transfusion compared to saline (ΔPCWP 0.3 ± 0.4 vs. 0.1 ± 0.4 mmHg). ΔEVLWI and ΔPVPI were significantly decreased after autologous RBC transfusion compared to saline (ΔEVLWI -1.6 ± 0.6 vs. 0.2 ± 0.4, $p = 0.02$; ΔPVPI -0.3 ± 0.1 vs. 0.0 ± 0.1, $p = 0.01$). Haemodynamic variables and colloid osmotic pressure were not different for autologous RBC transfusion versus saline.

Conclusion: Transfusion of autologous RBCs did not result in a more profound increase in PCWP compared to saline. RBC transfusion resulted in a decrease of EVLWI and PVPI compared to saline. Our data suggest that transfusing autologous RBCs may lead to less pulmonary oedema compared to saline. Future studies with

allogeneic RBCs are needed to investigate other factors that may mediate the increase of PCWP, resulting in TACO.

KEYWORDS
autologous blood transfusion, volume overload

Highlights

- Transfusion-associated circulatory overload (TACO) is the leading cause of transfusion-related morbidity and mortality. The onset mechanism has long been hypothesized as due to the capacity of red blood cells (RBCs) to increase hydrostatic pressure in the pulmonary vascular system.
- Transfusion of autologous RBCs does not result in a more profound increase in pulmonary capillary wedge pressure than transfusion of an equal volume of saline.
- Future studies should focus on other factors, including allogeneic RBC transfusion, and their role in the onset of TACO.

INTRODUCTION

Physicians consider blood transfusion a life-saving treatment, but transfusion has been associated with adverse events [1]. Transfusion-associated circulatory overload (TACO) is one of the major causes of severe transfusion-related morbidity and mortality [2], with an incidence of up to 6% in a critical care population [3]. Volume overload has a central role in the poorly understood pathophysiology of this clinical diagnosis [2, 4]. Hydrostatic pressure is a crucial component in the aetiology of the extravascular fluid accumulation in TACO [5], and an inflammatory process may contribute to this [6–8].

Multiple studies indicate that transfusion may lead to TACO through pathways other than hydrostatic pressure alone. Half of the reported TACO cases are diagnosed after transfusing only a single unit of red blood cells (RBCs), so volume overload is not to be expected [9]. The incidence of TACO is specific to the transfusion product [10, 11]. In a case–control study of critically ill patients, TACO patients received significantly less volume than patients diagnosed with circulatory overload in the absence of transfusion [3]. Furthermore, pro-inflammatory aspects of blood transfusion have gained more and more importance. One-third of TACO patients present with fever [8], and if leukocytes were reduced, this was associated with a decrease in the incidence of TACO [6]. Combined evidence suggests a transfusion-related acute lung injury (TRALI)-like inflammatory response, and enhanced vascular permeability, leading to pulmonary oedema [5, 6].

Previous studies assessing the effect of allogeneic RBC transfusion on hydrostatic pressure have found a pulmonary capillary wedge pressure (PCWP) rise of 3 mmHg in chronic anaemic patients following transfusion [12, 13], and suggested pulmonary artery pressure (PAP) increases after standard-issue versus fresh RBCs in critically ill patients [14]. A volunteer study showed an increase in the mean PAP following autologous transfusion of 1 unit of stored RBCs [15]. It remains to be determined whether hydrostatic pulmonary oedema is caused by the characteristics of the RBC product or by the allogeneic aspect of RBC transfusion. To exclude allogeneic aspects of RBC transfusion, a clinical study on the effects of autologous RBC transfusion versus crystalloid infusion on hydrostatic pulmonary pressure may help in identifying pulmonary oedema formation mechanisms after transfusion. In this randomized trial, we studied the effect of fresh autologous RBC transfusion versus saline infusion on PCWP change in cardiac surgery patients scheduled for bypass grafting with cardiopulmonary bypass (CPB) support. Systemic inflammation during extracorporeal circulation can lead to endothelial dysfunction. Therefore, these patients represent critically ill patients with endothelial dysfunction [16], at risk for TACO [11, 17, 18]. We hypothesized that transfusion of autologous RBCs would result in a more profound increase in PCWP and pulmonary oedema formation compared to transfusion of an equal volume of saline.

MATERIALS AND METHODS

The TACO crossover trial was an investigator-initiated, single-centre, prospective, crossover, randomized clinical trial. The study was performed at the Amsterdam University Medical Center, Location AMC, in the Netherlands. The local institutional review board approved the trial (NL59191.018.16), which was also registered at clinicaltrials.gov (NTC03135457) on 1 May 2017 and performed according to the CONSORT guidelines [19]. Written informed consent was obtained from all patients. Patient enrolment started in August 2017, and inclusion was completed in March 2020.

Study population

Patients (≥18 years old) scheduled to undergo elective non-redo on-pump coronary artery bypass graft surgery were eligible for enrolment. Patients undergoing emergency cardiac surgery, with severe arrhythmias, pulmonary hypertension, congenital heart disease, or

severe mitral or tricuspid valve disease, were excluded because of possible interference with pulmonary artery catheter (PAC) measurements. Patients with contraindications for PAC placement were excluded. Patients with chronic kidney disease ≥stage 4, those requiring massive transfusion during surgery or CPB duration of ≥2 h, or those on a high dose of corticosteroid infusion were not considered eligible because of the possible interaction with vascular permeability. Included patients with post-operative haemodynamic instability (defined as a mean arterial pressure <60 mmHg, central venous pressure >20 mmHg and noradrenaline dosage of >0.3 mcg/kg/min) were excluded before randomization.

Randomization intervention

A crossover design was used, in which patients were allocated to either infusion of 300 ml saline with a subsequent transfusion of 300 ml RBCs (from now on referred to as saline:RBCs) or the same in the reversed order (RBCs:saline), with a standardized wash-out period of 10 min. After admission to the intensive care unit (ICU), the patients were randomized by independent investigators in a 1:1 allocation ratio with the sealed-envelope method. Patients and care providers (with the exception of ICU nurses) were blinded to the treatment order. Blinding of the investigators was not possible because of the different aspects of the fluids given.

Intervention

According to local protocol, patients underwent coronary artery bypass graft surgery under general anaesthesia provided by anaesthesiologists who were not involved in the study. Induction of anaesthesia was achieved with midazolam, sufentanil, propofol, ketamine and rocuronium, followed by tracheal intubation. Maintenance of anaesthesia was achieved with sevoflurane, propofol and sufentanil. A PiCCO arterial line (Pulsion Medical Systems, Munich, Germany) and a PAC (Edwards LifeSciences, Irvine, CA) were placed under ultrasound guidance. Correct PAC placement was verified by transesophageal echocardiography intra-operatively and chest radiograph post-operatively and allowed the measurement of PCWP, which correlates closely with left atrial pressure, the gold standard to determine hydrostatic pulmonary pressure [20]. CPB was performed under mild hypothermia with a minimum temperature of 35°C employing a membrane oxygenator. A standard operating procedure during cardiac surgery is to use a blood salvaging device, a Fresenius Continuous Auto Transfusion System (Fresenius Kabi AG, Bad Homburg, Germany), whereby post-operative autologous transfusion was used as a non-investigational product. This autotransfusion system allows blood collection and washing with saline for transfusion. A high-quality wash was done to ensure a haematocrit of ≥60%. After surgery, patients were transferred to the ICU and sedated with propofol for the study duration. Patient in whom <300 ml of RBCs was obtained were excluded, and another patient was enrolled in their place.

The products were infused at a rate of 10 ml/min. This rate was based on previous studies that revealed an effect on hydrostatic pressure but not activation of mechanotransduction [13, 21]. Furthermore, this rate is representative of clinical practice and as advised in the British Society for Haematology Guideline on the administration of blood components. Haemodynamic measurements, including a passive leg raise (PLR) test, were performed before and after saline infusion and RBC transfusion to assess fluid responsiveness and volume status [22]. Hypovolemia may result in less increase in PCWP, although absolute haemodynamic values correlate poorly with volume status. A positive PLR test was defined as a cardiac output (CO) increase of >10%. Patients were prospectively screened for TACO criteria, according to the 2011 ISBT TACO definition, until 6 h post transfusion and for 12 h retrospectively using the 2018 revised surveillance case definition [23]. Patients who dropped out before the start of the second intervention, triggering exclusion criteria after randomization, were excluded, and another patient was enrolled in their place.

Patient data collection

Pre-operative patient characteristics were obtained from the electronic patient data system. PCWP was obtained through balloon inflation and wedging of the PAC, at the end of expiration, under positive end-expiratory pressure. Wedging was performed immediately before and directly following infusion at fixed time points. The PiCCO device uses transpulmonary thermodilution to measure CO, cardiac index (CI), extravascular lung water index (EVLWI), pulmonary vascular permeability index (PVPI), global end-diastolic volume index (GEDVI) and the systemic vascular resistance index (SVRI) [24]. Pulmonary vascular resistance index (PVRI) was calculated using standard formulas. Transpulmonary thermodilution was performed randomly throughout the respiratory cycle by three consecutive injections of 20 ml cold saline. All pressures were obtained after calibration conformed to standard ICU practice. Haemodynamic values were recorded in the electronic patient data system. Laboratory methods are described in Appendix S1. All data were collected using an electronic clinical report form built in Castor EDC, a Good Clinical Practice-compliant data capture system (Castor EDC, Amsterdam, the Netherlands).

Outcomes

The primary outcome was the difference in PCWP before and after transfusion (ΔPCWP). We compared RBC transfusion against saline infusion. Secondary outcomes included ΔEVLWI as a measure of pulmonary oedema, ΔPVPI as a measure of vascular permeability, as well as ΔSVRI, ΔPVRI and other haemodynamic variables. Furthermore, predefined secondary outcomes included colloid osmotic pressure (COP).

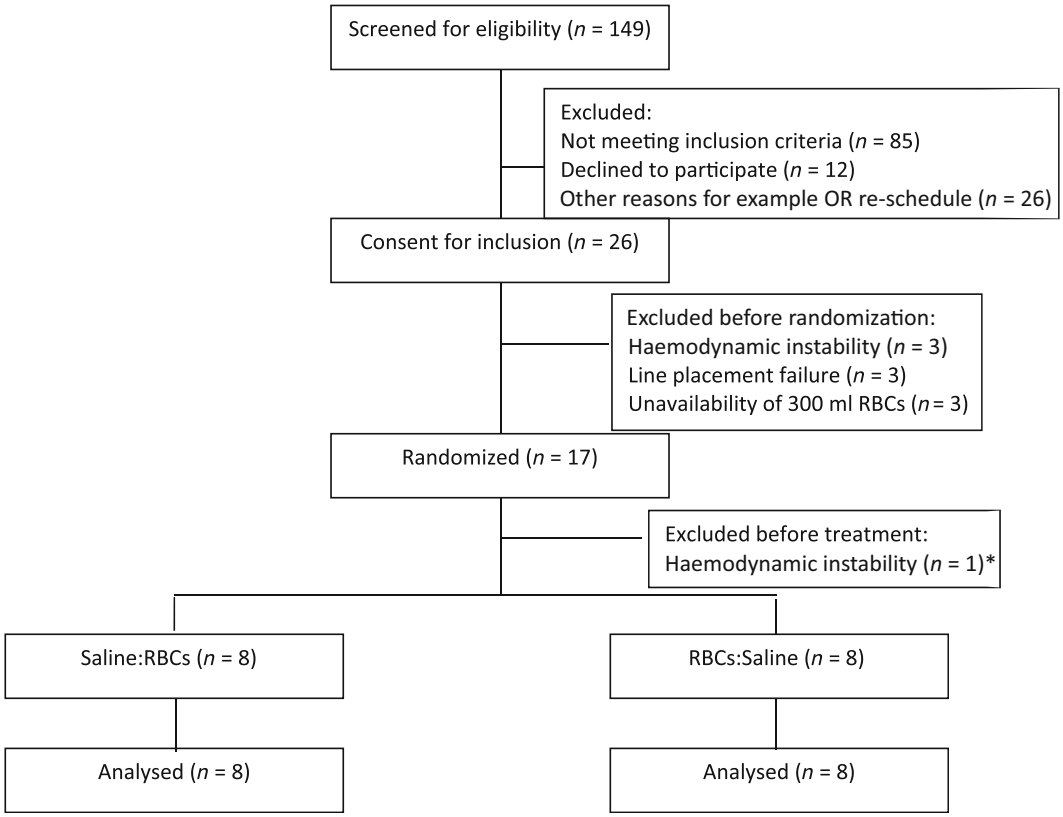

FIGURE 1 Flow-chart. *Measurements were not possible due to re-thoracotomy.

Sample size calculation

A sample size calculation was performed, indicating 8 patients per group, and a total of 16 patients were required to detect a difference in delta (Δ)PCWP of 4 mmHg, which is a clinically relevant difference with $\alpha = 0.05$ and a power of 95% (two-sided 2 × 2 ANOVA) [25]. ΔPCWP and variance were based on previous studies showing haemodynamic responses to RBC transfusion and saline loading [12, 13, 26].

Statistical analysis

Baseline assessments and outcome parameters were summarized using descriptive statistics. Data were analysed for normality. Wilcoxon signed rank tests were used for non-parametric related samples. Two-sample *t*-tests were used for testing the treatment effect in the presence of a period effect [25]. Wilcoxon rank sum statistics was used for non-parametric data in a crossover design [27]. Fisher's exact tests were used for binary outcomes with counts <5. Pearson's correlation coefficient was used to assess the linearity between ΔPCWP and PLR to assess the effect of volume status on ΔPCWP. A *p*-value of <0.05 was regarded as statistically significant. Analyses were performed with the SPSS software package (IBM SPSS Statistics, version 26; IBM Corporation).

RESULTS

Twenty-six patients were included between August 2017 and March 2020 (Figure 1). One-hundred and forty-nine patients were screened for eligibility. There was no screening for patients between March 2018 and March 2019 because of the unavailability of investigators. Nine patients were excluded before randomization. Seventeen patients were randomized, of whom one was excluded before initiation of treatment because of haemodynamic instability leading to re-thoracotomy. Sixteen patients completed the study and were included in the analysis. None of the included patients received an RBC transfusion during surgery. Three patients received platelet transfusion intra-operatively, one in treatment order saline:RBCs and two in treatment order RBCs:saline.

Demographic and baseline characteristics are shown in Table 1. Baseline differences were not assessed [19].

Primary endpoints

PCWP was not different before and after saline infusion or RBCs transfusion (PCWP before and after saline 13.0 ± 1.3 and 13.3 ± 1.3 mmHg vs. before and after RBC 12.9 ± 1.2 vs. 13.6 ± 1.5 mmHg, Table 2). Infusion of saline or RBCs did not increase ΔPCWP differently (ΔPCWP 0.3 ± 0.4 vs. 0.1 ± 0.4 mmHg, $p = 0.74$, Table 2, Figure S1). Crossover differences for the first

TABLE 1 Demographic and baseline characteristics of the study population

Parameter	Saline:RBC	RBC:Saline
Demographic data		
Patients (*n*)	8	8
Female/male (*n*)	0/8	2/6
Age (years)	68 ± 9	65 ± 11
Weight (kg)	87 ± 15	88 ± 22
Body mass index (kg/m^2)	27 ± 3	28 ± 6
Comorbidities		
Heart failure (*n*)	1	0
Diabetes (*n*)	2	2
Kidney disease (*n*)	0	0
COPD GOLD class ≤2 (*n*)	1	2
Medication		
Beta-blocker (*n*)	5	7
Acetylsalicylic acid (y/n)	8	8
Clopidogrel (y/n)	3	3
Vitals before surgery		
Heart rate (beats/min)	69 ± 11	64 ± 15
Systolic arterial pressure (mmHg)	134 ± 17	136 ± 19
Diastolic arterial pressure (mmHg)	80 ± 15	82 ± 15
Respiratory rate (breaths/min)	14 ± 1	15 ± 1
Post-operative data at ICU arrival		
Fluid balance (ml)	2810 ± 1218	2893 ± 961
Noradrenaline dosage (mcg/h)	390 ± 340	440 ± 270
Temperature (°C)	36.4 ± 0.2	36.4 ± 0.3
PEEP (cmH_2O)	5 ± 1	7 ± 2
Haemoglobin (mmol/L)	7.7 ± 1.1	6.4 ± 0.7
Positive PLR test 1	2	2
Positive PLR test 2	2	1

Note: Data are displayed as absolute numbers or mean ± standard deviation. Noradrenaline dosage is at time of intensive care unit (ICU) arrival.
Abbreviations: COPD, chronic obstructive lung disease; PEEP, positive end expiratory pressure; PLR, passive leg raise test; RBC, red blood cell.

allocation period did not differ compared to differences for the second allocation period (difference in PCWP per period −0.9 ± 0.6 vs. 0.2 ± 0.3, $p = 0.18$, Wilcoxon rank sum test).

Secondary endpoints

EVLWI and PVPI were significantly lower following RBC transfusion (EVLWI 7.0 ± 0.7 vs. 8.5 ± 0.6, $p = 0.02$ resp. PVPI 1.5 ± 0.2 vs. 1.8 ± 0.1, $p = 0.02$, before vs. after RBCs, Wilcoxon signed rank test). ΔEVLWI and ΔPVPI were significantly different following RBC transfusion compared to saline (ΔEVLWI −1.6 ± 0.6 vs. 0.2 ± 0.4, $p = 0.02$, and ΔPVPI −0.3 ± 0.1 vs. 0.0 ± 0.1, $p = 0.01$, ΔRBCs vs. Δsaline, Wilcoxon signed rank test Table 2, Figure S2). ΔSVRI and ΔPVRI were not different for infusion of saline or RBC transfusion (ΔSVRI 180 ± 85 vs. 0 ± 123, and ΔPVRI vs. 54 ± 37 vs. 25 ± 39, ΔRBCs vs. Δsaline, Wilcoxon signed rank test Table 2, Figure S2). Crossover differences for the first allocation period did not differ from the second allocation period for EVLWI, PVPI, SVRI and PVRI (EVLWI, $p = 0.25$; PVPI, $p = 0.59$; SVRI, $p = 0.9$; PVRI, $p = 0.19$, Wilcoxon rank sum test). Other haemodynamic variables, including SVRI, PVRI, MAP, CO, GEDVI and IBTVI, were the same before and after saline infusion or RBC transfusion (Table 2).

Passive leg raise test

Two patients in each treatment order had a positive PLR test before any transfusion or saline infusion. There was a poor correlation between PLR test and ΔPCWP after both saline infusion and RBC transfusion ($r = 0.14$, $p = 0.69$ and $r = -0.19$, $p = 0.57$).

Laboratory results

ΔCOP did not significantly differ after the intervention (ΔCOP 0.0 ± 0.7 vs. −0.5 ± 0.6 mmHg, $p = 0.25$, Table 2). ΔPV was significantly larger after saline infusion (−80 ± 30 vs. 40 ± 30 ml, $p = 0.02$, Table 2). Absolute COP and PV did not differ between before and after the intervention.

TACO criteria

The number of TACO diagnoses according to the 2011 ISBT TACO definition, as well as according to the 2018 revised surveillance case definition, was not different for the groups allocated to saline infusion first and RBCs transfusion second, versus the reversed order (Tables S1 and S2, TACO diagnosis 1 out of 8 vs. 0 out of 8, $p = 0.5$).

DISCUSSION

This study investigated pulmonary hydrostatic pressure after transfusing autologous RBC versus crystalloid infusion. Furthermore, we explored pulmonary oedema formation in coronary artery bypass graft surgery patients in the ICU. The main finding of this randomized clinical trial is that we found the same PCWP following transfusion of 1 unit RBC compared to saline infusion in these patients. Secondary findings are that RBC transfusion may decrease EVLWI and PVPI compared to saline infusion.

This study focused on pulmonary hydrostatic pressure following transfusion. We found no PCWP increase following autologous RBC transfusion compared to saline infusion. Previous observational and retrospective studies assessing the effect of allogeneic RBC transfusion on hydrostatic pressure had shown an increase in PCWP in chronic anaemic and critically ill patients [12, 13, 28]. These studies

TABLE 2 Haemodynamic variables before and after saline infusion and RBC transfusion

Parameter	Before saline	After saline	Δsaline	Before RBCs	After RBCs	ΔRBCs	*p*-Value
PCWP (mmHg)	13.0 ± 1.3	13.3 ± 1.3	0.3 ± 0.4	12.9 ± 1.2	13.6 ± 1.5	0.1 ± 0.4	0.74
EVLWI (ml/kg)	8.7 ± 1.3	7.9 ± 0.6	0.2. ± 0.4	8.5 ± 0.6	7.0 ± 0.7*	−1.6 ± 0.6***	0.02
PVPI	1.6 ± 0.1	1.7 ± 0.1	0.0 ± 0.1	1.8 ± 0.1	1.5 ± 0.2**	−0.3 ± 0.1****	0.01
CI (L/min/m^2)	2.4 ± 0.2	2.6 ± 0.2	0.1 ± 0.1	2.5 ± 0.2	2.5 ± 0.2	0.0 ± 0.1	0.44
GEDVI (ml/m^2)	713 ± 45	725 ± 41	11 ± 21	710 ± 37	741 ± 61	21 ± 23	0.57
MAP (mmHg)	71 ± 2	72 ± 3	2 ± 2	68 ± 3	72 ± 3	4 ± 3	0.31
SVRI (DS/cm^{-5}/m^2)	2025 ± 125	1903 ± 119	0 ± 123	1783 ± 150	2023 ± 157	180 ± 85	0.19
PVRI (DS/cm^{-5}/m^2)	277 ± 47	330 ± 68	25 ± 39	310 ± 68	298 ± 49	54 ± 37	0.56
ITBVI (ml/m^2)	840 ± 95	904 ± 56	78 ± 72	927 ± 48	926 ± 129	−24 ± 135	0.65
COP (mmHg)	19.9 ± 0.7	19.7 ± 0.7	0.0 ± 0.6	19.8 ± 0.7	19.5 ± 0.8	−0.5 ± 0.6	0.25
CBV (L)	5.4 ± 0.8	5.4 ± 0.8	–	5.4 ± 0.8	5.4 ± 0.8	–	–
PV (ml)	3760 ± 20	3670 ± 20	−80 ± 30	3700 ± 20	3710 ± 20	40 ± 30	0.02

Note: Δ = delta; data are displayed as mean ± SE.

Abbreviations: CBV, circulating blood volume; CI, cardiac index; COP, colloid osmotic pressure; EVLWI, extra vascular lung water index; GEDVI, global end-diastolic volume index; ITBVI, intra thoracic blood volume index; MAP, mean arterial pressure; PCWP, pulmonary capillary wedge pressure; PV, plasma volume; PVPI, pulmonary vascular permeability index; RBCs, red blood cells; SVRI, systemic vascular resistance index.

*$p = 0.02$ before versus after RBCs, Wilcoxon signed rank test; **$p = 0.02$ before versus after RBCs, Wilcoxon signed rank test; ***$p = 0.02$ ΔRBCs versus Δsaline, Wilcoxon signed rank test; ****$p = 0.01$ ΔRBCs versus Δsaline, Wilcoxon signed rank test.

were before the introduction of leukoreduction and were therefore performed with whole blood. There is emerging evidence that pulmonary oedema formation following transfusion may be explained by other mechanisms than solely an RBC transfusion effect [6–8]. These results may be important, as autologous RBC transfusion did not result in a more profound increase in PCWP compared to saline. However, the absence of change in the current study does not imply that in the setting of clinical TACO, an increase in hydrostatic pressure is present. However, our results suggest that factors other than just the volume of an RBC transfusion are needed to induce clinical TACO.

Storage lesion may be an alternative pathway leading to hydrostatic pulmonary oedema after transfusion. Baron-Stefaniak et al. compared fresh versus standard-issued RBCs and suggested PAP increase in the latter [14]. We transfused fresh RBCs and confirmed their finding that RBCs stored for 3 days did not alter PVRI. On the other hand, Berra et al. found a significant increase in PAP by estimating the mean PAP non-invasively during autologous transfusion of RBCs stored for 40 days in 14 volunteers with endothelial dysfunction [15]. The transfused products had increased levels of storage lesions such as cell-free haemoglobin. Increased levels of storage lesions, combined with ongoing haemolysis after transfusion, might have resulted in increased plasma nitric oxide (NO) consumption. In general, increased capillary pressure leads to enhanced NO release through mechanotransduction [21]. Reduced NO bioavailability may have led to vasoconstriction and PAP increase in a patient cohort with known decreased availability of endothelial NO before transfusion [15]. However, PVRI was not measured, and PAP is not a direct measure of hydrostatic pressure overload, as is PCWP. In our study, the absence of increased PCWP following autologous RBC transfusion was confirmed by PVRI, which was not different after the transfusion. Furthermore, increased PAP is not directly correlated to pulmonary oedema formation [29].

We found a decrease in pulmonary oedema measured by EVLWI and vascular permeability measured by PVPI after RBC transfusion compared to saline infusion. Various factors may explain these findings. First, the calculation of EVLWI includes GEDVI [24]. PVPI is calculated as the ratio of extravascular lung water (EVLW) over the pulmonary blood volume, so a decrease in EVLW will lead to a decrease in PVPI. Increasing preload and GEDVI should theoretically decrease EVLWI and PVPI. However, CO and GEDVI are the same before and after transfusion (Table 2). Second, the calculation of EVLWI and PVPI includes the downslope time of the thermodilution curve. Intrinsic specific density properties of an RBC transfusion and saline will affect the specific heat capacity differently. However, the manufacturer-recommended blood temperature differences were met during the thermodilution measurements; therefore, the algorithm calculating the haemodynamic variables from the downslope time should apply. Third, altered rheology by RBC transfusion versus saline infusion may affect the mean transit time of the cold fluid bolus [30]. Therefore, EVLWI and PVPI calculations may be affected. Last, COP differences may facilitate decreased vascular extravasation for RBCs compared to saline [31]. However, our study (not powered for COP differences) shows similar COP before and after RBC and saline infusion. Furthermore, COP measurement of RBCs in a previous study showed a pressure of 1.9 mmHg compared to a theoretical COP of 0 for saline [32]. Therefore, a large impact on plasma COP in vivo is not expected.

Several limitations apply to our study. First, our study included only one clinical TACO case. We cannot exclude that in clinical TACO other mechanisms may imply. However, this study focused on the effect of autologous RBC transfusion versus saline on the change in PCWP, which helps us to understand the factors involved in the onset of TACO. Second, this is a single-centre study with a small sample

size. Third, we cannot rule out a carry-over effect due to the nature of the crossover design [25]. However, since a standardized protocol was followed with a standardized wash-out period, PCWP did not change after RBC transfusion or saline infusion, and there was no correlation between PLR and PCWP, so carry-over effects should be minimal. Fourth, because autologous RBCs are not readily available for every patient, extrapolation to clinical practice is limited. Fifth, we performed measurements in anaesthetised patients in contrast to previous studies, which may affect volume compliance for saline and RBCs. Sixth, our study design lacks an allogeneic control group, which is due to ethical considerations. A lack of a representative study population is demonstrated by the preliminary termination of a recent study investigating fresh versus standard-issue allogeneic RBC transfusion [14]. Furthermore, allogeneic RBC transfusion for patients without any transfusion indication constitutes an ethical dilemma.

In conclusion, in this randomized clinical trial, we found the same PCWP following transfusion of 1 unit RBCs compared to saline infusion in critically ill patients. Our data suggest that transfusing fresh autologous RBCs may lead to less pulmonary oedema, and less vascular permeability than infusing saline. Future research should focus on other factors that may mediate the increase of PCWP resulting in pulmonary oedema, including allogeneic transfusion.

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CONFLICT OF INTEREST

Dr. Denise P. Veelo reported receipt of grants and consultancy fees from Edwards Lifesciences, and research grants from Philips and Hemologic. Dr. Markus W. Hollmann reported serving as executive section editor of pharmacology for *Anaesthesia & Analgesia* and as section editor of anaesthesiology for the *Journal of Clinical Medicine* and receipt of speakers fees from CSL Behring and Eurocept BV and consultancy fees from Eurocept BV. The other authors declare no conflict of interest.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Lack of alloimmunization to the D antigen in D-negative orthotopic liver transplant recipients receiving D-positive red blood cells perioperatively

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Abstract

Background and Objectives: D-negative patients undergoing orthotopic liver transplantation (OLT) might require a large number of red blood cell (RBC) units, which can impact the inventory of D-negative blood. The blood bank might need to supply these patients with D-positive RBCs because of inventory constraints. This study evaluates the prevalence of anti-D formation in D-negative OLT patients who received D-positive RBCs perioperatively, as this will assist in successful patient blood management.

Materials and Methods: This was a retrospective study performed at a single academic medical centre. Electronic medical records for all 1052 consecutive patients who underwent OLT from January 2007 through December 2017 were reviewed. D-negative patients who were transfused perioperatively with D-positive RBCs and had antibody screening at least 30 days after transfusion were included.

Results: Of a total of 155 D-negative patients, 23 (14.8%) received D-positive RBCs perioperatively. Seventeen patients were included in the study. The median age was 54 years (range 36–67 years); 13 (76.5%) were male. The median number of D-positive RBC units transfused perioperatively was 7 (range 1–66 units). There was no evidence of D alloimmunization in any patient after a median serologic follow-up of 49.5 months (range 31 days to 127.7 months). The average number of antibody screening post OLT was 7.29.

Conclusion: Our study showed that transfusion of D-positive RBCs in D-negative OLT recipients is a safe and acceptable practice in the setting of immunosuppression. This practice allows the conservation of D-negative RBC inventory.

KEYWORDS
alloimmunization, blood management, D antigen, liver transplant, RBC transfusion

Highlights

- Owing to increased transfusion demand and inventory constraints, the blood bank might be forced to transfuse D-positive red blood cells (RBCs) to D-negative patients undergoing liver transplant.

This work was presented, in part, at the American Association of Blood Banks Annual Meeting, 13–16 October 2018.

- Our study showed that transfusion of D-positive RBCs to D-negative liver transplant recipients is a safe and acceptable practice perioperatively; none of the studied patients had alloimmunization to the D antigen.
- The practice of transfusing D-positive RBCs to D-negative liver transplant recipients allows the conservation of D-negative RBC units for other patients in greater need of this resource.

INTRODUCTION

Liver transplantation has significantly improved the outcomes and life expectancy of patients with end-stage liver disease. Transplant surgeries in general and liver transplants specifically have been associated with significant blood loss and often require intensive transfusion support with blood products [1]. Advances in surgical techniques and anaesthetic management have contributed to an overall reduction in the transfusion needs during and following liver transplantation. Despite blood management, liver transplantation is still associated with high blood transfusion requirements perioperatively because of abnormal haemostasis and bleeding complications [2, 3].

D-negative patients are routinely transfused with D-negative red blood cells (RBCs) because of the high immunogenicity of the D antigen. The rate of alloimmunization to the D antigen in healthy volunteers following transfusion of D-positive packed RBCs can be as high as 80% [4]. However, immunosuppressed antigen-negative patients (such as organ transplant recipients and oncology patients) are much less likely to produce an alloantibody when exposed to a foreign RBC antigen [5–7].

When D-negative patients undergo liver transplant surgeries, it may not be possible for the blood bank to provide the required number of D-negative units because of inventory constraints; thus, the blood bank might transfuse these patients with D-positive RBCs. Though the process of providing D-positive RBCs to D-negative transplant recipients is accepted, the incidence of alloimmunization to the D antigen in D-negative liver transplant recipients has not been well defined. With a very active liver transplant programme at our institution, studying the prevalence of anti-D formation in D-negative liver transplant recipients receiving D-positive RBCs perioperatively will assist in successful patient blood management.

MATERIALS AND METHODS

This was a retrospective study performed at a single large academic medical centre. The study was approved by our Institutional Review Board, and informed consent was waived because of the study design. Electronic medical records and blood bank files for all 1052 consecutive patients who underwent orthotopic liver transplantation (OLT) with or without other organ transplants at Henry Ford Hospital in Detroit, Michigan, from January 2007 through December 2017 were reviewed. Patients were identified through the Liver Transplant Institute database. The transfusion records of D-negative OLT patients were reviewed for perioperative RBC transfusions. The perioperative period was defined as the period spanning 1 week before OLT until 2 weeks following OLT. Patients who were transfused perioperatively with D-positive RBCs and had antibody screening at least 30 days after the first D-positive RBC transfusion were included in the analysis.

Data collected for each patient included demographic details, clinical history, perioperative RBC transfusion and follow-up antibody screening. Blood bank records were last reviewed in December 2021 for updated antibody screening.

Blood bank testing

ABO and RhD group testing were performed using the following immunohaematology analysers utilizing solid-phase technology: Galileo (available till 2012), Galileo NEO (2012 to February 2020) and NEO Iris (since March 2020). Manual tube testing was used to resolve discrepancies in ABO forward and reverse grouping. ABO blood group testing was performed using monoclonal anti-A, anti-B and anti-D antisera for forward typing (Immucor, Norcross, GA) and pooled A1 and B cells for reverse typing (Immucor). Antibody screening was performed by indirect antiglobulin test (IAT) using a two-cell screen on the same analyser. Positive samples were investigated for antibody identification with a 14-cell panel. All tests were performed according to manufacturers' instructions.

Immunosuppression and blood management

The detailed immunosuppression protocol has been described previously [2]. Briefly, induction immunosuppression included rabbit antithymocyte globulin or basiliximab. The maintenance immunosuppression regimen consisted of tacrolimus, mycophenolate mofetil and steroids.

As soon as the blood bank is notified of a potential liver transplant, the recipient's transfusion records are reviewed. If the current and previous antibody screening are negative for clinically significant alloantibodies to RBC antigens, 20 ABO-compatible RBC units will be allocated for the recipient. If clinically significant alloantibodies were identified historically or on the current antibody screening, 30 antigen-negative serologically cross-matched RBC units were allocated. Ideally, blood would be stocked 24 h in advance of the day of transplant; however, blood bank is notified 6–12 h before most scheduled liver transplants. Our blood bank utilized leukoreduced RBCs collected by our blood supplier; we would start issuing irradiated units on the

day of transplant. The ABO group of each RBC unit and the RhD type of units labelled as D-negative were confirmed by the Henry Ford Hospital blood bank before going into inventory. The decision to transfuse D-positive RBC units to D-negative patients was based on both demand and inventory of D-negative RBC units in the blood bank.

Intraoperative blood product transfusion was managed by the anaesthesiologist; transfusion was dictated by the patient's haemodynamic status, intraoperative course, blood loss and oozing from the surgical field, and laboratory testing, mostly haemoglobin, platelet count, prothrombin time and activated partial thromboplastin time. Cell salvage and antifibrinolytics were utilized, as needed. The criteria for pre- and post-operative blood transfusion were referenced from current AABB clinical practice guidelines [8–10].

RESULTS

A total of 1052 patients underwent 1099 OLT during the study period. The number of transfused D-positive and D-negative patients was 809/897 (90.1%) and 140/155 (90.3%), respectively. Of the total 155 D-negative patients, 23 (14.8%) received D-positive RBCs perioperatively. Two patients did not survive surgery, and four patients did not have serologic follow-up after 30 days following transfusion and thus were excluded. The characteristics of the included 17 patients are presented in Table 1. The median age was 54 years (range 36–67 years); 13 (76.5%) were male. Twelve patients were Caucasian. The median number of RBC units transfused perioperatively was 19 (range 2–77 units), while the median number of D-positive RBC units transfused was 7 (range 1–66 units). None of the patients received Rh immune globulin prophylaxis. At the time of OLT, none of the patients had clinically significant RBC alloantibodies through antibody screening or a history of alloimmunization to RBC antigens. There was no evidence of D alloimmunization in any patient after a median serologic follow-up of 49.5 months (range 31 days to 127.7 months). The average number of antibody screening post OLT was 7.29.

We had 14 D-negative patients (14/155 = 9.0% of all D-negative liver transplant recipients) who had D alloimmunization before transplant; none of these patients was transfused with D-positive blood at our institution before alloimmunization.

DISCUSSION

In this study, we reviewed the medical records of OLT patients to determine the prevalence of anti-D formation in D-negative liver transplant recipients receiving D-positive RBCs. During the perioperative period, 17 eligible patients were transfused with a median of 7 D-positive RBC units (range 1–66 units). After a median serologic follow-up of 49.5 months, none of the patients developed anti-D antibodies.

OLT has developed throughout the years and become the standard of care for advanced liver disease. Although the use of blood components during OLT has considerably decreased over the past two decades, transfusion demands are still significant, especially

TABLE 1 Characteristics of the 17 D-negative liver transplant recipients who received D-positive red blood cells (RBCs) perioperatively

Patient	Age (years)/gender	Ethnicity	Pretransplant diagnosis	No. of D-positive RBCs	No. of D− RBCs	Serological F/U[a]
1	51/female	AA	HCV cirrhosis	2	0	21.23 months
2	44/male	AA	HCV and alcoholic cirrhosis	3	19	79.43 months
3	36/male	AA	Alcoholic cirrhosis	1	18	31.07 months
4	64/male	White	Autoimmune hepatitis	9	2	8.17 months
5	67/female	White	Liver cirrhosis secondary to NASH	4	0	127.70 months
6	53/male	White	Alcoholic cirrhosis	30	2	58.87 months
7	48/male	White	HCV and alcoholic cirrhosis	4	20	53 days
8	56/male	White	Metastatic neuroendocrine tumour	9	24	113.13 months
9	67/male	White	Alcoholic cirrhosis	25	8	95.23 months
10	59/male	White	Alcoholic cirrhosis	32	4	80.30 months
11	61/female	AA	HCV cirrhosis with HCC	4	4	83.67 months
12	53/female	White	Liver cirrhosis secondary to NASH	10	7	14.20 months
13	63/male	White	HCV cirrhosis with HCC	5	10	54.10 months
14	63/male	White	Polycystic liver disease	17	16	5.07 months
15	52/male	AA	Liver cirrhosis secondary to NASH	7	9	49.47 months
16	48/male	White	Alcoholic cirrhosis	3	5	4.13 months
17	54/male	White	Alcoholic cirrhosis	66	11	31 days

Abbreviations: AA, African American; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NASH, non-alcoholic steatohepatitis.
[a]Time from first D-positive RBC transfusion to last antibody screening.

during the perioperative period [2]. There is also wide variation in transfusion rates between transplantation centres, which can be attributed to specific patient population, surgical techniques, differences in institutional transfusion practices, variable use of antifibrinolytics and intraoperative blood salvage, among other variables [11].

Ensuring an adequate blood supply can be challenging because of the increased demand, increased number of donor deferrals, and sometimes decreased supply due to weather conditions and pandemics. Inventory concerns become more pronounced with D-negative units, which comprise only 10%–15% of donors. The Rhesus (Rh) blood group is the second most clinically important system after the ABO system. The D antigen is one of the Rh blood group antigens; it is highly immunogenic in healthy subjects as well as in immunocompromised patients [12, 13]. Knowing the high immunogenicity of the D antigen and the risks of delayed haemolytic transfusion reactions, D-negative patients are not usually transfused with D-positive blood. However, blood banks might be forced to transfuse D-negative patients with D-positive blood to preserve their D-negative RBC units, which are a scarce resource. Although it is not a common practice to transfuse D-negative males and females beyond the childbearing age with D-positive blood, this is considered an acceptable approach when the inventory of D-negative blood is small; however, the risks of delayed serologic/haemolytic transfusion reactions should be discussed with the treating physicians.

An adequate perioperative supply of D-negative blood for D-negative patients undergoing OLT is not always possible. We had a total of 23 D-negative OLT patients who were transfused with D-positive blood during the study period, 17 of whom were included in the analysis. All these patients were either males or females who were beyond the childbearing age. The decision to transfuse these patients with D-positive blood was dictated by the increased transfusion needs of these patients (refer to Table 1 for transfusion needs) and the sub-optimal inventory levels of D-negative blood. This cohort of OLT patients was transfused with a wide range of D-positive RBC units among the total number of units transfused.

There is a multitude of factors that could have contributed to the decision to transfuse D-positive RBC units and to this variation in transfused units. First, this study was not limited to evaluating intraoperative blood transfusions in OLT. We, instead, reviewed blood transfusions perioperatively, for a period spanning 1 week before OLT until 2 weeks following OLT. Transfusion needs post OLT surgery can persist because of many factors including coagulopathy, which can extend for months following transplantation [14]. In addition, up to 11% of OLT patients would require re-operation for bleeding within 2 weeks following OLT, which adds to their transfusion needs [15]. Second, although OLT is mostly well planned, some cases are relatively urgent with short notification to the blood bank, thus limiting our preparedness to provide D-negative blood. Evaluating the clinical situation of D-negative OLT candidates is crucial in making the decision to accept these patients for surgery. The decision to switch them to D-positive RBCs is necessary when the D-negative inventory is limited. Third, the demand for large numbers of RBC units may arise with traumas, massive transfusions and emergency surgeries, along with OLT cases complicated with intraoperative bleeding. In these situations, the decision to switch D-negative OLT patients to D-positive RBCs in order to save D-negative RBCs for patients with known anti-D, females of childbearing age and patients with no blood type on file would be appropriate.

Ramsey et al. evaluated 19 D-negative liver, heart and heart-lung transplant recipients who were transfused with D-positive RBCs [16]. Anti-D was detected in three liver transplant recipients; however, anti-D antibodies were detected at 3, 11 and 15 days following transfusion, which was not convincing of a primary immune response. In another study by Burin des Roziers et al., 20 D-negative OLT recipients transfused with D-positive RBCs perioperatively were evaluated [17]. None of the patients developed anti-D antibodies using the IAT. Two patients showed weak and transient anti-D reacting only with papain-treated RBCs at 10 and 11 days without any signs of haemolysis. Other investigators also have supported the safety of transfusing D-positive RBCs in D-negative patients during OLT surgery [5, 6, 18]. Table 2 summarizes the studies that evaluated alloimmunization to the D antigen in D-negative OLT recipients who

TABLE 2 Summary of studies evaluating the rate of alloimmunization to the D antigen in D-negative orthotopic liver transplantation recipients transfused with D-positive red blood cells (RBCs)

Study (year published)	Type of transplant	No. of patients	No. of D-positive units transfused; median (range)	F/U period; median (range), months	Number of patients that developed anti-D
Tiwari et al. [18]	Liver	21	7 (2–20)	11 (6–90)	None
Burin des Roziers et al. [17]	Liver	20	7 (1–40)	30 (9–120)	2 patients at 10 and 11 days after transfusion, transient
Casanueva et al. [5]	Liver	17	19 (5–41)	15 (2–70)	None
Ramsey et al. [16]	Liver, heart, heart-lung	19	10 (3–153)	2.5–51; median NA	3 patients at 3, 11 and 15 days after transfusion
Yuan et al. [6]	Liver	15	9 (2–39)	3.6 (1.7–13.5)	None
Current study	Liver	17	7 (1–66)	49.5 (1–128)	None

Abbreviation: NA, not available.

were transfused with D-positive blood. The lack of alloimmunization in D-negative OLT patients receiving D-positive blood can be explained by the fact that patients are maintained on immunosuppressive protocols to prevent the risk of graft rejection. None of the patients in our study developed anti-D antibodies on last follow-up.

Transfusion of D-positive RBCs in D-negative patients is unavoidable in surgeries such as OLT, which put an enormous pressure on limited blood inventory. Transfusing D-positive units to these patients is essential to prevent postponing these lifesaving procedures. This also helps in managing inventory and allocating D-negative units for other patients in whom the risk of alloimmunization would be higher, for example, D-negative females of childbearing age. In conclusion, transfusion of D-positive blood to D-negative OLT recipients is acceptable and safe in the setting of immunosuppression due to the low risk of alloimmunization.

One of our study limitations relate to its retrospective nature and being dependent on medical record documentation. The study was done at a single tertiary-care centre; thus, the results might not be generalizable to other institutions. In addition, the lack of systematic screening of OLT patients might have contributed to the relatively small sample size and decreased serologic follow-up period of some patients.

In conclusion, this study showed that transfusion of D-positive RBCs in D-negative OLT recipients is a safe and acceptable practice perioperatively in the setting of immunosuppression; none of the patients had alloimmunization to the D antigen following transfusion of D-positive RBCs. This practice allows the conservation of D-negative RBC units for other patients in greater need of this resource.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

An international inquiry into the contaminated blood tragedy

The recent publicity in the United Kingdom around the *Infected Blood Inquiry* highlights once again the tragedy that affected the haemophilia community worldwide. Sir Brian Langstaff and his team have collected an impressive amount of evidence on the situation in the United Kingdom 40 years ago (www.infectedbloodinquiry.org.uk/evidence). The question remains to what extent this Inquiry can dig deeper, to question the conduct of the pharma companies in the United States, and of the US Government, in the 1970s and early 1980s. Their actions led to thousands of people becoming infected with hepatitis C virus (HCV) and human immunodeficiency virus (HIV). Therefore, we call for an international investigation into the conduct of the pharma companies and the US authorities.

Since the 1990s, different countries have organized inquiries into the HIV and HCV infections caused by their national blood and plasma supply. These resulted in apologies, while compensation models were installed by national governments. In some cases, pharma companies donated small amounts. Only in Japan did the involved pharmaceutical companies make public apologies and paid substantial sums to the victims.

The UK Inquiry focuses on UK witnesses: haematologists, infected patients, their children, healthcare officials, and so on. Until now, none of the inquiries has laid emphasis on the role of the international plasma industry and the US authorities, even though there is strong evidence about their detrimental activities [1].

In our opinion, the US practices, with payment of donors and a high frequency of plasma donations (up to 102 donations a year in contrast to European regulations that establish a maximum of 33), have greatly contributed to the tragedy of HCV and HIV infections in the haemophilia community worldwide.

An international investigation into the conduct of the pharmaceutical companies and the US authorities might give answers to the many people who experienced the rollercoaster of emotions of past infections. But an even more important outcome can be a worldwide blood and plasma supply system that is safer for both donors and recipients.

Which organization has the authority to design and conduct such an investigation? Is it the WHO or the Joint United Nations Programme on HIV/AIDS (UNAIDS)? Or could it be Sir Brian Langstaff and his team, who have the experience, the information, and interviewed so many parties involved?

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The authors declare no conflicts of interest.

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LETTER TO THE EDITOR

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In favour of combining more than one alternate strategy

The interesting paper by Sivakaanthan et al. [1] explores the case for an alternate strategy for routine donor red cell antibody screening in Australian blood donors. According to their model, it would be cost effective to focus screening on first-time donors, donors returning to donation after more than 2 years and those with sensitizing events (pregnancies and transfusion) since their last donation, instead of routinely screening all donors. As the authors point out, their proposal resembles regulations in some European countries. However, according to their data, the economic benefit would be offset by the risk of haemolysis posed by the red cell antibodies that could evade this strategy.

We would like to make some suggestions that could to help their work come to fruition, coupling their proposal with other developments in the field.

First, to solve their dilemma we suggest dedicating plasma units specifically for transfusion and screening only those units for irregular antibodies. Thus, screening would still be performed on a small percentage of donations, rather than hundreds of thousands, resulting in reduced cost without hampering safety.

Second, this initiative would be further supported by the use of alternative plasma preparations that commit plasma units to transfusion. Freeze-dried plasma [2] and 'liquid plasma' (which has not been frozen) [3] are examples of plasma dedicated to transfusion, which are as effective and safe as fresh frozen plasma while being more convenient for transfusion services. Blood collection centres that supply plasma for transfusion could prepare that kind of products and specifically test them for irregular antibodies. If needed, stored frozen units could be tested within an acceptable time frame, allowing for the exclusion of any plasma unit with antibodies.

Third, for the sake of safety, donors at higher risk of developing antibodies could still be screened, even if their plasma was derived for the plasma fractioning industry and not destined for transfusion. Of note, the reported haemolytic reactions with plasma derivatives have been caused by anti-A or -B haemagglutinins but not red cell irregular antibodies [4].

Although those changes would need careful validation in each blood collection and transfusion centre before implementation, they could represent a welcome innovation to the transfusion community.

Finally, the authors' analysis focuses mainly on the savings calculated on the purchase value of reagents. However, manufacturers could respond by adjusting reagent prices to maintain their profits, so the final savings could be minimal. Nevertheless, the alternate strategy could still be justified through reduction in testing turnaround time, technicians' time for validation processes, and increased sustainability by reduction in resource use and wastage.

We congratulate Sivakaanthan and co-authors for their seminal work.

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The authors declare no conflict of interest.

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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

Reply to: In favour of combining more than one alternate strategy

We thank the authors for their interest in our paper and valuable input. We would like to reply to the suggestions by Romon et al. while considering current Lifeblood practices and the Australian context.

The initial proposal suggested by Romon et al. of dedicating plasma units specifically for transfusion, and screening only those units for irregular antibodies, is a practice that is currently implemented in Australia. As referred to in the original article, a modified test panel for apheresis plasma donations for fractionation was introduced in 2016. The modified panel meant that plasma for fractionation donations was no longer routinely screened. The authors have also considered the use of alternative plasma preparations, though we do not issue freeze-dried plasma or liquid plasma (that is never frozen) in Australia.

The third suggestion raised by Romon et al. refers to the ongoing screening of donors at higher risk of developing antibodies. As noted in the letter to the editor, the reported haemolytic reactions with plasma derivatives have been caused by anti-A and anti-B haemagglutinins. We routinely screen all plasma for transfusion for anti-A and anti-B titres to enable the use of low-titre products for minor ABO-incompatible transfusions.

While the adoption of all these suggestions may not be appropriate in the Australian setting and framework of practice, it is recognized that international practices vary widely and these suggestions should be considered by individual blood services.

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The authors declare no conflicts of interest.

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See also https://www.isbtweb.org/events/hvwebinars.html	
18.08.2022	Haemovigilance Webinars: Sharing international haemovigilance experiences. Session 1: International Programs
25.08.2022	Haemovigilance Webinars: Sharing international haemovigilance experiences. Session 2: Consumer Engagement
01.09.2022	Haemovigilance Webinars: Sharing international haemovigilance experiences. Session 3: Data
06.09.2022	Haemovigilance Webinars: Sharing international haemovigilance experiences. Session 4: Donors

