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Vox Sanguinis

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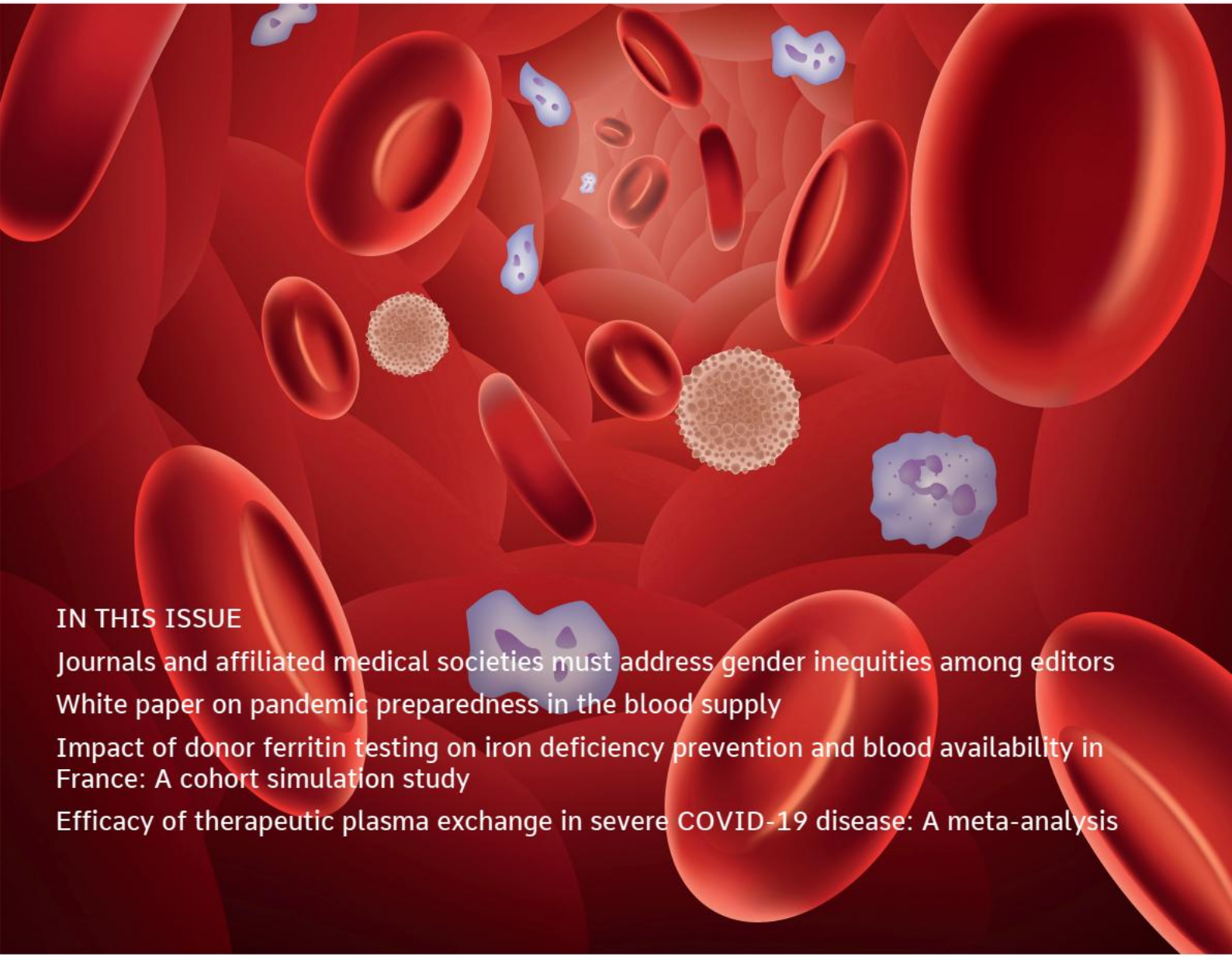

IN THIS ISSUE

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International Journal of Blood Transfusion

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Vox Sanguinis

International Journal of Blood Transfusion

Aims and Scope

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

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

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

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REVIEW

Vox Sanguinis ISBT International Society of Blood Transfusion

Patient blood management: Myths and facts about red blood cell transfusions

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Abstract

Transfusion medicine resembles all of medicine in that expert opinion predominates because hard data on clinical outcomes from randomized controlled trials and high quality observational data are simply unavailable. Indeed, some of the first trials evaluating important outcomes are barely two decades old. Patient blood management (PBM) depends on high quality data for assisting clinicians in making clinical decisions. In this review, we focus on several red blood cell (RBC) transfusion practices that new data suggest need reconsideration. The practices that may need revision include transfusion for iron deficiency anaemia, except in life threatening situations, toleration of anaemia as a largely benign condition and use of haemoglobin/haematocrit as primary indications for RBC transfusion, as opposed to adjuncts to clinical judgement. In addition, the long-standing notion that the minimum transfusion should be two units needs to be abandoned due to the danger to patients and a lack of clinical evidence of benefit. Finally, the difference in indications for leucoreduction versus irradiation needs to be understood by all practitioners. PBM is one of the strategies for managing anaemia and bleeding that holds great promise for patients, and transfusion is only one facet of the bundle of practices.

Keywords

blood safety, patient blood management, RBC components, transfusion strategy

Highlights

- A number of myths about red blood cells encountered by the authors are discussed here. This discussion is followed by evidence-based facts and expert opinion that disprove the myths and support evidence-based practice.
- This manuscript will serve to educate medical students, advanced practice providers, physicians in training and those in practice.
- Adequate patient blood management knowledge has also been linked to higher quality patient care and its implementation can improve important clinical outcomes such as decreased length of stay, a reduction in nosocomial infection and fewer intensive care unit admissions.

INTRODUCTION

Why is it important to address common misconceptions or 'myths'? Blood transfusion is one of the most common procedures performed in the inpatient setting [1,2]. Although ordering a transfusion is a part of routine practice for most physicians, the available literature has shown that non-transfusion medicine physicians have poor to intermediate transfusion medicine knowledge (TMK) [3–5].

Adequate physician TMK has also been linked to higher quality patient care [3,6]. As the composition of hospital medicine providers continues to evolve rapidly, attention is needed not only for physicians but also for advance practice providers (APPs) [3,7]. The growing involvement of APPs in the care of hospitalized patients raises the importance of adequate TMK among APPs. In one survey, 90% of APPs working in haematology and bone marrow transplant reported discomfort in their ability to practice in their specialty and wanted more training in transfusion medicine [3,8].

Unfortunately, medical education at both graduate and postgraduate levels includes minimal transfusion medicine instruction, the majority of which is in the form of passive lectures [3,9]. Therefore, hospital medicine providers are likely to have developed transfusion practices based on the limited knowledge taught in their training [3]. Transfusion decisions for acutely ill patients presenting with multiple co-morbidities may become complex and require a more in-depth understanding in order to minimize risks of transfusion and to identify, treat and report reactions for the health and safety at both the individual and population levels [3].

A study was conducted of 183 hospital medicine providers in the United States (US) (including 155 attending hospitalists and 28 APPs) who completed a 12-question online survey and 20 question exam [3]. The overall mean score was 52% (range 20%–85%) [3]. Forty-one percent of participants reported less than 1 h of training in transfusion medicine [3]. Five of the seven questions with the worst performance (<25% correct) focused on transfusion reactions [3]. Almost all respondents reported consenting a patient for blood transfusion and 60% believed that TMK was very or extremely important in order to provide appropriate care for patients [3]. More than 80% believed that having additional transfusion medicine education would be at least moderately helpful [3]. Although routinely consenting patients for transfusion, hospital medicine providers may have insufficient TMK [3].

A recent letter to the editor [10] made the following recommendations:

1. The best method to educate the physicians about patient blood management (PBM) is to oblige them to participate during their medical studies in courses focused on blood transfusion.
2. If this cannot be achieved, physicians should be educated. This would start with the attending physicians, as residents and fellows 'copy' their mentor's attitude, including their approach towards blood transfusion.
3. Blood myths exist and influence diverse populations. Increasing the level of general knowledge and education can reduce the influence of myths.
4. Finally, the various aspects and causes of blood transfusion overuse lead one to the conclusion that education is the key to success.

The purpose of this manuscript is twofold. First, it will show a number of myths in PBM that have been encountered by the authors and then provide the evidence-based facts that disprove these myths and support the correct practice for optimal patient care. Second, we hope that this manuscript will serve to educate medical students, APPs, physicians in training and those practicing. As stated above, adequate TMK (and PBM knowledge) has also been linked to higher quality patient care [3,6].

METHODS

A group of experienced and active practitioners of transfusion medicine and PBM convened a working group to develop a list of common clinician opinions encountered in daily practice that have dubious scientific evidence, henceforth, defined as 'myths', and which also have potentially adverse clinical consequences when actions are based on these notions. A focused literature review was performed to address the background of each 'myth' and provide evidence against it.

Iron deficiency should be treated with transfusion

Iron deficiency anaemia (IDA) is the most common nutritional disorder worldwide. The diagnosis of IDA is confirmed by the presence of low body iron stores and a haempglobin (Hb) level two standard deviations below normal [11]. The primary treatment for IDA is oral or intravenous iron, but in an emergency setting for patients who are actively bleeding or unstable, red blood cell (RBC) transfusion may be necessary. Choosing Wisely Canada guidelines state, 'Don't transfuse RBCs for iron deficiency without haemodynamic instability' [12]. The consensus is that transfusions should not be considered first line therapy in stable patients with an Hb ≥ 7.0 mg/dL. The question is whether these patients can be managed more efficiently and effectively than with RBC transfusion.

Current practice (myth)

Siddiqi et al. conducted an observational cohort study for a 6-year period following release of Choosing Wisely Canada guidelines [13]. A transfusion was considered outside of clinical guidelines if the patient's Hb was >7.0 mg/dL and if the patient had a heart rate <100 beats per minute and systolic blood pressure ≥100 mmHg [12]. The primary outcome was that the proportion of patients with IDA receiving transfusion outside of clinical guidelines. The rate of total transfusions in patients with IDA was 11.2%, and the rate of potentially avoidable transfusions was 18.7% [13].

Appropriateness of RBC transfusions ordered in the Emergency Department (ED) for management of patients with IDA was studied by Spradbrow et al. [14]. IDA was documented by an emergency physician in 61% of ED patients. Of these, 63% received RBC transfusions: 53% were deemed appropriate, 16% were appropriate for the indication, but received more than the required number of transfusions and 32% were deemed inappropriate [14].

Grey et al. conducted an audit to monitor transfusion practices of patients with confirmed severe iron deficiency. They found that RBC transfusions were commonly administered to elderly patients with severe IDA with Hb < 8.0 mg/dL and uncommon when the Hb was >9.0 mg/dL; and 75% of the patients in the audit were transfused to an Hb ≥ 10.0 mg/dL and 44% to an Hb ≥ 11.0 mg/dL, suggesting excessive transfusion [15]. RBC transfusion is taken as a 'quick fix' of haemoglobin, since it is relatively easy. There are non-evidence-based concerns regarding intravenous iron supplementation in surgical patients, such as increased risk of infection, iron overload or oxidative stress, which has been refuted time and time again [16].

Conclusion (fact)

In IDA, the role of RBC transfusion is controversial. The Association for the Advancement of Blood and Biotherapies (AABB) and other organizations do recognize that RBC transfusion may be indicated if the patient has haemodynamic instability due to anaemia as this helps to alleviate severe morbidity associated with microvascular hypoxemia until the time iron therapy becomes clinically effective [17]. The treatment of pre-operative IDA will improve haemoglobin before surgery, but good evidence exists that correcting anaemia by transfusing blood is detrimental to the outcomes of surgery, by increased risk for post-operative complications [18].

Historically, there has been a reluctance to use intravenous iron formulations due to concerns of hypersensitivity reactions, such as anaphylaxis. Newer preparations are far less likely to be associated with such reactions [19]. It is preferable to use preparations that can deliver a higher dose per infusion over the shortest time period possible.

General unfamiliarity with these newer preparations, the historical concerns regarding anaphylactoid reactions and time delays are likely barriers to the widespread use of these agents in the treatment of IDA [20]. RBC transfusions carry the risk of haemolysis, transfusion-associated circulatory overload (TACO), haemolysis and alloimmunization, and transfusion-related acute lung injury (TRALI) should be considered a less safe alternative to oral and intravenous iron [17].

Guidelines for management of IDA are necessary to reduce the knowledge-to-practice gap for IDA management and avoid inappropriate use of RBC transfusions. Anaemia in the geriatric population may be multifactorial, with nutritional deficiencies, senescence and chronic disease being the most common reason. Mild anaemia is rarely symptomatic and should not be transfused [21]. Symptomatic anaemia and severe anaemia where RBC transfusions may play a role, the emphasis should be on restrictive transfusion strategies and adequate dosing as compensatory mechanisms are overwhelmed [21]. Transfusion of single red cell units followed by clinical assessment including documented Hb increments should be done before ordering subsequent units to ensure that this valuable resource is appropriately and ethically used [22].

Mild anaemia is not clinically significant

Current practice (myth)

Anaemia is one of the most common clinical conditions worldwide and perhaps the single most common source of disability and poor quality of life. Indeed, the most common cause of anaemia is iron deficiency with anaemia being a part of late stage iron deficiency. There is abundant evidence that iron deficiency and IDA are associated with poor cognitive function in children as well as a cause of increased morbidity and mortality [23]. Blood loss anaemia is also quite common and associated with significantly increased risks of morbidity, mortality and hospital/ICU length of stay. Traditionally significant anaemia has been treated with blood transfusions, which are entirely inappropriate except in rare life threatening acute emergencies.

Conclusions (fact)

It is now clear that PBM needs to include pre-operative anaemia management programs and anaemia/iron deficiency screening programs for at-risk patients, such as women with abnormal menses and children with nutritional problems. Such approaches have been associated with improvements in quality of life, lessening of symptoms and a reduction in morbidity and mortality in various settings [24–26].

If the patient does not need two units you should not be transfusing

Current practice (myth)

Due to a lack of expertise and knowledge of transfusion practices in the majority of patients, RBC transfusion practices are driven by only laboratory-based Hb triggers. Some literature continues to suggest RBC transfusion thresholds for critically ill, clinically stable patients—Hb concentration <7 g/dL, for patients undergoing cardiac surgery Hb < 7.5 g/dL, for patients with hip fractures and cardiovascular disease or risk factors Hb < 8 g/dL and for haemodynamically stable patients with acute gastrointestinal bleeding Hb 7–8 g/dL [27]. It is a common myth among the treating physicians that we need to keep the Hb at a level of 9 gm/dL. So, estimating that for a target Hb level, they prefer to order two units of packed RBC transfusion.

Conclusion (fact)

Currently, transfusion practices have been moved to a more scientific and evidence-based approach that is known as PBM. PBM has emerged as an integral part of patient care. World Health Organization (WHO) defined PBM as 'PBM addresses the problems of anaemia, blood loss and coagulopathy. It is a patient centred, systematic, evidence-based approach to improve patient outcomes by managing a patient's own blood through diagnosis and aetiology specific treatment of anaemia and preserving the patient's own blood by minimizing blood loss and bleeding, while promoting patient safety and empowerment. It reduces the utilization of health care resources as well as expenditures, transfusion dependency, and the risks and complications of transfusion' [23]. The primary role of RBC transfusion is to provide adequate perfusion to the cells. The need for RBC transfusion is based on individualized clinical factors not merely on laboratory parameters such as Hb or haematocrit levels.

Keeping a focus on adverse incidents related to transfusion, it is better to avoid unnecessary transfusion. Overenthusiastic transfusions have been associated with increased ventilator days and prolonged ICU stays, and it has also been identified as an independent risk factor for mortality in critically ill patients [28,29].

The rise of Hb level following transfusion is not just a mere mathematical calculation anymore, that is, one unit raises the Hb level by 1.0 g/dL [30,31], whereas the rise in Hb level following transfusion is more dependent on characteristics of the patient such as height, weight, and body mass index as well as the underlying clinical condition [32]. So, transfusion of RBC should be judicious and evidence-based, and transfusion of one unit RBC may be sufficient. Retrospective observational studies done by Berger et al. and Bowman et al. mentions that transfusing malignant haematology and bone marrow transplant patients with single RBC units is safe and efficacious [33,34]. A recent clinical trial conducted on women with haemodynamically stable postpartum anaemia concluded that a single-unit transfusion protocol avoided a second unit of packed RBC in >80% of women without significant impact on morbidity [35]. The real evidence behind the one-unit orders is the fact that all the randomized clinical trials (RCTs) testing transfusion triggers employed a one-unit transfusion strategy and they showed no benefit to giving extra blood—more than is needed [36]. In reality it is necessary to reassess the patient, after one-unit red cell transfusion regarding the clinical condition of the patient. If there remains clinical need in terms of perfusion status and clinical condition, the patient should be scheduled for transfusion of another unit. So, in most cases of chronic anaemias and haemodynamically stable patients, transfusion of 1-unit red cell may be sufficient enough to meet this need and two units of RBC transfusion can be avoided calculating the risks associated [36]. While not widely used outside of paediatric patients, a recent study showed that patient's body weight differentially impacts the change in Hb after RBC transfusion. The authors suggested incorporating body weight into the clinical decision-making process when transfusing blood in adult surgical patients [37].

Transfusions of RBC should Be based on an Hb concentration of 7 g/dL

Current practice (myth)

A plasma Hb concentration of 7 g/dL has become a common 'trigger' for a blood transfusion, and the pervasiveness of this practice in diverse clinical scenarios can only be explained by it attaining mythical status. Figure 1 shows a histogram of the pre-transfusion Hb value for all transfusions of RBCs in one large multiple hospital medical system. The sharp peak centred at an Hb of 7 g/dL clearly illustrates that this laboratory assay value is the main driver of the clinical decision to transfuse in this system. If transfusions were based on individualized clinical factors, there would not be such a sharp peak at a laboratory value. A recent multi-national survey of ICU practice [38] provides additional evidence of the existence of the 'myth of 7 g/dL' in that it is widely used for actively bleeding patients in ICU, despite the complexity and variability of patient conditions there.

Getting to the origin of the myth is difficult. For many years, the practice was to use an Hb of 10 g/dL as a trigger, and this has been attributed [39] to a 1942 paper by Adams and Lundy. The text of that paper actually states 'When concentration of Hb is less than 8 to 10 grams per 100 cubic centimetres of whole blood, it is wise to give a blood transfusion before operation' [40]. The favouring of 10 as the value instead of 8 was likely re-enforced by the physiology studies of Case et al. [41] that demonstrated interference with cardiac function at haematocrit levels less than 30%. The fourth edition of Mollison's transfusion medicine textbook, the pre-eminent transfusion therapy textbook of its day advised 'Therefore, before surgery is undertaken the Hb should be raised above this level, even if only trivial haemorrhage is expected' [42].

The shift of the myth of 10–7 g/dL is easier to understand, as the numbers of clinical trials comparing the use of these two triggers are numerous and more contemporary. They all show either the non-inferiority or possibly superiority of the restrictive transfusion strategy of 7 g/dL as a trigger. We even have an overview of systematic reviews of meta-analysis of these trials, which fully explains the situation [43].

Conclusion (fact)

A trigger of 10 g/dL was never supported by expert practitioners of transfusion even when numerous physicians with less expertise were working under this myth assumption. A review article of the indications for transfusion appearing in JAMA in 1956 advised 'Anemia should be treated with red blood cell transfusion only after diagnostic procedures have excluded specific therapy or when anemia is so severe as to necessitate emergency treatment' as well as 'The frequency of transfusion of red blood cells in patients with marrow failure should be determined by the

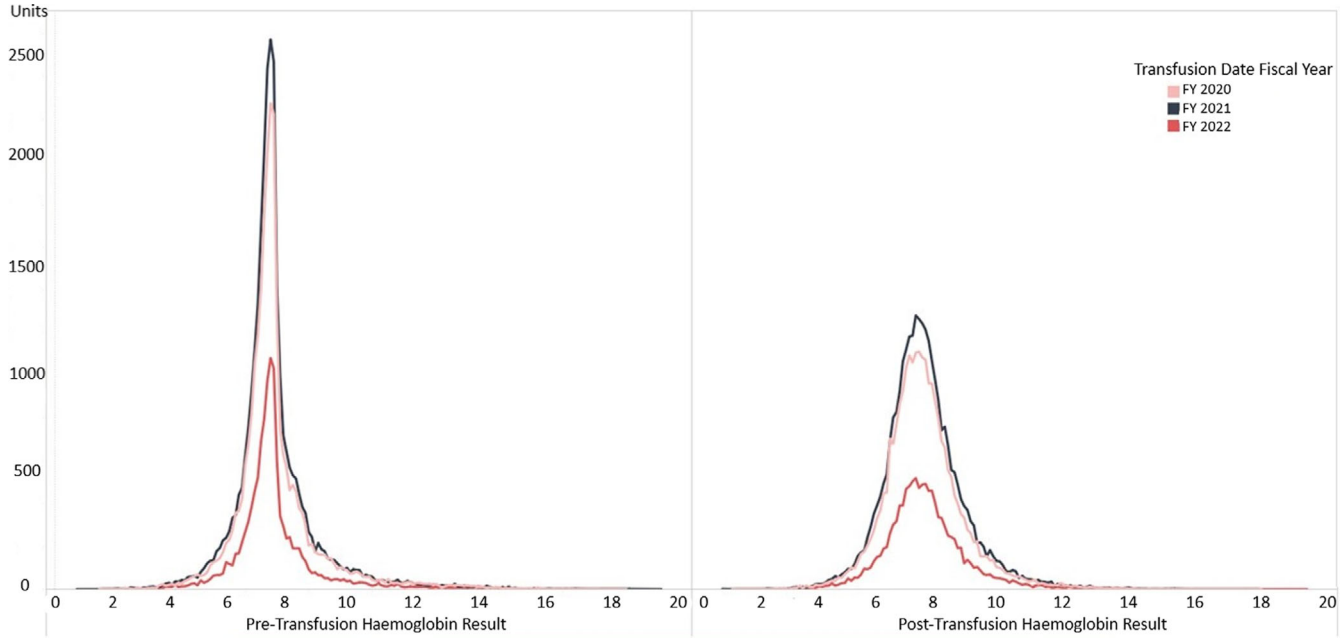

FIGURE 1 Histogram of the pre-transfusion Haemoglobin (Hb) value for all transfusions of red blood cells in one large multiple hospital medical system. The sharp peak centred at Hb of 7.0 g/dL clearly illustrates that this laboratory assay value is the main driver of the clinical decision to transfuse in this system, as opposed to clinical assessment based on other factors.

symptoms of the patient. The desired Hb level may vary from 6 to 11 gm per 100 cc in different persons' [44]. Other sections of the 1967 edition of Mollison included advice that patients with recent haemorrhage 'whose haemoglobin is as low as 7–8 g/100 ml should be transfused' as well as 'In patients with very severe anaemia transfusion may easily overload the circulation and precipitate cardiac failure. Thus, whenever it is probable that anaemia will respond to some other form of treatment transfusion should be avoided'. Also, 'To tide a patient over a short period of very severe anaemia inhalation of 100 percent oxygen can make an important contribution' is advised along with an explanation that plasma content of oxygen can be increased to make an Hb of 3 g/dL effectively 4.5 g/dL [42].

If the myth of 10 g/dL was never true, proving that 7 g/dL is 'non-inferior' does not make this notion true. As the JAMA authors point out by noting 'different persons', the transfusion decision is being made about one patient at a time. Randomized trials can be off-target to the circumstances of a particular patient. The Jehovah Witness population provides an example that most patients can tolerate haemoglobin levels significantly lower than 7.0 g/dL [45] and be supported by temporary oxygen supplementation, iron infusion and/or erythropoietin instead of transfusion. Clinical decision support may be perpetuating the myth by emphasizing a particular transfusion threshold rather than providing diagnostic algorithms to determine causes of anaemia and suggesting appropriate treatments based on diagnosis and symptoms. Monitors of a PBM program should not simply track pre-transfusion Hb levels and see Figure 1 as evidence of success, but perhaps should focus on the anaemia diagnosis and the use of iron when appropriate.

The only goal of transfusion of RBCs is to improve the delivery of oxygen to tissues

Current practice (myth)

Regarding off-target interpretation of results of randomized controlled trials, a straightforward example is the use of the Villanueva et al.'s [46] paper to support using a threshold of 7 g/dL for the broad category of bleeding ICU patients. This trial explicitly states the paradigm underlying the study as 'The goal of red-cell transfusions is to improve the delivery of oxygen to tissues'. As a result, the trial was designed to evaluate patients with an anatomic cause of bleeding and the ability of patients to tolerate an acutely progressive anaemia until a procedure could treat the site of bleeding. Greater than 97% of patients were not thrombocytopenic and required no platelet transfusions. Yet, this study is cited to support a restrictive threshold for bleeding ICU patients even if thrombocytopenic and without a readily correctable cause of bleeding. A common clinical plan seen in patient charts is 'transfuse for Hb < 7, platelets < 50', rather than have their bleeding potentially stopped earlier with only a few RBCs and many fewer platelets.

Conclusion (fact)

Multiple studies have definitively demonstrated that RBCs interact with platelets and play a role in haemostasis. A comprehensive review has been published [47]. Major mechanistic hypotheses include RBCs physically pushing platelets closer to the endothelium under flow

conditions, interaction of nitric oxide metabolism pathways and adenosine diphosphate augmentation of platelet function. The bleeding time has an inverse linear relationship to the haematocrit [48]. It is also becoming clear that RBCs participate in immunity by interacting with immune receptors in the spleen [49]. Better RCTs of RBC transfusions and patient outcomes need to also take into account platelet function and immunity.

Leucoreduction versus irradiation are the same

Current practice (myth)

Leucoreduction (LR) is the reduction of white blood cell (WBC) concentration in blood components, namely, RBC and platelets derived from the component preparation of whole blood or apheresis. There are many methods of LR, but, currently, this process may be performed using selective LR filters, which enable less than 1×10^6 or 5×10^6 residual WBC to be obtained in a RBC or 5×10^5 WBC in a whole blood derived platelet unit [50,51].

Over the past 30 years, it has been demonstrated that LR can reduce some adverse reactions due to blood component transfusion such as febrile non-haemolytic transfusion reactions, immunization against human leucocyte antigens and human platelet antigens, which may cause refractoriness to platelet transfusion and transmission of cytomegalovirus [53,54]. Furthermore, it is also claimed that LR also improves the clinical outcome of reducing post-operative surgical site infections or mortality in patients undergoing cardiac surgery and infection-related complications in trauma patients [52–54].

On the other hand, irradiated blood components are cellular blood components that have been exposed to irradiation to inactivate lymphocytes to stop their proliferation [55]. Irradiating blood components prevents the donor WBCs replicating and mounting an immune response against a vulnerable patient and recipient with the potential to cause transfusion-associated-graft-versus-host disease (TA-GvHD). Usually immunocompromised patients, foetus and premature neonates and patients who received haematopoietic stem cell transplant (HSCT) are prone to develop TA-GvHD. Evidence of TA-GvHD may include rash, fever, elevated liver enzymes, pancytopenia, diarrhoea, bone marrow aplasia or hypocellularity and hepatomegaly. It usually presents within 1–6 weeks after transfusion, with the median time from transfusion to first symptom being 11 days. Overall survival rate is reported to be 8.4% [56]. Patients receiving transfusions from a first-degree relative (e.g., parent, child or sibling) or second-degree relative (e.g., grandparent, grandchild, uncle, aunt, nephew, niece or half sibling), foetus and premature neonates or HSCT recipients and granulocyte transfusions should always be irradiated. The AABB *Standards for Blood Banks and Transfusion Services*, 33rd edition, recommends a dose of 25 Gy to the central area of the component with no portion receiving <15 Gy but sets no upper limit [51,56].

Conclusion (fact)

LR cannot prevent TA-GvHD as it contains a significant amount of residual lymphocytes. TA-GvHD continues to be reported with 66 out of the 348 (18.9%) cases who received nonirradiated LR components between 2000 and 2013 [56]. The British Society for Haematology recommendations note that the evidence is insufficient to recommend LR alone to prevent TA-GvHD in susceptible patients. So, irradiation and LR can never be used interchangeably.

Conclusions—The perpetuation of misconceptions and the ongoing need to address them

PBM has been recently recognized by WHO as an area for urgent intervention in current patient care practices [23]. The rationale is that prevention and appropriate treatment of anaemia and bleeding can lead to better outcomes. Transfusion plays a role in the care of anaemic and bleeding patients, but in general, expert opinion has traditionally been on the side of what we would now consider undue reliance of aggressive transfusion of RBCs and other components. Research in the last two decades has documented that it is unnecessary to transfuse most haemodynamically stable patients with anaemia, particularly those with IDA, for which safer and more precise treatments are available. Anaemia causes significant morbidity and mortality and avoiding transfusions is only part of the approach. Preserving the patient's own red cells is an important strategy. Early data suggest that the use of PBM may improve clinical outcomes such as length of stay, quality of life and even mortality [23]. And because RBC transfusions have serious complications including TRALI, infection, congestive heart failure and thrombosis, the minimum dose needed to achieve the desired clinical goals is more appropriate than arbitrary numbers of RBCs such as 'a minimum of two'.

Randomized trials of transfusion thresholds have been widely misinterpreted as suggesting that the Hb or haematocrit alone (usually 7/21) is a necessary transfusion threshold. Indeed, transfusion remains a clinical decision and laboratory values should be an adjunct. Haemodynamically stable, largely asymptomatic non-bleeding patients may not always need red cell transfusions unless they are unable to respond to normal erythropoietic stimuli, either endogenous or therapeutic. While RBC transfusions may have benefits beyond oxygen transport, high quality trials are needed to assess the risks and benefits of RBC transfusions in improving haemostasis. Finally, while LR and irradiation are important safety modalities and have some overlap in benefit, they have distinctly different indications and mechanisms of action.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

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Antiphospholipid antibodies in convalescent plasma of donors recovered from mild COVID-19 infection

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Abstract

Background and Objectives: Passive immunization by the infusion of convalescent plasma (CP) obtained from patients who have recently recovered from COVID-19, thus having antibodies to severe acute respiratory syndrome coronavirus 2, is a potential strategy to reduce the severity of illness. A high prevalence of antiphospholipid antibodies (APLA) in patients with COVID-19 has been reported during the pandemic, raising a concern whether the use of CP could increase the risk of thrombosis in transfused patients. We aimed to evaluate the prevalence of APLA in COVID-19 CP (CCP) in order to assess the potential prothrombotic influence of transfused CCP to COVID-19 patients.

Materials and Methods: We studied the prevalence of APLA in 122 CCP samples collected from healthy donors who recovered from mild-COVID-19 at two time periods: September 2020–January 2021 (defined as 'early period' samples) and April–May 2021 (defined as 'late period' samples). Thirty-four healthy subjects unexposed to COVID-19 were used as controls.

Results: APLA were present in 7 of 122 (6%) CCP samples. One donor had anti-β2-glycoprotein 1(anti-β2GP1) IgG, one had anti-β2GP1 IgM and five had lupus anticoagulant (LAC) using silica clotting time (SCT), all in 'late period' donors. In the control group, one subject had anti-β2GP1 IgG, two had LAC using dilute Russell viper venom time (dRVVT) and four had LAC SCT (both LAC SCT and LAC dRVVT in one subject).

Conclusion: The low prevalence of APLA in CCP donors reassures the safety of CCP administration to patients with severe COVID-19.

Keywords

anti-N antibodies, antiphospholipid antibodies, anti-S antibodies, convalescent plasma, COVID-19

Highlights

- A high prevalence of antiphospholipid antibodies (APLA) in patients with COVID-19 was reported.
- The presence of APLA is associated with a potential prothrombotic risk.
- We found a low prevalence of APLA in COVID-19 convalescent plasma, which suggests it can be safely transfused to COVID-19 patients with regard to prothrombotic risk.

INTRODUCTION

The coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is associated with coagulopathy through various mechanisms [1, 2]. Patients hospitalized with COVID-19 were observed to have an increased incidence of venous thromboembolism, pulmonary embolism and deep vein thrombosis, and an increased incidence of arterial thromboembolism—strokes, myocardial infarctions, systemic arterial embolism and acute limb ischaemia. These complications are major contributors to the high mortality rates associated with severe COVID-19 infection and have led to regular use of anticoagulant agents in hospitalized patients [3, 4].

COVID-19 patients generate antibodies against SARS-CoV-2 antigens including the spike (S) and nucleocapsid (N) proteins. The spike (S) protein, which is present on the envelope of SARS-CoV-2, is useful as an indicator of an effective immune response, including vaccine effectiveness. In contrast, nucleocapsid (N) proteins are biomarkers of natural infection [5].

Therefore, anti-S and anti-N antibodies are used for infection detection, as well as evaluation of responses to COVID-19 vaccinations. A variety of autoantibodies are also detected in COVID-19-infected patients. A recent study comparing patients with COVID-19 and healthy controls reported that about half of the patients had at least one type of autoantibody circulating in their bloodstream, compared to 15% of healthy controls [6]. The induction of autoantibodies was also previously described in association with other viral infections [7].

Studies have also reported a high prevalence of antiphospholipid antibodies (APLA) in COVID-19 patients [8, 9]. APLA are a panel of autoantibodies associated with a high prothrombotic risk composed of lupus anticoagulant (LAC), anticardiolipin antibodies (ACLA) and anti-β2-glycoprotein 1 antibodies (anti-β2GP1) of the immunoglobulin G and M classes. A recently published meta-analysis concluded that APLA were detected in nearly half of hospitalized patients with COVID-19 and even higher in patients with severe disease [10], suggesting a contributing role of APLA in the pathogenesis of thrombosis in COVID-19.

Passive immunization by the infusion of COVID-19 convalescent plasma (CCP) obtained from patients who have recently recovered from the disease, thus having antibodies to COVID-19, is a potential strategy to reduce the severity of illness and was studied prior to the development of anti-COVID-19 medications [11–14]. CCP is also an available therapeutic option in areas with no access to antiviral medications [15] or monoclonal antibody-resistant SARS-CoV-2 variants [16]. Approximately half of preserved CCP units collected from individuals recovered from wild type, alpha, beta and delta variants are also active against newer variants, that is, omicron [17]. Infusion of antibody-rich CCP can reduce the severity of acute viral disease through a direct antiviral effect (binding and neutralizing free virus) or by activating antiviral pathways (e.g., the complement cascade, phagocytosis and cellular cytotoxicity), as plasma, obtained by apheresis procedure, contains several kinds of antibodies and anti-inflammatory cytokines. On the other hand, there is also a possibility that antibodies could enhance disease severity by promoting viral entry or by proinflammatory mechanisms [18].

We studied the prevalence of APLA in CCP samples collected from otherwise healthy volunteer donors who recovered from mild COVID-19 and donated CCP in Magen David Adom National Blood Services (MDANBS) donation sites according to previously described criteria [19]. The results were compared with healthy individuals with no history of COVID-19 infection.

MATERIALS AND METHODS

CCP samples collected by MDANBS from volunteer donors who recovered from mild COVID-19 infection were tested. Mild COVID-19 was defined according to the World Health Organization (WHO) criteria as symptomatic patients meeting the case definition for COVID-19 without evidence of viral pneumonia or hypoxia [20].

Whole venous blood was collected into sodium citrate and heparin tubes and processed as follows [21]:

1. Plasma was obtained by double centrifugation at room temperature and frozen at −80°C.
2. Serum was obtained by centrifugation at room temperature and frozen at −20°C.
3. Frozen plasma samples were removed from the freezer, thawed in a 37°C water bath and then gently inverted three to five times prior to performing coagulation tests.
4. The frozen serum was removed from the freezer and allowed to acclimate to room temperature for approximately 10 min. They were then placed in a 37°C water bath with a gently swirl of tubes every 10–15 min until completely thawed.

Blood was collected from different donors in two time periods: between September 2020 and January 2021 (defined as 'early period' samples), and between April 2021 and May 2021 (defined as 'late period' samples).

Data on COVID-19 relevant signs and symptoms were self-reported by CCP donors at the time of donation [20]. Exclusion criteria for the CCP cohort included: prior COVID-19 vaccination, severe disease (hospitalization for symptomatic COVID-19), prior venous or arterial thrombosis, un-controlled or insulin-treated diabetes mellitus, congestive heart failure and un-controlled hypertension [19].

'Early period' donors were non-vaccinated individuals who recovered from COVID-19 before January 2021 and 'late period' donors recovered before April 2021 and were not vaccinated at this point.

Convalescence was supported by studying serum anti-COVID-19 antibodies that were in use at MDANBS in the defined time period: anti-N antibodies during the 'early period' and anti-S antibodies during the 'late period'.

Blood samples from healthy hospital care team members with no history of COVID-19 infection in the last 12 months (by self-report) or prior thromboembolism were collected in 2022 between February–March and June–July and used as control. Serum anti-N antibodies were studied in the control group in order to exclude prior COVID-19 infection.

The study was approved by the local ethics committee (approval date 10 February 2021, approval number 0399-20-ASF) and the Israel Ministry of Health (approval date 7 May 2021, approval number MOH-2021-05-07-009954).

All CCP donors and controls signed an informed consent.

Antiphospholipid antibody-positive participants were approached for repeat testing, were informed of the potential risks of APLA and instructed to have thromboprophylaxis in high-risk situations [22].

COVID-19 antibody tests

COVID-19 antibody testing was done according to the published guidelines [23]. Specifically, anti-N antibodies were tested by a chemiluminescent immunoassay (CMIA) and performed on the ARCHITECT i2000SR (Abbott, USA) instrument, according to the manufacturer's instructions. Positive results were defined as sample/cutoff (S/CO) ≥1.4.

Anti-S antibodies were tested using the same instrument (ARCHITECT i2000SR, Abbott, USA). The results ≥50 arbitrary units per millilitre (AU/mL) were considered positive.

Antiphospholipid antibody tests

Antiphospholipid antibody testing and interpretation were performed according to standard guidelines [24]. LAC testing was done using dilute Russell viper venom time (dRVVT) and silica clotting time (SCT). HemosIL dRVVT screen and HemosIL dRVVT confirm reagents (Instrumentation Laboratory) were used for the dRVVT assay. HemosIL silica clotting time screen/confirm reagents (Instrumentation Laboratory) were used for the SCT assay. Both assays were performed on the coagulation analyser ACL TOP-500 (Instrumentation Laboratory).

A mixing test on dRVVT was performed if the screening test of the sample was prolonged, according to International Society on Thrombosis and Haemostasis (ISTH) guidelines [24].

The results were reported as a normalized ratio (LAC dRVVT ratio normal range 0.91–1.42 and LAC SCT ratio normal range 0.7–1.3).

ACLA IgG and IgM and anti-β2-glycoprotein 1 (anti-β2GP1) IgG and IgM were detected using an ELISA method with AESKULISA Cardiolipin—GM kit (AESKU) and QUANTA Lite β2GP1 IgG and IgM ELISA kits (QUANTA Lite), respectively. Examinations were performed on the fully automatic TRITURUS analyser (Diagnostics Grifols). The results were reported as GPL U/mL and MPL U/mL for ACLA and SGU U/mL or SMU U/mL for anti-β2GP1 IgG/IgM (normal range 0–20 SGU/SMU U/mL).

Statistical analysis

We estimated that the prevalence of APLA in CCP samples was 30% (estimation deriving from the prevalence of APLA in hospitalized COVID-19 patients) and in controls 5%. One hundred and twenty-five samples of CCP were available for the study, and a 4:1 recruitment ratio was estimated since at the time of the study, a significant proportion of the population in Israel had already undergone COVID-19 infection, and we intended to include only subjects that did not have a history of COVID-19 in the control group.

The sample size required to detect a statistically significant difference in APLA prevalence was 145 subjects (CCP-116, controls—29), with power = 0.8 and alpha = 0.05 (two-tailed test).

Categorical variables are reported as frequency and percentage. Continuous variables are reported as the median and interquartile range (IQR). Chi-squared and Fisher's exact tests were used to compare categorical variables between CCP donors and healthy controls. Mann–Whitney and *t* tests were applied to compare continuous variables.

RESULTS

CCP study group included 122 participants, 80 samples were collected in the 'early period' and 42 during the 'late period'. Forty subjects were recruited as controls; however, anti-N antibodies were detected in six of them indicating previous asymptomatic COVID-19 infection, thus excluded from further analysis. Therefore, the final control group consisted of 34 subjects. At the time of plasma collection, none of the participants had a history of venous thromboembolism or arterial thrombosis. 'Early period' CCP donors were borderline older compared to 'late period' CCP donors, with a median age (IQR) of 35 years (25, 45) and 29 years (21, 41), respectively, $p = 0.07$. No other characteristic was significantly different between CCP donors during the two time periods.

Table 1 summarizes the demographic data and laboratory results of CCP plasma donors and controls. Compared to controls, CCP subjects were younger, with a median age (IQR) of 32 years (24, 43) compared to 37 years (29, 47), in the control group, $p = 0.04$. All but two subjects in the control group had received anti-COVID-19 (BNT162b2) vaccinations, most commonly three vaccines before APLA testing. There was a significant difference in the time interval between COVID-19 infection and CCP donation (tested for APLA) (median of 2.5 months), and the time between the last COVID-19 vaccination to APLA testing in controls (median of 7.8 months), $p < 0.001$.

Antiphospholipid antibody results

The results of APLA testing are given in Table 2. CCP donors had a significantly lower prevalence of any APLA (7 of 122, 6%) compared to controls (6 of 34, 18%), $p = 0.026$. All subjects (CCP donors and controls) were negative for anticardiolipin antibodies. In the CCP group, anti-β2GP1 IgG and anti-β2GP1 IgM antibodies were found in one participant each, both in 'early period' donors and five subjects had positive LAC SCT, all in 'late period' donors. In the control group, one subject was positive to anti-β2GP1 IgG antibody, two were LAC dRVVT positive and four had positive LAC SCT results (one of them

TABLE 1 Characteristics of study group and controls.

	Convalescent plasma (CCP)	Controls	*p*
N	122	34	
Age (y), median (IQR)	32 (24, 43)	37 (29, 47)	0.04
Gender (men), *N* (%)	97 (80)	27 (79)	>0.999
Time from recovery (CCP) to test/last vaccine (controls) to test (m), median (IQR)	2.5 (2, 3)	7.8 (5, 8)	<0.001
Number BNT162b2 vaccines 0/1/2/3/4	NA	2/0/2/28/2	

Abbreviations: CCP, COVID-19 convalescent plasma; IQR, interquartile range; m, months; N, number; y, years.

TABLE 2 Antiphospholipid antibodies results.

	Convalescent plasma (CCP) *N* = 122	Controls *N* = 34	*p*
APLA, *n* (%)			
Any	7 (6)	6 (18)	0.026
Anticardiolipin IgG /IgM	None	None	
Anti-β2GP1 IgG	1 (1)	1 (3)	
Anti-β2GP1 IgM	1 (1)	None	
Positive LAC dRVVT	None	2 (6)[a]	
Positive LAC SCT	5 (4)	4 (12)[a]	
APLA median (range)[b]			
Anticardiolipin IgG/IgM	–	–	
Anti-β2GP1 IgG SGU U/mL	54.6	40	
Anti-β2GP1 IgM SMU U/mL	68	–	
LAC dRVVT ratio	–	1.61 (1.56–1.66)	–
LAC SCT ratio	1.38 (1.35–2.37)	1.43 (1.32–1.62)	0.65

Note: Anti-β2GP1 IgG normal range 0–20 SGU U/mL, anti-β2GP1 IgM normal range 0–20 SMU U/mL, LAC dRVVT ratio normal range 0.91–1.42 and LAC SCT ratio normal range 0.7–1.3.
Abbreviations: anti-β2GP1, anti-β2-glycoprotein 1; APLA, antiphospholipid antibodies; CCP, COVID-19 convalescent plasma; dRVVT, dilute Russell viper venom time; LAC, lupus anticoagulant; SCT, silica clotting time.
[a]Positive LAC dRVVT and LAC SCT in one patient.
[b]In positive patients.

positive in both LAC tests). There was a trend towards older age in CCP subjects with positive compared to negative APLA, median (IQR) 41 years (27, 54), and 32 years (23, 43), respectively, $p = 0.17$ and no gender difference, $p = 0.56$. APLA-positive CCP donors tended to have longer times between COVID-19 recovery and CCP donation, a median of 3 months (IQR 1.75, 3.5) compared to APLA-negative subjects, a median of 2.5 months (IQR 2, 3), $p = 0.08$. In the control group, there was no difference between APLA-positive and APLA-negative subjects in age, gender, number of COVID-19 vaccination and the time period between the last vaccination to APLA testing.

There were no differences in the median titres of APLA between CCP donors and control subjects in the positive cases.

Among the CCP donors, there was no association between anti-COVID-19 antibodies (anti-N or anti-S) titre and the presence of either LAC or APLA (data not shown).

All APLA-positive subjects were approached to repeat the specific test. Two controls were lost to follow-up, one did not repeat the test, two were negative and one was positive (anti-β2GP 1 IgG 40.1 SGU U/mL). This decreased the total prevalence of APLA in controls to 3.22%. Only one CCP donor repeated APLA after 12 months and was positive to LAC SCT (ratio 1.43). We were unable to repeat testing in the other six positive CCP donors who did not confirm despite approaching them via email, as well as a phone call.

DISCUSSION

We found a low frequency (6%) of APLA in plasma samples collected from donors who have recovered from mild COVID-19 and donated CCP. This is in line with previous reports on the occurrence of LAC and ACLA in the young healthy population (1%–5%) [25–27] and the prevalence of APLA in healthy blood donors (10% ACLA-positive and 1% LAC-positive), with less than 1% remaining positive after 1 year [26]. A high prevalence of APLA has been previously reported in hospitalized patients with severe COVID-19. Since thromboembolic complications are common in severe COVID-19 cases, it was suggested that APLA participates in the pathogenesis of these complications. Therefore, it seemed plausible that infusion of CCP (containing APLA) as a treatment for severe COVID-19 patients could actually increase the risk of thrombotic complications and adversely affect infection outcome.

More recently, systematic reviews of papers reporting on the association between APLA and COVID-19 have called into question the role of APLA in the pathogenesis of COVID-19 and disease complications. The ambivalent results can be due to high heterogeneity amongst the studies resulting from the types and methods of APLA studied, different patient populations and timing of laboratory assessments, lack of data on the use of anticoagulation which can affect LAC testing, as well as additional confounders, that is, CRP levels [28]. Few studies have reported on the persistence of APLA in follow-up examinations. Accordingly, Favaloro et al. in a recent review on APLA and COVID-19 have concluded that at least some of the APLA appearing during acute COVID-19 can represent false-positive results, and therefore, the relationship between the detected APLA and COVID-19-associated coagulopathy remains to be resolved [29].

Regardless of the role of APLA in COVID-19 pathogenesis, we indeed detect a lower prevalence of APLA compared to other reports. Several explanations for this difference might be suggested. In our study, CCP was collected for antibody testing following mild COVID-19 infections, in contrast to APLA testing in hospitalized COVID-19 patients with severe disease, as previously reported [8].

The possibility of a rapid decline in autoantibodies after the acute infection could be another explanation for the low prevalence of APLA in the study cohort. APLA were tested during COVID-19 infection in hospitalized patients, while in our cohort, blood was drawn following a median recovery of 2.5 months, possibly at a time when APLA had already disappeared. Of note, the time point for CCP collection was directed by the Israeli MDANBS, enabling recovery of the donor prior to plasma collection while conserving an adequate anti-SARS-CoV-2 neutralizing antibody level. Although APLA may be transient and disappear within a few months [7], titres of the neutralizing antibodies also gradually decrease; therefore, collection of CCP at later time periods could result in sub-therapeutic levels of anti-SARS-CoV-2 antibodies [30].

We also found a trend for older age in APLA-positive CCP donors compared to negative ones. This is in line with previous reports on the higher prevalence of APLA in older compared to younger subjects and could also support a higher tendency to develop autoantibodies in the older population when exposed to infective agents [31].

Interestingly, we found only LAC in the plasma of 'late period' CCP donors and ACLA in 'early period' donors. We hypothesized that this could be explained by the difference in COVID-19 variants at the relative time periods, predominantly wild type and alpha strains and non-alpha variants in the 'early' and 'late' periods, respectively. Indeed, such associations were not reported from similar studies; however, most were done within a relatively short time period, presumably when only one SARS-CoV-2 variant was prevalent. Since molecular mimicry antibody response to different antigens is proposed as a possible underlying mechanism of the APLA development [32], COVID-19 variants could induce different APLA responses.

The median age of plasma donors in 'early period' was older compared to the age of 'late period' donors, 35 years and 29 years, respectively ($p = 0.07$), reflecting the fact that the alpha variant was more common in elderly people during the early pandemic.

In this study, we found a high prevalence of APLA (18%) in the control group. This relatively high rate could be explained by the small sample size. Reassuringly, the repeated test done after 3–5 months demonstrated a much lower prevalence (3.22%).

Another possible contributing factor is the specific vaccine used, that is, BNT162b2 in our control cohort.

A number of reports have shown that mRNA vaccines can trigger autoimmune syndromes in general [33] and APLA production in particular and can even lead to thrombosis which defines APLA syndrome [34–36]. A recent study reported on the prevalence of anticardiolipin IgG antibodies following COVID-19 or COVID-19 vaccinations (ChAdOx1-S $n = 37$ and BNT162b2 $n = 42$) [37]. The highest rate of positivity was in the COVID-19-positive group (18.9%). Following vaccination, BNT162b2 had the highest rate of positivity (11.9%), very similar to the rate in our study.

The number of vaccines given prior to APLA testing is also a potential factor in the rates of APLA in a healthy population. In a recent publication on 100 healthcare workers, 50 vaccinated with BNT162b2 and 50 with ChAdOx1 the authors concluded that vaccinations induced low titres of ACLA only occasionally [38]. Testing was done following one (ChAdOx1) or two (BNT162b2) vaccines compared to three vaccine doses of BNT162b2 in most of our control cohort.

Finally, the fact that convalescent donors had no thromboembolic disease at the time of plasma collection and the low prevalence of APLA in this cohort reassures the safety of continuous use of CCP to patients, in terms of thrombosis-inducing risk, according to recent publications [11–14].

In conclusion, we found a low rate of APLA in CCP samples collected after recovery from mild COVID-19. Using CCP from younger donors and fine-tuning of the time period between disease and plasma collection could lead to an optimal and risk–benefit balanced CCP product.

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D.B. initiated and designed the study, collected, analysed data and wrote the manuscript. M.K.M. analysed data and wrote the manuscript. M.I. and E.S. arranged the collection of plasma samples from CCP donors. T.F.R., O.G.J. and R. A.H. performed the laboratory tests. All authors provided input on the manuscript and approved the final version.

CONFLICT OF INTEREST STATEMENT

No conflict of interest to declare for all authors.

DATA AVAILABILITY STATEMENT

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

In vitro comparison of cold-stored whole blood and reconstituted whole blood

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Abstract

Background and Objectives: Cold-stored whole blood (CSWB) is increasingly used in damage control resuscitation. Haemostatic function of CSWB seems superior to that of reconstituted whole blood, and it is sufficiently preserved for 14–21 days. To provide evidence for a yet insufficiently studied aspect of prehospital CSWB use, we compared in vitro haemostatic properties of CSWB and currently used in-hospital and prehospital blood component therapies.

Materials and Methods: Blood was obtained from 24 O RhD positive male donors. Three products were prepared: CSWB, in-hospital component therapy (red blood cells [RBCs], OctaplasLG and platelets 1:1:1) and prehospital component therapy (RBCs and lyophilized plasma 1:1). Samples were drawn on days 1 and 14 of CSWB or RBC cold storage. On day 14, platelet concentrates at their expiry (5 days) were used for 1:1:1 mixing. Conventional clotting assays, rotational thromboelastometry, thrombin formation and platelet function were assessed.

Results: Haemoglobin, platelet count, fibrinogen and coagulation factor levels remained closest to physiological in CSWB. Factor VIII activity decreased markedly by day 14 in CSWB. The decline in platelet function was prominent in CSWB. However, CSWB on day 14 yielded physiological EXTEM MCF, suggesting haemostatically sufficient platelet function. Despite haemodilution and lower coagulation factor levels, in-hospital component therapy was haemostatically adequate. Prehospital component therapy formed the weakest clots. Thrombin formation potential remained comparable and stable in all groups.

Conclusion: Current prehospital component therapy fails to offer the clotting potential that CSWB does. CSWB and current in-hospital 1:1:1 component therapy show similar haemostatic potential until 14 days of storage.

Keywords

haemostasis, lyophilized plasma, platelet function, reconstituted whole blood, whole blood, whole blood storage

Highlights

- Cold-stored whole blood retains physiological coagulation factor levels during storage, except for factor VIII and von Willebrand factor activities.
- Platelet count and function declined during storage but remained haemostatically sufficient in cold-stored whole blood.
- Although coagulation factor levels were lower in reconstituted whole blood, it provides overall similar haemostatic potential to cold-stored whole blood.

INTRODUCTION

The most common preventable cause of death of trauma patients is massive haemorrhage [1]. Most deaths occur before reaching the hospital. A fifth of all trauma deaths could be prevented by better haemostatic control [1]. In developed countries, prehospital blood products are routinely used in damage control resuscitation (DCR). Blood products, typically red blood cells (RBCs) and fresh frozen plasma or lyophilized plasma (LP), may decrease mortality, although data are contradictory [2, 3].

Cold-stored whole blood (CSWB) has gained increasing interest in civilian DCR. CSWB, with easier logistics and thus potentially faster transfusions, can provide haemostatically active cold-stored platelets for prehospital transfusions. CSWB, compared to component therapy, contains less anticoagulant and additive solutions and may, therefore, reduce haemodilution in massive transfusion. CSWB may thus be superior to conventional component therapy in DCR. However, mortality and morbidity data are lacking as randomized controlled trials are still underway [4–6].

In previous studies [7–13], haemostatic function of CSWB has been superior to that of reconstituted whole blood (RWB) and sufficiently preserved up to 14–21 days. However, to the best of our knowledge, no studies have compared the haemostatic properties of CSWB to current prehospital and in-hospital component therapies for DCR. Here, prior to introducing CSWB to clinical use, we compared in vitro haemostatic properties of CSWB and the current component therapies used in massive haemorrhage.

MATERIALS AND METHODS

Twenty-four adult male O RhD positive regular donors were recruited through Finnish Red Cross Blood Service (FRCBS) electronic newsletter for blood donors. Standard FRCBS donor eligibility criteria were used, including haemoglobin level above 135 g/L. Use of non-steroidal anti-inflammatory drugs and herbal medications was prohibited 2 weeks prior to donation. Blood was collected following FRCBS standard procedures.

The study was approved by the ethics committee for Helsinki and Uusimaa Hospital District (HUS/699/2021). All participants signed an informed consent prior to donation.

Blood product preparation

We compared three different products (Figure 1), with eight donations allocated to each group: CSWB group, RWB mimicking hospital

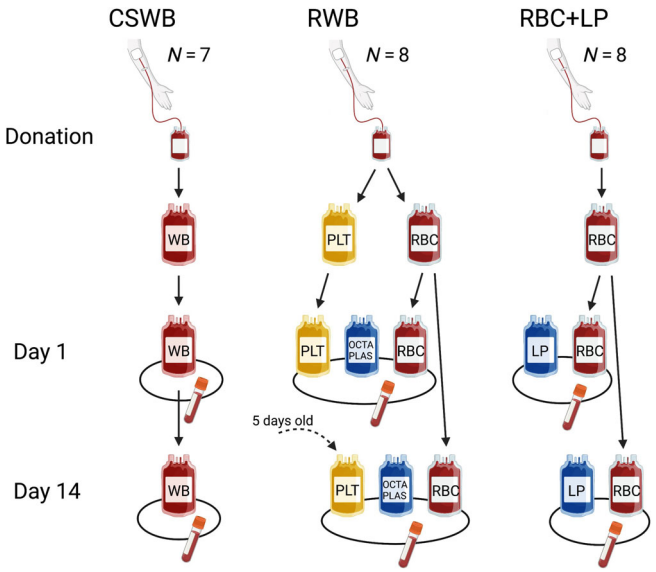

FIGURE 1 Preparation of blood products. Donated whole blood was leukoreduced 18–24 h after donation. Cold-stored whole blood (CSWB) and packed red blood cells (RBC) were stored at +5°C and PLT at +22°C. Unit volumes: CSWB 445 mL, RBC 260 mL, LP 200 mL, OctaplasLG® 200 mL and PLT 244 mL. Created with BioRender.com. LP, lyophilized plasma; PLT, buffy coat platelets; RWB, reconstituted whole blood, WB, whole blood.

massive transfusion protocol with 1:1:1 blood product ratio (RWB group) and RBCs and LP with 1:1 ratio used in prehospital setting (RBC+LP group). To determine baseline parameters, control samples were drawn from eight random study participants before donation. All donated units were stored at +22°C prior to processing.

In the CSWB group, whole blood was collected with a Terumo Imuflex® WB-SP collection set (Terumo Europe N.V., Leuven, Belgium) containing 63 mL of citrate phosphate dextrose (CPD). Whole blood was leukoreduced 18–24 h after donation with a platelet-sparing filter. One CSWB donation coagulated in the collection bag due to erroneous placing of the bag on the mixer scale and was discarded. CSWB units were stored at +5°C after leukoreduction.

In the RWB and RBC+LP groups, whole blood was collected with Fresenius CompoFlow® Quadruple T&B collection set (Fresenius Kabi AG, Bad Homburg, Germany) containing 63 mL of CPD and 100 mL of SAGM. Whole blood was leukoreduced 18–24 h after donation with a platelet-sparing filter, then separated into RBC, plasma and buffy coat. RBC units were stored at +5°C. Plasma was discarded, as only solvent/detergent-treated pooled plasma is used in Finland.

Buffy coats from four donors were pooled to form one platelet unit, stored in PAS-IIIM at +22°C.

In the RWB group, RBCs were mixed with blood group AB OctaplasLG® (Octapharma Nordic AB, Vantaa, Finland) and platelets in 1:1:1 ratio. On day 1 (d1), platelets were from the donations for this study. On day 14 (d14), platelets were O RhD positive buffy coat platelets from FRCBS surplus stock at unit expiry (5 days or within 12 h afterwards). In the RBC+LP group, RBCs were mixed with single-donor blood group AB LP (LyoPlas N-w®, DRK-Blutspendedienst West, Hagen, Germany) in 1:1 ratio.

Blood sampling and laboratory analyses

Control samples were drawn before blood donation to tubes with appropriate anticoagulants (BD Vacutainer® 3.2% Citrate and BD Vacutainer K2 EDTA, Becton Dickinson Finland, Vantaa, Finland; Vacuette Lithium Heparin, Greiner Bio-One GmbH, Kremsmünster, Austria). Samples from CSWB, RWB and RBC+LP groups were collected in test tubes without anticoagulants (Vacuette® Z No Additive, Greiner Bio-One GmbH, Kremsmünster, Austria).

Samples for laboratory analyses were obtained on d1 and d14 after donation. Samples were analysed within 3 h from collection. Citrated blood was centrifuged at 2500 g for 10 min before coagulation assays. For thrombin generation assay, citrated plasma was additionally centrifuged at 2500 g for 10 min, aliquoted and frozen at −80°C until analysis.

The blood count was analysed using Sysmex XN-9000® analyser (Sysmex Corporation, Kobe, Japan). Coagulation tests were done using routine analysers and methods; for fibrinogen, prothrombin time (PT), activated partial thromboplastin time (APTT), factor (F)V, FVIII, FIX, antithrombin, thrombin time (TT), D-dimer and von Willebrand factor glycoprotein Ib activity (VWF:Act) and antigen (VWF:Ag) with ACL TOP 500® (Instrumentation Laboratory, Werfen, Barcelona, Spain), for FII, FVII, FX, FXI, FXII, protein C, protein S free antigen with BCS XP® (Siemens Healthineers, Erlangen, Germany).

Platelet function analyser (PFA-200®, Siemens Healthineers, Erlangen, Germany) assay was performed from whole blood with collagen/epinephrine (PFA-EPI) and collagen/ADP (PFA-ADP) cuvettes in duplicate.

Multiple electrode aggregometry (MEA) for adenosine diphosphate (ADPtest) at 6.5 µM and thrombin receptor associated peptide-6 (TRAPtest) at 32 µM was done in duplicate from whole blood with Multiplate® analyser (Roche, Basel, Switzerland).

Thromboelastometry was done from whole blood using ROTEM® sigma analyser (Werfen, Barcelona, Spain), with cassettes containing extrinsic pathway (EXTEM), intrinsic pathway (INTEM) and fibrin formation (FIBTEM) tests. Clotting time (CT), clot formation time (CFT), amplitude at 5 min (A5), maximum clot firmness (MCF) and lysis index at 30 min (LI30) were reported.

Calibrated automated thrombogram® (CAT, Diagnostica Stago, Asnieres, France) was performed with 5 pM tissue factor, without thrombomodulin addition. Lag time, time to peak, thrombin peak and endogenous thrombin potential (ETP) were reported.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics, version 26. Non-parametric tests were used. As the control samples were not drawn from all donors, controls were treated as an independent group in statistical analyses. To quantify differences between the groups, ratios of group medians were calculated. Differences between the groups were tested with Kruskal–Wallis test. Time-dependent changes within groups were tested with Wilcoxon signed-rank test. Bonferroni correction was used to control the familywise error rate. A p-value of <0.05 was considered statistically significant.

RESULTS

Haemoglobin and haematocrit levels

Compared to controls, haemoglobin and haematocrit levels on d1 in the CSWB group were similar ($p = 0.094$), and in the RWB and RBC+LP groups significantly lower ($p < 0.001$) (Figure 2, Table S1). In the CSWB group, haemoglobin and haematocrit remained steady, and no clinically relevant changes occurred during storage. In the RWB and RBC+LP groups, haemoglobin concentrations and haematocrits were similar on d1 and d14 and were significantly higher in the CSWB group than in the RWB group.

Plasma coagulation factor levels

Compared to controls, coagulation factor levels on d1 in the CSWB group were similar ($p = 0.305$–0.903), except for lower FVIII activity ($p = 0.042$) (Figure 2, Table S2). All coagulation factor levels on d1 were significantly lower in the RWB ($p \le 0.010$) and RBC+LP ($p \le 0.001$) groups than in the controls.

In the CSWB group, coagulation factor levels decreased during storage, reflected in significantly decreased PT and increased APTT (Figure 2, Table S1). The storage effect was most prominent in FVIII levels (median 87 IU/dL on d1 and 43 IU/dL on d14, $p = 0.018$), which, in turn, prolonged APTT (Figure 2, Table S2). Fibrinogen level decreased during storage, but remained normal. Although FXII, FXI, FX, FV and FII levels decreased during storage, they remained within physiological range (Table S2). In the RWB and RBC+LP groups, the variation in coagulation factor levels between d1 and d14 was minimal. In the RBC+LP group, the FVIII level was significantly lower and thus APTT longer on d14 (Figure 2, Table S2). This was possibly due to variation in the single-donor LP composition.

Both on d1 and d14, coagulation factor levels were generally two-fold higher in the CSWB group than in the RWB and RBC+LP

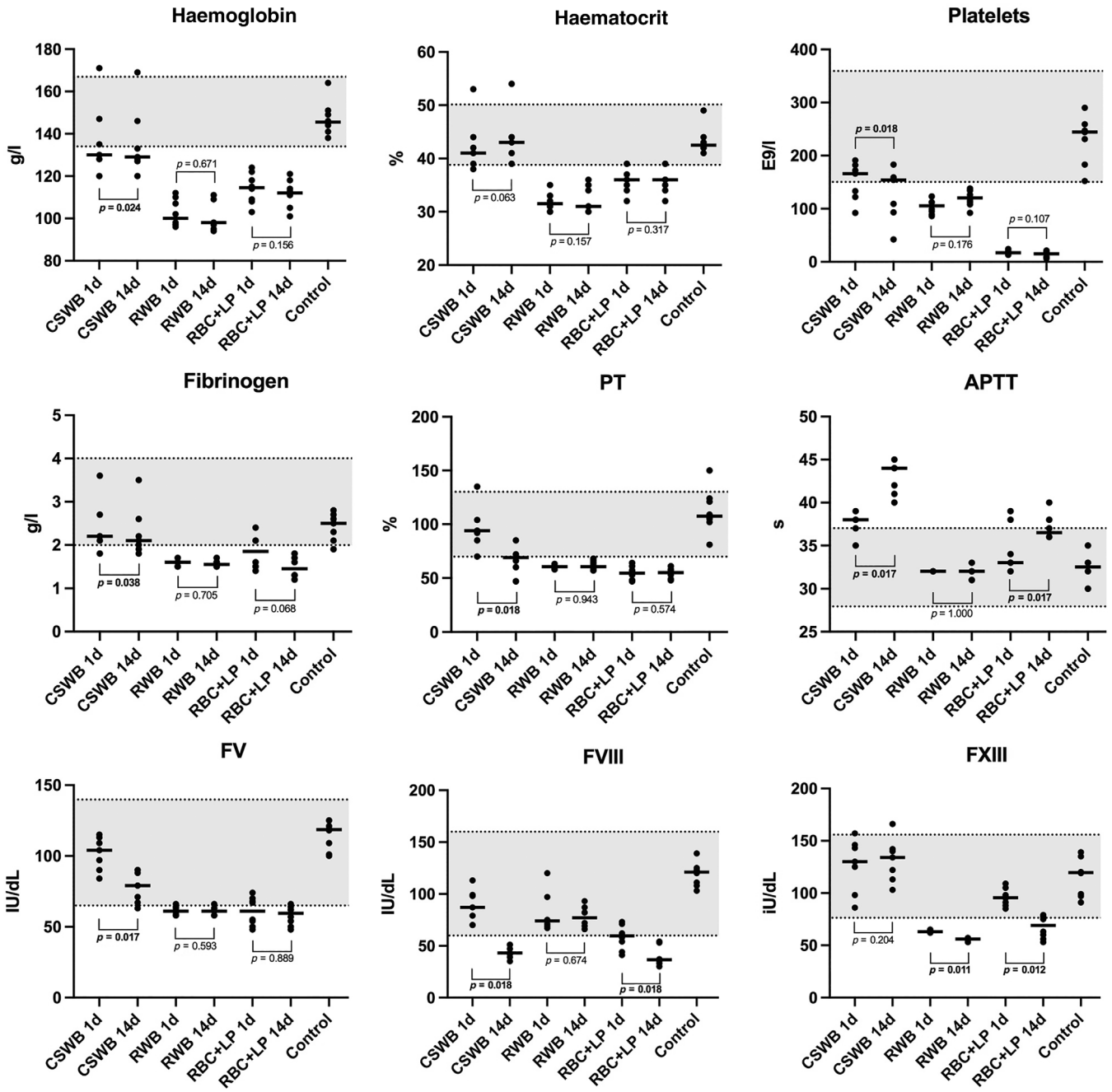

FIGURE 2 Blood count and coagulation assays. Individual measurements with median are shown. Laboratory reference range is shown in grey. Statistical comparison between groups is shown in Tables S1 and S2. CSWB, cold-stored whole blood; RBC+LP, packed red blood cells and lyophilized plasma; RWB, reconstituted whole blood.

groups (Figure 2, Table S2). In these groups, coagulation factor levels were approximately 50–70 IU/dL and thus below the physiological range. FVIII levels were significantly higher in the RWB group than in the RBC+LP group both on d1 ($p = 0.036$) and d14 ($p < 0.001$).

Thrombin generation

Compared to controls, CAT parameters on d1 in the CSWB group were similar, except for longer time to peak ($p = 0.010$) (Figure 3). In the RWB and RBC+LP groups, CAT parameters on d1 were comparable to controls, except for shorter lag time in the RBC+LP group ($p = 0.006$).

In the CSWB group, lag time increased during storage (Figure 3). Concomitantly, time to peak slightly decreased and peak height slightly increased. However, ETP in the CSWB group was similar to that in controls and did not change during storage, indicating overall normal thrombin generation in CSWB. In the RWB and RBC+LP groups, the variation in CAT parameters between d1 and d14 was minimal (Figure 3).

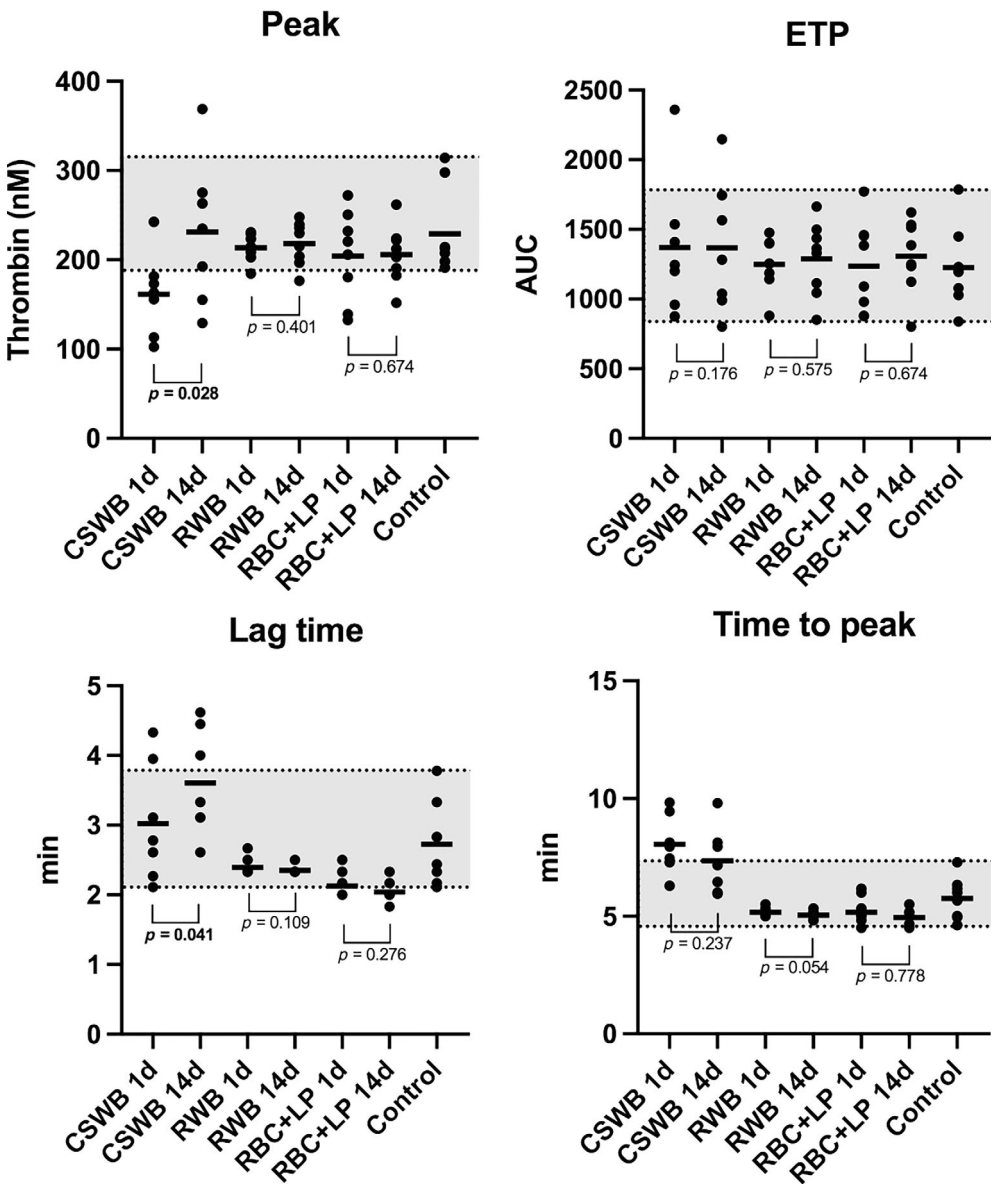

FIGURE 3 Thrombin generation assessed with Calibrated automated thrombogram. Individual measurements with median are shown. Control sample range is shown in grey. AUC, area under the curve; CSWB, cold-stored whole blood; ETP, endogenous thrombin potential; RBC+LP, packed red blood cells and lyophilized plasma; RWB, reconstituted whole blood.

Both on d1 and d14, time to peak was significantly longer in the CSWB group than in the RWB ($p = 0.007$ and $p = 0.004$, respectively) and RBC+LP ($p = 0.001$ and $p = 0.002$, respectively) groups (Figure 3). Likewise, on d14, lag time was significantly longer in the CSWB group than in the RWB ($p = 0.038$) and RBC+LP ($p < 0.001$) groups. However, ETP was similar in all groups on d1 and d14, indicating similar thrombin generation.

Viscoelastic properties

Compared to controls, EXTEM CT ($p < 0.001$) and INTEM CT ($p = 0.011$) on d1 were longer in the CSWB group (Figure 4). In the RWB and RBC+LP groups, viscoelastic properties were generally inferior compared to controls ($p \leq 0.018$, except for EXTEM CT in the RWB group $p = 0.168$).

In the CSWB group, clotting times and EXTEM CFT increased during storage (Figure 4, Table 1). EXTEM A5 decreased significantly, but FIBTEM A5 and EXTEM MCF were comparable on d1 and d14. In the RWB group, EXTEM A5 and EXTEM MCF increased during storage. FIBTEM A5 was comparable on d1 and d14. In the RBC+LP group, clotting times increased and clot strength decreased during storage. These effects were likely due to variation in the single-donor LP composition.

Both on d1 and d14, EXTEM CT was longer in the CSWB than in the RWB group, and on d14 also, EXTEM CFT was longer (Figure 4, Table 1). EXTEM A5 and EXTEM MCF were the lowest in the RBC+LP group, explained by the fact that RBC+LP samples contained only some residual platelets.

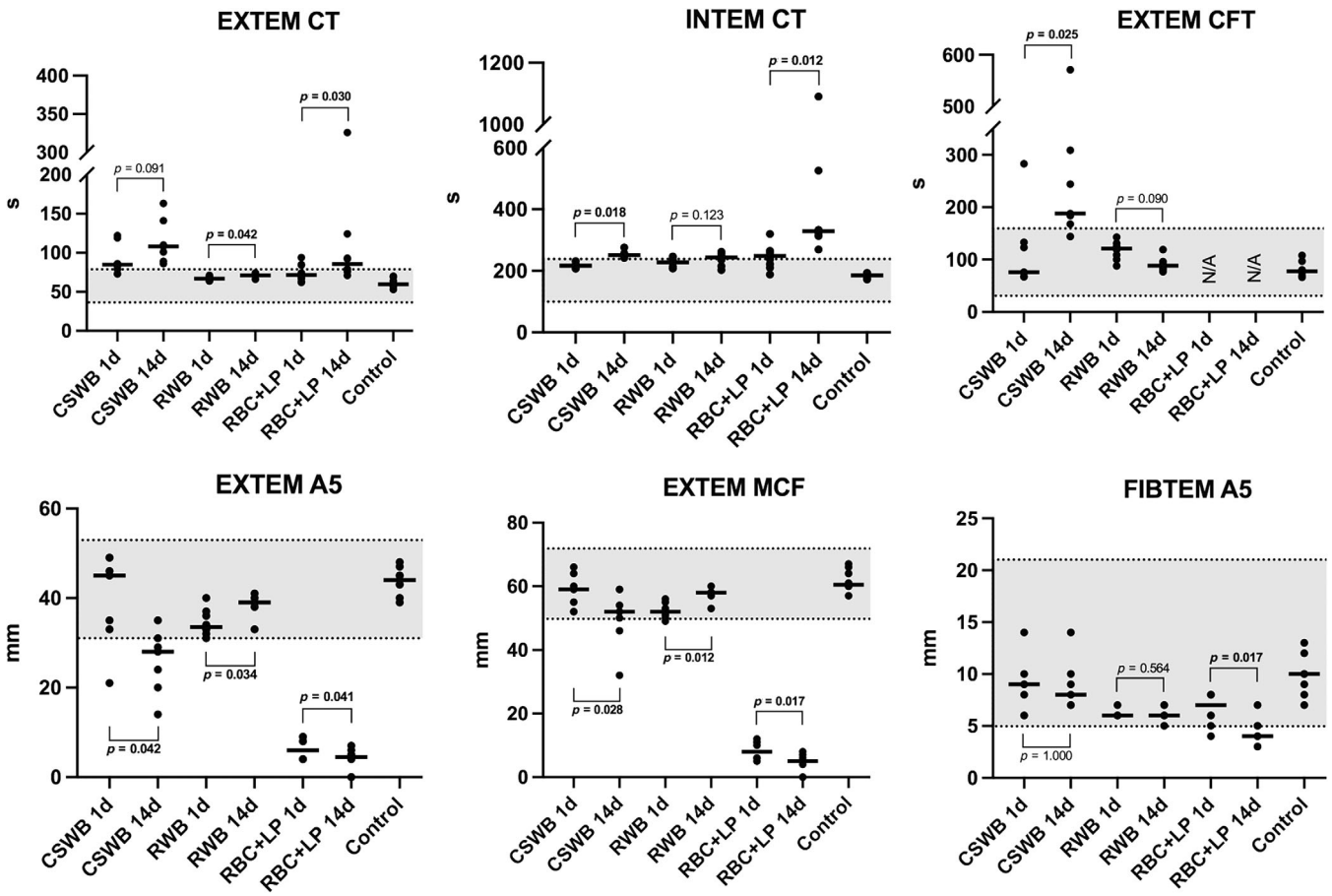

FIGURE 4 Viscoelastic properties assessed with rotational thromboelastometry (ROTEM). Individual measurements with median are shown. Laboratory reference range is shown in grey. Statistical comparison between groups is shown in Table 1. CFT, clot formation time; CSWB, cold-stored whole blood; CT, clotting time; RBC+LP, packed red blood cells and lyophilized plasma; RWB, reconstituted whole blood.

Platelet counts, function and VWF levels

Compared to controls, platelet count on d1 was lower in both the CSWB ($p = 0.011$) and RWB ($p < 0.001$) groups (Figure 2), whereas VWF:Ag and VWF:Act were similar (Table 2, Table S1). Compared to controls, platelet function on PFA and MEA on d1 was reduced both in the CSWB ($p \leq 0.029$) and RWB ($p < 0.001$) groups. Interestingly, in controls, collagen/ADP closure time was slightly above laboratory reference.

Platelet count in the CSWB group decreased during storage but remained at an adequate haemostatic level (Figure 2, Table S1). Both on d1 and d14, platelet counts were slightly, yet not significantly, higher in the CSWB than in the RWB group (Figure 2, Table S1). In the RWB group, platelet counts achieved adequate haemostatic levels (>100 × 10E9/L) both on d1 and d14.

The platelet function declined significantly during storage in the CSWB group. In PFA, collagen/ADP closure time prolonged (Table 2). In MEA, the platelet function in both ADP and TRAP channels declined. In the RWB group, storage had no effect on the platelet function. On d1, the platelet function was comparable in CSWB and RWB groups. On d14, the platelet function was better preserved in the RWB than in the CSWB group.

VWF:Ag levels remained stable during storage in the CSWB group, whereas VWF:Act decreased significantly (Table 2). In the RWB group, both VWF:Ag and VWF:Act remained stable during storage. Both on d1 and d14, VWF:Ag levels were slightly, yet not significantly, higher in the RWB group than in the CSWB group. On d14, VWF:Act was higher in the RWB group.

DISCUSSION

CSWB is considered a superior prehospital blood product due to the availability of platelets and easier logistics. Most in vitro research has focused on how storage time influences CSWB haemostatic potential. To the best of our knowledge, only a few such studies compare CSWB and RWB, and none compare CSWB and the current common prehospital practice of RBC+LP. We, therefore, studied the haemostatic potential of CSWB, RWB and RBC+LP with up to 14 days of CSWB and RBC storage time.

TABLE 1 Rotational thromboelastometry (ROTEM) results.

	Parameter	Laboratory reference range	Controls	CSWB	RWB	RBC+LP	Differences between groups		
							CSWB versus RWB	CSWB versus RBC+LP	RWB versus RBC+LP
Day 1	EXTEM CT (s)	38–79	60 (57–68)	85 (79–119)	67 (64–70)*	68 (63–84)*	1.27††	1.25	0.99
	EXTEM CFT (s)	34–159	78 (68–93)	76 (68–133)	121 (102–130)*	N/A	0.63	N/A	N/A
	EXTEM A5 (mm)	31–53	44 (41–47)	45 (33–49)*	34 (32–37)*	8 (4–9)*	1.34	5.63†††	4.19††
	EXTEM MCF (mm)	50–72	61 (60–66)	59 (52–64)*	52 (50–55)*	11 (6–11)*	1.13	5.62†††	4.95†
	EXTEM LI30 (%)	<15	100 (100–100)	100 (100–100)	100 (100–100)	100 (100–100)	1.00	1.00	1.00
	FIBTEM A5 (mm)	5–21	10 (8–12)	9 (6–10)	6 (6–6)	8 (5.5–8)*	1.67†	1.25	0.75
	INTEM CT (s)	100–240	185 (177–191)	217 (216–219)*	228 (211–241)	230 (210–260)*	0.95	0.94	0.99
Day 14	EXTEM CT (s)			108 (89–141)	71 (71–72)*	79 (75–92)*	1.51††	1.37	0.9†
	EXTEM CFT (s)			188 (168–309)	89 (82–95)*	N/A	2.12††	N/A	N/A
	EXTEM A5 (mm)			28 (20–31)*	39 (38–40)*	5 (4–6)*	0.72	5.60	7.8†††
	EXTEM MCF (mm)	See above	See above	52 (46–59)*	58 (57–58)*	6 (5–7)*	0.90	9.45†	10.55†††
	EXTEM LI30 (%)			100 (100–100)	100 (100–100)	100 (100–100)	1.00	1.00	1.00
	FIBTEM A5 (mm)			8 (7–10)	6 (5.3–6.8)	4.5 (4–5.5)*	1.33	1.78†††	1.33
	INTEM CT (s)			251 (248–256)*	244 (226–253)	320 (313–333)*	1.03	0.79†	0.76†††

Note: Median values and interquartile range are shown. Values outside laboratory reference are in grey. * $p < 0.05$ between days 1 and 14 within each group. Differences between groups are shown as a ratio of medians. † $p < 0.05$, †† $p < 0.01$ and ††† $p < 0.001$ (Bonferroni corrected) between the groups at each time point.

Abbreviations: CFT, clot formation time; CSWB, cold-stored whole blood; CT, clotting time; MCF, maximum clot firmness; N/A, not applicable; RBC+LP, packed red blood cells and lyophilized plasma; RWB, reconstituted whole blood.

TABLE 2 Platelet function assessed by platelet function analyser (PFA), multiple electrode aggregometry (MEA) and VWF assays.

	Parameter	Laboratory reference range	Controls	CSWB	RWB	CSWB versus RWB
Day 1	PFA ADP (s)	62–100	106 (89–110)	150 (126–165)*	>300 (>300–>300)	N/A†††
	PFA EPI (s)	82–150	118 (114–144)	>300 (270–>300)	>300 (>300–>300)	N/A
	Multiplate ADP (U)	40–110	67 (62–80)	19 (11–22)*	14 (12–20)	1.36
	Multiplate TRAP (U)	70–130	92 (88–99)	30 (16–43)*	23 (18–30)	1.33
	VWF:Ag (IU/dL)	50–190	110 (98–112)	99 (90–118)	119 (114–120)	0.83
	VWF:Act (IU/dL)	50–190	97 (84–104)	87 (75–99)*	87 (85–88)*	1.01
Day 14	PFA ADP (s)			>300 (>300–>300)*	>300 (>300–>300)	N/A
	PFA EPI (s)			>300 (>300–>300)	>300 (>300–>300)	N/A
	Multiplate ADP (U)	See above	See above	5 (4–11)*	11 (7–17)	0.48
	Multiplate TRAP (U)			10 (4–16)*	25 (21–28)	0.40††
	VWF:Ag (IU/dL)			99 (85–110)	118 (117–119)	0.84
	VWF:Act (IU/dL)			54 (42–58)*	93 (90–97)*	0.58††

Note: Median values and interquartile range are shown. Values outside laboratory reference are in grey. *($p < 0.05$) between days 1 and 14 within each group. Comparison between groups is shown as ratio of medians. †† ($p < 0.01$) and ††† ($p < 0.001$) between the groups at each time point.
Abbreviations: ADP, adenosine diphosphate; CSWB, cold-stored whole blood; EPI, epinephrine; N/A, not applicable; RWB, reconstituted whole blood; TRAP, thrombin receptor activating peptide-6; VWF:Act, von Willebrand factor activity; VWF:Ag, von Willebrand factor antigen.

In line with previous data [14], we demonstrated that platelets are essential for well-maintained in vitro haemostatic function. CSWB platelet count depends primarily on storage time and leukoreduction, and in the latter case, whether a platelet-sparing filter was used [7–10, 12, 14–18]. In our study, median platelet count was slightly higher in CSWB (leukoreduced with a platelet-sparing filter) than in RWB despite the decrease in platelet count in CSWB over time. Due to the single-donor nature of CSWB, the variance in platelet count is wide and CSWB units may display divergent haemostatic function, which may be clinically relevant.

Platelet function was abnormal in both CSWB and RWB groups. As fibrinogen concentration and FIBTEM A5 remained stable throughout storage in CSWB, the observed increase in EXTEM CFT and decrease in EXTEM A5, both clearly outside the reference range on day 14, are likely due to impaired platelet function in CSWB. Indeed, in line with previous studies [9, 10, 16, 18], both ADP- and TRAP-induced platelet aggregation in CSWB decreased already during 14-day storage. As platelets in CSWB are exposed to cold storage, this impaired aggregation is seemingly in contradiction with increased platelet activation and enhanced haemostatic capacity of cold-stored platelet concentrates [19, 20], but in accordance with the finding of attenuated aggregation responses to ADP and TRAP in CSWB as compared to cold-stored platelet concentrates [10]. This implies that other CSWB components, namely, RBCs and plasma, may influence platelet functionality. We observed a significant decrease in VWF activity, which possibly contributed to decreased CFT and firmness (EXTEM A5) through impaired platelet-to-platelet adhesion. Nevertheless, the platelet function in CSWB seemed haemostatically sufficient, as MCF remained within the physiological range throughout storage. This also supports that, instead of measuring coagulation factor concentrations or platelet function separately, whole blood-based assays such as viscoelastic tests probably give the most accurate estimate of the total CSWB haemostatic capacity.

Although CSWB can provide platelets for prehospital DCR, platelet concentrates are commonly used for in-hospital DCR. We demonstrated that platelet count in RWB, in contrast to CSWB, was more uniform and achieved good haemostatic levels (median 121 × 10E9/L) even when RWB was prepared with platelets at the end of their shelf life. Interestingly, in RWB with stable fibrinogen content and older platelets, we detected enhanced CFT and MCF, suggesting platelet activation during traditional storage contributed to enhanced haemostasis [21].

An unwanted but unavoidable by-product of blood product storage is dilution due to anticoagulant and additive solutions. We demonstrated that CSWB, with retained physiological haematocrit, is less diluted than in-hospital (RWB) or prehospital (RBC+LP) component therapy. Accordingly, despite individual variation in CSWB units, coagulation factor levels were generally twice as high in CSWB than in RWB or RBC+LP. However, both in the RWB and RBC+LP groups, fibrinogen concentration, APTT and PT were within the currently recommended levels in traumatic bleeding [22]. FVIII is labile and, affirming previous studies [8, 12, 14, 15, 23], decreased considerably during CSWB storage. This, and the decrease in other intrinsic pathway coagulation factors, was reflected in prolonged APTT, INTEM CT and thrombin generation lag time. Despite this, overall thrombin generation in CSWB remained robust and similar to that of RWB and RBC+LP. Finally, FXIII and natural anticoagulants were well preserved in CSWB compared to RWB and RBC+LP, which may be beneficial in maintaining the stability of the formed clots and limiting hypercoagulation after traumatic bleeding.

Blood group affects haemostasis, a feature largely undiscussed in the CSWB literature. Surprisingly, few in vitro studies clearly state having used universal donor group O CSWB [8, 9, 11, 14]. Group O

blood has lower VWF [24] and FVIII [25] levels and longer PFA closure times [26]. Indeed, this effect on the platelet function is evident in our control group, where ADP response in PFA was attenuated. The clinical haemostatic effects of transfusing group O, as opposed to group-specific, CSWB remain unclear.

There are several limitations to this study. First, the storage time was only 14 days. Although we show that CSWB retains haemostatic potential for at least 14 days, the effects of continued storage up to 21–28 days cannot be predicted. Studies with longer storage times have reported stable haemoglobin and fibrinogen concentrations but continued decrease in platelet count and function and clot firmness after 14 days [7–10, 12, 15–17]. Based on these studies, a CSWB shelf life of 14 [9] to 21 [7, 10] days has been suggested. The length of storage in this study was based on the planned 14-day storage time for the FRCBS CSWB product. Second, the sample size of eight donors per group is relatively small but likely sufficient to detect major differences between groups and time points. We considered it unethical to recruit a large number of donors for research purposes only. The small sample size is reflected in the marked variation in especially the CSWB platelet count. However, this accurately mimics clinical transfusion of non-pooled blood products, where the achieved response may vary significantly between units. A third, intrinsic limitation is that in vitro conditions do not imitate in vivo circumstances. Effects of acidosis, hypothermia, inflammation and endotheliopathy, all important in haemostasis and trauma-induced coagulopathy, were not addressed in this study. Also, post-transfusion platelet function remains unclear, as storage-induced platelet dysfunction may be restored after transfusion [21]. Similarly, this study cannot elucidate whether the patient's own platelets along with transfused RBC and LP are able to support haemostasis sufficiently.

Taken together, our study suggests that the coagulation properties of CSWB are superior to those of RWB or RBC+LP and supports its use in prehospital care where platelets are otherwise unavailable. Leukoreduced CSWB retains its haemostatic function over storage time of 14 days and is comparable with that of RWB. After initial DCR in prehospital care, in-hospital RWB seems a viable option when continued blood product use is warranted.

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S.S., J.L. and M.I. designed the study. J.L. recruited the blood donors. S.S. and M.I. collected the blood samples and data and wrote the paper. S.S. analysed the data. T.H., L.J.-K. and M.I. designed the laboratory assessments. J.L., T.H. and L.J.-K. reviewed the article.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Migration of di(2-ethylhexyl) phthalate, diisononylcyclohexane-1,2-dicarboxylate and di(2-ethylhexyl) terephthalate from transfusion medical devices in labile blood products: A comparative study

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Abstract

Background and Objectives: Polyvinyl chloride (PVC) plasticized with di(2-ethylhexyl) phthalate (DEHP) is a widely used material for medical transfusion devices. Not covalently bound to PVC, DEHP can migrate into blood products during storage. Recognized as an endocrine disruptor and raising concerns about its potential carcinogenicity and reprotoxicity, DEHP is gradually being withdrawn from the medical device market. Therefore, the use of alternative plasticizers, such as diisononylcyclohexane-1,2-dicarboxylate (DINCH) and di(2-ethylhexyl) terephthalate (DEHT), as potential candidates for the replacement of DEHP in medical transfusion devices has been investigated. The purpose of this study was to evaluate the quantity of PVC-plasticizers in the blood components according to their preparation, storage conditions and in function of the plasticizer.

Materials and Methods: Whole blood was collected, and labile blood products (LBPs) were prepared by the buffy-coat method with a PVC blood bag plasticized either with DEHP, DINCH or DEHT. DINCH and DEHT equivalent concentrations were quantified in LBPs by liquid chromatography–tandem mass spectrometry or coupled with UV and compared to DEHP equivalent concentrations.

Results: The plasticizer equivalent concentration to which a patient is exposed during a transfusion depends on the preparation of LBPs as well as their storage conditions, that is, temperature and storage time. At day 1, for all LBPs, the migration of DEHP is 5.0 and 8.5 times greater than DINCH and DEHT, respectively. At the end of the 49 days storage period, the DEHP equivalent concentration in red blood cells concentrate is statistically higher when compared to DINCH and DEHT, with maximal values of 1.85, 1.13 and 0.86 μg/dm^2/mL, respectively.

Conclusion: In addition to lower toxicity, transfused patients using PVC-DEHT or PVC-DINCH blood bags are less exposed to plasticizers than using PVC-DEHP bags with a ranging exposure reduction from 38.9% to 87.3%, due to lower leachability into blood components.

Keywords
di(2-ethylhexyl) phthalate, di(2-ethylhexyl) terephthalate, diisononylcyclohexane-1,2-dicarboxylate, labile blood product, metabolites, plasticizer migration

Highlights

- The ability of the plasticizers diisononylcyclohexane-1,2-dicarboxylate (DINCH) and di(2-ethylhexyl) terephthalate (DEHT) to migrate into blood products was studied and compared to di(2-ethylhexyl) phthalate (DEHP).
- The equivalent concentration of plasticizer to which a patient is exposed during a transfusion depends on the preparation of the labile blood product transfused, as well as its storage conditions, plasticizer's nature and the storage period.
- In addition to lower toxicity, transfused patients are less exposed using PVC plasticized with DEHT or DINCH bags rather than PVC plasticized with DEHP due to their lower ability to migrate into blood components.

INTRODUCTION

Polyvinyl chloride (PVC) is a polymer widely used in the manufacture of medical devices, particularly in the field of labile blood products (LBPs) collection, preparation and storage for transfusion. This transparent and inexpensive material with very high resistance, especially to temperature, made the PVC material of choice in the development of transfusion bags and tubing. Because of its rigidity, PVC needs to be plasticized to obtain more flexible materials. However, these plasticizers are not covalently bound to PVC, resulting in their migration within blood products, that is, whole blood (WB), plasma, red blood cell (RBC) concentrate and platelet concentrate (PC) [1–8].

Currently, di(2-ethylhexyl) phthalate (DEHP) is the most common plasticizer used in transfusion medical devices. Indeed, it has the major advantage to extend the preservation of RBCs by interacting with their membrane [1]. This phthalate, which was recognized as a reproductive toxicant and potential carcinogen in animal models for several years, belongs to category 1B of Regulation (EC) No. 1272/2008 because of similar effects demonstrated in humans [9–12]. Recently, IARC has considered that there is sufficient evidence in experimental animals for the carcinogenicity of DEHP. Thus, DEHP has been classified as possibly carcinogenic to humans (Group 2B) [11]. Moreover, its membership in an endocrine disruptor family has contributed to its gradual withdrawal from the market [11, 13]. However, it is still present in blood bag devices, potentially exposing transfused patients to significant concentrations of DEHP [5]. In addition, DEHP is metabolized in vivo to form mono(2-ethylhexyl) phthalate (MEHP), which is regarded as the main toxic substance [14, 15].

In this context, the use of alternative plasticizers, such as diisononylcyclohexane-1,2-dicarboxylate (DINCH) and di(2-ethylhexyl) terephthalate (DEHT), has been investigated as potential candidates for the DEHP replacement in transfusion medical devices. Biologically, these plasticizers have been shown to provide adequate storage of LBPs within the specifications set by the European Directorate for the Quality of Medicines and Health Care [16]. For instance, RBCs stored in PVC-DINCH and PVC-DEHT devices maintain a haemolysis rate between 0.14% and 0.57% depending on the plasticizer and the storage solution considered, that is, below the set threshold of 0.8% [1, 2, 4, 17, 18]. Furthermore, with a non-observed adverse effect level of 40 mg/kg bw/day and 500–700 mg/kg bw/day for DINCH and DEHT, respectively, these plasticizers showed lesser toxicity than DEHP (4.8 mg/kg bw/day) [19].

In the present investigation, we aim to evaluate and compare the release of DEHP, DINCH and DEHT from the PVC transfusion medical devices in WB, plasma, PC and RBC during their respective storage conditions. Particular attention was also paid to the quantitation of their first breakdown product to estimate the equivalent plasticizer concentration (plasticizer + first breakdown product concentration) to which blood recipients may be exposed.

MATERIALS AND METHODS

Preparation of blood samples

Blood collection

WB was collected in PVC-DEHP or PVC-DINCH bags (LQT system, Macopharma) by the French Blood Establishment (Lille, France) according to the procedure described in Figure 1a ($n = 3$/condition). In the absence of official registration, the donors' WB could not be collected directly using the PVC-DEHT medical devices. Therefore, WB previously collected in a PVC-DEHP bag was transferred to a PVC-DEHT device 1 day after collection and stored at room temperature (20–24°C) for 24 h. At the end, all WB bags were in contact with their respective plasticizers for 24 h before processing.

Preparation of LBPs

The buffy-coat method was applied to prepare the different LBPs, that is, the preparation of RBC, plasma and mini-platelet concentrate

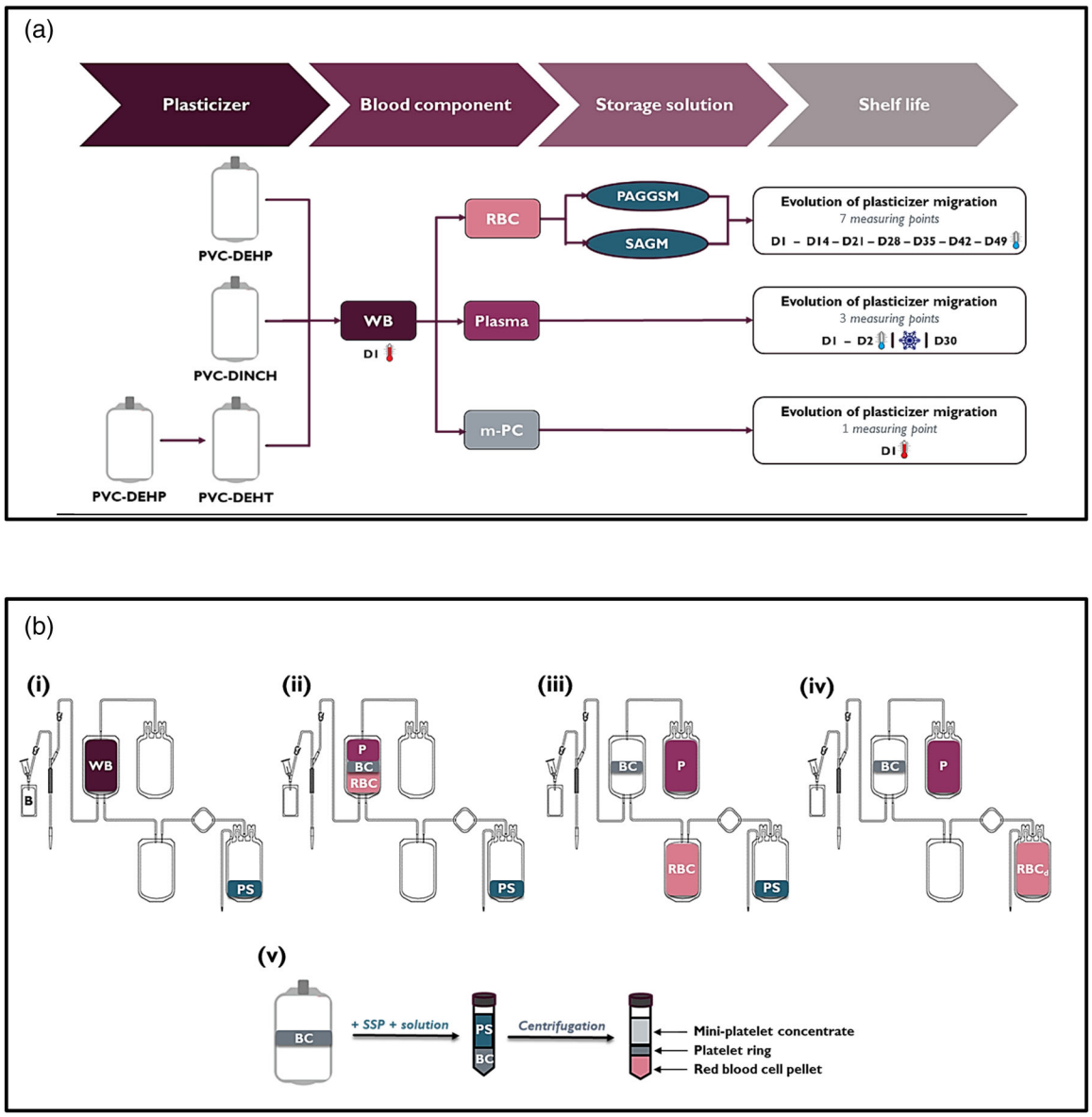


FIGURE 1 (a) Study design to evaluate the migration of di(2-ethylhexyl) phthalate (DEHP), diisononylcyclohexane-1,2-dicarboxylate (DINCH) and di(2-ethylhexyl) terephthalate (DEHT) from the transfusion medical devices in the four blood products during their respective storage conditions and (b) preparation of labile blood products with a medical device composed of four bags. B, Bactivam; BC, buffy coat; m-PC, mini platelet concentrate; P, plasma; PAGSSM, SAGM, RBC storage solutions; PS, preservation solution (PAGGSM, SAGM, SSP+); PVC, polyvinyl chloride; RBC, red blood cells; SSP+, platelet storage solution; WB, whole blood. Shelf life of blood products: RBC—42 days (France), 49 days (Germany); Plasma—at least 1 year (freezing); PC—7 days. Storage temperature of blood products: 20–24°C; 4°C; −40°C. Number of replicates for each condition: $n = 3$.

(m-PC), as described in Figure 1b. Briefly, the WB bag was centrifuged at 5000*g* for 15 min at 22°C (Figure 1b.i). The three phases obtained, from top to bottom, correspond to plasma, buffy coat and RBC, respectively (Figure 1b.ii). Plasma and RBC were then transferred into their storage and transfer bag, respectively (Figure 1b.iii). RBC was leucoreduced (RBC_d) and transferred in its storage bag containing SAGM or PAGGSM as the preservative solution (Figure 1b.iv). Finally, platelet preservative solution (SSP+, Macopharma, Mouvaux, France) was added to the RBC in a propylene tube and the mixture was centrifuged to obtain an m-PC (Figure 1b.v).

Sampling of LBPs

First, a blood sample was taken from the sample pouch (Bactivam), and used for donation qualification, to quantify the plasticizer and metabolite potentially present in the blood of each donor, so as to not overestimate the concentration of plasticizers released from the medical device. For DINCH and DEHT plasticizers, it should be noted that no contamination was detected at their respective detection limits of 2 and 110 nM (data not shown). Each LBP was then sampled, at different times, during its storage according

to the schedule depicted in Figure 1a. In this manuscript, 'Dx' denotes 'Day x', where D1 (and not D0) stands for the first day of follow-up. All samples were collected in polypropylene tubes and frozen immediately after collection at −80°C until analysis by liquid chromatography coupled with mass spectrometry (LC-MS/MS) or UV (LC-UV).

Determination of DEHP, DINCH, MEHP and MINCH by LC–MS/MS analysis

DEHP, DINCH, MEHP and monoisononylcyclohexane-1,2-dicarboxylate acid ester (MINCH) concentrations were determined by LC–MS/MS using an internal calibration method after the liquid–liquid extraction procedure, as described by Descat et al. [20]:

LC–MS/MS analyses were performed using a UFLC-XR system (Shimadzu, Kyoto, Japan) coupled to a QTRAP® 5500 MS/MS hybrid system triple quadrupole/linear ion trap mass spectrometer (Sciex, Foster City, CA, USA), equipped with a Turbo VTM ion source operating in positive ion mode. Three liquid–liquid extractions were successively applied with a mixture of n-heptane/ethyl acetate (1/2 v/v) (Merck, Guyancourt, France) to extract the plasticizers from the biological matrix. DEHP-d_4 (Sigma-Aldrich, Saint-Quentin Fallavier, France) was used as an internal standard to quantify DEHP and MEHP. DINCH-d_6 (Alsachim, Illkirch-Graffenstaden, France), and MINCH-D_{19} (synthetized) was used as an internal standard to quantify DINCH and MINCH, respectively. The concentration ranged from 25 to 300 nM for analytes and each calibration standard was supplemented with 100 nM of internal standard. Analytes were quantified using 1/*x* weighted linear models for DINCH, MINCH and MEHP and with a 1/*x* weighted linear quadratic model for DEHP. The mean recovery data of DEHP, MEHP, DINCH and MINCH were all around 80% in plasma and m-PC and 52%, 70%, 65% and 90% in RBC and WB (relative standard deviation < 10%), respectively. Each sample was analysed in duplicate.

Determination of DEHT and MEHT by HPLC-UV analysis

DEHT and mono(2-ethylhexyl) terephthalate (MEHT) concentrations were determined by high-performance liquid chromatography coupled with a UV detector (HPLC-UV) using an internal calibration method after the liquid–liquid extraction procedure, as described by Thelliez et al. [21]:

HPLC-UV analyses were performed using an Alliance system (Waters, Milford, MA, USA) equipped with a gradient quaternary pump, an online degasser apparatus, an autosampler and a 996-photodiode array detector. Three liquid–liquid extractions were successively applied with a mixture of n-heptane/ethyl acetate (2/1 v/v) (Merck, Guyancourt, France) to extract the analytes from the biological matrix. Dicyclohexyl phthalate (DCHP) was used as an internal standard (Sigma-Aldrich, Saint-Quentin Fallavier, France). The concentration ranged from 500 to 3000 nM for each analyte. Each calibration standard was supplemented with 15 µM of DCHP and analytes were quantified using linear models. The mean recovery data of DEHT and MEHT in the four biological matrices were 105% and 80% (relative standard deviation < 10%), respectively. Each sample was analysed in duplicate.

Calculations and statistics

The plasticizer equivalent concentration for calculation of the total plasticizer migration rate in blood products was calculated using the following equation [22]:

$$[\text{Plasticizer}]_{\text{equivalent}} = [\text{Plasticizer}] + \frac{[\text{Plasticizer breakdown product}]}{M_{\text{Plasticizer breakdown product}}} \times M_{\text{Plasticizer}}$$

where $[\text{Plasticizer}]_{\text{equivalent}}$ is the equivalent concentration of plasticizer in blood product (in µg/mL), [Plasticizer] and [Plasticizer breakdown product], the respective plasticizer and its first breakdown product concentrations in blood product (in µg/mL), respectively, and *M*, the molecular mass (in g/mol).

Plasticizer equivalent concentrations were expressed in µg/dm^2/mL of blood product considering the internal surface of the medical device (Table 1).

Univariate distributions of continuous variables are presented using the mean ± standard deviation. Group differences for plasticizer concentrations were analysed using a repeated measure two-way analysis of variance (ANOVA). If the ANOVA result was statistically significant, post hoc pairwise comparisons using Tukey's test were performed. All statistical tests were two-sided and statistical significance was considered as a p value <0.05. A correction of type-1 error was applied to post hoc tests. The data analyst was blinded to the plasticizer. All data analyses were performed using R and RStudio [23].

RESULTS

Influence of the LBP preparation and storage conditions on plasticizer's migration

On D1, whatever the plasticizer nature, plasticizer equivalent concentrations in plasma and WB (0.30–3.59 µg/dm^2/mL) are higher than those determined in m-PC and RBC (0.05–1.80 µg/dm^2/mL) (Figure S1).

The storage conditions of the blood product, that is, the temperature, also seem to influence the migration of plasticizers (Figure 2). For plasma, an increase in the equivalent concentration of DEHP (+0.61 µg/dm^2/mL) and DINCH (+0.20 µg/dm^2/mL) is noted between D1 and D2, followed by a concentration steady-state until D30. For DEHT, a constant equivalent concentration of

TABLE 1 Internal surface of the medical devices in contact with each labile blood product.

	Internal surface (dm^2)				
Medical device	**WB**	**RBC—SAGM**	**RBC—PAGGSM**	**Plasma**	**m-PC**
PVC-DEHP	5.37	16.32	16.19	10.31	5.37
PVC-DINCH	5.32	16.15	16.15	10.27	5.32
PVC-DEHT	5.75	16.49	16.99	10.53	5.75

Abbreviations: m-PC, mini platelet concentrate; PAGGSM and SAGM, RBC storage solutions; RBC, red blood cells; WB, whole blood.

0.41 ± 0.04 μg/dm^2/mL in plasma has been observed during the first 30 days of storage.

Influence of the plasticizer nature and the storage period on plasticizer's migration

On D1, for all LBPs, the migration of DEHP is in mean 5.0 and 8.5 times greater compared to DINCH and DEHT, respectively (Figure 2). The same statement was observed on D30 regarding plasma throughout its storage reaching a mean equivalent plasticizer concentration of 3.53, 0.87 and 0.45 μg/dm^2/mL for DEHP, DINCH and DEHT, respectively. For RBCs, whatever the preservative solution is, the DEHP equivalent concentrations are higher than those of DINCH and DEHT, throughout the storage, reaching a mean value in SAGM and PAGGSM of 1.68 ± 0.60, 1.12 ± 0.10, 0.84 ± 0.10 μg/dm^2/mL at D49, respectively, for DEHP, DINCH and DEHT.

In addition, whatever the nature of the RBC preservative solution (PAGGSM, SAGM), the equivalent concentrations of DEHP (1.50 ± 0.39 vs. 1.85 ± 0.43 μg/dm^2/mL), DINCH (1.11 ± 0.07 vs. 1.13 ± 0.10 μg/dm^2/mL) and DEHT (0.86 ± 0.01 vs. 0.81 ± 0.01 μg/dm^2/mL) have not been found statistically different at the end and throughout the storage of RBC (Figure S2, $p > 0.1$).

Regarding the corresponding breakdown product (MEHP, MINCH, MEHT) of each plasticizer, they are present in the LBPs but at lower concentrations than plasticizers, corresponding to 17%, 31% and 47% of the equivalent concentration, for DEHP, DINCH and DEHT, respectively, on D1 (Figure 2).

An increasing change in the concentration of MEHP, MINCH and MEHT is observed during the storage of RBCs stored in SAGM or PAGGSM solution ranging from 0.01 to 0.27 μg/dm^2/mL, on D1 and D49, respectively (Figure S3). Whatever the plasticizer is, a similar amount of plasticizer breakdown products ranging from 0.20 to 0.32 μg/mL/dm^2 was found in RBC stored in SAGM or PAGGSM solution, on D49. Conversely, no change in the concentration of MEHP (0.34 ± 0.01 μg/dm^2/mL), MINCH (0.19 ± 0.02 μg/dm^2/mL) and MEHT (0.12 ± 0.01 μg/dm^2/mL) was observed during the 30 days of plasma storage. Predictably, the short storage time (1 day) at 4°C and subsequent storage at −40°C of the plasma does not favour the degradation of the diesters into monoesters.

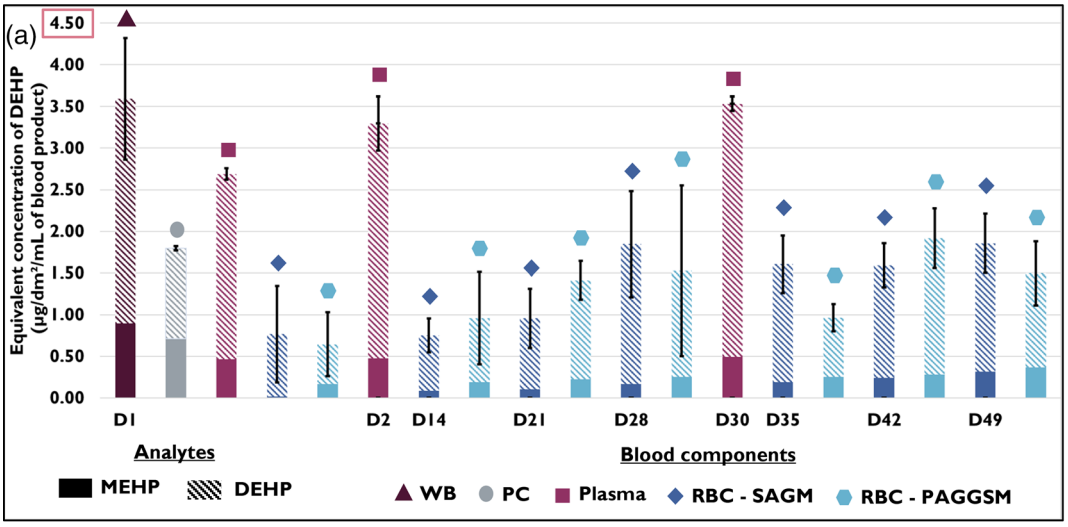
We then assessed the impact of the plasticizer nature and the duration of storage on the concentrations of plasticizers released in LBPs. For example, the statistical results obtained for equivalent plasticizer migration in the plasma matrix are displayed (Figure 3). We observe that the plasticizer concentration quantified during storage depends on the medical device used (PVC-DEHP, PVC-DINCH or PVC-DEHT) and on time (Figure 3a, $p < 0.05$). The migration of the plasticizer is higher when a PVC-DEHP device is used compared to a PVC-DINCH or PVC-DEHT device, regardless of the day of sampling. The metabolite concentration is only influenced by the nature of the medical device (Figure 3b, $p < 0.005$) and has been found steady during the 30 days of plasma storage ($p > 0.1$). Consequently, the plasticizer equivalent concentration depends on the nature of the plasticizer (Figure 3c, $p < 0.005$) and the day of sampling ($p < 0.05$) as demonstrated for the plasticizer alone since its concentration is preponderant to that of the metabolite.

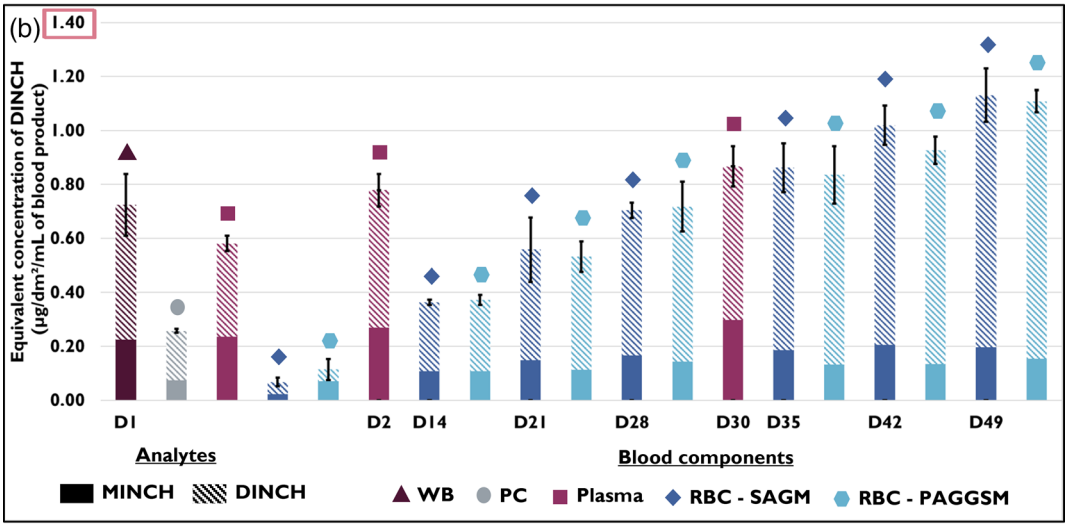
DISCUSSION

When quantifying the analytes of interest, variability in results was found for DEHP. This variability is inherent to the ubiquitous presence of DEHP in the environment which induces contamination along the blood preparation and/or analytical processes, which is not the case for the DINCH or the DEHT [20, 24, 25]. Although some strategies were implemented to avoid DEHP contamination from the environment, the variability of the results remains, especially for WB and RBC which are complex biological matrices.

Influence of the LBP preparation and storage conditions on plasticizer's migration

Three hypotheses can be highlighted to explain the differences shown on D1 between the different blood components. The first one can be related to the dilution factor applied during the preparation of RBC and m-PC. Indeed, whereas RBC (250 ± 50 mL) was prepared in a PAGGSM or a SAGM solution (105–110 mL), and buffy coat (16 mL) was diluted in SSP+ solution (24 mL) and centrifuged to obtain m-PC (supernatant). The difference in contact surface area between plasticized PVC from the medical device (tubes, bags and filter) and the blood product combined with the contact time with each of these surfaces could be the second reason to explain the difference in migration rate. Truly, the contact surface areas encountered by plasma (abt. 10 dm^2) and RBC (abt. 16 dm^2) are greater than those come across by WB and m-PC (abt. 5 dm^2), as shown in Figure 1b. On the other hand,





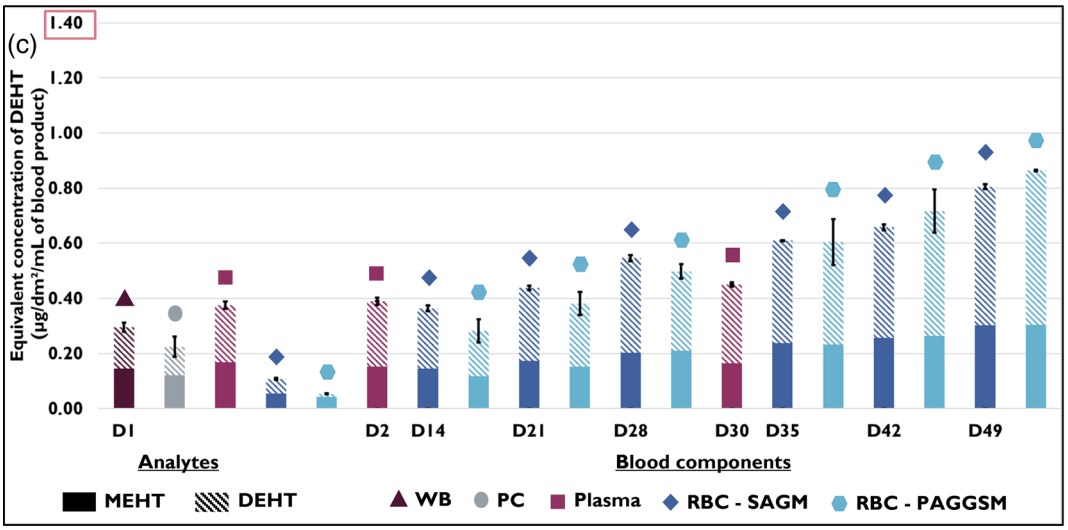


FIGURE 2 Equivalent concentrations of (a) di(2-ethylhexyl) phthalate (DEHP), (b) diisononylcyclohexane-1,2-dicarboxylate (DINCH) and (c) di(2-ethylhexyl) terephthalate (DEHT), in the four blood products, during their respective storage period. m-PC, mini platelet concentrate; P, plasma; PAGSSM, SAGM, RBC storage solutions; RBC, red blood cells; WB, whole blood. Number of replicates for each condition: $n = 3$.

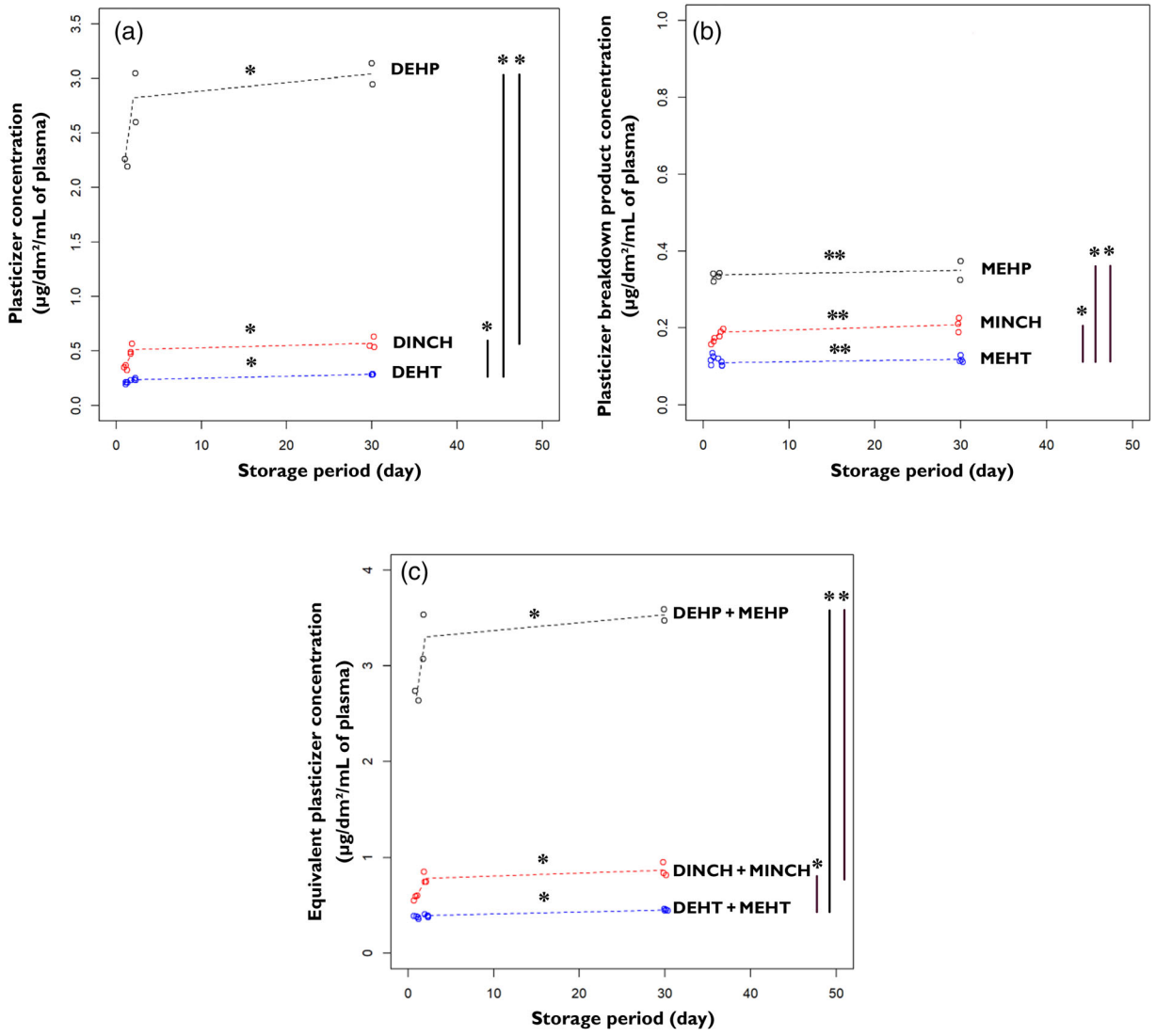


FIGURE 3 Evolution of the concentration of (a) plasticizer (di(2-ethylhexyl) phthalate [DEHP], diisononylcyclohexane-1,2-dicarboxylate [DINCH] and di(2-ethylhexyl) terephthalate), (b) plasticizer breakdown product (mono(2-ethylhexyl) phthalate [MEHP], monoisononylcyclohexane-1,2-dicarboxylate [MINCH] and mono(2-ethylhexyl) terephthalate [MEHT]) and (c) equivalent plasticizer (plasticizer + plasticizer breakdown product), during the 30 days of plasma storage. Dotted lines join the means and have been drawn to help with reading. Number of replicates for each condition: $n = 3$. *Statistically different; **non-statistically different.

the WB is stored 24 h in the collection bag, whereas the other LBPs are made from WB in a short period (1 h) before the sampling (D1). Indeed, prolonged contact with a larger plasticized PVC contact surface such as a bag will further promote the leaching of plasticizers. Finally, the lipophilicity of matrices such as plasma enhances the leachability of plasticizers from PVC, as previously described by Descat et al. [20].

The migration difference observed during plasma's storage is probably due to the change in storage conditions with a temperature of 4°C on D1 and D2 and freezing at −40°C from D2 to D30 (Figure 1a). It has already been shown that an increase in temperature conditions favours the migration of plasticizers [4, 26]. In addition, the lack of difference in equivalent DEHT concentrations along the plasma storage is probably due to its lesser capacity to migrate into the bag contents compared to DEHP and DINCH [19, 27–30]. This could be explained by the fact that the two-carbonyl functions of DEHT are in para-position on the aromatic ring.

Influence of the plasticizer nature and the storage period on plasticizer's migration

The relationship between storage period and plasticizer migration in blood products has already been demonstrated in several studies for DEHP and DINCH [4, 5, 7, 31]. Lagerberg et al. showed an increasing plasticizer concentration along the storage of RBC (from D1 to D42) ranging from 4.1 ± 0.9 to 33.0 ± 11.0 µg/mL for DEHP and from 0.2 ± 0.1 to 4.5 ± 0.9 µg/mL for DINCH [4]. The same observation was noted by Morishita et al. during the storage of RBC in PVC-DEHP bags with DEHP concentrations from 2.60 ± 0.72 µg/mL to 22.0

± 4.4 μg/mL at D1 and D42, respectively [31]. Our study highlights the same phenomenon for DEHT in RBC, namely, a concentration increases from 0.50 ± 0.46 to 7.12 ± 0.74 μg/mL, along the storage period.

Furthermore, our results for RBC on D42 are consistent with those stated above for DEHP ([DEHP] = 24.3 ± 3.2 μg/mL) and slightly higher for DINCH ([DINCH] = 13.0 ± 0.3 μg/mL). Only few studies concerning the release of DEHT from medical devices have been published to date and are focused on the field of infusion. Bernard et al. showed that DEHP migrates three times more than DEHT in an ethanol/water mixture (50/50 v/v) [27]. Faessler et al. found that DEHT was 16 times and 2 times less released in lipid emulsions than DEHP and DINCH, respectively [32]. The same trend is showed in our study.

In aqueous solutions, phthalates, including DEHP, are inert compounds [33]. As such, DEHP does not spontaneously react with any of the constituents of the preservation solutions. Therefore, no impact of the RBC preservative solution (PAGGSM, SAGM) was highlighted. We have found the same outcome with DINCH and DEHT.

Based on the ratios of breakdown products (Figure S3), it is difficult to clearly define the origin of the breakdown products present in each of the LBPs. In line with the literature data, this phenomenon remains unsolved. It seems to be linked either to the in vivo metabolism of these plasticizers by enzymes (lipases and esterases) present in LBPs [34–40], the degradation of the plasticizers during the autoclave sterilization stage of medical devices [37, 41] or the presence of the breakdown product in the plasticized PVC device during its production [37, 41]. In our study, in situ degradation seems to be the main source of degradation products considering the increasing concentrations of diesters and monoesters observed during the RBC storage.

Plasticizer exposure in patients with thalassemia, thrombotic thrombocytopenic purpura or sickle cell disease

When long-term suppression of circulating sickle cells is required, chronic RBC transfusion therapy is indicated and usually repeated every 3–4 weeks in the most severe cases [42]. In patients with severe beta-thalassemia, the frequency of leucoreduced RBC transfusion is usually every 2–4 weeks [43]. On the other hand, thrombotic thrombocytopenic purpura (TTP) front-line therapy includes typically 1–1.5 plasma volume exchange, with fresh frozen plasma replacement and immunosuppression with corticosteroids for the first 3 days, followed by 1× plasma volume exchange each day thereafter and continued daily until the clinical response is achieved and sustained for 2 days [44]. In these cases, equivalent DEHT and DINCH concentrations were reduced by 53.5% and 38.9% for patients with sickle cell disease and beta thalassaemia and by 87.3% and 75.4% for patients with TTP, respectively, compared to DEHP. These reductions in plasticizer concentrations represent a significant improvement in transfusion safety, especially in those cases that require a significant number of procedures.

Thus, our study showed that a patient transfused with a PVC-DINCH or PVC-DEHT device is much less exposed to plasticizers than when using a PVC-DEHP device, regardless of the LBP transfused. This reduction in exposure needs to be even re-assessed by considering the toxicological profile of each DEHP alternative plasticizers and their respective derivatives.

In conclusion, our study showed that the equivalent concentration of plasticizer to which a patient is exposed during a transfusion depends on the nature of the LBP transfused as well as its storage conditions and period. In addition, no influence of the preservative solution (SAGM, PAGGSM) on the migration of plasticizers was shown during the storage of RBC. Furthermore, the increasing concentration of the first breakdown product (MEHP, MINCH and MEHT) was revealed during the storage of the RBC but remains partially explained. Finally, DEHT and DINCH migrate less than DEHP in blood products, resulting in lesser patient exposure to plasticizers during blood transfusion using PVC-DEHT or PVC-DINCH blood bags. Due to the lower toxicity of the plasticizers DINCH and DEHT as well as their lower ability to migrate into LBPs, the use of PVC-DINCH or PVC-DEHT devices is a safer alternative to PVC-DEHP devices.

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A.T., B.D. and M.L. designed the research study; A.T. performed the research, acquired and analysed the data; E.C. carried out the statistical analysis; A.T., B.D. and M.L. wrote the first draft of the manuscript; A.T., B.D. and M.L. designed the research study; B.D. and M.L. supervised the research. B.D., M.L., C.S., E.C. and S.R. reviewed the manuscript. B.D. and M.L. edited the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors have declared no conflict of interest.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Proof of concept for detection of staphylococcal enterotoxins in platelet concentrates as a novel safety mitigation strategy

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Abstract

Background and Objectives: *Staphylococcus aureus* is a predominant contaminant of platelet concentrates (PCs) that can evade detection during screening with culture methods. Importantly, *S. aureus* produces staphylococcal enterotoxins (SEs) during PC storage, which are linked to slow growth and enhanced biofilm formation. This study investigated timing of SE production during PC storage and feasibility of SE detection as a PC safety strategy.

Materials and Methods: Genomic and transcriptomic data of transfusion-relevant *S. aureus* PS/BAC/169/17/W, PS/BAC/317/16/W, CI/BAC/25/13/W and CBS2016-05 were used to determine the presence and differential expression of exotoxin genes in PCs. Trypticase soy broth (TSB) and PCs were inoculated with 1.0E+06 cfu/mL of *S. aureus* PS/BAC/169/17/W and CBS2016-05. Expression of SEs at different growth phases was confirmed with Western blotting. PCs were inoculated with 30 cfu/unit of the same strains, and SE detection during PC storage was optimized with a sandwich dot-ELISA assay.

Results: *S. aureus* genomes contain multiple exotoxin genes including those encoding for SEs. Transcriptome data revealed significant upregulation (0.5–6.7-fold, $p < 0.05$) of SE genes in PCs versus TSB. Western blots demonstrated SE production at all growth phases. Notably, dot-ELISA detected clinically relevant concentrations of SEs (~0.2 μg/mL) at 32 h of PC storage when *S. aureus* PS/BAC/169/17/W and CBS2016-05 counts were 1.8E+04 and 1.4E+04 cfu/mL, respectively.

Conclusion: Genomic analyses revealed that staphylococcal exotoxins are widely distributed and highly conserved among transfusion-relevant *S. aureus* isolates. Furthermore, SEs are significantly upregulated in PCs and detected at 30 h of PC storage. Therefore, bacterial toxin detection could supplement mitigation strategies to enhance PC safety.

Keywords

enterotoxins, platelet concentrates, staphylococcal exotoxins, *Staphylococcus aureus*

Highlights

- Genomes of transfusion-relevant *Staphylococcus aureus* isolates encode for a variety of staphylococcal exotoxins, including superantigens such as staphylococcal enterotoxins (SEs), which are likely responsible for septic shock symptoms of patients transfused with contaminated platelet concentrates (PCs).
- Expression of SE genes is significantly upregulated in PCs contaminated with *S. aureus* in comparison to *S. aureus* grown in laboratory media.
- Detection of SEs in *S. aureus* contaminated PCs is feasible as early as after 30 h of PC incubation. Importantly, SE concentration at 32 h of PC incubation is approximately 0.2 μg/mL, which is considerably higher than 0.1 pg/mL, an SE concentration reported to cause septic shock symptoms in immunocomprised patients.

INTRODUCTION

Contamination of donated blood with bacteria remains a significant concern in transfusion medicine. Platelet concentrates (PCs), which are therapeutic for preventing or treating thrombocytopenia, are at higher risk of bacterial proliferation, compared to other transfusable blood components such as plasma and red blood cell concentrates. PCs are stored in glucose-rich, neutral pH solutions and gas-permeable plastic bags at 22 ± 2°C under agitation for up to 7 days [1]. These storage conditions are essential for platelet viability; however, they are also amicable for the proliferation of the majority of PC bacterial contaminants. To enhance the safety of donated blood, mitigation strategies have been implemented prior to and during blood collection, including donor selection, disinfection of donor's arm at the venipuncture area and diversion of the first aliquot of collected blood [2, 3]. Additionally, post-collection safety protocols are applied in different centres, which comprise PC screening for bacterial contamination or PC treatment with pathogen reduction (PR) technologies (PRTs) [1, 4]. Over the years, a remarkable success in decreasing bacterial contamination of PCs has been achieved worldwide, especially in high income countries [5].

Bacterial testing of PCs can be performed using culture-based approaches or rapid methods [6]. During the last two decades, automated culture-based systems have been widely implemented worldwide. PC culture diagnostic systems are credited with reducing septic events from PC transfusion by 69.7% [7]. Notwithstanding, there is no absolute guarantee of PC sterility since culture systems only validate the release of PC units on a negative-to-date basis, without considering the inoculated volume, sampling time, presence of slow growing pathogens or formation of surface-attached aggregates known as biofilms during PC storage [1, 8].

Gram-positive and gram-negative bacteria have been isolated from PCs with *Staphylococcus aureus* ranking as a major cause of transfusion related sepsis and fatalities [2, 9–14]. This species can evade detection during routine PC screening with automated culture systems due to slow growth and ability to form biofilms in PCs [13, 14].

Remarkably, *S. aureus* can secrete a compendium of exotoxins such as exfoliative toxins, staphylococcal superantigens (SAgs), and haemolysins among others [15]. SAgs are potent immunostimulatory proteins that include the toxic shock syndrome toxin-1 (TSST-1), staphylococcal enterotoxins (SEs), which have been involved in septic transfusion cases, and superantigen-like toxins (SSLs) [13, 14, 16]. Within all SEs, SE-type G (SEG) and SE-type H (SEH) are relevant for this study. While SEG was detected in a contaminated PC unit involved in a septic transfusion reaction, the *seh* gene was found in the genome of another *S. aureus* strain reported to be involved in a septic transfusion event [9, 13]. Furthermore, we have recently demonstrated that SEG and SEH production during PC storage increases the formation of biofilms and impairs growth of *S. aureus*, likely contributing to missed detection of this pathogen with culture methods [17]. In this study, we investigated time of SE production during PC storage, which could be used as a supplementary test to enhance transfusion patient safety.

MATERIALS AND METHODS

PC units

PC units were manufactured at the Canadian Blood Services netCAD Blood4Research Facility (netCAD, Vancouver, Canada) in agreement with standard procedures. PCs were shipped to the Canadian Blood Services Microbiology laboratory in Ottawa, Canada. Ethical approval for this study was granted by the Canadian Blood Services Research Ethical Board.

S. aureus isolates

Four *S. aureus* strains listed in Table 1 were used in this study, including one isolated in Canada [9] and three isolated in England [10–12]. Two of the English isolates (PS/BAC/169/17/W and PS/BAC/317/16/W) were detected during routine PC screening using the BACT/ALERT culture system at the National Health Service Blood and Transplant (NHSBT) (i.e., confirmed positives). The third English strain (CI/BAC/25/13/W) and the Canadian isolate (CBS2016-05) were missed during BACT/ALERT PC screening (i.e., false negative screening). While the PC unit contaminated with *S. aureus* CI/BAC/25/13/W

TABLE 1 Staphylococcal exotoxin genes encoded by the genomes of four *Staphylococcus aureus* strains.

S. aureus strain	BACT/ALERT PC screening result	Staphylococcal enterotoxins and superantigen-like genes
CBS2016-05	False negative[a]	*seg*, *seh*, *sei*, *sem*, *sen*, *seo*, *ses*, *selu*, *selw*, *selx*, *sely*, *exo*, *ssl1*, *ssl3*, *ssl4*, *ssl5*, *ssl7*, *ssl8*, *ssl9*, *ssl10*, *ssl11*, *ssl12*, *ssl13*, *ssl14*
CI/BAC/25/13/W		*seb*, *selp*, *selw*, *selx*, *selz*, *ssl1*, *ssl2*, *ssl3*, *ssl4*, *ssl5*, *ssl7*, *ssl8*, *ssl9*, *ssl10*, *ssl11*, *ssl12*, *ssl13*, *ssl14*
PS/BAC/169/17/W	Confirmed positive[b]	*seg*, *sei*, *sem*, *sen*, *seo*, *selu*, *selw*, *ssl1*, *ssl2*, *ssl3*, *ssl4*, *ssl5*, *ssl7*, *ssl9*, *ssl10*, *ssl11*, *ssl12*, *ssl13*, *ssl14*
PS/BAC/317/16/W		*selw*, *ssl1*, *ssl2*, *ssl3*, *ssl4*, *ssl5*, *ssl7*, *ssl9*, *ssl10*, *ssl11*, *ssl12*, *ssl13*, *ssl14*

Abbreviation: PC, platelet concentrate.
[a]False negative BACT/ALERT screening results refers to bacteria missed during routine screening of PCs and detected during investigation of transfusion reactions or near-misses [1].
[b]Confirmed positive BACT/ALERT screening results indicate the same bacterial species identified during initial and confirmatory PC screening [1].

was identified prior to transfusion by visual inspection due to clot formation ('near-miss') [12], the PC unit carrying CBS2016-05 was transfused causing a septic transfusion reaction [14].

Staphylococcal exotoxin gene identification

The genomes of all four *S. aureus* isolates were next-generation sequenced. Illumina paired end DNA libraries of the isolates were sequenced using the Miseq platform, assembled, annotated at the Health Canada genomics facility, and published at the NCBI database [9–12]. The presence of SE genes in the next-generation sequences of all studied strains was further confirmed by Sanger sequencing. Information from the genome sequences was used to design primers for specific PCR amplification of genes encoding for SEs and SSLs. OneTaq DNA polymerase kit (New England Biolabs, Ipswich, MA, USA) was used as follows: each reaction tube containing a mixture of 2 µL (10 ng) genomic DNA, 5.0 µL buffer (1×), 0.5 µL dNTPs (200 µM), 1.0 µL primer pair (0.4 µM), 0.125 µL polymerase (0.625 unit) and nuclease-free water summing to 25 µL reaction volume was run on a thermal cycler with initial denaturation (94°C); annealing and extension 30 cycles (94°C, 30 s: 45–68°C, 1 min; 68°C, 1 min); final extension (68°C, 5 min). Following the PCR run, the amplicons were resolved on 1% agarose gel (Sigma-Aldrich, Oakville, ON, Canada).

Differential expression of SE genes

RNAseq assays and comparative transcriptome analyses were performed using protocols recently reported [17]. Quantitative reverse transcription PCR (RT-qPCR) [18] verification of upregulated SE genes was performed in duplicate and repeated once. Approximately 1 µg total RNA was subjected to reverse transcription (RT) with QuantiTect Reverse Transcription kit protocol (Qiagen, Germantown, MD, USA) after treatment with TURBO DNAse enzyme (Invitrogen, Waltham, MA, USA). The RT reaction mixture, composed of the purified RNA, RT buffer, RT primer mix, and quantitect reverse transcriptase, was incubated at 42°C for 30 min. Two microliters (10 ng) of the cDNA was subsequently added to a qPCR mixture comprising 5 µL of 2× SYBR Green PCR master mix from QuantiNova SYBR Green PCR kit (Qiagen, Germantown, MD, USA), 1 µL of forward and reverse gene specific primers (0.7 µM) and 2 µL nuclease-free water. Each gene and condition were run in duplicate using equal amounts of cDNAs in trypticase soy broth (TSB) and PCs. For controls, *gyrA* (positive) and nuclease-free H_2O (negative) were used. CFX96 Touch™ Real-Time PCR Detection System (BioRad, Hercules, California, CA, USA) was used to run the experiments. Transcript copy numbers were determined using concentrations generated by mean Cq (quantification cycle) values of the samples obtained by standard curve analyses based on 10-fold dilutions of their respective PCR amplicons of known concentrations.

Western blotting detection of staphylococcal SE types G (SEG) and H (SEH) at different growth phases of *S. aureus*

TSB and PC cultures were inoculated with *S. aureus* CBS2016-05 and PS/BAC/169/17/W, representing strains that missed detection or were captured during BACT/ALERT screening, respectively, at a target concentration of approximately 1.0E+06 cfu/mL. TSB cultures were incubated at 37°C/18 h with agitation, and PC cultures were incubated under PC storage conditions (20–24°C under agitation for 120 h [5 days]). *S. aureus* spiked TSB or PC cultures obtained at mid-exponential, late-exponential, and stationary growth phases were analysed by Western blotting [19, 20]. Secreted proteins in TSB supernatants were precipitated for a minimum of 1 h at −20°C in 25% trichloroacetic acid [21], and the pellet was washed in 500 µL ice-cold acetone, dried and rehydrated in nuclease-free H_2O. PC supernatants were obtained by centrifugation of samples at 10,000 rpm for 15 min. Both TSB and PC protein samples were resolved on 12% SDS-PAGE, transferred onto polyvinylidene difluoride membrane on iBlot™ 2 Gel Transfer Device (Invitrogen, Waltham, MA, USA). Thereafter, the membranes were blocked with 5% bovine serum albumin (BSA) buffer for 1 h, followed by incubation with mouse polyclonal antibodies (1:5000) against enterotoxin SEG (GenTex.com, Irvine, CA, USA) or against enterotoxin SEH (Abcam, Waltham, MA, USA) and washed in Tris-buffered saline (TBS) supplemented with 0.1% Tween® 20 (TBST).

Membranes were then incubated with horseradish peroxidase (HRP) conjugated goat anti-mouse IgG secondary antibody (1:10000) (Abcam, Waltham, MA, USA). The protein bands were visualized in the ChemiDoc imaging system (BioRAD, Hercules, California, CA, USA) after incubating for 5 min in detection solution, Pierce ECL Western Blotting Substrate (ThermoScientific, Waltham, MA, USA).

Semi-quantitative sandwich dot-ELISA assay for detection of SEs during PC storage

The limit of detection of SEs was investigated using a semi-quantitative sandwich dot-ELISA assay as described by Venkataramana et al. [22], with some modifications. PC cultures were inoculated with *S. aureus* CBS2016-05 and PS/BAC/169/17/W at a target concentration of approximately 30 cfu/PC and incubated under PC storage conditions. In brief, 10 µL aliquots of combined mouse polyclonal antibody anti SEG and SEH (1:1000) were dotted on 1.0 × 1.0 cm nitrocellulose membrane pieces that had been placed in a 12-well plate. To optimize SE detection, antibodies against SEG and SEH were combined. Blocking of unbound sites was achieved with addition of 5% BSA and incubation for 1 h at 45°C in TBS followed by washing with TBST. Subsequently, 500 µL of PC samples (initially inoculated with 30 cfu/PC), which were collected at 0, 24, 30, 32, 48, 72, 96 and 120 h of incubation, were added to different wells. Five hundred microliters of non-spiked PCs and 5 µL of a 20 µg/mL solution of purified enterotoxin SEG protein (0.1 µg) (Antibodies-online, Limerick, PA, USA) were included as negative and positive controls, respectively. The plate was incubated for 1 h

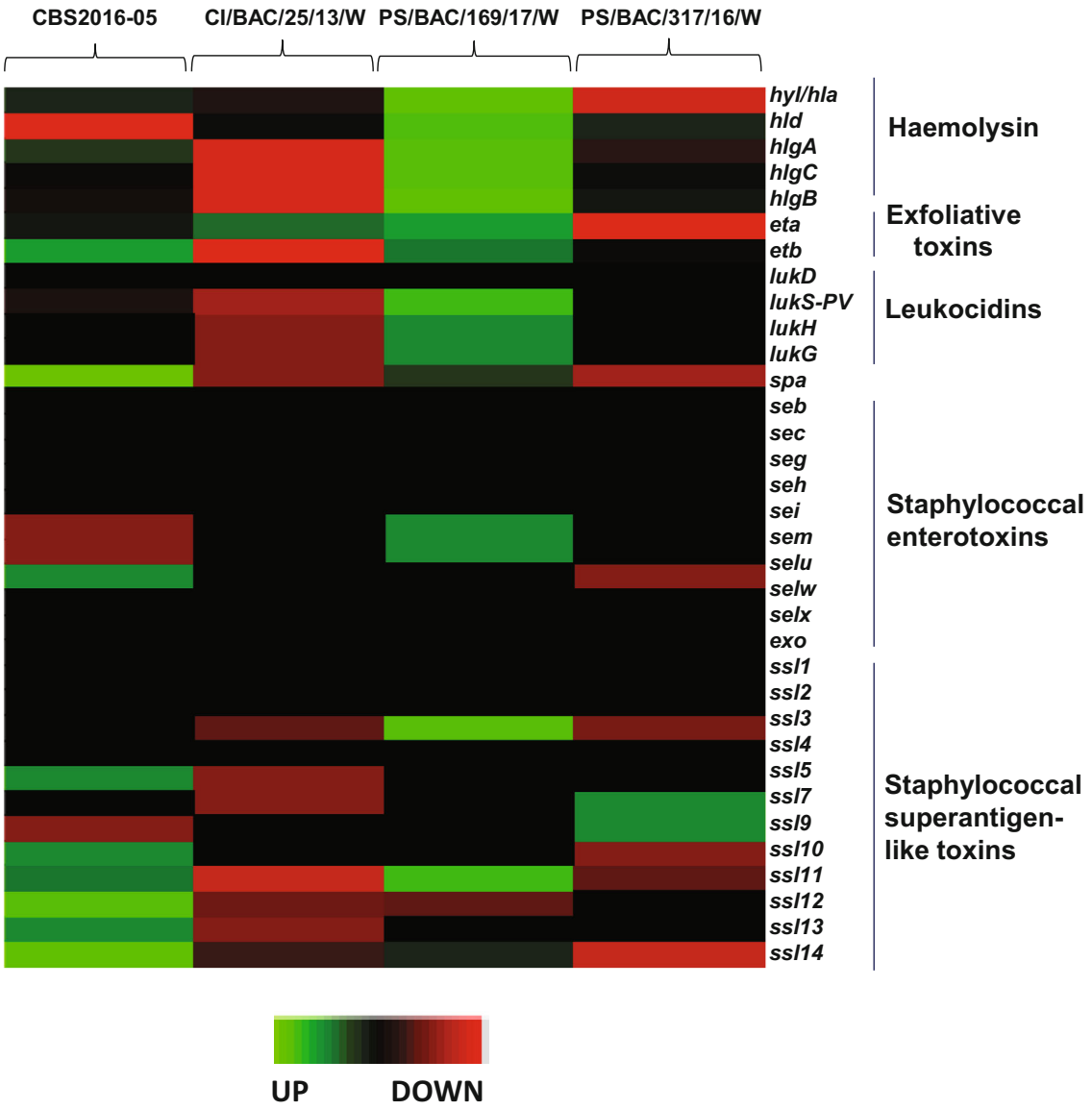


FIGURE 1 Heatmap of differentially expressed *S. aureus* exotoxin genes in PCs compared to TSB as revealed by RNAseq analyses. Experiments were performed in triplicate. The differential expressions were based on fold change values of ≥0.5 with TSB as the baseline for all strains. The statistical significance of RNAseq-based foldchange DE genes ($p < 0.05$) was determined using two-tailed *t*-tests on gene raw transcript counts performed in triplicate between the study conditions.

at 37°C, washed and further incubated with 300 µL of 1:500 monoclonal SEs antibody cocktail under the same conditions. After washing, HRP conjugated goat anti-mouse IgG (Abcam, Waltham, MA, USA) was added onto the wells, incubated for 45 min at 37°C, washed and colour developed with TMB + 0.4% H_2O_2 (3,3′,5,5′-tetramethylbenzidine substrate plus hydrogen peroxide). This assay was performed twice.

Statistical analyses

Statistical tests were performed to determine the difference in gene expression between PCs and TSB inoculated with *S. aureus*. The mean quantification cycle numbers obtained in the RT-qPCR analyses were used to calculate the standard error mean deviation over the biological and technical replicates. Normalized count values of the RNAseq transcripts were used to calculate differential gene expression between the PC and TSB conditions for each bacterial strain. Data were provided with a *p* value and a FDR/q value that correct the *p* value for multiple testing using the Benjamini–Hochberg approach. This analysis was done in *R*. Normalized data were further used to determine the statistical significance of the RNAseq-based foldchange differentially expressed (DE) genes using two-tailed *t*-tests on gene raw transcript counts performed in triplicates between the study conditions. A *p* value <0.05 was considered significant.

RESULTS

S. aureus genomes encode multiple exotoxin genes

Whole genome sequence analyses of the four *S. aureus* studied herein revealed an arsenal of genes coding for exotoxins in all strains. Genes of all staphylococcal exotoxin classes consisting of pore-forming toxins (haemolysins, leukocidins and phenol soluble modulins), cytotoxic enzymes (exfoliative toxins), and SAgs, including staphylococcal protein A (*spa*), SSLs, and SEs, were present in the genomes of the *S. aureus* isolates (Table 1). The presence of genes coding for SE and SSL superantigens was confirmed by PCR, as shown in Figure S1.

SSLs and SEs are upregulated in PCs compared to TSB

Comparative RNAseq analyses performed on the *S. aureus* strains grown in PCs and TSB revealed differential expression of exotoxin genes (Figure 1). Among the upregulated genes are haemolysins and leukocidins in strains CBS2016-05 and PS/BAC/169/17/W, exfoliative toxins in all strains but PS/BAC/317/16/W, and superantigens in all strains (0.5–6.7-fold change, $p < 0.05$). In general, SEs had increased transcripts in PCs compared to TSB (Figure 2a), with enterotoxin type H (*seh*), enterotoxin-like Z (*selz*) and an uncharacterized enterotoxin gene (*exo*) being significantly upregulated (2.3–3.6-fold

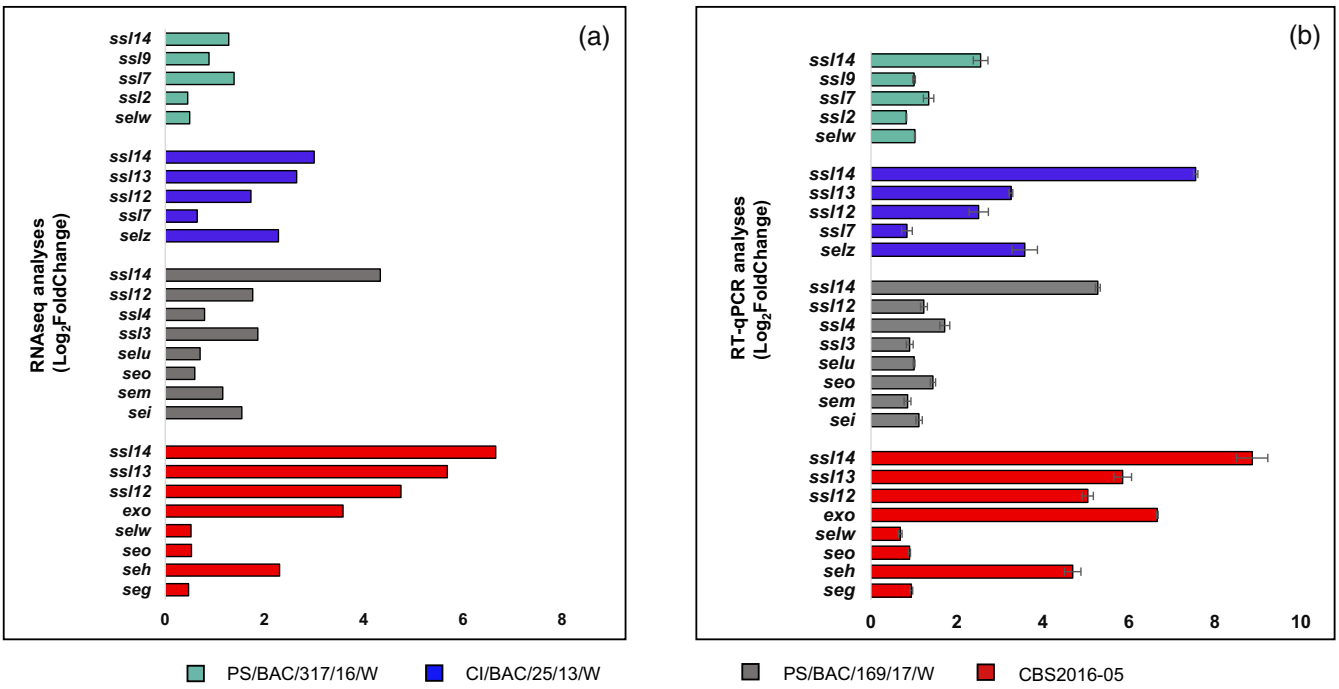
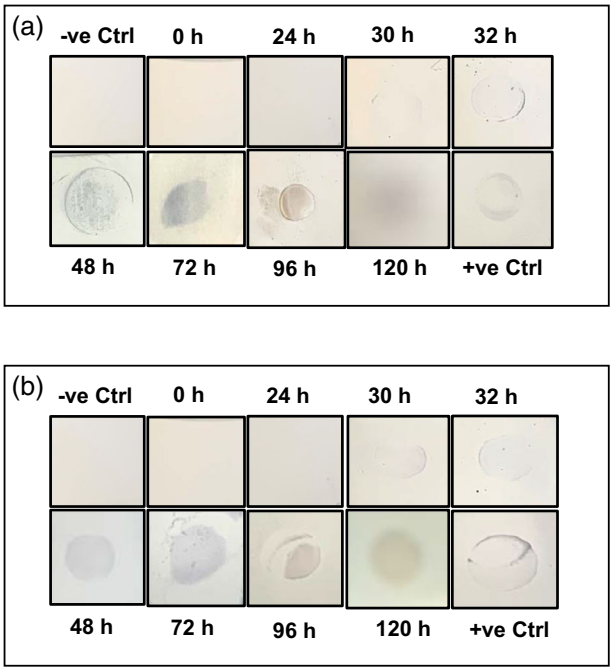


FIGURE 2 Upregulated staphylococcal SAg genes in PCs compared to TSB (baseline). (a) RNAseq data analyses. (b) Quantitative reverse transcription PCR (RT-qPCR) validation of the RNAseq results. Differential expression fold change of ≥0.5 was considered for all strains. For RT-qPCR, each gene and conditions were analysed in duplicate, and assays were repeated twice. *ssls* are superantigen-like exotoxin genes while *ses* are staphylococcal enterotoxin genes. The standard error mean (SEM = 0.01–0.37) (represented by the bars) of upregulated genes between PCs and TSB was determined based on the mean quantification cycle numbers obtained in the RT-qPCR.



(c)

PC incubation time (h)	Bacterial counts (CFU/mL)	
	S. aureus CBS2016-05	*S. aureus* PS/BAC/169/17/W
0 h	<1.0E+00	<1.0E+00
24 h	1.5E+02	2.0E+02
30 h	1.1E+04	1.6E+04
32 h	1.4E+04	1.8E+04
48 h	1.8E+05	2.0E+05
72 h	3.7E+05	3.9E+05
96 h	1.6E+06	1.8E+06
120 h	2.5E+07	2.8E+07

FIGURE 3 Determination of the limit of detection of SEs in PCs by semi-quantitative sandwich dot-ELISA. Enterotoxins from *Staphylococcus aureus* CBS2016-05 (a) and PS/BAC/169/17/W (b) were detected on samples collected at different times during PC incubation (c). Negative control (−ve Ctrl) is non-spiked PCs and positive control (+ve Ctrl) corresponds to 0.1 μg of commercially obtained staphylococcal enterotoxin SEG. ($N = 2$).

change, $p < 0.05$). Remarkably, the greatest fold differences were observed for superantigen-like toxin genes with *ssl14* being significantly upregulated in *S. aureus* CBS2016-05 and marginally upregulated in the CI/BAC/25/13/W strain ($p = 0.04$ and $p = 0.05$, respectively) (Figure 2a). The DE candidate genes selected and validated by RT-qPCR (Figure 2b) corroborated the RNAseq results.

Detection of SEs during PC storage

Western blotting was used to detect the presence of secreted SEG and SEH in TSB and PC cultures inoculated with high bacterial counts (approximately 1.0E+06 cfu/mL) of *S. aureus*. The SEs were secreted in mid-exponential, post-exponential and stationary growth phases in TSB and PC cultures of CBS2016-05 and PS/BAC/169/17/W as shown in Figure S2.

After the initial assessment and confirmation of SE production in PCs by Western blotting, semi-quantitative sandwich dot-ELISA assays were performed to determine the analytical sensitivity of SEs in contaminated PC samples collected at different time points. The results of the immune-dot assays revealed time-dependent SE production. No SEs were detected at 0 or 24 h of PC incubation when *S. aureus* concentration was approximately 1.0E+02 cfu/mL (Figure 3). SE detection was apparent at 30 h of PC incubation when bacterial counts had reached approximately 1.0E+04 cfu/mL (Figure 3). Intensity of the dots increased with time and bacterial concentration indicating greater SE concentration at 120 h of PC storage when the bacterial concentration was approximately 1.0E+07 cfu/mL (Figure 3). Dots observed at 32 h of PC incubation and beyond were similar or darker than the dot of the positive control (0.1 μg of SEG) indicating that SE concentration in the PCs was ≥0.2 μg/mL (calculated based on 0.5 mL PCs blotted on the membranes).

DISCUSSION

Detection of PC contaminated with *S. aureus* is sometimes challenging with automated culture systems due to slow growth and ability to form biofilms by this species during PC storage [1, 13]. The safety risk of transfusing PCs contaminated with *S. aureus* is exacerbated by SE production during PC storage as exemplified by several reports of transfusion reactions with the detection of staphylococcal superantigens in the transfused units [13, 14, 16]. Remarkably, we have recently shown that SE production during PC storage enhances biofilm formation and decreases growth of *S. aureus* [17], which is likely linked to missed detection of this species with culture methods. We have previously demonstrated that transfusion-relevant *Serratia marcescens* strains form biofilms in PCs, resulting in decreased detection by the BACT/ALERT automated culture system [23].

The results of this study show that SEs exhibit biomarker characteristics for detection of *S. aureus* in PCs stored for 30 h. First, we showed that different SE genes are present in the genomes of *S. aureus* isolated from contaminated PC units. Findings by previous studies have also shown that nearly 80% of methicillin-susceptible

S. aureus and >90% of methicillin-resistant *S. aureus* isolates secrete several superantigen toxins [24, 25]. The wide distribution and high sequence conservation of SEs in transfusion-relevant *S. aureus* isolates endorse their nomination as indicators of the presence of this bacterium in PCs. Second, we observed significant upregulation of SE gene expression in PCs compared to TSB cultures in all *S. aureus* isolates, corroborating the recommendation by the Food and Drug Administration (FDA) stating that ideal biomarkers must have increased expression in testing conditions [26]. Furthermore, the immunological detection of SEs in stored PC cultures complies with the FDA requirement for ease of detection of toxins in biological fluids as an important biomarker characteristic [26, 27].

Notably, we detected SEs from PC samples taken as early as 30 h of storage when bacterial counts were approximately 1.0E+04 cfu/mL, which is below the concentration that has been reported to be clinically significant (1.0E+05 cfu/mL) [28]. This result also indicates that SEs could be detected before culture results are obtained when PCs are screened using a large volume delayed testing algorithm, which requires PC sampling at ≥36 h post-blood collection [29, 30]. Recipients of PCs contaminated with *S. aureus* are at elevated risk of sepsis as SE concentrations ≥0.2 μg/mL were detected after 32 h of PC incubation, which are considerably higher than the 0.1 pg/mL concentration reported to cause toxin shocks in susceptible patients [31]. Hence, ideally, an immunoassay developed to detect SEs or other staphylococcal exotoxins in PCs should have high sensitivity and be able to detect lower levels of toxins earlier during PC storage. Others have optimized detection of SEs using dot-ELISA assays in the range of 32 pg/mL to 50 ng/mL [32, 33]. Our data provide a proof of principle of the importance of detecting SEs in PCs. Further complementary studies are needed to expand detection of enterotoxins by other *S. aureus* clinical isolates. Similarly, it would be interesting to optimize detection of toxins secreted by other bacteria such as superantigens produced by streptococci.

Exemplifying SEs secreted by *S. aureus* as potential biomarkers, we introduce the idea of supplementing bacterial detection with screening for bacterial toxins. Alternatively, toxin detection could also be a stand-alone point-of-care strategy to detect toxins in PCs that have been treated with PRT, as these methods have not been demonstrated to inactivate endotoxins or other pyrogens [34]. It is not known if whole blood derived PCs would contain bacterial toxins secreted prior to PC manufacturing that could resist PR treatment. Having a strategy to detect bacterial toxins in PCs will, therefore, further decrease the residual safety risk of PCs that are either screened with culture methods or pathogen reduced.

Investigating the presence of SEs is not common practice when septic transfusion reactions involving *S. aureus* are reported [35–37]. Therefore, the applicability of the proof of concept developed in this study is directly related to patient safety as it has been proven that staphylococcal superantigens such as TSST-1, SEG and SEU have been implicated in transfusion septic reactions [13, 14, 16]. It is recognized that the positive impact of implementing this additional safety measure cannot be quantified due to lack of data on the incidence of enterotoxin production when septic transfusion reactions are investigated. Patients receiving PCs contaminated with *S. aureus* develop septic shock symptoms likely due to the secretion of exotoxins in PCs and adding bacterial toxin detection will supplement current mitigation strategies to prevent transfusion of bacterially contaminated PC units. It is, however, acknowledged that implementing bacterial toxin detection should be feasible and efficient for blood banks.

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S.C.I. and S.R.-A. designed the study. J.B. and C.M. isolated and characterized English *S. aureus* strains. B.Y. and C.P. prepared RNA from PCs and TSB cultures and obtained RNAseq. S.C.I. performed RNAseq data analyses and experimental work related to immunological assays for detection of staphylococcal exotoxins. S.C.I. and S.R.-A. wrote the manuscript, which was reviewed by B.Y., C.P., J.B., and C.M.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The genome sequences that have been used to design primers are available; they have been published as Genome Sequence Announcements (references 9-12 of the manuscript). The transcriptome data is available at the NCBI site, BioProject: PRJNA915492 found at https://dataview.ncbi.nlm.nih.gov/object/PRJNA915492?reviewer=413fkhgmqdmj42p7u0ursh41tt.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Monitoring viral genomic sequences in transfusion-transmitted viruses

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Abstract

Background and Objectives: Monitoring genomic sequences of blood-borne viruses infecting blood donors enables blood operators to undertake molecular epidemiology, confirm transfusion transmission and assess and characterize molecular and serological screening assays. The purpose of the study was to determine how blood operators globally value viral diversity surveillance and to assess its impact.

Materials and Methods: An electronic questionnaire was developed and circulated to members of the International Society of Blood Transfusion-transmitted infectious diseases working party. Responses were compiled and complete data sets were analysed.

Results: Ninety-seven percent of respondents agreed that monitoring viral genomic sequences was important to blood operators and the transfusion community. However, only 47% of respondents are currently doing this monitoring. The main limitations reported were a lack of financial resources and expertise. Sequencing techniques, primarily next-generation sequencing and also Sanger sequencing, were considered most appropriate, with the preferred option for testing being regional or national reference centres. Respondents agreed that engagement with public health authorities needs to be enhanced.

Conclusion: Monitoring genomic sequences of blood-borne viruses is widely considered important by the transfusion community because of its direct applications for transfusion safety, and beyond for public health in general. Therefore, there is a need to strengthen collaboration between blood operators and public health authorities. While national and regional reference centres may be the most appropriate structure for such testing, international collaborations should not be overlooked. Overcoming financial barriers will be an important hurdle for many.

Keywords

diversity, public health, sequencing, transfusion safety, virus

Highlights

- Blood operators and the transfusion community favoured the implementation of monitoring genomic sequences of blood-borne viruses.

- Lack of financial resources and experienced staff are the main limitations.
- Enhanced collaboration between blood operators and public health authorities is needed.

INTRODUCTION

Rapid advances and continuous improvements in serology assays and nucleic acid amplification testing (NAT) have reduced the risk of transfusion-transmitted infectious diseases (TTIDs). In addition, the introduction of molecular screening has stimulated the development and use of increasingly sophisticated molecular methods to confirm initial screening results. Concomitantly, the development of molecular biology has led to an unprecedented increase in the knowledge of the genetics of TTIDs including viruses, and, in particular, their diversity.

Differences in the nucleic acid sequences of a viral genome are driven by genetic variability leading to phenotypic changes. This is the result of various drivers, such as the natural history of viral infection, high replication rates and host–pathogen interactions [1]. This can occur through a range of mechanisms including the high intrinsic error rate of the viral reverse transcriptases or the RNA-dependent RNA polymerases, reassortment or template switching [2]. RNA viruses tend to display greater amounts of genetic diversity than DNA viruses, while diversity is higher in single-stranded compared with double-stranded viruses [2, 3]. Viruses with a smaller genome also tend to exhibit more genetic diversity than those with larger genomes [2, 4]. There is wide variability in the genetic diversity of blood-borne viruses, such as the single-stranded RNA viruses, hepatitis C virus (HCV) [5] and human immunodeficiency virus (HIV) [6], and the partially double-stranded DNA virus, hepatitis B virus (HBV) [7, 8]. Based on genetic diversity, viruses can be separated into genotypes and eventually sub-genotypes. HIV-1 is broken down into four groups (main [M], outlier [O], non-M [N] and P), with group M subdivided into nine subtypes [9], while HCV is classified into at least eight genotypes, each with several subtypes (over 80 in total) [10, 11], and HBV into nine genotypes with subtypes identified in all genotypes except E and G [12]. Inter-genotype genetic recombination, leading to new viral circulating recombinant forms (CRFs), has also been documented for HIV and HBV [13, 14].

Different methodological approaches are used to characterize and monitor genomic sequences in viruses infecting blood donors and eventually recipients. First, direct amplification of viral DNA or RNA by nested (RT)-PCR methods is usually needed to generate viral amplicons in sufficient quantity and length to obtain informative viral sequences. It might be necessary to increase the amount of viral nucleic acid template in the amplification reaction by increasing the volume of plasma in the nucleic acid extraction procedure or by concentrating viral particles in the sample prior to extraction [15–17]. Whole or partial genome sequencing is then performed by standard Sanger or next-generation sequencing (NGS) methods. Sanger sequencing is based on the random incorporation of dideoxynucleotides. NGS methods, however, allow for higher throughput, automated sequencing. Non-sequencing approaches, such as strain-specific nucleic acid tests or proteomics can also be used [18, 19]. Viral genetic characterization can be performed by blood transfusion centres either in-house, using technologies adapted to local resources and infrastructure, through regional or national reference centres, or, via international collaborative networks.

The analysis of viral genomic sequences in blood donors can have both direct and indirect impacts on transfusion safety. It may shed light on nucleic acid and amino acid variations that can reduce the performance of NAT or serological detection by altering primers/probes hybridization or antigenicity [20–22]. Genetic polymorphism may also affect the natural history of infection and negatively impact viraemia and antigenemia, which may challenge the analytical sensitivity of detection assays [23]. Identification of the molecular features responsible for detection failure has proven essential to improve not only molecular and serological blood screening tests but also both qualitative and quantitative viral diagnosis. Monitoring viral genomic sequences can also allow investigation of cross-reactive samples leading to false-positive results, as shown in donors vaccinated against the Japanese encephalitis virus and tested falsely reactive for West Nile virus (WNV) RNA [24]. Characterization of TTIDs is essential for assessing the transmissibility of (re)emerging viruses, and the effectiveness of new screening strategies on the residual risk to blood safety. However, in the absence of a recipient pre-transfusion sample free of viral markers, a high genetic similarity of the viral strains found in both donor and recipient is required to definitively differentiate between transfusion transmission, reactivation of persistent viral infection and iatrogenic infection, especially in highly endemic areas [25]. In addition, molecular epidemiology is essential for monitoring the constant genetic evolution of viruses that may result in changes in genotype geographical distribution and the emergence of inter-genotype CRFs and viral variants with potential differences in their replicative and infectious properties, pathogeny and sensitivity to antiviral treatments or vaccines [26–28]. Examining diversity in specific genes can provide insight into single or multiple nucleotide polymorphisms associated with phenotypic changes. For example, a single amino acid change to the WNV NY99 genotype resulted in the development of the WN02 genotype, which had a shorter extrinsic incubation period leading to rapid spread across the United States eventually displacing the original NY99 strain [26, 29].

Monitoring the prevalence of viral variants in blood donors appears to be important to assess the risk of transfusion transmission and future epidemiological changes in order to continuously evaluate and improve the performance of screening tests to ensure blood safety. Blood operators are in a unique position to enable investigations for variants of transfusion-transmitted viruses.

However, the place of this type of molecular investigation in the blood transfusion field is still a matter of debate. An interactive session focused on monitoring transfusion-transmitted virus diversity was developed by the Virology subgroup of the International Society of Blood Transfusion TTID Working Party (ISBT TTID WP) during the 31st regional congress of the ISBT—ISBT in Focus!—in June 2021. Following on from widespread interest during and after this session, the present study was conceived to understand how blood operators globally value monitoring for viral diversity and assess the potential impact of monitoring for viral diversity in the transfusion and blood operator fields.

MATERIALS AND METHODS

A survey, which contained 19 questions, was developed to gather data on the value of monitoring for viral diversity and its possible impact on blood operators (see Data S1). The electronic link to the survey was first sent to members of the ISBT TTID WP on 27 October 2021, with a reminder sent on 4 February 2022. No new responses were accepted after 28 February 2022.

Response data were analysed, and basic graphs were generated using the GraphPad Prism v9.4.1 software.

RESULTS

Thirty-two questionnaires were received that contained sufficient data to be analysed. Twenty-nine respondents provided information about the World Health Organization (WHO) income classification of their country, with 13 (45%), 10 (35%) and 6 (21%) classified as high, middle, and low income, respectively. Of all respondents, 11 (34%) identified themselves as blood service/operator, 7 (22%) as transfusion medicine laboratory, 9 (28%) as both transfusion medicine and blood service/operator, 4 (13%) as other (including virology research laboratory, reference laboratory, industry and medical practitioner) and 1 (3%) did not provide the information. Among the 28 respondents whose activities are related to blood donation qualification, preparation and distribution of blood products as well as post-donation expertise, 46%, 14% and 29% were from national, regional and local organizations, respectively (Table 1). One (4%) was from a regional (blood screening)/national (reference laboratory) hybrid structure, and no information was provided for two (7%). National organizations were more frequent in high-income countries (80%) compared to middle- (33%) and low-income countries (50%). There was no difference between middle- and low-income countries with local organizations accounting for 44%–50% (data not shown). Four respondents had activities not linked to blood donations.

TABLE 1 Organizational status of 28 respondents with blood transfusion activities.[a]

Status	Blood services/ operators	Transfusion medicine laboratories	Blood services/ operators + transfusion medicine laboratories	Reference laboratory	Total (%)
National	6	5	1	1	13 (46.4%)
Regional	1	–	3	–	4 (14.3%)
Local	3	1	4	–	8 (28.6%)
Other[b]	1	–	–	–	1 (3.6%)
NA[c]	–	1	1	–	2 (7.1%)

[a]Excluding four respondents identified as virology research laboratory, industry, medical practitioner or without identification.
[b]Hybrid regional (blood screening)/national (reference laboratory) structure.
[c]Not available (information not provided by the respondent).

TABLE 2 Estimating the potential impact of viral diversity monitoring on blood service/operator policy and public health activities.

	Participants			WHO national income classification			
Impact estimation	Total ($n = 32$)	Monitoring viral diversity ($n = 15$)	Not monitoring viral diversity ($n = 17$)	Low ($n = 6$)	Medium ($n = 10$)	High ($n = 13$)	NA ($n = 3$)
Blood service/operator policy							
Yes	12 (37%)	7 (47%)	5 (29%)	5 (83%)	3 (30%)	3 (23%)	1 (33%)
Maybe	15 (47%)	5 (33%)	10 (59%)	1 (17%)	4 (40%)	9 (69%)	1 (33%)
No	5 (16%)	3 (20%)	2 (12%)	–	3 (30%)	1 (8%)	1 (33%)
Public health activities							
Yes	16 (50%)	7 (47%)	9 (53%)	6 (100%)	5 (50%)	3 (23%)	2 (67%)
Maybe	11 (34%)	6 (40%)	5 (29%)	–	4 (40%)	6 (46%)	1 (33%)
No	5 (16%)	2 (13%)	3 (18%)	–	1 (10%)	4 (31%)	–

Abbreviations: NA, information not provided; WHO, World Health Organization.

Thirty-one participants (97%) indicated that monitoring for viral diversity in blood donors is important. Only one participant from a reference laboratory in a high-income country indicated that this was not important but acknowledged that monitoring viral diversity was still relevant to public health. Most participants (84%) estimated that monitoring viral diversity had or could have an impact on both blood service/operator policy and public health activities, particularly in low- and middle-income settings (Table 2). Irrespective of whether viral diversity monitoring was performed, 69% (22/32) of participants stated that national or local blood services/operators were involved in informing and/or developing the public health strategy, and 64% (18/28 reporting) considered this contribution important in their respective countries regardless of national income classification. However, 97% of responders still highlighted the need for improved engagement between blood operators and public health authorities.

Participants noted a range of benefits from monitoring viral diversity, including identification and monitoring of viral variants that may compromise the performance of molecular and serological screening tests, surveillance of circulating viral strains evading therapeutic and immunoprophylactic treatments, investigation of TTID, monitoring national and/or international molecular epidemiology, and improving knowledge of the natural history of infections (Figure 1). A similar importance was attributed to these different benefits, with the exception of the last. However, just under half of the participants (47%, $n = 15$) reported that they currently monitor viral diversity, with 40% (6/15) indicating that this monitoring was conducted in a national/regional reference centre, followed by 40% (6/15) in-house at the local level. One participant reported the viral diversity to be monitored both in-house and in a national/regional reference centre, and the remaining two in a consortium of blood services and a public

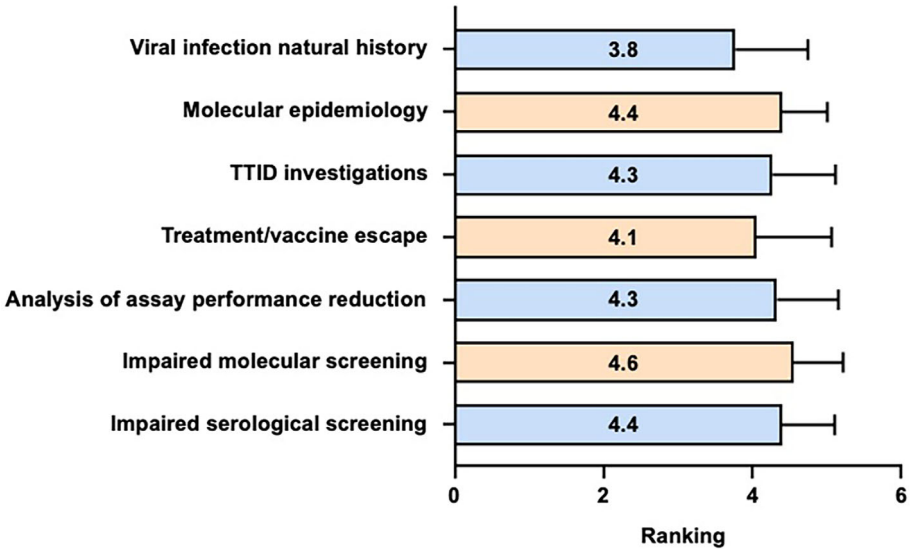

FIGURE 1 Perceived benefits of monitoring viral diversity. Participants ranked benefits from 1 (not relevant) to 5 (high importance). Columns represent means, with error bars showing standard deviations. TTID, transfusion-transmitted infectious disease.

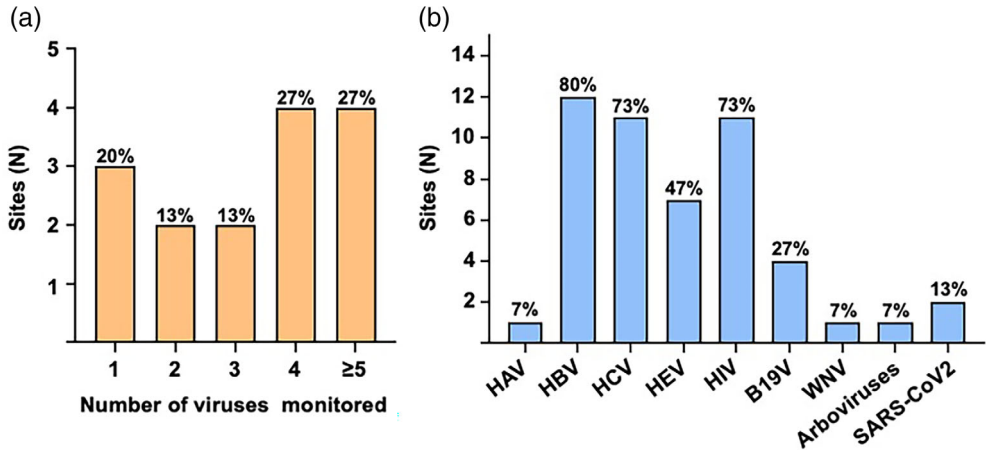

FIGURE 2 Number (a) and types of viruses (b) reported by 15 participants who monitor viral diversity.

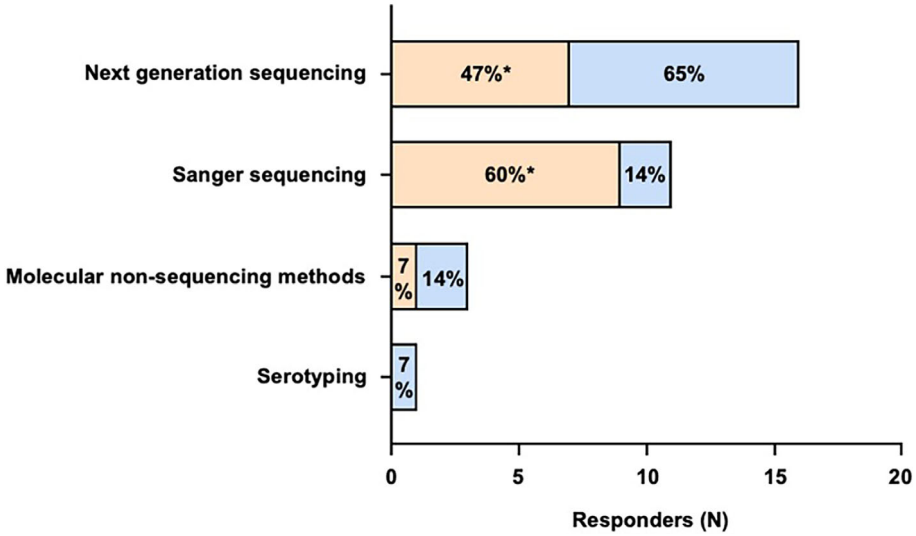

FIGURE 3 Methods to monitor viral diversity in blood donors currently used (grey bars) or wishing to be used (white bars) by 15 and 14 participants, respectively. *Two participants reported to use both sequencing (Sanger and NGS) and non-sequencing methods. NGS, next-generation sequencing.

FIGURE 4 Limitations to implementing viral diversity monitoring. Participants ranked benefits from 1 (not relevant) to 5 (high importance). Columns represent means, with error bars showing standard deviations.

health institute. Of those 15 responders, monitoring 4 or more viruses was the most common approach (54%), followed by monitoring for a single virus (20%) (Figure 2a). HBV genetic diversity was monitored by the majority of participants (80%), followed by HCV and HIV (73%), then HEV (47%) (Figure 2b). Sanger sequencing and NGS were the methods used by 60% (9/15) and 47% (7/15) of respondents, respectively, including two respondents reporting the use of both methods (Figure 3). One participant reported using non-sequencing molecular methods (e.g., genotype-specific nucleic acid tests). NGS was used exclusively in national/regional reference centres or public health institutes, whereas Sanger sequencing was mainly used in-house locally. Although the use of NGS was limited to high- and middle-income countries, there was no significant difference overall compared with the use of Sanger sequencing that was also used in three low-income settings (data not shown). Of 14 respondents interested in implementing viral diversity monitoring, 9 (65%), 2 (14%), 2 (14%) and 1 (7%) preferred NGS, Sanger sequencing, non-sequencing molecular methods and serotyping, respectively (Figure 3).

Overall, the main limitations to implementing viral diversity monitoring were reported to be financial, followed by inadequate infrastructures, and lack of political support (Figure 4). When examining the responses in relation to the WHO national income classification, financial resources and inadequate infrastructures were the main limitations for those categorized as low- or middle-income (data not shown). While financial reasons were still the main limitation for high-income countries, lack of qualified staff and lack of expertise were also key limitations. Various operational models for monitoring viral diversity with viruses were proposed, with 54% of responses

TABLE 3 Best-fit model of viral diversity monitoring based on replies from 28 participants, with blood transfusion activities,[a] stratified according to WHO national income classification.

	WHO national income classification			
Models	**Low ($n = 6$)**	**Medium ($n = 10$)**	**High ($n = 12$)**	**Total ($n = 28$)**
Regional/national reference centre	4 (67%)	4 (40%)	7 (58%)	15 (54%)
In-house local resources	2 (33%)	2 (20%)	1 (8%)	5 (18%)
International collaborative network	–	4 (40%)	2 (17%)	6 (21%)
Other[b]	–	–	2 (17%)	2 (7%)

Abbreviation: WHO, World Health Organization.
[a]Excluding four respondents identified as virology research laboratory, industry, medical practitioner or without identification.
[b]Including collaboration with local research groups or different models used to address different questions.

favouring the use of regional or national reference centres (Table 3). Although developing an international collaborative network was the next most frequent response (24%), no responder classified as low by the WHO national income classification favoured this option. The development of suitable in-house testing based on local resources was also on the radar of 17% of participants.

DISCUSSION

Blood safety is directly challenged by the continuous, and mostly unpredictable, emergence of new viruses (e.g., SARS-CoV-2) and variants of well-characterized viruses that are not limited to those viruses known to be highly variable (e.g., parvovirus B19). Indeed, viral variants have been repeatedly identified in blood donors in recent years [28, 30–32]. Therefore, monitoring viral genomic sequences appears clearly an important initiative for blood operators as agreed by 97% of participants to the present survey. This figure may be biased by the number of participants in the present survey ($n = 32$) who may represent primarily individuals already actively involved in viral genetic surveillance and may constitute a limitation of the study. However, only slightly less than half of the respondents (47%) indicated that they were currently monitoring viral genomic sequences. An imbalance between participants from countries with different resource levels could also introduce a bias. Although the majority (45%) of respondents were from countries classified as high income, countries classified as middle (35%) and low (21%) income were also represented.

The reasons for the limited monitoring of viral genomic sequences appear to be primarily the lack of financial resources, irrespective of income classification. Limited financial resources result in a lack of adequate infrastructure, and limited access to advanced technologies, but possibly also the lack of qualified staff. Viral genetic monitoring and characterization require a sufficient number of qualified, trained and competent staff. It might be particularly challenging for blood operators to establish training programmes and develop valorization measures to retain experienced staff [32]. Expertise in viral genetics was also reported as a key limiting factor, suggesting that efforts should be made to more actively involve clinicians and researchers with expertise in molecular virology, epidemiology and infectious diseases in the blood transfusion field. Ultimately, the development of methods for monitoring viral genomic sequences will depend heavily on political will to allocate the necessary resources, as participants indicated.

Different methods exist for monitoring viral genomic sequences. Genotype-specific (RT)-PCR amplification is a relatively inexpensive approach that does not require very advanced technology. However, the level of information provided remains limited. Sanger sequencing of PCR amplicons of full-length or partial viral genomes directly or after cloning remains a method of choice to characterize viral diversity. NGS is becoming an attractive alternative for 47% of participants. Compared to Sanger sequencing, NGS can identify a greater diversity of variants and provide the information to enable the broader comparison of genetic relationships in a population of viruses [33]. However, NGS requires costly sophisticated equipment, not only for sequencing itself but also for sample preparation and expensive maintenance. The development of portable third-generation sequencing, based on nanopore technology, is a promising alternative, which applies long-read single-molecule sequencing directly to amplified whole viral genomes, allowing also for the direct identification of recombination events within and between virus species [34]. This technology has been rapidly implemented successfully in complicated clinical situations to provide rapid viral genome consensus sequencing from field-collected samples in resource-limited settings [35]. However, genomic data analysis is complex and may require additional bioinformatics resources and staff training, regardless of the NGS methodology used [36]. Despite these limitations, NGS-based approaches may offer a greater level of data enrichment that may allow for more substantial analyses [18]. Ultimately, the choice of methodological approach will depend on the type of infrastructure available.

Various operational models have been proposed for monitoring viral genomic sequences, ranging from in-house to national and regional reference centres. The majority of participants favoured the use of regional or national reference centres, which present the advantage of re-grouping resources and expertise, and potentially allow for the implementation of the most advanced technologies. However, this option requires initially expensive infrastructure, adequate centralized management and a strong political will to provide financial resources over the long term. Small local facilities may be

more affordable when combined with the use of less sophisticated but nonetheless valuable sequencing facilities, particularly in resource-limited settings that may face practical difficulties in transporting samples to centralized sequencing facilities. The establishment of international collaborations to pool resources and expertise can be an interesting approach. Collaborative networks such as those developed within the ISBT TTID WP have proven to be effective in sharing knowledge, providing practical training and securing national and international funding. However, only 21% of participants, mainly from middle-income settings, favoured this option. Although limited in number, no participant from a low-resource region chose this option. The reasons why are unclear but may be related to difficulties in sample transport and delays in getting information back, local regulations or concerns around being too dependent on other organizations. Although analysing donor viral sequences has many applications, there are limitations to relying only on such sequences, and future studies should utilize viral sequences from donors and the general population. Ultimately, the approach used will depend on individual settings or regional circumstances.

It is clear that blood operators and public health are inherently linked. The present survey showed that there is a dominant feeling among blood operators that collaboration between the two needs to be improved. Efforts should be made to break down any silos between the two. It would be interesting to hear from public health actors about this question. Nevertheless, blood operators often have national coverage, have access to specimens from a healthy subset of the population and have experience undertaking large-scale donor surveillance using the most sensitive methods available [37]. The role of blood operators as members of the one-health community became evident with the SARS-CoV-2 pandemic, which saw blood operators undertake large seroprevalence studies to inform public health policy [38]. Moreover, blood donor viral sequences have informed various public health initiatives [39, 40]. In front of the globalization of human and animal population travel, human activities with increased risk of zoonotic infections, and the environmental changes leading to the geographical expansion of viral vectors, blood operators and the transfusion medicine community should be prepared to actively engage in surveillance of emerging viral infections as a major actor in public health and in order to ensure optimal blood safety.

In conclusion, monitoring for genetic diversity of blood-borne viruses appears important given the many applications for blood safety and global public health. It is important for enhanced collaboration between blood operators and public health authorities to enable the greatest benefits to be afforded. While national and regional reference centres may provide the most suitable place for such monitoring, international collaborations should not be overlooked. Overcoming financial hurdles will be important. This review of practices inevitably leads to questions regarding the next steps that blood operators and the transfusion medicine community can play in the genetic characterization of blood-borne viruses. There are three core options including (1) conceptualization of specific multi-regional studies to characterize blood-borne viruses identified in blood donors; (2) development of networks to support the implementation of viral molecular characterization, especially in limited-resourced settings; and (3) creation of grant-writing teams to identify and compete for research grants focused on this theme.

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CONFLICT OF INTEREST STATEMENT

S.J.D. has been a paid consultant on transfusion-transmitted arboviruses and malaria for Roche. He has functioned as a content expert for respiratory viruses for Johnson & Johnson (Janssen) and has received funding in-kind from Abbott. D.C. has received research funding from Grifols Diagnostic Solutions Inc. H.F. has received research funding and/or honoraria from Grifols Diagnostic Solutions Inc. and Roche Diagnostic Solutions.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Intensity of endogenous thrombocytopenia after autologous stem cell transplantation in patients prophylactically transfused with platelets

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Abstract

Background and Objectives: Large clinical trials have demonstrated that some patient groups with hypoproliferative thrombocytopenia benefit from prophylactic platelet transfusions, while in others, a therapeutic transfusion regimen might be sufficient. The remaining capacity to generate endogenous platelets might be helpful to select the platelet transfusion regimen. We assessed whether the recently described method of digital droplet polymerase chain reaction (PCR) can be used to assess the endogenous platelet levels in two groups of patients undergoing high-dose chemotherapy with autologous stem cell transplantation (ASCT).

Materials and Methods: Multiple myeloma ($n = 22$) patients received high-dose melphalan alone (HDMA); lymphoma patients ($n = 15$) received BEAM or TEAM (B/TEAM) conditioning. Patients with a total platelet count <10 G/L received prophylactic apheresis platelet concentrates. Daily endogenous platelet counts were measured by digital droplet PCR for at least 10 days post-ASCT.

Results: Post-transplantation B/TEAM patients received their first platelet transfusion on average 3 days earlier than HDMA patients ($p < 0.001$) and required about twofold more platelet concentrates ($p < 0.001$). The endogenous platelet count fell ≤5 G/L for a median of 115 h (91–159; 95% confidence interval) in B/TEAM-treated patients compared to 12.6 h (0–24) ($p < 0.0001$) in HDMA-treated patients. Multivariate analysis confirmed this profound effect of the high-dose regimen ($p < 0.001$). The CD-34$^+$-cell dose in the graft was inversely correlated with the intensity of endogenous thrombocytopenia in B/TEAM-treated patients.

Conclusion: Monitoring endogenous platelet counts detects the direct effects of myelosuppressive chemotherapies on platelet regeneration. This approach may help to develop a platelet transfusion regimen tailored to specific patient groups.

Keywords

autologous blood stem cell transplantation, endogenous platelets, platelet transfusion, thrombocytopenia, transfusion strategy

Highlights

- Digital polymerase chain reaction (PCR) allows endogenously produced platelets and transfused platelets to be differentiated in patients with hypoproliferative thrombocytopenia.
- After high-dose chemotherapy with autologous haematopoietic stem cell transplantation in myeloma or lymphoma patients, the time span with endogenous platelet counts ≤5 G/L (a threshold for elevated bleeding risk) varies substantially between the two conditioning regimens studied.
- Digital PCR may allow the identification of treatment regimens that maintain the platelet count predominantly at >5 G/L during the aplasia period, possibly making those patients suitable for a therapeutic platelet transfusion strategy.

INTRODUCTION

Hypoproliferative thrombocytopenia is common in haemato-oncological patients. Bleeding risk and quality of life have to be balanced against rare but serious hazards of platelet transfusions, for example, transfusion-related sepsis [1]. Furthermore, supply issues may limit patient treatment in medical settings with the restricted availability of platelet concentrates. The two main strategies are therapeutic platelet transfusions only in case of symptomatic bleeding and prophylactic platelet transfusion when the threshold of ≤10 G/L platelets has been reached, independent of bleeding symptoms.

Wandt et al. [2] directly compared both strategies in a randomized prospective trial in 391 haemato-oncology patients. The therapeutic transfusion strategy reduced the number of platelet transfusions by 33.5% relative to a prophylactic transfusion strategy triggered by a platelet count of 10 G/L without an overall increase in major bleeding. The investigators concluded that therapeutic transfusions should become the standard of care for patients with high-dose chemotherapy and autologous transplantation [3] treated in experienced centres. Stanworth et al. [4] assessed clinical bleeding (WHO stage 2–4) in a prospective trial in 598 thrombocytopenic patients with characteristics similar to the Wandt trial. They also randomly assigned patients to either a prophylactic (platelet trigger: 10 G/L) or a therapeutic platelet transfusion regimen. The pre-defined subgroup analysis [5] indicated that prophylactic platelet transfusions were somewhat more effective in patients undergoing allogeneic stem cell transplantation compared to patients undergoing autologous stem cell transplantation (ASCT). A major dilemma of these studies is that the total platelet count cannot differentiate between transfused platelets and endogenous platelets produced by the bone marrow of the patient. This is probably highly important, as endogenous platelets have a better haemostatic capacity compared to stored, transfused platelets [6]. Hanson and Slichter [7] estimated from the kinetics of radioactively labelled platelets in healthy volunteers and thrombocytopenic patients a daily need of up to 7 G/L platelets to maintain the integrity of the vascular lining. By monitoring faecal blood loss after injections of ^{51}Cr-labelled autologous red blood cells [8], a sharp increase in blood loss was detected when platelet counts declined ≤5 G/L. Based on this observation, patients in whom the endogenous platelets decrease ≤5 G/L might be at a higher risk for bleeding than patients in whom this threshold is maintained independently of platelet transfusions.

Digital polymerase chain reaction (PCR) reliably detects low platelet counts as validated by flow cytometry [9] and differentiates between endogenous and transfused platelets. We applied digital PCR to daily monitor the endogenous platelet counts in patients undergoing ASCT for multiple myeloma or malignant lymphoma and found substantial differences between the two groups in the duration the endogenous platelet counts decreased below 5 G/L. This information can be used for the design of future trials.

MATERIALS AND METHODS

Patient population

All ASCT patients recruited into the CAPTURE study [10] at the Oldenburg site with the diagnosis of a multiple myeloma or a

TABLE 1 Characteristics of patients according to the high-dose (HD)-treatment group (melphalan alone vs. B/TEAM).

	Melphalan alone ($n = 22$)	B/TEAM ($n = 15$)
Median age, years (range)	59 (45–74)	58 (37–65)
Male/female, *n* (%)	16 (73)/6 (27)	10 (67)/5 (33)
Median number of prior treatments (range)	2 (1–3)	2 (1–5)
Prior radiotherapy		
Yes, *n* (%)	6 (27)	2 (13)
Prior HD with ASCT		
Yes, *n* (%)	5 (23)	0 (0)
Febrile neutropenia		
Yes, *n* (%)	16 (73)	14 (94)
Median number of days with neutropenia (range)	6.5 (5–10)	8 (5–15)
Median number of days with fever (range)	2.5 (0–10)	5 (0–14)
Stem cell dose infused: median number of CD-34^{+}-cells $\times$ 10^{6}/kg (range)	3.6 (2.1–8.6)	3.5 (2.2–10.8)

malignant lymphoma and allocated to ASCT were included. After completion of the CAPTURE trial, seven additional lymphoma patients were included to adequately represent both patient groups. These patients were treated according to the CAPTURE protocol and received standard apheresis platelet concentrates.

Ethics approval and informed consent

The study was approved by the institutional ethics review board (Medical University Oldenburg). Each patient provided written informed consent at least 48 h before the collection of the first blood sample. All blood donors provided informed consent.

Study design

During the study period of at least 10 days after transplantation, a daily blood sample was collected from each participant to determine the morning platelet and white blood cell (WBC) counts in 3 mL samples of EDTA anticoagulated blood with the ADVIA 2120i (Siemens Healthineers AG, Erlangen, Germany) in the optical mode. Fever was attributed to an increase in the body temperature to ≥38°C.

All patients received G-CSF (approx. 5 µg/kg bodyweight s.c.) from day +1 after ASCT until stable WBC counts of ≥1 G/L were observed. Following the first platelet transfusion, an additional 3 mL daily EDTA blood sample was taken daily to measure endogenous platelets.

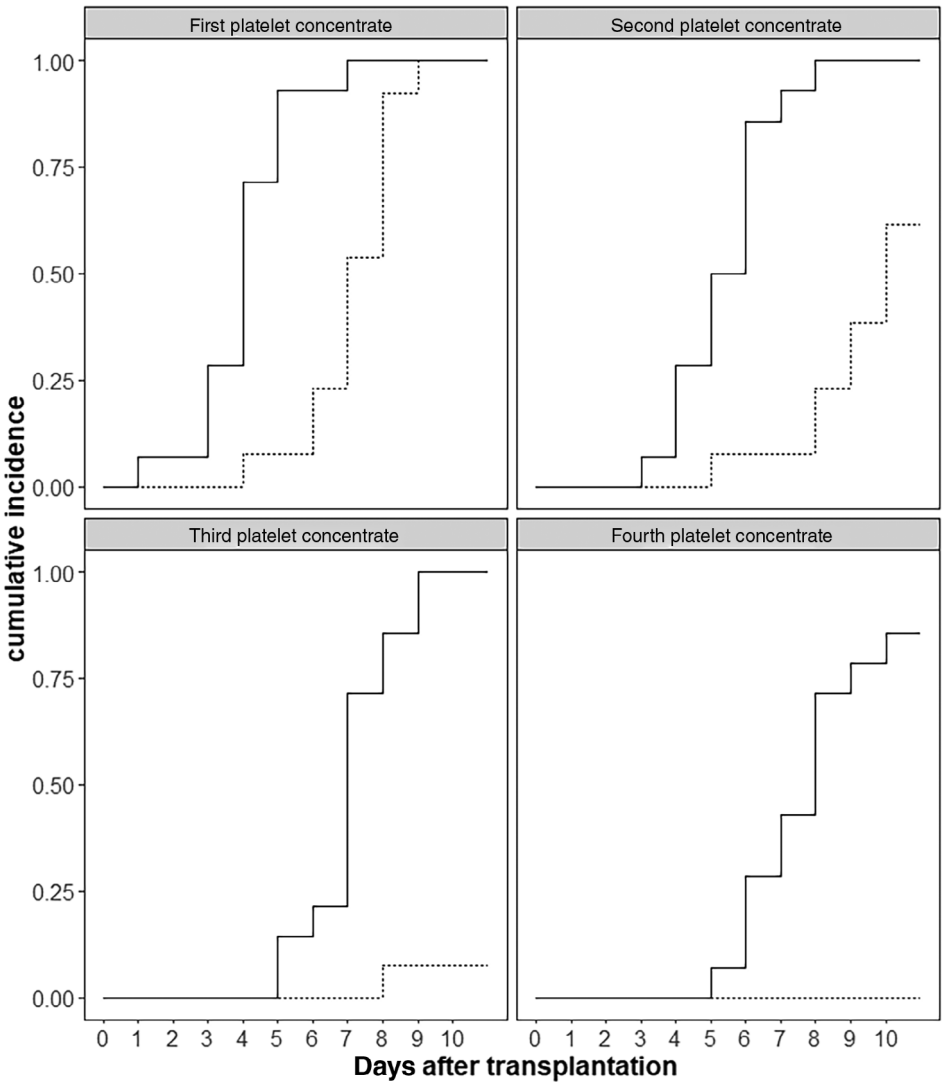

FIGURE 1 Time to consecutive platelet transfusions within 10 days after autologous stem cell transplantation (ASCT) for 13 melphalan alone (dotted line) and 13 B/TEAM-HD patients with ASCT (solid line). Data from all patients in the study supported with standard single donor platelet concentrates (not irradiated with UVC for pathogen reduction). The cumulative incidences differed between both patient groups for each transfusion series ($p < 0.001$; log-rank test).

Diagnosis and chemotherapy regimen of the patient groups

Multiple myeloma patients ($n = 22$): 16 patients were treated with the standard dose of melphalan (200 mg/m^2) and 6 patients were treated with a melphalan regimen adapted to age or renal insufficiency (1 pt 100 mg/m^2 and 5 pts 140 mg/m^2).

Malignant lymphoma patients ($n = 15$): six patients with relapsed diffuse large B-cell lymphoma, four with T-cell lymphoma, two with Hodgkin's disease, two relapsed follicular lymphoma and one mantle cell lymphoma. All received high-dose chemotherapy BEAM (BCNU, etoposide, cytarabine and melphalan) with the exception of the relapsed follicular lymphoma patient who received TEAM (thiotepa, etoposide, cytarabine and melphalan) due to pulmonary comorbidity.

For all patients, peripheral blood stem cells had been collected by standard apheresis procedures in the JACIE-accredited centre in Oldenburg. The number of CD-34$^+$-cells in each stem cell preparation was measured with the BD stem cell enumeration kit (344563) and the FACSCanto II system (BD Biosciences, Heidelberg, Germany).

Endogenous platelet measurements

Sample preparation, DNA extraction, primers, probes, PCR conditions and instrumentation for digital PCR were reported [9]. A patient sample collected after study inclusion and before the first platelet transfusion, as well as a sample from each platelet concentrate administered, were genotyped for seven mitochondrial markers [9]. An endogenous platelet count versus time post-ASCT curve was interpolated in R (loess function automatically optimized for span) for each individual patient. From these curves, the time points (in hours) when the endogenous platelet count declined below and returned above a threshold (5 and 10 G/L, respectively) were derived and expressed as duration in hours and as cumulative area AUT in h G/L of the endogenous platelet count versus time curve spent below these threshold levels. All parameters were calculated from the daily endogenous platelet counts by procedures in R (version 4.2.2).

Platelet transfusion

Single donor platelet concentrates with a mean platelet number of 3.2×10^{11} (range: 2.4–4.3 $\times$ 10^{11}) were collected by apheresis. All patients received unselected single donor platelet concentrates.

Some patients with myeloma (9/22) or lymphoma (2/15) were randomized to the group transfused with UVC-treated platelet concentrates instead of standard (i.e., non-UVC-treated) platelet concentrates.

Statistics

Statistical comparisons (log-rank, Mann–Whitney *U*, Kruskal–Wallis or multivariate analysis) were calculated in R. The relation between the parameters for patient characteristics (Table 1) as exploratory variables and the intensity of endogenous thrombocytopenia as the independent variable was assessed by multivariate analysis. Augmented backward elimination [10] was applied to assess whether the number of exploratory variables could be reduced.

RESULTS

The clinical characteristics of the 37 patients treated with high-dose chemotherapy and ASCT are summarized according to their conditioning regimen in Table 1.

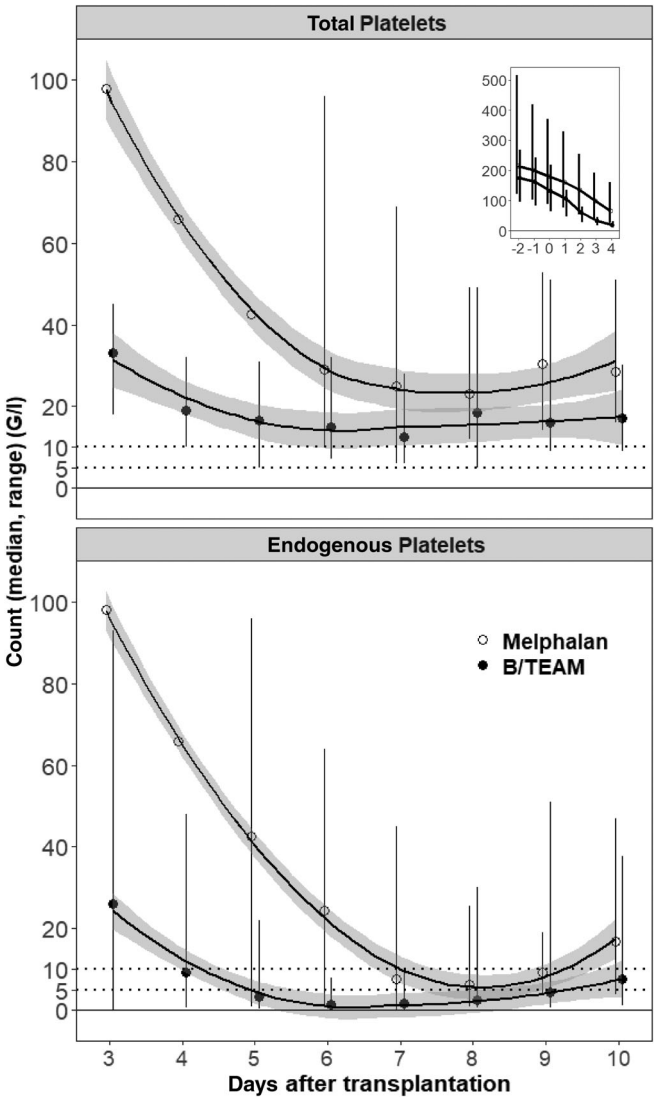

FIGURE 2 Total (upper panel and insert) and endogenous (lower panel) platelet counts (median with range in G/L) after HD treatment with melphalan alone (o, $n = 22$) or B/TEAM (•, $n = 15$) with autologous stem cell transplantation (ASCT). The dotted horizontal lines represent the thresholds of 5 G/L (associated with an increased risk for bleeding) and 10 G/L (trigger for platelet substitution). The grey area indicates the 95% confidence interval.

TABLE 2 Parameters to assess the intensity of endogenous thrombocytopenia by daily measurements of endogenous platelets according to the high-dose (HD)-treatment group.

HD with:	Melphalan alone ($n = 22$)		B/TEAM ($n = 15$)		
Endogenous platelet counts	**Median**	**95% CI**	**Median**	**95% CI**	***p***
Absolute nadir					
Counts (G/L)	4.21	(3.46–6.46)	0.59	(0.46–1.81)	<0.0001
after ASCT (h)	192	(192–216)	144	(144–168)	<0.0001
≤5 G/L threshold					
Duration (h)	12.6	(0–24.5)	115	(91–159)	<0.0001
AUT (h·G/L)	6.47	(0–32.3)	317	(232–491)	<0.0001
≤10 G/L threshold					
Duration (h)	59.2	(42.3–75.0)	169	(136–222)	<0.0001
AUT (h·G/L)	183	(108–325)	1036	(833–1472)	<0.0001

Abbreviations: ASCT, autologous stem cell transplantation; AUT, area under the threshold; CI, confidence interval.

TABLE 3 Statistical model of patient characteristics (Table 1) predicting the intensity of endogenous thrombocytopenia (assessed as the area under the 5 G/L threshold (AUT) [h·G/L]).

		AUT 5: mean (SD)	Difference (univariate)	Difference (multivariate)	Difference (multivariate reduced by ABE[11])
HD with	Melphalan alone	30.4 (70.8)	–	–	–
	B/TEAM	340 (161)	309 (231 to 388, $p < 0.001$)	265 (154 to 376, $p < 0.001$)	**292 (224 to 360, $p < 0.001$)**
Gender	Female	222 (208)	–	–	–
	Male	128 (182)	−94 (−232 to 44, $p = 0.18$)	−102 (−177 to −26, $p = 0.010$)	**−100 (−170 to − 31, $p = 0.006$)**
Age	(37.0, 74.0)	156 (192)	1.0 (−6.2 to 8.2, $p = 0.78$)	3.2 (−0.8 to 7.1, $p = 0.11$)	3.5 (−0.04 to 7.1, $p = 0.053$)
Number of previous treatments	(1.0, 5.0)	156 (192)	85 (21 to 148, $p = 0.01$)	26 (−33 to 85, $p = 0.38$)	–
Days of neutropenia	(5.0, 15.0)	156 (192)	44 (15 to 74, $p = 0.004$)	14 (−7 to 35, $p = 0.18$)	16 (−3.1 to 35, $p = 0.098$)
Prior radiation	No	168 (193)	–	–	–
	Yes	111 (192)	−57 (−214 to 99, $p = 0.46$)	−56 (−171 to 62, $p = 0.34$)	–
Second ASCT	No	175 (202)	–	–	–
	Yes	72 (117)	−103 (−265 to 59, $p = 0.20$)	11 (−100 to 122, $p = 0.85$)	–
Days of fever	(0.0, 14.0)	156 (192)	22 (7.6 to 37, $p = 0.004$)	2.6 (−6.9 to 12, $p = 0.58$)	–
CD-34$^+$-cell dose	(2.1, 10.8)	156 (192)	−32 (−63 to −0.6, $p = 0.046$)	−17 (−36 to 2.8, $p = 0.091$)	**−19 (−38 to − 0.8, $p = 0.041$)**

Abbreviations: ABE, augmented backward elimination; ASCT, autologous stem cell transplantation; CI, confidence interval; HD, high dose.
Bold values with a $p < 0.05$.

Timing and frequency of platelet transfusions

All platelet transfusions were administered prophylactically. The median number of platelet concentrates transfused within 10 days after ASCT was 2 (mean: 2.0; range: 1–5) for the 22 melphalan HD patients versus 5 (4.7; 3–7) for the 15 B/TEAM-treated patients ($p < 0.001$, Mann–Whitney U test). Figure 1 summarizes the time course of consecutive transfusions for all patients treated with standard platelet preparations.

The melphalan alone HD patients received their first transfusion later than B/TEAM patients (Figure 1; $\chi^2 = 18.1$; $p < 0.001$). Only 62% of melphalan alone-treated HD patients received a second transfusion, 8% a third and none received a fourth transfusion. All B/TEAM patients, however, required a third and 79% even a fourth transfusion. The cumulative transfusion incidences observed for the standard platelet preparation (administered to 26 patients) did not differ from those for any platelet preparation, that is, including those exposed to UVC for pathogen reduction, in all 37 patients ($\chi^2 \leq 1.9$; $p > 0.2$).

No red cell concentrate was administered to treat overt bleeding. Major bleedings of WHO grade 3 or 4 [1] [10] did not occur in any of the 37 patients.

Duration and intensity of severe thrombocytopenia

The two melphalan alone subgroups of 16 patients treated with the standard high dose of melphalan and 6 patients receiving an adapted melphalan high dose did not differ in their total platelet and their endogenous platelet counts. Therefore, the results are represented in the following for the group of all 22 patients.

The median platelet counts on day 2 before transplantation were in the normal range and comparable for both treatment groups (data not shown). They steadily declined until day 7 and day 4 after transplantation in the melphalan alone and the B/TEAM HD patients, respectively (Figure 2).

In the patients exposed to HD melphalan alone, the median endogenous platelet counts remained above 5 G/L during the entire post-transplant period. They fell below the threshold of 10 G/L only from day 7 to day 9 after ASCT. In contrast, in all B/TEAM-treated patients, the endogenous platelet count decreased below 5 G/L already on day 5 post-transplantation. Their daily median endogenous platelet count remained ≤5 G/L until day 9 after ASCT.

Curves were fitted to the time series of the daily endogenous platelet counts for each individual patient to characterize the individual intensity of the endogenous thrombocytopenia by the parameters as summarized in Table 2.

In 10 of the 22 melphalan alone HD patients, the endogenous platelet count did not fall below 5 G/L. In 11 of the remaining 12 patients of this HD group, the endogenous platelet count declined to ≤5 G/L for less than 48 h. A single patient had an endogenous platelet count ≤5 G/L for 84 h. The treatment course of this individual had been complicated by a respiratory syncytial virus (RSV) pneumonia, likely explaining the low platelet counts.

In contrast, the endogenous platelet counts decreased below 5 G/L in all 15 B/TEAM-HD patients. They remained below this level for a median period of almost 5 days (115 h, Table 2). The integral parameter AUT of the time and the extent of platelet counts ≤5 G/L were 49-fold lower for the melphalan alone- than for the B/TEAM-HD patients.

The highly significant differences in the time endogenous platelet count decreased below 5 G/L (Table 2) observed between both HD treatment groups were further evaluated by multivariate analysis including all the variables summarized in Table 1. For the threshold of 5 G/L, multivariate models of the AUT (adjusted $r^2 = 0.78$; Table 3) and the duration (adjusted $r^2 = 0.75$; data not shown) as parameters indicating severe endogenous thrombocytopenia consistently revealed the HD-treatment regimen as the most important prognostic factors ($p < 0.001$). In addition, the patients' gender ($p = 0.006$) and the dose of CD-34$^+$-cells ($p = 0.041$) were significant predictors in the reduced model obtained by augmented backward elimination [10]. The platelet nadir, however, as an indicator of severe endogenous thrombocytopenia did not provide an adequately powered statistical model (adjusted $r^2 = 0.35$; data not shown) for such predictions.

Next, we assessed, whether the time spent below an endogenous platelet count of ≤5 G/L depends on the transplanted CD-34$^+$-cell dose. This analysis was only performed for the B/TEAM-HD-treated patients as the melphalan alone HD patients barely fell below this threshold. Figure 3 shows that the AUT values significantly declined with an increase in CD-34$^+$-cell dose (slope: −54.7; $r^2 = 0.55$; $p = 0.0015$). In the absence of the data pair from the patient with the highest CD-34$^+$-cell dose, the negative slope of the regression line (−65.5; $r^2 = 0.35$; $p = 0.026$) was still maintained.

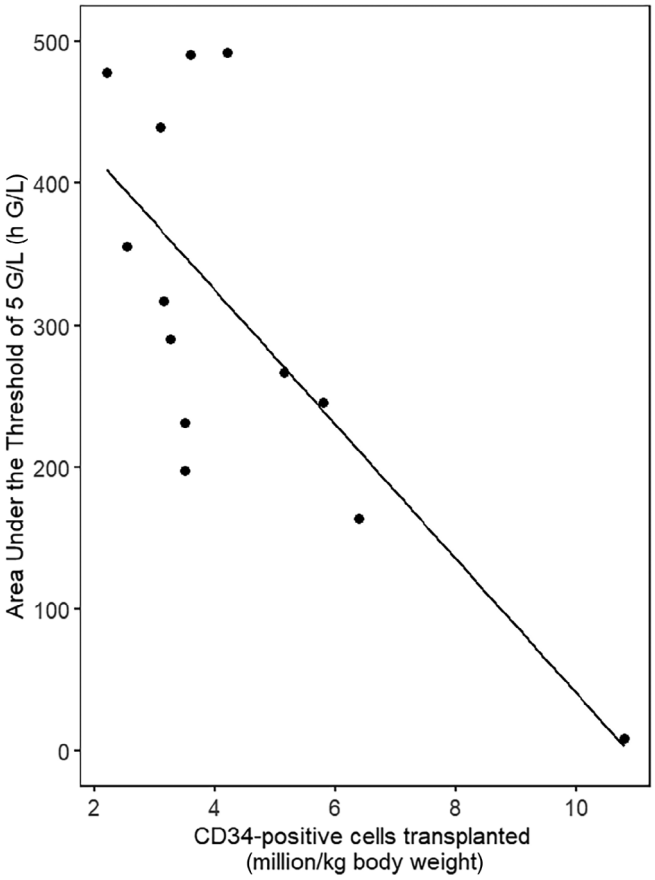

FIGURE 3 Intensity of the endogenous thrombocytopenia (assessed as area under the 5 G/L threshold [h·G/L]) in patients after B/TEAM-HD with autologous stem cell transplantation (ASCT) ($n = 15$) in relation to the CD-34$^+$-cell dose (10^6/kg) transplanted.

DISCUSSION

This study demonstrates consistent and profound differences between the melphalan alone HD and the B/TEAM-HD regimen in the decline in endogenous platelet counts ≤5 G/L. An endogenous platelet count ≤5 G/L is a risk factor for increased bleeding according to the studies of Slichter et al. [8] and from the evaluation of over 16,000 patient-days in more than 1000 patients with secondary hypoproliferative thrombocytopenia [12], including patients after high-dose chemotherapy with ASCT.

The endogenous platelet count remained ≤5 G/L for a median time of almost 5 days in the B/TEAM HD and, thus, approximately 10-fold longer than in the melphalan alone-treated HD patients. This

difference suggests that a therapeutic platelet transfusion strategy may be most appropriate for the melphalan alone-patient group, but could be less safe in patients treated with B/TEAM-HD therapy. Determining the duration the endogenous platelet counts decrease below 5 G/L in different patient and treatment regimen groups by digital droplet PCR may provide the tool to identify patient groups in whom therapeutic platelet transfusion strategy is likely safe.

Multivariate analysis of clinical parameters potentially affecting endogenous thrombocytopenia (Table 3) identified the conditioning regimen as the primary determinant for these differences. As the underlying disease determines the conditioning regimen, we cannot separate the effects of the disease (multiple myeloma vs. malignant lymphoma) from the type of HD chemotherapy (melphalan alone vs. BEAM or TEAM). Our findings cannot rule out that patients with the same underlying disorders but receiving other treatment regimens show a different kinetic of the endogenous platelet count.

The second largest difference in the intensity of endogenous thrombocytopenia was observed between female and male patients. Women developed a significant and almost twofold higher intensity of endogenous thrombocytopenia in response to HD therapy. This gender difference remains to be confirmed by larger populations, as female patients represented only about one third of the participants in our study.

Another limitation of our study is that a majority of patients were randomly assigned to either standard or UVC-treated platelet concentrates [11]. However, the average number of platelet concentrates transfused in the UVC group of the CAPTURE trial was only about 0.25-fold higher than in the control group [11]. This relatively minor effect does not account for the 2.5-fold higher median number of platelet concentrates transfused to B/TEAM- versus melphalan alone HD patients. On the other hand, enrolment into the CAPTURE trial strengthens our study because patients were systematically followed and managed within a pre-defined study protocol with the same transfusion trigger [11]. Thus, different transfusion regimens could not attribute to the differences in the endogenous platelet count.

At present, clinical routine application of the ddPCR method to differentiate between endogenous and transfused platelets is hampered by a delay of about 5 h from blood sampling to the results [9]. The measurement of the immature platelet fraction (IPF) by haematology analysers offers an intriguing option to more rapidly assess recovery of endogenous platelet production in patients after ASCT. In 112 patients with immune thrombocytopenia, Greene et al. observed a significant inverse correlation between the absolute IPF and an acute bleeding score [13]. However, it needs to be shown whether IPF is as sensitive as ddPCR to detect a low signal given by an endogenous platelet count of 5–10 G/L. Most attempts to predict clinically relevant bleeding events in individuals by daily monitoring signs of minor bleeding have been disappointing so far with the potential exception of haematuria [14, 15].

We fully acknowledge that other parameters [12] like a reduced haematocrit and altered coagulation parameters may further contribute to the risk of bleeding and have to be considered for deciding on the transfusion regimen. An additional example is provided by the only patient with a RSV pneumonia in our study presenting with an intensity of the endogenous thrombocytopenia characteristic for B/TEAM-HD patients despite the melphalan alone treatment. RSV pulmonary infections have been reported to be associated with lower platelet counts [16]. But such cofactors can also be considered in the design of an appropriate clinical trial.

In addition to the already established subgroup analysis [5], platelet trials comparing bleeding risks for transfusion strategies similar to the Wandt [2] and the TOPPS [4] trials may benefit from stratification of ASCT patients according to their HD regimen. Our investigation furthermore demonstrates that monitoring endogenous platelet counts in transfused patients provides a new tool in the search for the optimal platelet transfusion strategy and to design future clinical trials on platelet transfusion strategies for chemotherapy-induced thrombocytopenia not only in haematologic but also in non-haematologic malignancies [17].

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A.V., A.D. and H.K. performed the research; A.S., A.G., B.M. and T.H.M. designed the research study; and all authors analysed the data and wrote this article.

CONFLICT OF INTEREST STATEMENT

The authors have nothing to disclose.

DATA AVAILABILITY STATEMENT

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

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ORIGINAL ARTICLE

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Temporal trends and geographic variations in perioperative red blood cell transfusion in major surgical procedures from 2013 to 2018 in China

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Abstract

Background and Objectives: Transfusion-related guidelines promote restrictive blood transfusion. However, whether these guidelines have been successfully translated into clinical practice in China is unknown. This study aimed to provide updated information about the temporal trends in the prevalence of perioperative red blood cell (RBC) transfusion in China.

Materials and Methods: We analysed data from the Hospital Quality Monitoring System database (2013–2018) to investigate the prevalence of perioperative RBC transfusion in patients undergoing craniotomy for cerebral aneurysms or arteriovenous malformations, sternotomy for mitral valve replacement, open thoracotomy lobectomy, open gastrectomy and hip arthroplasty. Mixed-effects logistic regression models quantified the likelihood of RBC transfusions.

Results: The study included 438,183 patients, with 44,697 (10.20%) receiving perioperative RBC transfusions. Introducing transfusion-related guidelines in China markedly decreased the prevalence of RBC transfusion among patients who underwent major surgical procedures in the following years. The prevalence of RBC transfusion for hip arthroplasty was 17.34% in 2013 and 7.03% in 2018. After adjusting for patient risk factors, the odds ratio of RBC transfusion for hip arthroplasty was significantly lower in 2018 (0.74, 95% confidence intervals [CI] 0.53–1.02) than in 2013 (1.84, 95% CI 1.37–2.48).

Conclusion: The prevalence of perioperative RBC transfusion decreased from 2013 to 2018 in China, supporting the potential beneficial effects of transfusion-related guidelines. Considering the geographic variations in RBC transfusion, reducing heterogeneity may impact public health by improving surgical outcomes.

Keywords

patient blood management, transfusion strategy, transfusion-surgery

Bo Tang and Yuelun Zhang contributed equally to this work.

Highlights

- We analysed the prevalence of perioperative red blood cell (RBC) transfusion from the national database in China from 2013 to 2018 and assessed 438,183 patients who underwent index surgical procedures.
- The prevalence of perioperative RBC transfusion markedly declined in the years after the transfusion-related guidelines were formally introduced.
- Heterogeneity in the prevalence of RBC transfusion was high among the seven major geographic regions of China.

INTRODUCTION

Red blood cells (RBCs) are commonly transfused in surgical patients during the perioperative period. More than 304.7 million units of blood products were needed by patients worldwide in 2017 [1]. Although RBC transfusion can save lives, it can also result in adverse outcomes, including fatal complications (e.g., transfusion-associated circulatory overload and transfusion-related acute lung injury) [2, 3]. Furthermore, the overuse of RBC products consumes the reserve for patients who require RBC transfusion [1, 4]. These factors jointly increase the risk of morbidity and mortality among surgical patients [5, 6]. Thus, reducing unnecessary RBC transfusions is an appropriate strategy to improve the clinical outcomes of surgical patients.

Restrictive RBC transfusion strategies, by lowering transfusion thresholds, can effectively decrease the likelihood of exposure to RBC products for patients [7, 8]. These strategies are safe for haemodynamically stable surgical patients [8]. Several guidelines and recommendations (issued by the American Association of Blood Banks, UK National Clinical Guideline Centre, etc.) further support restrictive RBC transfusion as a standard of care for surgical patients [9]. Evidence from national data has shown that introducing transfusion-related guidelines is associated with a decline in perioperative RBC transfusions (e.g., in the United States [10], Canada [11] and Sweden [12]). Although adherence to the guidelines varies [13–16], current evidence supports the notion that transfusion-related guidelines are effective in promoting restrictive RBC transfusion in clinical practice.

In China, approximately 47.9 million surgeries were performed in 2016 [17], and more than 43.2 million units of blood products were required [4]. Given that the practice of perioperative RBC transfusion varies among clinical centres [18–22], restrictive transfusion strategies were formally emphasized in the national perioperative transfusion guideline based on evidence of high-quality clinical trials and meta-analyses in 2014 [23]. This guideline listed several specific transfusion indications in the perioperative period (i.e., haemoglobin <7 dg/L, refractory anaemia patients with symptoms, pre-existing cardiovascular disease and considering transfusion for patients with symptoms or a haemoglobin level of 8 g/dL or less, etc.) and, thus, tends to be more pragmatic than former guidelines. Currently, whether transfusion-related guidelines have been successfully translated into clinical practice in China is unknown. This study aimed to provide updated information about the temporal trends of perioperative RBC transfusion in China from 2013 to 2018. Furthermore, we investigated the geographic variations in perioperative RBC transfusions among multiple surgical specialties.

MATERIALS AND METHODS

Ethics

This retrospective observational study followed the Standard of Reporting of Observational Studies in Epidemiology guidelines [24]. It was approved by the ethics committee of the Peking Union Medical College Hospital, and the requirement for written informed consent was waived by the institutional review board (S-K1047).

Data source

Data for this study were collected from the Hospital Quality Monitoring System (HQMS) database of the People's Republic of China in September 2018. The dataset of the main page information on inpatient medical records available among hospitals in all 31 provincial-level administrative divisions is collected by the National Health Commission. An official data collection system is used to acquire the data from each hospital's information systems. Data on demographic characteristics, surgical procedures based on *ICD-9-CM* (*International Classification of Diseases, 9th Revision, Clinical Modification*) and RBC transfusion information are stored in a specified data management institution. As of August 2018, this database has included inpatient medical records from 1067 (45.6%) tertiary hospitals in China [25]. Details of the database have also been provided in previous studies [26, 27].

Study population and data collection

In this study, we focused on neurological, cardiac, thoracic, abdominal and orthopaedic surgeries, as the patients usually require RBC transfusion [28]. One representative major procedure was selected for each surgical specialty according to the *ICD-9-CM* procedure codes (Table S1) (i.e., craniotomy for cerebral aneurysm or arteriovenous malformations, sternotomy for mitral valve replacement, open thoracotomy lobectomy, open gastrectomy and hip arthroplasty, with

perioperative RBC transfusion prevalence ranging from 5% to 34%) [29–33]. We then collected RBC transfusion data between 1 January 2013 and 1 August 2018, representing the most contemporary data available, and investigated the effect of transfusion guidelines [23]. Patients with incomplete clinical data (i.e., age, sex, admission date, surgical procedures and transfusion-related information) were excluded.

Statistical analysis

Perioperative RBC transfusion is defined as the transfusion of packed RBC products (≥1 unit) during hospitalization [5]. The Agresti–Coull proportion prevalence estimates and 95% confidence intervals (CI) were computed for perioperative RBC transfusion [34].

To investigate the dynamic changes in the likelihood of RBC transfusion, we used mixed-effects logistic regression models to estimate the adjusted odds ratio (aOR) of RBC transfusion in each year compared with the overall prevalence of RBC transfusion (adjusted for age, sex and geographic factors).

To study the effect of geographic region on the likelihood of RBC transfusion, we estimated the aOR of RBC transfusion in various geographic regions of China (adjusted for age, sex and time). Statistical analyses were conducted using R statistical package (version 4.0.3, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

We assessed 36,092,159 patient records from the data management institution between 1 January 2013 and 1 August 2018, among which 438,183 records from 31 provincial-level administrative divisions in China fulfilled the inclusion criteria (Figure 1). The characteristics of the included population are detailed in Table 1. The mean age of the included population was 40.5–61.5 years (craniotomy for cerebral aneurysms or arteriovenous malformations: 40.5 years, sternotomy for mitral valve replacement: 52.0 years, open thoracotomy lobectomy: 57.5 years, open gastrectomy: 60.1 years and hip arthroplasty: 61.5 years). The overall prevalence of perioperative RBC transfusion ranged from 4.68% to 19.57% among the individuals who underwent these five major surgical procedures (Table 1).

Temporal trends in perioperative RBC transfusions

We found declining trends in the prevalence of perioperative RBC transfusions among the major surgical procedures (Figure 2). Notably, the prevalence of RBC transfusion was reduced after 2014, when transfusion-related guidelines were introduced. For example, among 151,041 patients undergoing hip arthroplasty, the RBC transfusion prevalence decreased from 17.34% (95% CI, 16.85%–17.85%) in 2013 to 6.49% (95% CI, 6.22%–6.77%) in 2017. Furthermore, the declining trend of RBC transfusion prevalence was significant after adjusting for age, sex and geographic factors (aOR 1.84 [95% CI, 1.37–2.48] in 2013 vs. aOR 0.62 [95% CI, 0.46–0.84] in 2017) (Figure 3). We found that the population undergoing major surgical procedures in 2016 and 2017 was less likely to receive RBC transfusions than the population in 2013 and 2014 (Figure 3). Notably, the prevalence of RBC transfusion was higher in 2018 than in 2017 (although lower than in 2013), possibly because the RBC transfusion data available did not cover the entire year of 2018. Studies including the complete

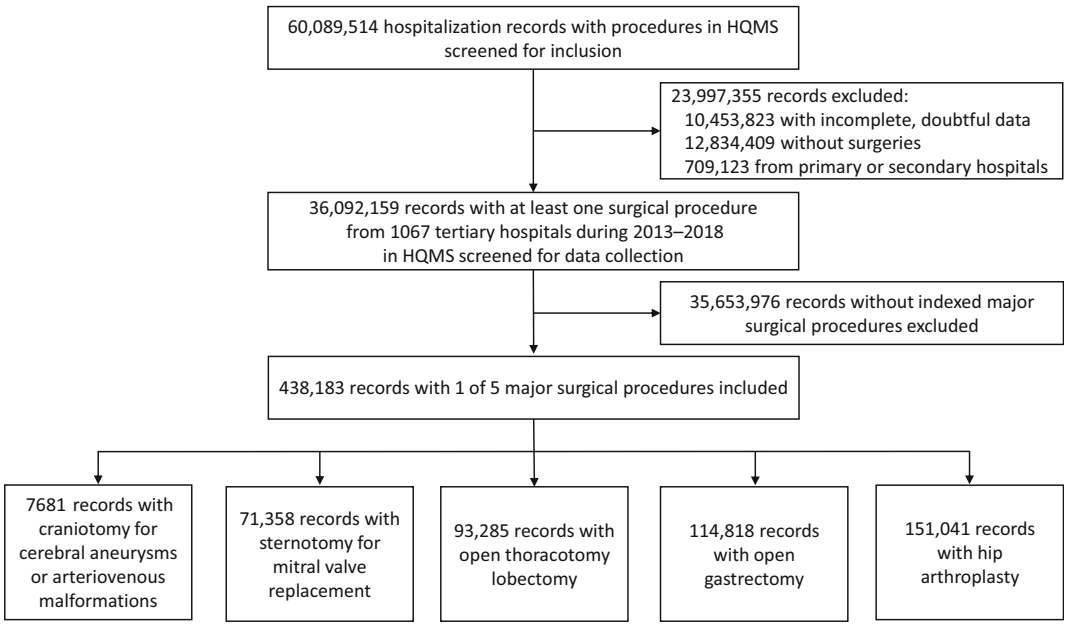

FIGURE 1 Flow diagram for population inclusion. HQMS, Hospital Quality Monitoring System.

TABLE 1 Demographic and clinical characteristics of the populations.

	Craniotomy for cerebral aneurysms or arteriovenous malformations ($n = 7681$)	Sternotomy for mitral valve replacement ($n = 71,358$)	Open thoracotomy lobectomy ($n = 93,285$)	Open gastrectomy ($n = 114,818$)	Hip arthroplasty ($n = 151,041$)
Age, years	40.5 (18.7)	52.0 (11.4)	57.5 (11.9)	60.1 (11.8)	61.5 (13.9)
Female, *n* (%)	3556 (46.30)	43,338 (60.73)	33,632 (36.05)	33,500 (29.18)	83,755 (55.45)
RBC transfusion at procedure year, *n* (%)					
2013	142 (10.40)	2949 (21.56)	1145 (6.70)	1591 (7.87)	3860 (17.80)
2014	152 (9.31)	3000 (20.66)	1038 (5.06)	1843 (7.49)	4409 (15.19)
2015	138 (9.11)	3133 (20.77)	790 (3.99)	1913 (7.63)	3696 (11.45)
2016	139 (8.07)	2769 (18.04)	775 (4.15)	1728 (6.93)	3022 (8.54)
2017	73 (5.48)	1964 (16.47)	569 (3.56)	1336 (7.21)	1999 (6.67)
2018	8 (7.08)	147 (18.31)	50 (4.09)	127 (8.63)	192 (7.14)
RBC transfusion at geographic region, *n* (%)					
Central	286 (12.89)	2878 (26.96)	1536 (10.32)	1127 (9.80)	4791 (20.79)
East	65 (4.07)	2314 (11.05)	386 (1.12)	2105 (4.21)	2679 (4.57)
North	73 (6.02)	3943 (24.85)	559 (5.01)	732 (6.28)	3818 (21.69)
Northeast	21 (3.43)	283 (12.35)	384 (3.12)	1659 (9.59)	856 (6.83)
Northwest	27 (8.26)	1455 (50.31)	259 (11.85)	1007 (16.98)	1167 (27.19)
South	47 (6.69)	1303 (11.74)	133 (2.67)	299 (3.40)	989 (6.54)
Southwest	133 (13.17)	1786 (23.55)	1110 (8.37)	1609 (16.73)	2878 (14.55)

Note: Data were collected from the Hospital Quality Monitoring System database in China. As of August 2018, this database had included inpatient medical records from 45.6% of tertiary hospitals in 31 provincial-level administrative divisions in China. Values are mean (standard deviation) or number (percentage).
Abbreviation: RBC, red blood cell.

data of this year are warranted to further investigate this declining trend.

Geographic variations in perioperative RBC transfusions

Considering the marked diversity of geography and socioeconomic circumstances in China, we investigated whether the prevalence of RBC transfusions varied among distinct geographic regions of China. We found prominent geographic variations in RBC transfusions among the seven regions of China. In Northwest China, the prevalence of RBC transfusion was highest for sternotomy for mitral valve replacement (48.20% [95% CI 46.39%–50.02%]), open thoracotomy lobectomy (11.68% [95% CI 10.41%–13.08%]), open gastrectomy (14.38% [95% CI, 13.57%–15.24%]) and hip arthroplasty (23.05% [95% CI, 21.88%–24.27%]). In comparison, the prevalence was lowest for sternotomy for mitral valve replacement (10.29% [95% CI 9.90%–10.70%]), open thoracotomy lobectomy (1.11% [95% CI 1.01%–1.23%]) and hip arthroplasty (3.71% [95% CI, 3.57%–3.85%]) in East China and lowest for open gastrectomy (2.84% [95% CI 2.54%–3.17%]) in South China. The prevalence of craniotomy for cerebral aneurysms or arteriovenous malformations was highest in Southwest China (14.19% [95% CI 12.13%–16.54%]) and lowest in Northeast China (4.26% [95% CI 2.95%–6.11%]). Consistently, these findings remained significant after adjusting for age, sex and time (Figure 4). Given the marked geographic variations in RBC transfusion data, our findings provide a foundation for improving homogeneity in perioperative RBC transfusion practices.

DISCUSSION

Exploring the HQMS national database from 2013 to 2018, this study investigated the perioperative RBC transfusion data of 438,183 patients undergoing major surgical procedures in China. Our results showed a significant declining trend in perioperative RBC transfusion among patients (relatively decreased by 62.56%–5.99%). In economically privileged regions (East and South China), the populations showed a lower prevalence of RBC transfusions during the perioperative period. Our study suggests that after the introduction of transfusion-related guidelines in 2014, the prevalence of perioperative RBC transfusion in China has markedly decreased. Data regarding the heterogeneity of RBC transfusion among various regions of China have the potential to guide further clinical improvements in RBC transfusion.

Our study found that the prevalence of RBC transfusion among major surgical procedures reduced from 2013. Given the restrictive strategies introduced by the transfusion-related guidelines in 2014, the results indicate that RBC transfusion practices have potentially improved in China. Evidence has shown that the national guidelines on restrictive transfusion strategies are linked to reduced risks of

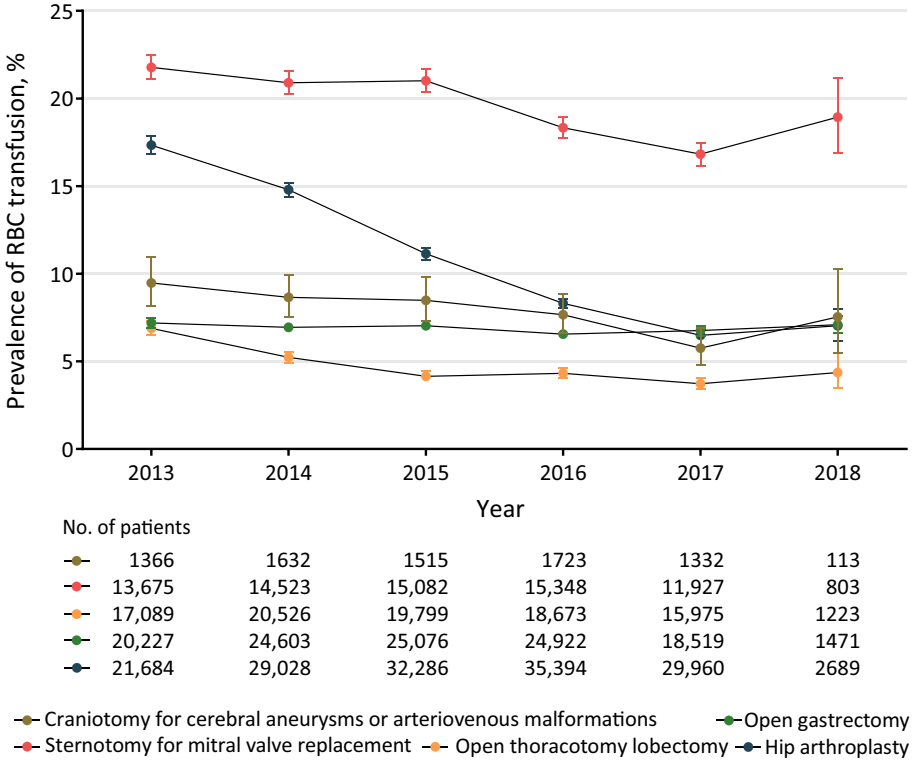

FIGURE 2 Temporal trends in the prevalence of perioperative red blood cell (RBC) transfusion among major surgical procedures. The Agresti–Coull proportion prevalence estimates were computed for perioperative RBC transfusion. Note that the prevalence of RBC transfusion reduced after 2014 when the transfusion-related guidelines were introduced [23]. The prevalence of RBC transfusion was higher in 2018 than in 2017 (although lower than in 2013), possibly because the RBC transfusion data available did not cover the entire year of 2018.

unnecessary exposure to blood products in the United States [35] and Canada [11], among other countries [8]. Educational campaigns improve blood transfusion practice [22, 35, 36]. Furthermore, the strength of evidence partially influences the translation of guidelines into clinical practice [37]. In line with prior studies, the decline in RBC transfusion in this study was most prominent in hip arthroplasty (62.56% relative decrease) (robust evidence supporting restrictive strategies in hip fracture) [8, 37] but less significant in open gastrectomy (5.99% relative decrease) (weak evidence for oncological patients) [8]. Notably, caution is needed regarding the inference about improved RBC transfusion practice, as the results may be influenced by confounders (e.g., change in patient characteristics, severity of disease, decreased rate of preoperative anaemia [38], improved surgical techniques [35] and use of tranexamic acid [39]). Further studies should consider these confounders to determine whether transfusion-related guidelines could indeed improve clinical practice.

Aiming to investigate the effects of geographic regions on RBC transfusion in China, we estimated the RBC transfusion prevalence among the seven geographic regions of China. We found that the populations in East and South China were less likely to receive RBC transfusions than those in the other regions. Geographic disparities in RBC transfusion among surgical specialties (total hip and knee arthroplasty [40], among others [15]) were found in the United States. For example, the prevalence of RBC transfusion in joint arthroplasty showed heterogeneity across various regions (i.e., 15.31% in the Northeast, 14.66% in the South and 9.08% in the Midwest) [40]. A possible explanation for this geographic variation is socioeconomic status. The population in privileged regions tends to have access to more advanced healthcare services (thus, less likely to be exposed to RBC transfusion). Although transfusion-related guidelines have been introduced, adherence to these guidelines may differ among various regions [18]. Another explanation for the geographic variation is the heterogeneity of preoperative anaemia in populations among distinct regions [38]. Thus, studies are warranted to further investigate the effects of geographic regions on transfusion practice.

This study supports the notion that the introduction of restrictive transfusion strategies into guidelines potentially confers beneficial effects on perioperative RBC transfusion practices. The reduction in unnecessary transfusion can decrease transfusion-related complications and prompt patient outcomes, as well as conserve blood resources and lower the economic burden [41]. Aiming to standardize transfusion practices, restrictive transfusion strategies were conceptualized in the patient blood management (PBM) programme in 2005, which aims to conserve the patient's own blood [37]. PBM programmes have been initiated in Australia, followed by the United States and European nations and are increasingly being implemented globally [42]. In China, the National Health Commission has supported the implementation of the PBM

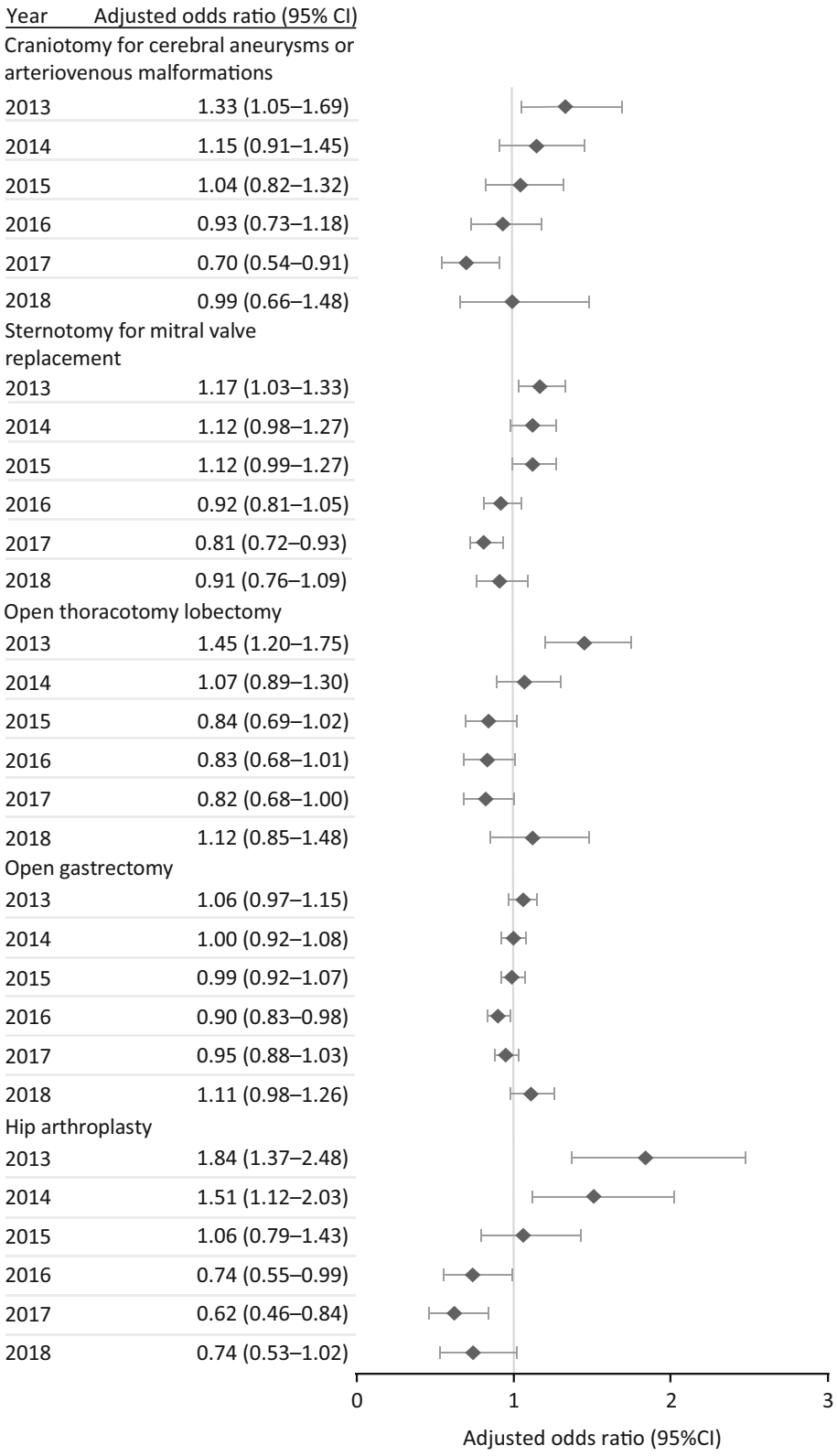

FIGURE 3 Adjusted odds ratio (aOR) of perioperative red blood cell (RBC) transfusion among major surgical procedures. Mixed-effects logistic regression models were used to calculate the aOR of RBC transfusion each year (adjusted for age, sex and geographic factors). Note that the aOR of RBC transfusion was higher in 2018 than in 2017, potentially due to the lack of RBC transfusion data of the entire year of 2018.

programme [43] and related guidelines [44–46] in recent years. It should be noted that the patients included in this study were likely from economically and socially privileged regions; therefore, the implementation of transfusion guidelines is expected to be insufficient in the general population. Thus, a public health benefit can be realized from the recognition that restrictive strategies, as well as

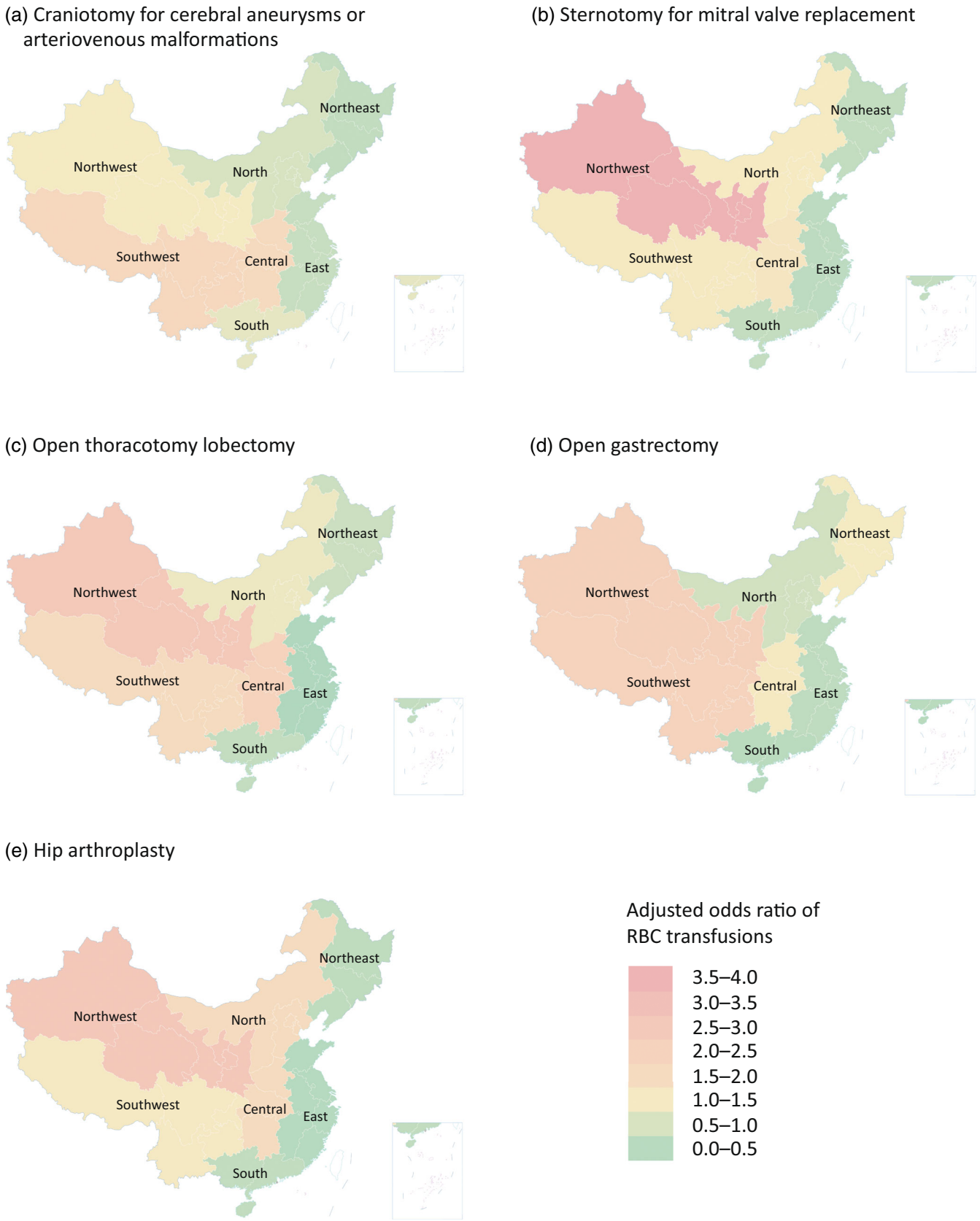

FIGURE 4 Geographic variations in perioperative red blood cell (RBC) transfusion among major surgical procedures. Mixed-effects logistic regression models were used to calculate the adjusted odds ratio (aOR) of RBC transfusion in various geographic regions (adjusted for age, sex and time). The results showed marked geographic variations in perioperative RBC transfusion among the seven geographic regions in China.

PBM, should be adopted as a standard of care, particularly for the general population. However, caution should be exercised regarding the potential risks of restrictive transfusion. An important concern is that patients with severe baseline illness (e.g., myocardial infarction [8]) may develop serious outcomes due to anaemia. Another concern is the safety of patients with acute ongoing blood loss [47]. Future studies should resolve these concerns to guide safe and effective RBC transfusions in clinical practice.

This study has several limitations. We were unable to determine the causal relationship between the introduction of transfusion-related guidelines in China and the decline in the prevalence of perioperative RBC transfusion. Our findings require validation in longitudinal research and data on the adherence to the RBC transfusion guidelines. Moreover, the apparent declining trend in the prevalence of RBC transfusion is likely to be confounded by the severity of anaemia and the advancement of surgical techniques. Selection bias is another limitation that should be considered. Given that the available data allowed us to include patients in 45.6% of tertiary hospitals in China and considering that the included patients are likely from privileged regions, the estimates of RBC transfusion prevalence in China may be higher than the observed data. In addition, we did not collect complete data in 2018; therefore, the composition of hospitals that had uploaded data to HQMS in 2018 might be different from that in previous years, and our data may be less comprehensive. Thus, this limitation should be considered when interpreting the transfusion prevalence in 2018. A further limitation is the geographic variation in RBC transfusion. The heterogeneity of RBC transfusion among distinct regions could be confounded by various factors, including preoperative haemoglobin level, disease severity and blood loss during surgery. Further studies are warranted to address these important questions, and such research would provide insights into the effects of transfusion-related guidelines.

In summary, evidence from this study suggests that the prevalence of perioperative RBC transfusion in China has declined in multiple major surgical procedures after the year when the transfusion-related guidelines were introduced. In light of the geographic variations in RBC transfusion practice and the risks of transfusion-related complications, the findings support the view that transfusion-related guidelines should be adopted, particularly for the general population. Although the findings suggest that transfusion-related guidelines may accrue substantial health and economic benefits from restrictive strategies, future studies are warranted to explore the safety issues regarding restrictive transfusion in populations with various medical conditions.

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L.M. and Y.H. conceptualized, designed, supervised and provided administrative, technical or material support. B.T. drafted the manuscript and performed statistical analysis. Y.Z. performed statistical analysis and provided administrative, technical or material support. All authors acquire, analysed or interpreted the data and provided critical revision of the manuscript for important intellectual content.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding authors.

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SUPPORTING INFORMATION

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

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SHORT REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

Prevalence of weak D phenotypes in the general population of Québec, Canada: A focus on weak D type 42

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Héma-Québec

Abstract

Background and Objectives: Weak D type 42 accounts for an unusually high proportion of weak D phenotypes in Québec (Canada), which contrasts with other predominantly White populations. However, its prevalence in the general population is unknown. We estimated the prevalence of weak D type 42 and other common weak D phenotypes in Québec.

Materials and Methods: We screened for *RHD*01W.42* alleles among 1000 individuals of CARTaGENE—a cohort representative of Québec's population. The prevalence of weak D type 42 was calculated based on the allele frequency of *RHD*01W.42* and *d* (i.e., all recessive alleles that confer a D− phenotype), assuming a Hardy–Weinberg equilibrium. This prevalence was then leveraged to calculate that of other common weak D phenotypes, using published prevalence estimates among weak D phenotypes.

Results: Two individuals harboured the *RHD*01W.42/RHD*01* heterozygous genotype. Assuming an allele frequency of 38.19% for *d*, the overall prevalence of weak D type 42 was 0.08%. The following prevalence estimates were also obtained: 0.44% for all weak D phenotypes and 0.07%, 0.01% and 0.04% for weak D types 1, 2 and 3, respectively.

Conclusion: Québec has the highest documented prevalence of weak D type 42, which was estimated at 0.08%.

Keywords

genetics, genotyping, RBC antigens and antibodies

Highlights

- Québec has the highest documented prevalence of weak D type 42, which was estimated at 0.08% in this study.
- The two individuals from the CARTaGENE cohort who harboured the *RHD*01W.42* allele were White and had Canada-born parents and grandparents, consistent with a founder effect.
- Despite the high prevalence of weak D type 42, the prevalence of all weak D phenotypes (0.44%) is at the lower end of those obtained in previous European studies (0.42%–0.67%); other weak D phenotypes may, therefore, exhibit a lower prevalence that offsets the high prevalence of weak D type 42.

INTRODUCTION

Rh incompatibilities must be prevented to avoid alloimmunization and subsequent complications [1]. While most individuals are unequivocally classified as D+ or D−, some harbour D variants are associated with weaker agglutination reactions or discrepant results with various anti-D reagents [1]. D variants are serologically categorized as weak D, partial D or DEL based on cell-surface-exposed epitopes and cell-surface expression of the D antigen [2].

In predominantly White populations, weak D types 1, 2 or 3 are generally the most prevalent weak D phenotypes [3–6]. One exception is Québec (Canada), where weak D type 42 (conferred by *RHD*01W.42*) is the most common weak D phenotype [7]. This variant is characterized by a K409M mutation at the C-terminus of RhD [8], which is predicted to be located intracellularly [3]. A cross-sectional study of 215 women with weak D type 42 found no evidence of alloanti-D [9], whereas another study of samples with an ambiguous D serology found 17 patients with weak D type 42, including 2 patients with alloanti-D [10]. Current clinical guidelines recommend managing these patients as D− individuals [11].

The epidemiology of this phenotype is, however, little documented. In a recent study conducted in Québec, 17.48% of women aged ≤45 with an atypical D typing result had weak D type 42, whereas only 15.3% had type 1, 3.3% had type 2 and 8.6% had type 3 [7]. Furthermore, Leiva-Torres et al. recently showed that the regional prevalence of weak D type 42 was negatively correlated with the proportion of minorities consistent with a founder effect [7].

While informative, the Leiva-Torres et al. study did not estimate the prevalence of weak D type 42 in the general population. Thus, we estimated the prevalence of weak D type 42 in the Québec general population.

MATERIALS AND METHODS

Data source and study population

A total of 1000 individuals were selected among CARTaGENE individuals (www.cartagene.qc.ca). CARTaGENE is a two-phase (A and B), population-based biobank and a cohort representative of Québec residents aged 40–69 years who live in urban regions [12]. In phase A, individuals living in Montreal, Québec city, Sherbrooke and Saguenay were recruited, and phase B additionally recruited individuals living in Gatineau and Trois-Rivières. Individuals were selected so that the age, sex and regional distributions matched those of the overall cohort. The ethics committee of Héma-Québec approved the study protocol, and informed consent was obtained from all subjects.

DNA extraction

Genomic DNA was extracted from CARTaGENE's 10 µL dried blood spots by adding 100 µL of QuickExtract™ Solution (Lucigen, Middleton, WI, USA) per well, following the manufacturer's instructions. The extracted DNA was then diluted 1:5 in 10 mM Tris-Cl pH 8.5 to prepare polymerase chain reaction (PCR) reaction.

PCR-based screening of *RHD*01W.42* alleles

PCRs were performed using the Veriti thermal cycler (Applied Biosystems) and the AmpliTaq Gold™ (ThermoFisher Scientific) according to the manufacturer's instructions. Reactions were carried out as follows: heating to 94°C for 9 min followed by 33 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s with a final extension of 5 min. Reactions were carried out simultaneously for *RHD*01W.42* and a control (human growth hormone) [13]. Amplicons were visualized on a 2% agarose gel.

qPCR-based screening of wild-type *RHD*01* alleles

Quantitative polymerase chain reaction (qPCRs) were performed in 20-µl reactions using the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Using a CFX96 thermal cycler (Bio-Rad), reactions were carried out as follows: 98°C for 2 min, followed by 40 cycles of 98°C for 10 s and 60°C for 30 s. A standard melting curve cycle was then performed. The primer sequences used for wild-type *RHD* were the same as those for *RHD*weak D type 42*, albeit without the *D42* single nucleotide polymorphism (Table S1).

Prevalence of weak D type 42

Three genotypes confer weak D type 42: *RHD*01W.42/d*, *d/RHD*01W.42* and *RHD*01W.42/RHD*01W.42*, where *d* comprises all recessive alleles that confer a D− phenotype. Assuming the Hardy–Weinberg equilibrium, the prevalence of weak D type 42 can be deduced by summing these genotype frequencies, which are themselves the product of the allele frequencies. The prevalence of weak D type 42 in the general Québec population was thus calculated as follows:

$$\begin{aligned} Prev\,(\text{weak D type 42}) &= G_f\,(RHD^*01W.42/d) + G_f\,(d/RHD^*01W.42) \\ &\quad + G_f\,(RHD^*01W.42/RHD^*01W.42) \\ &= 2 \times (A_f\,[RHD^*01W.42] \times A_f\,[d]) \\ &\quad + (A_f\,[RHD^*01W.42] \times A_f\,[RHD^*01W.42]) \end{aligned}$$

where *Prev* is the prevalence, G_f is the genotype frequency and A_f is the allele frequency.

The allele frequency of *d* was estimated based on the prevalence of the D− phenotype, again assuming the Hardy–Weinberg equilibrium. One hundred and ninety-two of the 1000 study samples were randomly chosen and tested for a D+/D− associated allele by qPCR. Test-negative samples were assumed to be D−, and the allele frequency of *d* was, thus, the square root of the estimated proportion of D− individuals.

TABLE 1 Estimated prevalence of weak D type 42 in Québec, overall and among subgroups of interest.

Genotype	Allele frequency 1, % (95% CI) [A]	Allele frequency 2, % (95% CI) [B]	Genotype frequency, % (95% CI) [A × B]
Overall population			
*RHD*01W.42/d*	0.10 (0.00–0.29)	38.19 (35.19–41.19)	0.04 (0.00–0.14)
*d/RHD*01W.42*	38.19 (35.19–41.19)	0.10 (0.00–0.29)	0.04 (0.00–0.14)
*RHD*01W.42/RHD*01W.42*	0.10 (0.00–0.29)	0.10 (0.00–0.29)	0.0001 (0.00–0.01)
Total			0.08 (0.00–0.25)
White, with Canada-born parents			
*RHD*01W.42/d*	0.13 (0.00–0.38)	38.19 (34.76–41.62)	0.05 (0.00–0.21)
*d/RHD*01W.42*	38.19 (34.76–41.62)	0.13 (0.00–0.38)	0.05 (0.00–0.21)
*RHD*01W.42/RHD*01W.42*	0.13 (0.00–0.38)	0.13 (0.00–0.38)	0.0002 (0.00–0.01)
Total			0.10 (0.00–0.32)
White, with Canada-born parents and grandparents			
*RHD*01W.42/d*	0.15 (0.00–0.44)	38.19 (34.53–41.85)	0.06 (0.00–0.24)
*d/RHD*01W.42*	38.19 (34.53–41.85)	0.15 (0.00–0.44)	0.06 (0.00–0.24)
*RHD*01W.42/RHD*01W.42*	0.15 (0.00–0.44)	0.15 (0.00–0.44)	0.0002 (0.00–0.01)
Total			0.11 (0.00–0.37)

Abbreviation: CI, confidence interval.

Prevalence of other weak D phenotypes

Leiva-Torres et al. reported the prevalence of weak D types 1, 2, 3 and 42 among all weak D phenotypes [7]. Together with the herein calculated prevalence of weak D type 42, this information was leveraged to derive the prevalence of weak D phenotypes (overall) and those of weak D types 1, 2 and 3 in the general Québec population.

Statistical analysis

Prevalence rates were calculated for weak D type 42 and other common weak D phenotypes. Clopper–Pearson confidence intervals were reported since the outcome of the study experiment (absence/presence of *RHD*01W.42*) is binary and does not include a time component.

RESULTS

Prevalence of D−

Of 192 randomly chosen samples, 28 (14.58%) were D− (*d/d*) as determined by qPCR. The estimated allele frequency of *d* was, therefore, 38.19%.

Prevalence of weak D type 42

Two individuals harboured the *RHD*01W.42/RHD*01* genotype. These individuals were White, had Canada-born parents and grandparents and lived in Laval or the North Shore of Montreal. The allele frequency of *RHD*01W.42* was, therefore, 0.10% in the overall population ($N = 1000$), 0.13% among White individuals with Canada-born parents ($N = 765$) and 0.15% among White individuals with Canada-born parents and grandparents ($N = 672$; Table 1). Assuming an allele frequency of 38.19% for *d*, the estimated prevalence of weak D type 42 in the general Quebec population was 0.08% overall, 0.10% in White individuals with Canada-born parents and 0.11% in White individuals with Canada-born parents and grandparents (Table 1).

TABLE 2 Estimated prevalence of weak D phenotypes in Québec.

Phenotype	Prevalence among weak D phenotypes, % (95% CI)[a]	Prevalence in the general population, % (95% CI)[b]
Weak D type 42	17.48 (15.18–19.98)	0.08 (0.00–0.25)
Weak D type 1	15.34 (13.12–17.56)	0.07 (0.00–0.23)
Weak D type 2	3.28 (2.18–4.38)	0.01 (0.00–0.09)
Weak D type 3	8.55 (6.83–10.28)	0.04 (0.00–0.16)
Other weak D phenotypes	55.34 (52.28–58.41)	0.24 (0.00–0.55)
Total	100%	0.44 (0.13–1.08)

Abbreviation: CI, confidence interval.
[a]Prevalence among all weak D phenotypes in Québec reported by Leiva-Torres et al. [7].
[b]Prevalence of weak D types 1, 2 and 3 and other weak phenotypes was estimated by cross-multiplicity. For example, the prevalence of weak D type 1 in the general population was obtained as follows: 15.3444% × 0.0765%/17.4821% = 0.0671%.

Prevalence of other weak D phenotypes

Having estimated the prevalence of weak D type 42 at 0.08% (this study) and knowing that weak D type 42 accounts for 17.48% of all weak D phenotypes (as reported by Leiva-Torres et al.) [7], the prevalence of all weak D phenotypes was, therefore, 0.44% in the general Québec population (Table 2). Leveraging the other estimates of Leiva-Torres et al. for weak D phenotypes [7], we estimated the prevalence of weak D type 1 at 0.07%, weak D type 2 at 0.01%, weak D type 3 at 0.04% and other less prevalent weak D phenotypes at 0.24% (Table 2).

DISCUSSION

With an estimated prevalence of 0.08%, Québec has the highest documented prevalence of weak D type 42. In other populations, the prevalence of weak D type 42 was estimated at 0.0008% in the rest of Canada (5 out of 608,486) [6], 0.006% in the Greater Toronto area (2 out of 33,342) [8] and 0.007% in Denmark (1 out of 100 weak D tested, in a population for which the prevalence of all weak D phenotypes is 0.7%) [5].

The high prevalence observed in Québec may be driven by a founder effect dating back to the foundation of New France, as previously suggested [7–10]. Consistent with this explanation, both individuals with weak D type 42 were White and had Canada-born parents and grandparents. Furthermore, in three studies conducted in France, no individuals with weak D type 42 have been found [14–16]. The prevalence of weak type 42 might, therefore, have been higher among the first settlers of New France than the general French population, resulting in a founder effect in Québec, although this is speculative.

Although few individuals harboured the *RHD*01W.42* allele (i.e., 2 of 1000), a sample size of 1000 provides a good level of confidence and reliability. Based on a post hoc power analysis (detailed in Data S1), a sample of 1000 individuals with an expected proportion of 0.002 (i.e., 2 of 1000) can detect this expected proportion with a margin of error of ±6% with a level of confidence of 95%.

Our study also provides prevalence estimates for other weak D phenotypes in the general population of Québec, thereby building on the prevalence estimates obtained by Leiva-Torres et al. among weak D phenotypes [7]. The prevalence of all weak D phenotypes was 0.44%, which is at the lower end of previous estimates in Germany and Denmark (range: 0.42%–0.67%) [3–5]. Therefore, the high prevalence of weak D type 42 in Québec does not appear to increase the overall prevalence of weak D phenotypes. Instead, other weak D phenotypes seemingly exhibit a lower prevalence that offsets the high prevalence of weak D type 42 (e.g., the prevalence of weak D type 1 among weak D phenotypes: 15.3% in the Leiva-Torres et al. study [7] and 24.9%–79.0% in other studies). These results may prove useful to conduct risk analyses that will inform the clinical management of patients with weak D phenotypes.

We conclude that Québec has the highest documented prevalence of weak D type 42, which we estimated at 0.08%. This prevalence was higher among White individuals with Canada-born parents and grandparents, consistent with a previously described founder effect.

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M.S.L. and J.L. designed the study. M.D. and J.L. acquired the data. M.D., S.R., A.L. and J.L. analysed the data. M.D., S.R., A.L. and J.L. interpreted the data. M.D., S.R. and J.L. drafted the manuscript. All authors critically reviewed the manuscript for intellectual content.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

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

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SHORT REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

Two cases of *Streptococcus dysgalactiae* subspecies *equisimilis* infection transmitted through transfusion of platelet concentrate derived from separate blood donations by the same donor

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Abstract

Background: Transfusion-transmitted bacterial infections (TTBIs) in Japan have been largely prevented due to a short shelf life of 3.5 days after blood collection for platelet concentrate (PC) and washed PCs (WPCs; PC in which 95% plasma is replaced by platelet additive solution).

Case Presentation: *Case 1*: In January 2018, a woman in her 50s with aplastic anaemia who received WPC transfusion and developed a fever the next day and *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE) was detected in the residual WPC. *Case 2*: In May 2018, a man in his 60s with a haematologic malignancy who received PC transfusion and developed chills during the transfusion. SDSE was detected in the patient's blood and residual PC.

The contaminated platelet products were both manufactured from blood donated by the same donor. The multi-locus sequencing typing revealed that SDSE detected in case 1 was identical to that from case 2; however, whole blood subsequently obtained from the donor was culture negative.

Conclusion: WPC and PC produced from two blood donated 106 days apart by the same donor were contaminated with SDSE of the same strain and both caused TTBIs. Safety measures should be considered regarding blood collection from a donor with a history of bacterial contamination.

Keywords

bacterial infection, blood donors, blood safety, platelet transfusion, washed platelet

Highlights

- Two transfusion-transmitted bacterial infections (TTBIs) were caused by *Streptococcus dysgalactiae* derived from consecutive blood collections from the same donor.
- This is the first case in Japan in which washed platelet concentrate was the causative product for TTBI.
- Management of donors with a history of bacterial contamination in their donated blood is crucial.

INTRODUCTION

Bacterial contamination of platelet concentrates (PCs) remains a serious problem in transfusion. In Japan, although bacterial cultures of PCs are not screened, transfusion-transmitted bacterial infections (TTBIs) are prevented by shortening the PC shelf life to 3.5 days. However, several cases of bacterial contamination of PCs have been found each year. We encountered two cases of TTBIs caused by *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE) in two blood products from the same donor.

CASE PRESENTATION

Case 1

In January 2018, a woman in her 50s with aplastic anaemia received 2-day-old irradiated leukocyte-reduced washed PC (WPC). The next day (day 1), the patient experienced chills, severe fatigue, decreased awareness levels and fever (39.5°C). She was administered antibiotics (meropenem), and 90 min later blood samples were taken for culture. The patient recovered consciousness on day 2, and her fever decreased on day 3. Although the patient's blood culture was negative, group G streptococci were detected in the residual WPC culture in the hospital laboratory. The WPCs were not subjected to a culture test in the laboratory of the Japanese Red Cross (JRC) because almost no residue was left when the blood centre recovered the WPC bag. The empty bag was stored frozen at the JRC laboratory. A fresh frozen plasma separated from the WPC was culture negative at a public health institute 9 days after blood collection.

Case 2

In May 2018, a man in his 60s with diffuse large B-cell lymphoma received 3-day-old regular PC that was irradiated and leukocyte-reduced. Seventy-five minutes after transfusion started, the patient developed chills, and blood samples were collected for culture. Since symptoms improved, the transfusion was restarted and completed. Ten minutes after the transfusion ended, the patient developed a fever (41.8°C) and was administered antibiotics (cefepime). His fever subsided 6 h after transfusion, and he showed signs of recovery on day 3. SDSE was detected in the patient's blood culture in the hospital laboratory. A culture test of the PC was performed in the JRC.

Donor investigations

The donor, a man in his 40s, had donated blood >80 times. His white blood cell (WBC) and platelet (PLT) counts were consistently high over the past 3 years, with a mean WBC count of 10,380/μL (median 10,410/μL) and mean PLT count of 406 × 10^9/L (median 401 × 10^9/L) in 29 measurements. The WBC and PLT counts at the time of collection of the causative products were 9920/μL and 453 × 10^9/L in January 2018 and 9430/μL and 429 × 10^9/L in May 2018, respectively. No infection-related symptoms were reported during the medical interview before donation. In June 2018, the blood centre called the donor and collected whole blood and a pharyngeal swab specimen (Figure 1).

MATERIALS AND METHODS

Ethics statement

This study was approved by the Ethics Review Committee of the Japanese Red Cross Society (approval number: 2018–052), and we obtained consent from the donors for interviews, sample collection and analysis.

WPC

WPC is a platelet concentrate in which >95% of the plasma have been replaced by a platelet additive solution. This product is indicated for (1) cases with multiple transfusion-related adverse reactions that were unpreventable with premedication or cases with serious adverse reactions such as anaphylactic shock and (2) cases in which only ABO-incompatible unit is available for HLA-matched PC.

Culture of WPC and PC samples suspected of TTBI

In case 1, the empty WPC bag after transfusion was refrigerated for 6 days and subsequently stored at −30°C. However, when a second TTBI case occurred with PC collected from the same donor, a culture test was performed using this empty bag, which had been kept frozen for 130 days. After thawing at room temperature (20–24°C), a lavage suspension of the bag with 30 mL saline was used as a sample for culture. A PC sample from case 2 was obtained from the remaining PC that was stored at 4°C for 8 days after transfusion. These component samples were cultured using the BACT/ALERT 3D system (bioMérieux Inc., Durham, NC, USA) and agar plates. BACT/ALERT BPA and BPN bottles (bioMérieux) were each inoculated with 3.5 mL WPC samples or 1 mL PC samples. Bottles were subsequently incubated in the BACT/ALERT 3D system at 36°C under aerobic or anaerobic conditions for 10 days. Trypticase soy agar (TSA; Nippon Becton Dickinson Company, Ltd., Tokyo, Japan), TSA with 5% sheep blood (TSAB) and modified Gifu anaerobic medium (GAM) (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) agar plates were inoculated with 100 μL samples and incubated at 25 and 35°C for 7 days. TSA and TSAB plates were incubated aerobically, while modified GAM plates were incubated anaerobically. Bacterial concentrations were determined by serial dilution plating of the samples on TSA plates.

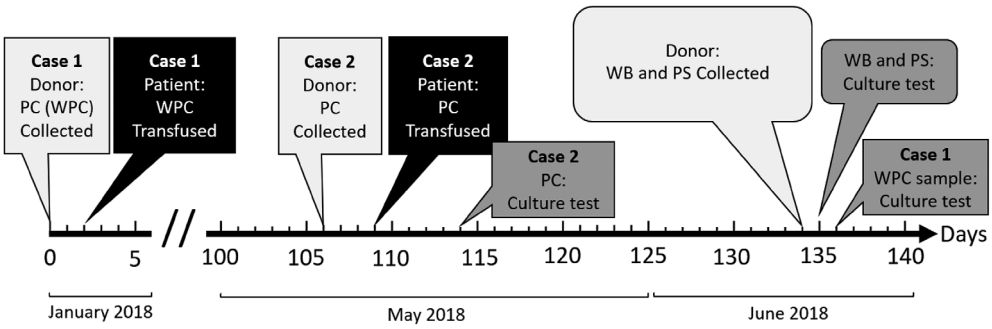

FIGURE 1 Timeline of donor blood and sample collection, transfusion and culture test events in two transfusion-transmitted bacterial-infection cases. PC, platelet concentrate; PS, pharyngeal specimen; WB, whole blood; WPC, washed platelet concentrate.

Culture of whole blood and pharyngeal specimen of donor

Whole blood and pharyngeal swabs from the donor were collected 28 days after the case 2-related blood donation and subjected to culture the next day. Ten millilitres of the whole blood samples were inoculated into each of the BPA and BPN bottles and incubated using the BACT/ALERT 3D system. The pharyngeal specimen was collected using a Seed Swab No. 3 (Eiken Chemical Co., Ltd., Tokyo, Japan), and a 3 mL saline suspension of the swab specimen was cultured using TSA and TSAB plates. Bacterial concentrations were determined by serial dilution plating of the samples on TSA and TSAB plates.

Bacterial isolates and identification

All colonies with different morphologies were isolated from the TSA and TSAB plates of cultured pharyngeal specimens. All β-haemolytic colonies were isolated from TSAB plates. Bacterial species were identified using 16S rRNA gene amplification and sequencing according to the protocols previously described [1].

Multi-locus sequencing typing

MLST was conducted using five strains including WPC and PC isolates, patient isolates from case 2, and two strains from donor pharyngeal swab isolates. The SDSE isolates were genotyped using seven housekeeping genes: glucose kinase (*gki*), glutamine transport protein (*gtr*), glutamate racemase (*murI*), DNA mismatch repair protein (*mutS*), transketolase (*recP*), xanthine phosphoribosyl transferase (*xpt*) and acetoacetyl-coathioloase (*atoB*); sequencing was performed according to the previously described protocol [2]. Amplicons were sequenced and analysed as described in Section 3.5. Sequencing results of the seven housekeeping genes were submitted to the SDSE MLST database (PubMLST; https://pubmlst.org/), and sequence types (STs) were defined.

RESULTS

All WPC and PC cultures in the BACT/ALERT 3D system, and plate cultures were positive. All isolates were identified as SDSE by 16S rRNA gene analysis and ST8 by MLST. Bacterial counts were 9.2×10^3 colony forming units (CFU)/mL and 1.3×10^7 cfu/mL for WPCs and PCs, respectively.

The subsequent whole blood sample of the donor was culture negative. The bacterial counts of the pharyngeal specimen were 3.1×10^4 CFU/mL on TSA and 5.7×10^4 CFU/mL on TSAB. The following 19 colonies were isolated and identified from both mediums: seven colonies of *Streptococcus salivarius*, three colonies of *Rothia dentocariosa*, two colonies of SDSE and one colony each of *Rothia mucilaginosa*, *Streptococcus equinus*, *Streptococcus infantis*, *Streptococcus oralis*, *Streptococcus parasanguinis*, *Streptococcus pyogenes* (*S. pyogenes*) and *Neisseria* species. Of these, three colonies showed β-haemolysis on TSAB medium, two corresponded to SDSE and one corresponded to *S. pyogenes*. The two SDSE colonies isolated from the pharyngeal specimen were ST34 by MLST, which was different from ST8 isolated from WPC in case 1 and PC and the patient blood in case 2.

DISCUSSION

SDSE has low pathogenicity and generally inhabits the upper respiratory of healthy people as resident bacteria. However, it occasionally causes purulent diseases such as cellulitis, purulent arthritis and sepsis [3]. Since SDSE shares a common ancestor with *S. pyogenes*, which is highly virulent, it has similar virulence factors and may cause streptococcal toxic shock syndrome [2, 3]. In Japan, SDSE was the causative agent of 4 of 17 (24%) of TTBI cases in 2011–2020 (including our cases). SDSE has been reported to multiply rapidly within PCs, meaning that it can reach the number of bacteria that can cause TTBI even during the short shelf life of PCs in Japan [4].

In the two cases of TTBI caused by SDSE reported in France and USA, the causative products were pooled PC, and SDSE was detected in the pharyngeal swab of one of the multiple donors [5, 6]. It is presumed that pharyngeal mucus splashes contaminated the PCs.

The case reported in the United States was a fatal one after transfusion of PC, wherein bacteria was undetected by culture screening [5].

In our cases, the same genotypic strain was detected in blood products collected from the same donor at different times in January and May 2018. This strain may have been present persistently as part of the resident flora in the donor's body. Because the genotypes of SDSE strains isolated from the donor pharyngeal swab differed from TTBI-related SDSE strains, the source of SDSE contamination in blood products was unknown.

SDSE is frequently detected as a causative bacterium in patients with recurrent bacteraemia [3, 7, 8]. Interestingly, it has been reported that SDSE is endogenous to vascular endothelial cells and persistently infects the bloodstream, causing recurrent infections [7, 8]. An in vitro study has reported that SDSE strains from patients with recurrent bacteraemia tend to be more efficiently internalized in vascular endothelial cells than SDSE strains from wounds. Strains with some specific properties, such as retention of cell invasion ability by zipper mechanism, are likely to cause bacteraemia [8]. The blood donor's WBC and PLT count in our cases has remained relatively high over the past 3 years, and SDSE of the same genotype was detected twice in the series of blood donations. These facts suggest that PC-derived SDSE strains could have settled somewhere that is more likely to be exposed to blood in the body other than the pharynx, for example, in vascular endothelial cells.

Moreover, the association between bacteraemia and asymptomatic colonic disease has been reported in species closely related to SDSE, such as *Streptococcus agalactiae* and *Streptococcus gallolyticus* [9]. However, there are few such reports in cases of SDSE, and the association between SDSE carriage and asymptomatic disease are subject to further investigation.

This is the first case in Japan where WPC was the causative product for TTBI. JRC started issuing WPC in 2016 to mitigate non-haemolytic transfusion reactions. WPC contains <5% plasma, indicating a very low amount of complement contained in WPC [10]. SDSE can aggregate human platelets and the tendency may be augmented by the low plasma protein level in WPC.

Culture methods are useful for detecting bacteria in blood products, and PC culture screening has been introduced in several countries. However, culture screening still does not detect all bacterial contamination in PCs [5, 11]. A comprehensive implementation of safety measures integrating multiple strategies, including donor health questionnaires, compliance with proper skin disinfection technique, initial flow diversion, visual inspection of PC and platelet pathogen inactivation, is needed. An additional strategy for blood safety could be to avoid blood collection from a donor for whom a bacterial species such as *Staphylococcus aureus* and *Streptococcus* spp., which tend to escape detection through culture methods, is repeatedly identified. It is reasonable to consider that such a donor harbours some pathological status that allows the occurrence of intermittent bacteraemia. In Canada, *Escherichia coli* was detected twice in PC collected from the same donor, and the donor was permanently deferred after identifying multiple diverticula in his colon [12]. In our cases, two TTBIs were caused by the same bacteria derived from consecutive blood collections from the same donor. The JRC considered the high virulence of SDSE and the possible liability to intermittent SDSE bacteraemia in blood donors and decided to exclude those donors from the platelet donor registry whose donated blood was verified to be contaminated with SDSE.

In conclusion, this is the first study in Japan to report two cases of TTBI caused by two blood products (WPC and PC) from the same donor. These cases highlight that the management of donors with a history of bacterial contamination of their donated blood is crucial.

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M.K. designed the study and wrote the manuscript. M.K., M.M. and A.T. performed the donor investigations and acquired the data. N.G. provided clinical details of the cases and reviewed the manuscript. R.A.F., K.M. and M.S. reviewed and edited the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

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

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GUIDELINES

Vox Sanguinis ISBT International Society of Blood Transfusion

International Society for Blood Transfusion Guidelines for Traceability of Medical Products of Human Origin

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Contents

This document has been written by the International Society of Blood Transfusion Working Party on Information Technology Traceability Task Force.

INTRODUCTION

Medical products of human origin (MPHO) are products that come from a human donor and are intended for clinical application in a human recipient. Due to their human source, they carry the risk of infectious disease transmission, incompatibility and other adverse reactions. This makes MPHO products of an exceptional nature and strict traceability requirements must be maintained to identify the MPHO and its constituents every step of the way until final disposition and to ensure traceability from donor to recipient and vice versa. In some instances, traceability records are required to be maintained for long periods that can exceed 30 years based on country or local regulations. Some MPHO may be deemed not suitable for clinical application, and there may be additional traceability requirements for these products. It is important that all those involved in the chain of custody of MPHO, at all levels, understand the importance of traceability and their role in maintaining the traceability pathway. Traceability regulations do differ between countries, and requirements may be different for MPHO shipped internationally. Contractual expectations between supplier and customer may also differ.

These guidelines were developed by the Traceability Task Force of the International Society of Blood Transfusion Working Party on Information Technology (ISBT WPIT). The purpose of these guidelines is to provide recommendations on ensuring that MPHO can be traced regardless of the extent of computerization used in record keeping. The ability to accurately trace the products from donor to recipient and from recipient back to the donor is a cornerstone of patient safety and involves those collecting, testing, modifying, distributing, shipping and using the MPHO in patient care. Technology is rapidly advancing. These guidelines should serve as a basis for evaluating the current system and developing plans to ensure changes to system maintain traceability.

SCOPE OF GUIDANCE

This guideline is focusing on the traceability of blood and cellular therapy products. However, much of the information and guidance provided is applicable across all areas of MPHO. It is intended to provide best practices in capturing and maintaining a robust record of processes involving the collection, manufacture and disposition of MPHO. It does not replace local or national regulations. Such regulations would supersede these guidelines. Accreditation institutions may have standards that also affect traceability requirements.

WHAT IS TRACEABILITY?

Traceability of MPHO is defined as the maintenance of a permanent continuous information trail beginning with the selection of donors of MPHO and continuing through procurement, processing, testing, distribution and recipient matching to the final disposition of all the sub-products, ensuring timely tracing from donor to recipient and vice versa is possible.

TABLE 1 Workflow showing points where additional data can be collected—An example.

Activity recorded for each plasmapheresis collection
Identification of the machine (model and serial number)
Confirmation of the state of the machine
Kit batch identification
Solutions batch identification
Identification of the donor
Identification of the containers traceable to the donation
Test tube identification
Arm venipuncture identification
Start time of apheresis procedure
End time of apheresis procedure
Possibility to register adverse events
Final procedure parameters

This core level of traceability is essential for all MPHO products and is often required by regulation. It is necessary to support biovigilance activities including product recall and lookback.

Traceability is also a tool in quality management that provides detailed information regarding who did what, where and when on a particular process, and what supplies, reagents and equipment were used. This additional traceability information provides lookback information that is useful in performance monitoring and conducting root cause analysis.

Traceability information may be captured manually, electronically or by a combination of electronic and manual processes. Traceability steps should be embedded in the workflow of all critical processes.

WHO IS RESPONSIBLE FOR TRACEABILITY?

Responsibility for traceability lies with both the organizations responsible for any part of the MPHO chain of custody and also with each individual responsible for handling these products or the information associated with them.

Each organization working with MPHO should have an established and actively managed and audited quality system. Traceability requirements should be integrated into this system and all relevant procedures should specify the traceability steps associated with the process. End-to-end traceability must be ensured and verified through traceability audits (end-to-end within the organization including interfaces with other organizations). Additional information can be captured to further enhance traceability and an example is shown in Table 1.

Organizations are responsible for the long-term retention of traceability information and for ensuring procedures that are maintained for retrieving this information promptly. If organizational change such as acquisition, merger or closure occurs careful attention must be paid to the ongoing security of traceability information.

At the individual level, staff must

- only perform tasks for which they have been trained,
- follow procedures and
- ensure accurate and complete records are maintained.

Particular care must be taken in the transcription of traceability information as an error can result in a breakdown in the traceability chain. Any perceived failure in traceability should be immediately reported and investigated.

IMPORTANCE OF TRACEABILITY

Good traceability is essential to patient safety and is required by law in many countries (e.g., for blood and blood products, USA: 21 CFR 606 [1], EU: EC directive 2002/98/EC) [2]. There are several reasons why good traceability is essential:

- Mitigation of transfusion-transmitted diseases: If the donor has a disease transmissible by transfusion or transplantation that was undetected at the time of donation or has a high-risk behaviour that could have affected his/her eligibility to donate that was only discovered after donation, good traceability records allow facilities to quickly:
  - Identify the current and any prior donations from the donor;
  - Locate and quarantine any products in inventory from the current donation and any prior donations;
  - Identify the recipient(s) of the product(s) of the current donation, as well as recipients of previous donations, and notify their physicians. Early identification and treatment of patients exposed to transfusion-transmitted diseases may allow mitigation of the disease in the recipient;
  - Exclude the donor from further donations;
  - Encourage the donor to consult a doctor for investigation and treatment of the disease.
- Archive retrieval: In case of a newly discovered transmitted disease, it is useful to have traceability to archive samples from prior donations from the same donor. Those samples can be re-tested with more sensitive tests (e.g., nucleic acid amplification test) to determine if something may have been missed in prior tests [3].
- Follow-up of adverse reactions: Should a recipient be found to have a problem that could be related to having received the MPHO (e.g., Transfusion-Related Acute Lung Injury), traceability records allow facilities to quickly identify the donor and quarantine any products still in inventory. If the product was the cause of the problem in the recipient, the donor may need to be deferred, and other donations from the same donor identified. If a determination of causality is made, traceability records allow facilities to identify the recipients of other products from the donor and notify their physicians. Again, early identification and treatment of patients may allow mitigation, or even prevention, of adverse events.
- Mitigation of risks related to critical product issues: Traceability records should indicate which critical supplies and equipment were used in the collection and processing of the MPHO. Should a problem be subsequently identified with a supply or item of equipment, the associated MPHO can be identified and evaluated for suitability for use in transfusion/transplantation. If appropriate, recipients of any adversely affected products must be identified, and their physicians notified. Instruments and consumable supplies used in the manufacture of MPHO should have the manufacturer's name, product identifier (catalogue number) and lot or serial number recorded to uniquely identify them. The Global Trade Item Number, where available, is suitable for capturing the manufacturer and catalogue number.
- Recall defective products: Suppliers must have the ability to identify the constituents of their product batches to support rapid recall if defects are identified. Suppliers should be audited and qualified, for example, a blood pack manufacturer would be expected to have records identifying every constituent element used for the blood bags and the production line used. Table 2 provides examples of critical products and equipment. Following qualification, a yearly supplier audit is recommended.
- Haemovigilance and biovigilance: Haemovigilance in the case of blood, and biovigilance in the case of cells, tissues, organs, vaccines and other MPHO, comprise surveillance procedures covering the whole transfusion/transplantation chain from collection to follow-up of recipients and donors. It assesses information on undesirable transfusion/transplantation effects and reactions to prevent their re-occurrence. Such activity is dependent on traceability records. For example, good traceability records are required for impacted blood centres and manufacturers to be able to retrieve all components derived from the same donation or donor when a product derived from that donor or donation has been implicated in a patient adverse event (WHO Guide to establishing a national haemovigilance system) [4].
- Litigation: Should legal action be taken against a facility based on the claim that the recipient was injured because of receiving the MPHO, all records should be maintained and protected from alteration or destruction.

TABLE 2 Examples of traceable products and equipment.

• Blood bags (lot number)
• Containers
• Blood collection mixer (identification number/serial number)
• Centrifuge (identification number/serial number)
• Blood component separator (identification number/serial number)
• Controlled temperature devices (identification number/serial number)
• Solutions (lot number NaCl, dextrose, Composol, DMSO, RPMI, etc.)
• Syringes (identification number/serial number)
• Sterile welder and disposables (identification number/serial number)

FUNDAMENTALS/PRE-REQUISITES OF TRACEABILITY

The purpose of traceability is to ensure that an information trail is maintained between the donor and the recipient(s), processes related

TABLE 3 Traceability requirements that should be included in procedures.

- What information is to be recorded
  - Date and time of event
  - Person responsible
  - Key identifiers (donation numbers, product codes, batch numbers, etc.)
- How the information is to be captured (automated/manual)
- Any verification steps required (e.g., double entry for manual transcription)

to preparation are retained and the information can be retrieved quickly. This requires a system to ensure the unique identification of each MPHO product, a secure mapping from the donation to the donor and mechanisms to accurately capture and store critical information. It is preferable to use electronically readable information (e.g., barcodes) and to have data saved electronically in a well-structured Information Technology (IT) system. It is possible to perform traceability in a manual capture and paper-based system; however, it is more laborious and open to errors, requires space for record preservation and storage and is slower.

All organizations involved in the MPHO chain of custody should have an established and well-controlled quality system. Procedures should identify the elements of traceability that must be documented (see Table 3) and staff should be trained, and their competency assessed.

Traceability should be achieved by real-time tracking and recording each step of the lifecycle, from donor screening to the MPHO being transfused/transplanted into the patient or allocated for any other intended use.

COMPLEXITIES OF TRACEABILITY

It is recognized that traceability of MPHO from the donor to the recipient and vice versa is necessary for biovigilance. Many factors impact the effectiveness of traceability.

Each collection of an MPHO, each step in the manufacturing process and each movement of an MPHO from one location to another should be clearly and concisely documented. Where possible, identifiers in records should be electronically readable/storable to avoid potential transcription errors.

In some situations, the MPHO product is collected and infused/transplanted within the same organization. Traceability in this instance is straightforward. In other instances, a single MPHO collection can result in multiple products that may be further divided and/or pooled with components from different MPHO collections. These products may then be distributed to various unrelated sites, further modified or distributed to additional sites. For example, pooling platelets or cryoprecipitate would require both a new product identifier and a confirmed link to the components that were used to create the pool. To further complicate the scenario, components may be combined in large batches for further manufacturing of lot number-based products. Starting material for a cellular therapy treatment for a patient may be collected at one site, sent to one or more sites for processing and ultimately sent to another site for infusion.

The examples below detail a few scenarios that demonstrate the complexity involved with the traceability of an MPHO product.

Red blood cells example

Blood Donor Centre (whole blood collected, processed to produce red blood cell [RBC], tested and distributed) → Transfusion Laboratory X (received RBC and transferred out) → Transfusion Laboratory Y (received RBC, divided into four parts) → Parts A and B transfused to patient 1, Part C transfused to Patient 2 and Part D discarded. Donation is recalled due to information obtained on subsequent donation. In this instance, it must be possible to track the donor and the products made from the donation, from the Blood Centre to the Transfusion Laboratories and then to the point of use, accounting for each product and identifying all recipients.

Platelets example

Transfusion Laboratory receives platelet products from two different blood centres. The transfusion service pools six platelets each from a different donor: three platelets originating from Blood Centre A and three from Blood Centre B. The pool is given a new identification number (pool number). The pooled product is transfused. The patient contracts an infectious disease. In this instance, the Transfusion Laboratory must notify each Blood Centre of the donation identification numbers in the pooled unit. The Blood Centres must be able to trace back to each source donor, all products derived from the current donations and any previous donations from the donors.

Manufactured cellular therapy product example

Cells are collected, split and sent to three manufacturing facilities. Facility A makes three different products from the original product. The first of these manufactured products is further spit into 100 vials. The second product, from Facility B, is further manufactured and is split into 15 containers. The final product that was sent to Facility C is further processed and sent to six different distributors.

At the core, each institution must have a mechanism to track and trace each MPHO from its point of origin or receipt to its final disposition within that institution. Each step in the collection, manufacturing and distribution processes must be documented transparently. Each holder of the product must be able to provide relevant information to either the previous holder of the product or the subsequent holder of the product.

Traceability records need to be retained for long periods often specified in the regulation. For example, the European Directives require traceability information for blood, cells and tissues to be

TABLE 4 Information to retain for traceability.

Information to retain	Purpose
Information linking the donor to the donation identification number.	To link the MPHO to the donor.
Information linking the recipient of the product to the donation identification number, the product description code and the division identifier of the MPHO.	To identify the specific portion of the MPHO and link it to the recipient.
Information describing the identity of processing facility (if different from collecting facility).	To identify facility(ies) involved in the creation of final MPHO.
Information linking a pooled product to each component in the pool.	To link the pooled product to each MPHO collection in the pool.
All steps in the handling of the product.	To allow investigation of an MPHO if a problem is discovered in the handling and storage of a product.
For all processing steps, a link between the input product and the output products.	To ensure traceability of all products prepared from the starting material.
For all transfers between organizations, retain the sending/receiving organization details.	To ensure traceability when responsibility moves from one organization to another.
Final disposition of the product.	To allow traceability of each product and division of an MPHO.
Location and methods of access to those records for traceability.	To allow traceability information to be queried in an efficient manner.

Abbreviation: MPHO, medical products of human origin.

retained for 30 years following clinical application. Over long periods, information storage systems change and may become unusable. Information management plans need to be in place to ensure that storage media are updated regularly and information retrieval procedures are kept up to date. Organizational change (takeovers, amalgamation and closure) can also impact traceability and plans need to be in place to manage archive information. If an electronic system that contains traceability data is replaced with another, it is important to keep traceability in mind when deciding which data need to be converted from the old system to the new one.

RISK MANAGEMENT

Risk is defined as the chance or possibility of some process going wrong with an undesirable outcome. The resources for identifying the risk are various, such as:

- Expert judgement
- Benchmarking
- Customer complaints
- Brainstorming

Risk management is the process of having a contingency plan to prevent risk occurrence or reduce undesirable outcomes as much as possible. According to the International Organization for Standardization (ISO) 14971 for Medical Devices Risk Management Assessment [5], risk management is described as the systematic application of management policies, procedures and practices to the tasks of analysing, evaluating, controlling and monitoring risk. Each facility is responsible for evaluating the risks of its traceability processes and procedures and taking action to reduce those risks.

INFORMATION TO RETAIN

Once key traceability information is established for each MPHO, the ability to trace an MPHO from its origin to its final disposition and vice versa is only as good as the records retained. Retained records should be electronically readable to avoid transcription errors.

To ensure accuracy, permanent records should be created concurrently with collection, processing and distribution steps. At a minimum, sufficient information should be captured to ensure continuity of the traceability pathway between donor and recipient, including key identifiers and links to previous and next points in the pathway (i.e., donation identification number, product code, where received from, where distributed to and date of record). Organizations responsible for collection and clinical application must retain information linking the donation to the donor and recipient, respectively. Additional desirable information includes who created the record, and identification of operators, critical supplies, equipment, testing and critical steps used in the manufacturing process of each product.

National regulations and accrediting agency standards will describe the specific documentation to save and the retention periods. These vary from country to country. For example, the specific time to retain MPHO-related information ranges from 5 years to at least 30 years depending on the specific kind of MPHO, national regulations and accrediting agency standards. Table 4 describes key traceability information to retain and the reason for retention.

WHAT TO DO WHEN DATA IS LOST

If current data (data in use) is lost, a plan should be in place to retrieve data from backup resources, validate it to ensure accuracy and restore it

for ongoing use. The restoration and validation should be fully documented.

Policies, processes and procedures should be in place to assure that backup data is: always readily available, stored appropriately (locally and remotely) to preserve the data and tested periodically to assure integrity. Any legacy equipment required to access data should be available and in working order. Procedures and effective risk management should ensure that catastrophic data loss does not occur. However, if all data is lost and there is no backup or the backup cannot be restored for some reason (disaster, lack of legacy equipment, legacy equipment failure, etc.) the entire process must be documented as to why the backup failed or could not be restored and what will be done to assure that this will not happen again. All policies, processes and procedures should be reviewed to make sure that they are updated and reflect changes for accurate future data restoration.

In some cases, it may be possible to reconstruct the traceability path by a reverse search. For example, if a blood centre loses information on where a product was sent, it may be possible to query all likely recipient organizations to locate the receiving facility indirectly.

DATA MIGRATION

In its simplest form, data migration is the movement of data from one storage area to another where no change to the data is needed, for example, where one storage disc is replaced with another identical disc and data is copied from one to another. However, data migration is generally a lot more complex as manipulation of the data is usually required to move it from one system to another (e.g., when IT systems are upgraded or replaced with a new system).

Moving large amounts of data

When large amounts of data are involved, automated data migration processes should be employed to ensure standardization of the process. Minimizing the amount of human intervention will reduce the potential for error and cut down the time it takes to complete the migration. Data migration can adversely impact operations by causing extended downtime, data integrity issues (which may impact donor, component and patient safety) and costly rework if the migration steps are not adequately designed, controlled and documented.

Gaining an understanding of the data to be migrated and the relationships between data items will help identify which items of data are to be migrated. It is vital to identify all data needed to maintain the traceability chain and ensure those items are included in the migration. Where partial migrations are being run it is possible to miss data essential for traceability which will cause future issues should tracing be required and may irretrievably break the chain. It is less of an issue when all data is being migrated. The main objective then is to ensure that data relationships and context are maintained.

Common issues in data quality

It is common to find some issues with data quality in any IT system, for example, duplication, redundancy and miscoding. It is therefore recommended that a data cleansing exercise is undertaken before data migration. Removing unnecessary data and correcting data values and representation will simplify the design process, reduce the likelihood of errors and cut down the time needed to migrate.

Data mapping/conversion

It is unlikely that two systems will hold exactly the same data in the same format therefore moving data from one system to another usually requires some degree of data mapping and/or conversion. Where data mapping is required, for example, where one coding system is being replaced by another, a one-to-one link has to be created between the old and new code. This mapping will be held in a table and used during the migration process to convert items to the new coding system. If the data has to be traced back, this table will be a vital tool to help understand the history of the data.

Where data has to be converted from one format to another, for example, date format, the rules for the conversion need to be written in such a way that all data variations are considered, and the conversion process results in a standard output. Again, these rules need to be documented as they could be instrumental in understanding what has happened to the data sometime in the future.

Data migration utility

Once the data to be migrated is identified and mapping and conversion rules have been established the utility to carry out the migration needs to be designed and created. The objective should be to create as automated a process as possible that can be run and re-run giving consistent results each time. The data migration utility is essentially an ETL process (extract, transform and load) that pulls the data to be migrated from the original system, transforms it using the mapping and conversion rules to the new system presentation and loads it into the new system. Responsibility for each step may reside with different parties (e.g., multiple system suppliers, IT departments and user departments). Communication, activity and expectations all need to be carefully managed. It is very unlikely that a data migration will be correct on the first run; therefore, this utility should be documented in detail, version controlled and placed under change management from the outset. It could be vital for traceability that the processes, mappings and conversions used in this utility can be retrieved and understood at a future date.

Management of displaced data

As previously noted, systems can differ in the data they hold and need. It is quite usual for the receiving system not to have a defined space for

all the incoming data. Judgements will need to be taken on the criticality of such data and decisions made as to how it will be handled.

If the 'extra' data is deemed to be non-critical, it can be simply discarded when the old system is decommissioned. If, however, it is essential to maintain the traceability chain it can be:

1. left in the old system which then needs to be maintained with read-only access for look-up purposes,
2. moved out to an existing data warehouse-type application or another custom-built data repository,
3. taken to the new system by identifying or creating a suitable space in the new system for the data to reside.

Option 1 will cause issues at some point in the future as technology changes and becomes outdated. Choosing option 3 can add complexity to system development, configuration, data migration and validation, but it has the advantage of keeping all data together in one place.

Management of unavailable data

Another equally common problem for data migration is that the new system may have the ability to hold data items that the old system did not. For a migration, this is not a problem as these data fields are just not populated during the migration. A decision is then needed to determine if they need to be populated going forward and if a historical update is needed and feasible, and if not, they remain empty. A potential problem arises, however, if the population of the data field is mandatory for the new system. Failure to populate may cause operational issues going forward. The solution here is to derive and populate the necessary data item as part of the migration process if possible or to agree on a standard default value for migrated records, ensuring the rules are documented for each data item in question.

Validation

Initial migration runs should be performed on a test system and a thorough validation should be performed. This should involve data checks on a select number of records that cover all critical data combinations, and statistical checks to ensure pre- and post-migration statistics match.

Once the data migration utility has been run and the data is available in the new system, statistical checks should be repeated to ensure that all expected data has been moved. Record sampling should take place to ensure mapping, conversions, derivations and defaults are accurately in place. At this point, backward traceability should be tested to ensure that the traceability chain is not broken. Any issues discovered at this stage should be addressed as it may not be possible to address them once the migration is complete and operation on the new system begins.

TABLE 5 Considerations for good data migration practice.

- Understand the data and how it will fit in its new environment.
- Clean the data removing duplicates, correcting presentation where possible before migration.
- Develop a structured, repeatable and testable migration approach and process.
- Validate and adjust the migration process before operating on the new system.
- Document and record the entire data migration process to aid traceability in the future.

Documentation

Documenting the data migration approach, rules, processes and outcomes is essential to ensure that going forward traceability is supported. Detailed documentation will reduce the risk of data being lost or misunderstood in the future, and it will greatly reduce the cost and effort of traceability exercises as people and years move on; see Table 5 for considerations for good data migration practices.

ARCHIVING

Regulations in many parts of the world require that traceability information be retained for very long periods, for example, in the European Union, for at least 30 years for blood, tissue and cell products [6, 7].

This creates a significant challenge for information systems as developments in technology mean that both information systems and storage technologies are likely to undergo multiple transformations within the lifetime of the data. To meet traceability obligations, organizations are faced with a choice between:

- migrating large amounts of historical data each time they upgrade their computerized systems,
- retaining superseded systems as 'read only' archive storage and
- creating distinct archive storage and retrieval systems.

Historical data need to be managed and protected to meet the same standards of confidentiality and security as the live database.

Uniqueness of identifiers

Effective traceability depends upon key identifiers remaining unique throughout the domain that the MPHO circulate in and across the period of record retention. The donation identification number provides the key identifier for an MPHO donation, its samples and test results, and for each of the products derived from the donation. The donation identification system must be able to ensure that donation identification numbers are not repeated within the data retention period. The ISBT 128 international standard uses a donation identification number structure that ensures uniqueness at the global level for a period of 100 years [8]. If

historic records do not use such a numbering system, there may be duplication of identifiers over time. The archive system needs to accommodate this and ensure the integrity of the distinct records.

Inter-organization tracking

When MPHO are shipped to one facility and then transferred to another facility, there is the possibility of breaking the chain of identity when products are renumbered and the link between the two numbers is broken. Policies and procedures that ensure complete data recording and methods to search for renumbered units should be included in the policies and procedure manual of the facility responsible for the renumbering.

Mergers/acquisitions/closure

The ability to trace MPHO may be lost when facilities are merged or closed. To ensure traceability, the facility must have a plan for data preservation. In some countries, a governmental agency may be responsible for the data. In other situations, it may be the responsibility of the management of the facility to have a plan for the storage of, and access to, the data from the closed or merged facility.

Managing historical data

Approaches to managing historical data include:

- purging—removing the date entirely,
- reversible archiving—removing data from immediate access with the ability to restore access and revise the information,
- irreversible archiving—removing data from immediate access, viewable only with no ability to modify the data,
- archiving into a repository readable by the original application,
- archiving into a different format, like a data warehouse,
- maintaining legacy systems—systems that the software vendor no longer supports.

Where data must be converted from one format to another (e.g., date format), the rules for the conversion need to be written in such a way that all data variations are considered, and the conversion process results in a standard output. Again, these rules need to be documented as they could be instrumental in understanding sometime in the future what has happened to the data.

Consideration needs to be given to deleting archived data once the need for retention has passed. Data protection regulation permits data to be retained when there is a legitimate need. Once the regulatory retention period has expired, there is no longer a legitimate need to hold the data and failing to delete it may lead to infringement of data protection regulation.

Technology obsolescence

Archived data is usually stored on media, and using software applications, that are current at the time of archiving. Over the long periods required for storage, technology will have moved on and systems capable of reading the media may be rare or unobtainable. The archiving strategy should be reviewed at defined regular intervals and data transfer to modern media undertaken promptly. Such data transfer may be complex, and sufficient time and resources will need to be provided.

Knowledge retention

When archived data is being maintained on older systems, such as read-only legacy systems, it is important to ensure that the skill set required to operate the system is retained. At all times, there should be at least two individuals in the organization who are competent to perform the necessary lookback operations. If the maintenance of the legacy system is contracted to a third party, the same requirement will need to apply, and contracts should specify the need for a minimum number of contractor staff with the necessary skills. Standard Operating Procedures (SOPs) and staff training for utilization of the legacy systems mitigate this problem. Consideration should be given to alternative archiving methods as new technology becomes available.

Security

All systems containing sensitive information should be covered by the organization's data protection and security policies and should be subject to security audits. These policies and procedures must meet the same regulatory requirements as live data. Where data is transferred to more modern archive systems, the old database will need to be handled in a manner that ensures that all sensitive data is removed before disposal. Authority to access or restore information should be limited and may be different from the live system.

Timeliness

Consideration needs to be given to the time required to initiate and perform a traceability action in the archived system. In some cases, particularly where the implicated product may still be in stock, a delay in the traceability search could result in the additional transmission of infection. In general, data will not be archived whilst the product remains in stock, but products with very long shelf lives (e.g., frozen red cells) may remain in the supply chain for very long periods after they were distributed.

The recovery time for archived information will depend upon the archiving strategy employed. Organizations should adopt a risk-based approach to archiving and should establish target recovery times for

archived data that can be verified through a local audit programmed at defined intervals.

TRACEABILITY AUDIT

Periodic audits of IT documents and processes are an essential part of a quality management system. Audits provide evidence that regulatory requirements are being met. The focus of the audit should include high-risk activities as identified in a risk assessment or those critical items in the operational qualification (OQ) and performance qualification (PQ) of the user requirements.

Audit plans

Many regulatory agencies and standard-setting organizations require that there be a written process in place to assure that traceability is defined and audited to assure that MPHO can be traced from donation to final disposition. Requirements include unique identifiers, critical equipment, materials used in processing, lab samples, donor records and patient records. Both internal and external audits should be performed periodically to assure the robustness and continuity of traceability. External audits may be performed as a part of ordinary inspections by accrediting organizations, regulatory bodies, competent authorities or external consultants. The frequency and type of audit may be determined by regulatory or standard-setting organizations.

Audits may be performed once, periodically or may be performed on an ongoing basis. The scope may be broad or limited to one element. Commonly performed audits include lookback tracing, policy and procedure audits, operations audits and tracer audits. An audit schedule and plan should be documented.

Considerations in establishing a traceability audit:

- Responsibility—who will be the signature authority for the audit,
- Scope—what is to be included and what is to be excluded from the audit—be prepared to justify exclusions to regulatory/standard-setting organizations,
- Audit criteria—what goals should be fulfilled to pass the audit,
- Audit checklist—data collection form,
- Resources—qualified people, policies, processes and procedures to be involved,
- Timeline—how long is the audit estimated to take,
- Stakeholders—what other key areas might be involved or disrupted during the audit,
- Audit instructions—how to perform the audit,
- Report—written documentation of the audit and any findings,
- Review—who will be involved in reviewing the audit report once it is complete,
- Corrective action—what needs to be done to bring traceability back into compliance if there are any findings. The corrective action should have a closing date,
- Preventive action—if previously unrecognized areas may cause problems in the future, how can they be changed to prevent the occurrence,
- Lessons learned—what can be done to make traceability more robust going forward,
- Monitoring—scheduled follow-up to assure that the corrective action(s) is/are being followed and are effective.

Barriers/complexities to a traceability audit

Several factors add complexity to auditing information about MPHO. These include:

- Pooled products/divided products—add to the difficulty in tracing the final disposition,
- End-of-life data storage—the lack of availability of needed obsolete equipment, knowledge retention, stability/degradation of data, electronic e-records and accessibility,
- Data migration issues,
- Interface issues,
- Paper records—the inability to locate records promptly, readability after storage, searching a large volume of paperwork and lack of indexing making records difficult to search,
- Restructuring—facilities have relocated, closed, merged, divided or have been taken over; where are the records located that have been held over the required retention period?
- Traceability chain may involve multiple facilities in multiple geographic locations,
- Audit trail vulnerability—single trail (no backup), any break in the chain can disrupt or prevent traceability, linking donors to previous donations at other sites,
- Required uniqueness—identifiers may not be unique (donor identification, donation identification, equipment identification, employee identification, etc.).

What to look for in a traceability audit

Questions that may be asked in an audit include:

- When was the system last audited and what were the findings?
- Has anything changed since the last audit—critical equipment, materials or data storage?
- Are there procedures for this process?
- Is it reviewed regularly?
- Was the process tested?
- Were recommendations in previous audit reports implemented?
- Are there up-to-date contracts with suppliers in place?
- Is there a policy for data retention?
- What is the plan for media/technology obsolescence?
- What is the plan for the next interface or software update?
- Is there a plan for data migration including the resources needed?

Tracer audits

Tracer audits in a hospital would include lookback audits where both the donor and the final disposition of all parts of the product may be tracked and traced from the patient back to the physician order and receipt of the product from the supplier. Tracer audits can be very time-consuming as every aspect of the process is reviewed for appropriate documentation, personnel qualifications and competency assessment, quality control and equipment maintenance records, as well as matching policies and procedures to documentation. Examples of records that may be reviewed as a part of a tracer audit are listed below:

- Order—physician and authorized caregiver privileges,
- Specimen collection—phlebotomist, date, time and tube type,
- Receipt in the laboratory—assessment of suitability, storage,
- Testing—manual, automated and following reagent/equipment manufacturer's instructions,
- Personnel—qualifications, training and competency assessment,
- Equipment—receipt, quality control, calibration, IQ, OQ, PQ and maintenance,
- Supplies and Reagents—lot numbers, receipt and disposition of unacceptable,
- Proficiency testing,
- Reporting—validation of transfer of information,
- Computer—validation, version control and problems,
- Critical value notification—notification and all elements documented,
- Caregiver response—action was taken as needed,
- Policies and procedures—up to date, followed.

Audit report

After the audit, an audit report summarizing the finding should be written and include:

- Summary of the data reviewed—inclusive of dates and types of records reviewed,
- Acceptance criteria,
- Total number of patient records, units or products reviewed,
- How many (and percentage) met the acceptance criteria,
- Comparison with previous audit results,
- Incidental findings.

It is the management's responsibility to review the report, make recommendations and ensure action was taken to correct existing problems and implement preventative actions.

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CONFLICT OF INTEREST STATEMENT

M.C. is the CEO of Swiss SCWeb company and the scope of the company is 'Design and development of specific application software for tracking of healthcare professionals' activities'.

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Vox Sanguinis ISBT International Society of Blood Transfusion

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

DOI: 10.1111/vox.13492

CORRIGENDUM

Vox Sanguinis ISBT International Society of Blood Transfusion

We would like to draw the reader's attention to an error in the following publication:

Abstracts of the 33rd Regional Congress of the ISBT Gothenburg, Sweden 17–21 June 2023. Vox Sang. 2023;118(Suppl 1): 119–379. https://onlinelibrary.wiley.com/doi/10.1111/vox.13433_1

Abstract P158 should be added to the abstract publication.

Evaluation of three different platelet pooling sets at Croatian Institute of Transfusion Medicine

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Background: Preparation of platelet concentrates (PC) from buffy coats (BC) at Croatian Institute of Transfusion Medicine (CITM) accounts for more than 80% of total PC production. Platelet recovery and in vitro quality of PCs during their shelf life can vary depending on the blood bank setting and the PC pooling set used, so in-house evaluation of different sets for platelet recovery and quality parameters is important before choosing the most appropriate set.

Aims: To examine the recovery of platelets and quality of platelet products in different pooling sets.

Methods: In CITM, four BC are used for PC pool. All products are leukoreduced and stored in platelet additive solution. Shelf life of pooled PC is 7 days. Results for sets from two producers were compared with results of currently used sets (sets named A, B, C). One set was excluded early from further analysis due to very low recovery and haemolytic appearance of PCs and is not part of this analysis.

Results: The testing was done on 48 PC in total. The main results are summarized in Tables 1 and 2. All products had preserved swirling (3+) and no visible aggregates till the end of the shelf life. Change in metabolic parameters was expected and in day 7 in comparison with day 1 was as follows: pH (set A + 3%, set B + 2%, set C + 2%); glucose (set A−41%, set B−44%, set C−47%); lactic acid (set A + 102%, set B + 117%, set C + 143%). Significant difference was observed in the recovery of platelets and platelet number. Set A had lower recovery than sets B and C ($t = -6.4$ and -8.8, respectively; $p < 0.001$, t test). Set B had higher platelet number (1st day) than sets A and C ($t = 3.2$ and 2.8, respectively; $p < 0.05$, t test).

TABLE 1. Results of recovery and platelet quality; numbers are expressed as average (minimum–maximum), 1st day of storage.

Parameter	Set A $N = 20$	Set B $N = 20$	Set C $N = 8$[a]
Recovery (%)	73 (69–78)	79 (70–89)	79[a] (73–87)
Platelet number $\times 10^{11}$	2.8 (2.05–3.58)	3.13 (2.45–3.79)	2.89[a] (1.99–3.66)
WBC $\times 10^{6}$	0.255 (0.004–0.824)	0.373 (0.06–0.772)	0.032[a] (0.003–0.244)
pH	7.14 (7.1–7.18)	7.13 (7.07–7.17)	7.14 (7–7.19)
Glucose (mmol/L)	7 (6.4–7.6)	6.9 (6.5–7.8)	6.95 (6.7–7.3)
Lactic acid (mmol/L)	5 (4.1–5.9)	4.7 (4.2–5.1)	4.4 (4–4.8)

[a] Set C recovery, platelet number ($N = 56$) and WBC ($N = 52$) data were taken from prior verification.
Abbreviation: WBC, whiste blood cell count.

TABLE 2. Platelet quality; numbers are expressed as average (minimum–maximum), 7th day of storage.

Parameter	Set A $N = 20$	Set B $N = 20$	Set C $N = 8$
Platelet number $\times 10^{11}$	2.2 (1.63–2.8)	2.59 (2.17–3.16)	2.62 (2.32–3.14)
pH	7.34 (7.25–7.39)	7.27 (7.19–7.34)	7.28 (7.08–7.41)
Glucose (mmol/L)	4.1 (3.4–5.2)	3.9 (3–5.3)	3.7 (2.6–4.6)
Lactic acid (mmol/L)	10.1 (8.6–12.3)	10.2 (8.9–12.1)	10.7 (9.1–13.3)

Summary/Conclusions: Metabolic parameters at the end of the shelf life were comparable for all the examined sets, but owing to a higher number of platelets, set B was considered the most relevant choice. However, there are also other factors that need to be considered individually in every blood bank when choosing the most appropriate materials for component production (e.g., compatibility of new material with existing equipment and processes, methods of component production, etc.).