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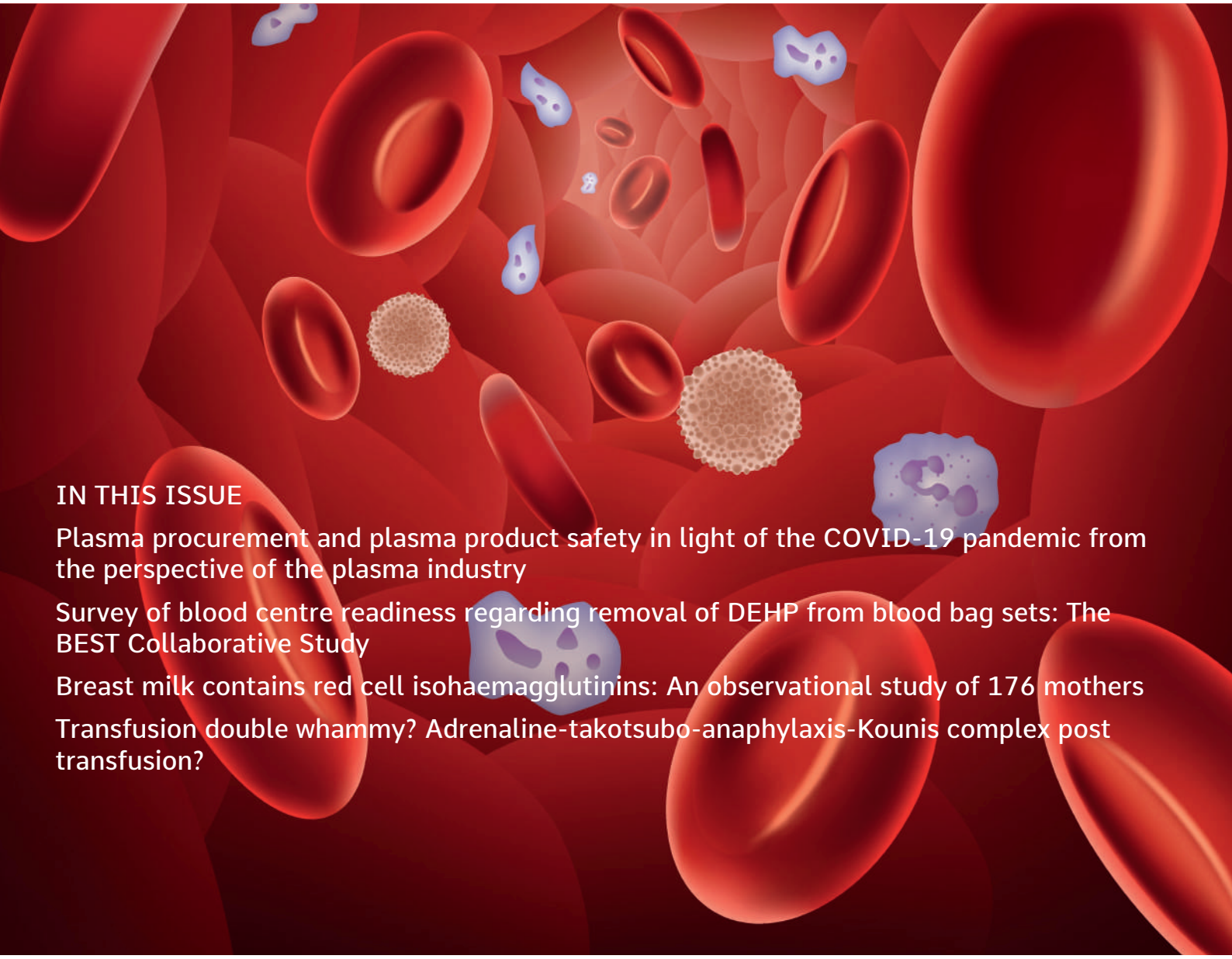

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ISBT International Society of Blood Transfusion

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International Journal of Blood Transfusion

Official Journal of the International Society 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 ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

A review of electronic medical records and safe transfusion practice for guideline development

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Abstract

Background and Objectives: Electronic medical records (EMRs) are often composed of multiple interlinking systems, each serving a particular task, including transfusion ordering and administration. Transfusion may not be prioritized when developing or implementing electronic platforms. Uniform guidelines may assist information technology (IT) developers, institutions and healthcare workforces to progress with shared goals.

Materials and Methods: A narrative review of current clinical guidance, benefits and risks of electronic systems for clinical transfusion practice was combined with feedback from experienced transfusion practitioners.

Results: There is opportunity to improve the safety, quality and efficiency of transfusion practice, particularly through decision support and better identification procedures, by incorporating transfusion practice into EMRs. However, these benefits should not be assumed, as poorly designed processes within the electronic systems and the critically important electronic–human process interfaces may increase risk while creating the impression of safety.

Conclusion: Guidelines should enable healthcare and IT industries to work constructively together so that each implementation provides assurance of safe practice.

KEYWORDS
decision support, electronic medical records, patient identification, transfusion

Highlights

- Incorporating transfusion into electronic medical records may provide opportunities to improve practice through implementation of decision support and robust patient identification procedures.
- There are risks if implementation does not consider and integrate with local workflow and culture.
- Guidelines should assist healthcare organizations and vendors to develop and implement systems to ensure electronic systems and their integration into clinical environments mandate safe practice.

INTRODUCTION

The transfusion of blood and blood products are common procedures required in a diverse range of medical and surgical specialties. Systems have been developed to improve the quality and safety of blood transfusions. The integration of blood product administration into institution-wide electronic medical record (EMR) systems has the potential to improve safety. However, variability in electronic systems and their integration into institutions may pose risks. Guidance on how information systems should be designed and implemented to retain and maximize transfusion safety can assist software developers and institutions to build and maintain safe transfusion infrastructures.

Double independent checking of patient identification and of blood products is recommended immediately prior to transfusion [1]. There is evidence for this approach from medication administration, particularly where calculations of doses and rates are required. However, it is acknowledged that the evidence base for this approach in transfusion is weak. By contrast, the evidence suggests that patient safety is improved with the use of electronics-facilitated identification.

In 2019, transfusion practitioners of the Australian and New Zealand Society of Blood Transfusion (ANZSBT) expressed concern about the implementation of transfusion practices into EMRs [2]. Electronic systems were sometimes perceived as sub-optimal, being mostly sourced from the United States with variable adaptation to local healthcare systems. Frequently, serious concerns were raised regarding the discrepancy between the expectations of clinician users, what was provided by software vendors and the implementations of these systems within institutions. Electronic systems may be purchased as 'solutions', but their implementation must account for variances in clinical workflow practices and human factors, and integrate into human systems.

Limited guidance creates difficulties for the implementation and maintenance of information technology (IT) solutions. Vendors developing products may be unclear of the expectations and standards they need to fulfil. Hospitals may inadvertently overlook the importance of their own processes and human components of the system in achieving the desired outcomes. Both may underestimate the importance of integrating clinical workflow practices and the need to invest to ensure safe patient care.

For these reasons, local 'Guidelines for the Implementation of Electronic Medical Records for Transfusion' were proposed by the ANZSBT. A draft guideline was developed by the Clinical Practice Improvement Committee, which was then subjected to widespread consultation, including an online forum facilitated by the Australian Red Cross Lifeblood. Through this process, common themes emerged in different institutions regarding problems with IT solutions for transfusion practice. It was apparent that practicing clinicians were concerned that safety gains in transfusion may be lost and that despite the best intentions, poor implementation could embed risk-prone practices, leaving room for significant variability in the information documented.

EVOLUTION OF MEDICAL RECORDS

Medical records are primarily used to record information about patients so that healthcare practitioners can maintain continuity of care serving as an aid memoire to individual clinicians and the transfer of information to other practitioners. Although a blank paper patient progress note serves this purpose with a high level of flexibility, an organized platform for cumulative measurements, such as observations or fluid balance, enables clinicians to compare and detect changes not easily seen among free text. Similarly, having specific processes for recording medications or infusions provides a single location to check treatments and alerts, improving prescribing and administration safety in an institutional setting. The recording of information also has secondary purposes, whereby it provides indelible evidence of that episode of patient care. This may be for internal audit, research, legal proceedings or for demonstrating compliance with clinical standards.

With dedicated processes for recording certain information, institutions can standardize procedures around these to improve patient care. In the case of observations, early warning systems to detect deteriorating patients can be implemented by using the forms recording data to help interpret and guide clinicians at the bedside [3, 4]. In the case of transfusion, unique processes for prescription or administration may be opportunities to ensure that consent has been obtained, to remind administering staff of transfusion processes or to detect and manage transfusion reactions. The medical record is therefore evolving to facilitate or enforce best practice to improve patient care rather than simply record the events as they occur.

The development of EMRs has often involved harnessing these benefits by individual clinical areas with technology tailored to their particular needs. These may allow systems to be developed that optimize specialty-specific functionality; however, procedures such as transfusion, which cross many clinical areas, may not be well served. Transfusion-specific software has therefore been developed [5, 6], which may increase the complexity of the information systems required in a work environment.

CURRENT GUIDANCE FOR TRANSFUSION IN EMRs

In the United States, blood bank IT systems are regulated by the Food and Drug Administration (FDA), whereas EMRs are specifically excluded from FDA regulation [7, 8]. FDA advises that users validate systems and not just their software [9]. The former includes the transfer of information between software components and the integration into the human environment. Despite concerns regarding the risks associated with electronic prescribing of blood products, they are yet to come under FDA oversight [10]. The Office of the National Coordinator for Health IT provides certification of the technical requirements of EMRs, but they are unable to assess the safety of these systems when integrated into clinical environments [8]. Outside the United States, there is even less regulation or guidance. Japanese

guidelines for blood product administration using EMRs have been published and require double independent checking of blood products prior to transfusion [11]. This may discourage the use of point-of-care identification technologies, which have proven ability to reduce identification errors [6, 12–18].

OPPORTUNITIES

EMRs potentially enable access to patients' clinical histories from multiple locations in an organized and rapidly accessible format, with data access restricted by appropriate delegation. Information may be recorded instantaneously and indelibly and accessed without the need to trace paper-based notes. EMRs have been implemented in many centres, and as technology progresses, their continuing adoption appears inevitable.

Decision support

The provision of advice about the appropriateness of transfusion at the time of request may be used to support best practice and has been implemented in paper-based formats. Electronic decision support has also been used for transfusion prescribing, comparing the clinical details, typically the haemoglobin concentration, with accepted transfusion guidelines and allowing or requesting further information before ordering blood [19–23]. Decision support should guide appropriate care but needs to be carefully implemented so as not to prohibit practices that may be appropriate under some circumstances, or to allow dangerous practices. These processes may be used to indicate to prescribers when product modifications, such as irradiated or washed products, are required. Linking this to comprehensive EMRs opens the possibilities to improve patient safety, for example, suggesting irradiation when there is a history of purine analogue prescription or Hodgkin lymphoma, as situations where failure to irradiate has been commonly reported [24].

Decision support may also be applied at the point of care to assist in other aspects of transfusion, such as the investigation and management of adverse transfusion reactions [25]. Paper-based systems may already suggest appropriate investigations of suspected transfusion reactions and these may be mirrored in electronic systems. There may be opportunities in fully integrated systems to capture changes in patients' observations and suggest interventions [26]. The use of electronic systems in this way is not new to healthcare and has been used in a variety of settings [27]. Bringing these quality management processes into clinical transfusion practice offers potentially further improvement in patient safety.

Identification safety

Incidents of wrong blood in tube are a significant source of error in transfusion practice and have been refractory to many interventions [28]. Transfusion of blood to the wrong patient is an uncommon, yet recurring, system failure. Both these events can lead to transfusion of ABO-incompatible blood and the risk of acute haemolytic transfusion reactions, and even death. Invariably, ABO-incompatible transfusions result from human error. Electronic identification using patient-specific barcodes or radio frequency identification may minimize errors both at the point of collection and the point of transfusion [14–16, 29, 30]. Systems have shown reliability, and there are products developed primarily to verify patient identity and the blood product specificity for patients. These systems may also help track blood outside the transfusion laboratory and monitor time out of refrigeration.

RISKS

Patient identification

Correct patient identification is seen as a benefit of electronic systems, but it may also pose risks. Positive patient identification is essential for safe transfusion practice and is an ongoing concern for most transfusion practitioners [2]. It needs to be acknowledged that electronic processes are not able to identify patients—only their identification bands. Misplaced wristbands are a well-recognized cause of misidentification errors [31]. Positive patient identification must therefore remain the first essential step in the process wherever possible. Relying on the wristband may transfer the site of human error from the transfusion process to an earlier process, such as the admitting person placing the wrong identification band on the patient.

Electronic processes may also fail. Wristbands may become wet, worn or displaced [32]. The implementation of EMRs needs to include clear guidance on how electronic process failures are managed. Where systems, such as barcode scanning, are overridden or bypassed, this must be acknowledged and additional safety measures may be required to establish and record patient identification. Ideally, process failures should be tracked, and causes investigated and addressed.

Feedback from end users during our consultations highlighted specific examples of risk. Patient identification was assumed by clinicians using one system that had no linkage between the EMR and the transfusion laboratory information system [2]. In this process, a blood bag barcode was scanned to record it as transfused within the EMR. This was misconstrued by clinical staff, thinking it had electronically verified that the laboratory had issued to the patient. As the electronic process had no capability to do that, it created a risk with the manual independent check seen to be unnecessary.

Another institution reported concerns about sample identification in the emergency department where long-standing processes had developed for nurses to place a cannula and collect blood before a medical practitioner had requested investigations. Samples were being taken unlabelled to a central area to print labels, which was a common cause for wrong blood in tube collections. An electronic ordering process with label printing at the patient's side was proposed; however, examination of the workflow suggested the electronic system could

increase errors, as no labels would be available because they are printed only for tests already requested. Similar issues arise when labels are not immediately printed at the point of collection or when scanning and printing devices are not taken to patients in isolation.

Multiplicity of systems

The different vendors of EMRs who include patient-side blood transfusion checking processes have different strengths and weaknesses that institutions need to consider, both in the software and support. Where a transfusion package is implemented in conjunction with a revised EMR, the transfusion component may only have a single function and ensuring safe transfusion practices may not be prioritized among other EMR features. This highlights the need for an accepted minimum standard for all systems.

Within hospitals, there may be multiple systems used in different departments, as each clinical unit seeks electronic systems to optimize their own workflow using vendors specializing in their field. Furthermore, even when a software vendor is the same, there may be differences in the way it is seen and operates between institutions. For a health workforce that is mobile between facilities, this may create confusion. A particular software package could be interfaced to the laboratory information system in one institution and not in another, for example, creating differences in how transfusions are administered through the software.

Alert fatigue

There is an advantage in electronic systems being able to detect potential errors or risks and alert the user; however, excessive alerts may lead to staff dismissing these as routine [33, 34]. Importantly, the alerts do not have to relate to transfusion processes to induce reduced attention to issues during the transfusion process. Setting alert levels to draw attention to appropriate risks may be difficult. There is no standardized approach, and it requires ongoing review [34]. For example, in medication prescribing, theoretical drug interaction alerts based on pharmacopoeia-reported interactions are common even with combinations that are known to be safe. Too frequent and irrelevant alerts were implicated as a source of misidentification error in our feedback, as was practitioner bias. Having selected a patient (unwittingly incorrectly) in the electronic system, the alert was interpreted as 'not logical' and ignored [2]. Affected healthcare professionals suggested that critical processes should have barriers to progression until serious errors are corrected.

Unfamiliar terminology and processes

Many information systems are developed for larger markets, particularly the United States and Europe. Other jurisdictions have similarities, but the differences can lead to risks. These may be minor but confusing, such as different terminology and purposes for billing-associated numbers. However, others may have a more substantial impact, such as the supply of fractionated blood products through blood banks or pharmacies, which may cause significant difficulties for integrating software into the workflow. These variations are also seen between institutions within the same jurisdiction, and a failure to account for them may lead to misinterpretation and unnecessary ambiguity and risk.

The combination of these issues has led to a perceived increase in errors and near-miss events in our feedback. Electronic systems were seldom isolated as a cause for these errors by institutions. Despite the complexity of changes being faced as new processes were implemented or trialled, the tendency was to see the failure as due to healthcare professionals not following procedures. Proposed corrective actions therefore may seek to address the staff as 'the problem', with education frequently identified as the remedy. Examining human factors would identify the need for electronic and human systems to integrate [35]. Clinicians need to keep pace with advances in their own fields. An EMR that creates additional training needs in order to be used safely poses an additional risk. Electronic systems may remind and prompt correct practice to improve safety; however, if poorly implemented, they may make the task more complex, prevent appropriate care or increase the risks. System design, as we have come to expect from many of our personal electronic tools and devices, should make safe operations easy and intuitive.

IMPLEMENTATION RECOMMENDATIONS

Co-design

Known risks of using an EMR to facilitate an 'end-to-end' transfusion process can be mitigated by ensuring that intended users are included during the design process and pilot phases of implementation. Failure to address this and ensure adequate engagement can lead to failure, which is as high as 50% for all new EMR implementations [36]. The inclusion of end users in the design process and the need to tailor systems have been reported as crucial by one hospital, and simplification of some pathways was needed to enhance the user experience [37]. However, they did report a reluctance of the program design team to modify the program, and this may create a barrier for organizations implementing 'straight out of the box' systems [37]. Options for tailoring and creation of bespoke modules should form part of negotiations to ensure that any program is fit for the purpose. Having accepted standards for vendors and hospitals could reduce the need for excessive design changes.

Education and training

The provision of adequate education and training is also of importance to support the uptake of any new system. Traditional models may not be appropriate for EMR systems, which are inherently

TABLE 1 Summary of Australian and New Zealand Society of Blood Transfusion practical guidelines

Transfusion process	Summary of guidance
Decision to transfuse	Prescription of blood products through an EMR is an opportunity to offer decision support. Decision support, standardized prescription protocols and product information need to be regularly reviewed and updated by transfusion professionals.
Consent	EMRs must have a process for documenting informed consent or refusal in line with institutional policies.
Blood product prescription	Prescriptions must be in accordance with national guidelines. The EMR must have a complete and up-to-date list of blood products, to be prescribed by the product form (e.g., units of red cells) or weight. EMRs should alert prescribers to special transfusion requirements. Special transfusion requirements should be communicated between clinical and laboratory systems. EMRs may use standardized prescriptions for rates of administration but need to maintain flexibility for individual patient needs.
Electronic requests	Requests may include requests for blood sample collection and testing, requests for blood products to be prepared or requests for blood to be delivered. Sample collection requires positive patient identification and labelling at the bedside immediately after collection. Where EMRs assist with patient identification, they must be identified by a barcode or radio frequency identification chip specific to the patient and distinguishing them from the patient record.
Storage and collection	Where blood is issued to a refrigerator external to the laboratory, processes for blood product tracking, identification and collection are required.
Blood product administration	Positive patient identification is required prior to administration of blood products. Independent confirmation of patient and product identification needs to be performed by a second practitioner or the EMR. If performed by the EMR, it must be able to identify the patient, confirm the blood product and group and that it has been specifically issued to the patient.
Special circumstances	Processes must be in place for issuing un-crossmatched emergency blood for critical bleeding and massive transfusion.
Adverse events	Adverse events should be recorded in the EMR and warnings provided to clinicians where a patient has special transfusion risks. Decision support for adverse transfusion reactions should be considered. The role of EMRs in adverse events should be captured and evaluated to determine whether systemic improvements are required.

Abbreviation: EMR, electronic medical record.

TABLE 2 Summary of clinical governance recommendations for EMRs

User roles	User roles must be defined and limited to functions for which users are qualified and authorized. EMRs must record the fate of blood products and maintain traceability of each unit.
Common functions and processes	Sample and product labels must always have written identification in addition to machine-readable identification. Each process should be indelibly recorded. Where a process has more than one operator, all should be recorded.
Overrides	Overrides should be recorded when they are needed. All overrides should be evaluated to determine whether systemic improvements are required. Wherever possible, overrides should not create a process that is easier for the electronic process to avoid shortcuts that may affect safety.
Implementation	Implementation must include integration into human healthcare systems, including the following: • Clear policies and standard operating procedures; • Directions within the EMR to guide correct performance of processes; • Involvement of users in design and implementation; • Maintaining downtime procedures. Systems must be designed for safety, mandating best practice where possible. Appropriate education and training should be provided, but critical safety features should be designed and not dependent on EMR-specific training to maintain safety. Integration between electronic systems should be bidirectional to minimize the chance of error.
Validation and maintenance	Validation is required for all transfusion processes. Interfaces between electronic systems must be specifically validated. Validation needs to consider software, hardware and integration into the healthcare setting, workflow and culture. Validation should ensure that forcing functions operate within a clinical workflow to achieve the intended aims. Validation should include override and partial system failure procedures. Maintenance and review of policies and procedures should occur regularly and involve staff in all stages of the transfusion process.

Abbreviation: EMR, electronic medical record.

complex and may not be intuitive to the user. Often, 'train the trainer' processes are relied upon, whereby a small pool of 'super-users' are provided with comprehensive systems training, who are then responsible for training remaining staff in the use of the EMR [37]. This has been demonstrated to be insufficient, and organizations should consider individualized training for all intended end users to maximize the chances of successful implementation. Alternative and customized training that embraces technology should be considered to help ensure relevant support for end users. E-learning modules that are clinically relevant and/or videos can offer a cost-effective way of providing training [36, 38]. However, it is critical that education and training is not used to replace good system design and that safety requirements should be embedded into the IT and workflow processes.

Technological support

Further to providing sufficient education and training, support is needed to assist with troubleshooting and identification of non-functioning equipment. A systematic review published in 2020 reported widespread agreement in the literature that timely and easy access to support is critical to the success and correct use of new EMR systems [39]. Equipment failure or being too cumbersome to

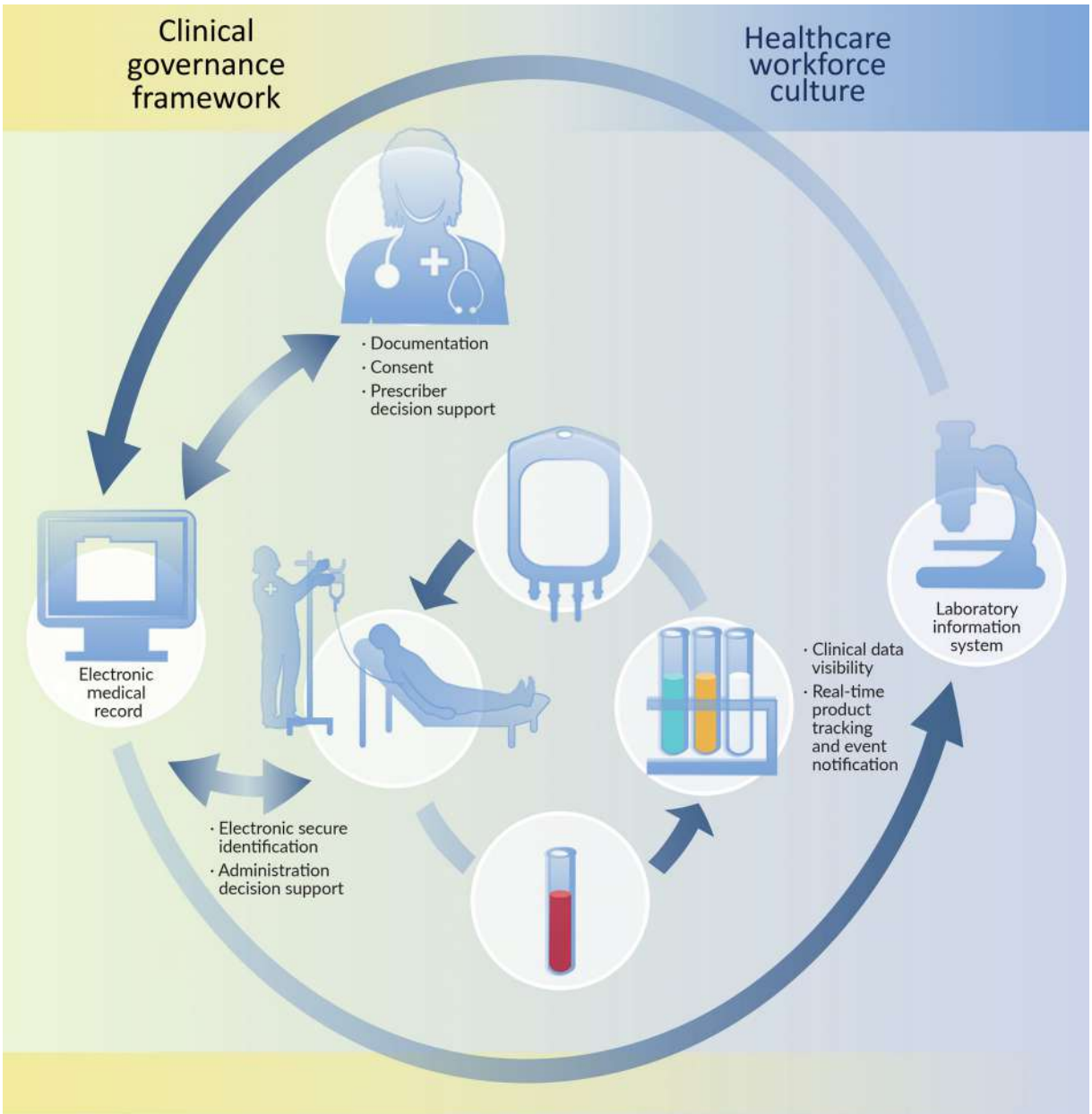

FIGURE 1 Electronic medical records offer potential benefits of targeted decision support for prescribing and administering, and with essential linkages to laboratory information systems, can enhance safety through verification of patient identification. Implementation needs to consider the local workforce culture and be supported by robust clinical governance

operate encourages reversion to traditional paper-based systems. Prioritization of support to the new system is therefore needed to ensure functionality and increase confidence of end users [37].

GUIDELINES

Guidelines promote a set of expectations around electronic system design and clinical practice workflow. Having a set of standards enables vendors to design IT fit for purpose rather than having individual institutions bear the onus and costs of meeting minimum safety design. A summary of key practice points from the ANZSBT is included in Table 1. Guidelines can also create expectations around implementation into the clinical environment, enabling organizations to understand the scope required when considering implementation plans and clinical governance (Table 2).

CONCLUSION

EMRs have many potential advantages to improve hospital efficiency and safety. An inexorable movement to an increasingly sophisticated approach where the data are not simply stored but are interpreted and flagged in order to improve the quality and safety of patient care is to be expected. There are benefits to the introduction of EMRs for transfusion practice. Just as blood bank information systems have transformed blood issuing through the electronic cross-match, widespread adoption in clinical areas is expected also to improve safety and efficiency. However, the software packages themselves are not the complete solution. Vendors and institutions need to incorporate safe system design, understand variances in clinical workflows and the human element in healthcare, and design and implement processes that guide safe and appropriate patient care, while warning and at times prohibiting processes that may be unsafe (Figure 1).

These are goals the transfusion community should embrace. As more holistic systems are developed, transfusion practices will be a small part of larger systems. Institutions and vendors need guidance on how systems should be built and implemented in order that the goals of more efficient and safer transfusion practices are incorporated into all stages of the development process. Guidelines have been developed by the profession for blood banking systems in order to drive change appropriately in the laboratory. Guidelines for EMRs to facilitate clinical transfusion should likewise signpost the future of digitally assisted practice improvement.

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P.C. led the consultation and wrote the initial draft of the paper. T.V. led the consultation and revised the manuscript. C.A., K.B., A.D., A.K., F.K. and A.O. reviewed and wrote the manuscript.

CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare in relation to this manuscript.

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REFERENCES

1. Australian and New Zealand Society of Blood Transfusion. Guidelines for the administration of blood products. Sydney, Australian and New Zealand Society of Blood Transfusion (2019).
2. Verrall T. Electronic medical records: implications for clinical transfusion practice. Adelaide, BloodSafe eLearning (2019).
3. Bell D, Baker J, Williams C, Bassin L. A trend-based early warning score can be implemented in a hospital electronic medical record to effectively predict inpatient deterioration. Crit Care Med. 2021;49: e961–e967.
4. Hall KK, Lim A, Gale B. The use of rapid response teams to reduce failure to rescue events: a systematic review. J Patient Saf. 2020;16: S3–7.
5. Albornoz MA, Marquez S, Rubin L, Luna D. Design of a mobile application for transfusion medicine. Stud Health Technol Inform. 2017; 245:994–8.
6. Miller K, Akers C, Magrin G, Whitehead S, Davis AK. Piloting the use of 2D barcode and patient safety-software in an Australian tertiary hospital setting. Vox Sang. 2013;105:159–66.
7. Sharma G, Parwani AV, Raval JS, Triulzi DJ, Benjamin RJ, Pantanowitz L. Contemporary issues in transfusion medicine informatics. J Pathol Inform. 2011;2:3.
8. Food and Drug Administration Guidance for Industry and Food and Drug Administration Staff: Changes to existing medical software policies resulting from section 3060 of the 21st century cures act. Maryland US Department of Health and Human Services. (2019).
9. Food and Drug Administration: Guidance for industry: blood establishment computer system validation in the user's facility. Maryland, US Department of Health and Human Services (2013).
10. Schiff G, Wright A, Bates D, Salazar A, Amato MG, Slight SP, et al. Computerized precriber order entry medication safety (CPOEMS). Maryland, Food and Drug Administration (2015).
11. Ohsaka A, Kato H, Kino S, Kawabata K, Kitazawa J, Sugimoto T, et al. Recommendations for the electronic pre-transfusion check at the bedside. Blood Transfus. 2016;14:419–24.
12. Davies A, Staves J, Kay J, Casbard A, Murphy MF. End-to-end electronic control of the hospital transfusion process to increase the safety of blood transfusion: strengths and weaknesses. Transfusion. 2006;46:352–64.
13. Uriz MJ, Antelo ML, Zalba S, Ugalde N, Pena E, Corcoz A. Improved traceability and transfusion safety with a new portable computerised system in a hospital with intermediate transfusion activity. Blood Transfus. 2011;9:172–81.
14. Nuttall GA, Abenstein JP, Stubbs JR, Santrach P, Ereth MH, Johnson PM, et al. Computerized bar code-based blood identification systems and near-miss transfusion episodes and transfusion errors. Mayo Clin Proc. 2013;88:354–9.
15. Murphy MF, Jayne Addison J, Poles D, Dhiman P, Bolton-Maggs P. Electronic identification systems reduce the number of wrong components transfused. Transfusion. 2019;59:3601–7.
16. Chan JC, Chu RW, Young BW, Chan F, Chow CC, Pang WC, et al. Use of an electronic barcode system for patient identification during blood transfusion: 3-year experience in a regional hospital. Hong Kong Med J. 2004;10:166–71.
17. Khammarnia M, Kassani A, Eslahi M. The efficacy of patients' wristband bar-code on prevention of medical errors: a meta-analysis study. Appl Clin Inform. 2015;6:716–27.

18. Askeland RW, McGrane SP, Reifert DR, Kemp JD. Enhancing transfusion safety with an innovative bar-code-based tracking system. Healthc Q. 2009;12:85–9.
19. Derzon JH, Clarke N, Alford A, Gross I, Shander A, Thurer R. Restrictive transfusion strategy and clinical decision support practices for reducing RBC transfusion overuse. Am J Clin Pathol. 2019;152:544–57.
20. Dunbar NM, Szczepiorkowski ZM. Hardwiring patient blood management: harnessing information technology to optimize transfusion practice. Curr Opin Hematol. 2014;21:515–20.
21. Goodnough LT, Hollenhorst MA. Clinical decision support and improved blood use in patient blood management. Hematology Am Soc Hematol Educ Program. 2019;2019:577–82.
22. Kassakian SZ, Yackel TR, Deloughery T, Dorr DA. Clinical decision support reduces overuse of red blood cell transfusions: interrupted time series analysis. Am J Med. 2016;129:e13–e20.
23. Shah N, Baker SA, Spain D, Shieh L, Shepard J, Hadhazy E, et al. Real-time clinical decision support decreases inappropriate plasma transfusion. Am J Clin Pathol. 2017;148:154–60.
24. Elliot J, Narayan S, Poles D, Tuckley V, PHB B-M. Missed irradiation of cellular blood components for vulnerable patients: insights from 10 years of SHOT data. Transfusion. 2021;61:385–92.
25. Shah NK. Decision support in transfusion medicine and blood banking. Clin Lab Med. 2019;39:269–79.
26. Escobar GJ, Liu VX, Schuler A, Lawson B, Greene JD, Kipnis P. Automated identification of adults at risk for in-hospital clinical deterioration. N Engl J Med. 2020;383:1951–60.
27. Varghese J, Kleine M, Gessner SI, Sandmann S, Dugas M. Effects of computerized decision support system implementations on patient outcomes in inpatient care: a systematic review. J Am Med Inform Assoc. 2017;25:593–602.
28. Bolton-Maggs PH, Wood EM, Wiersum-Osselton JC. Wrong blood in tube - potential for serious outcomes: can it be prevented? Br J Haematol. 2015;168:3–13.
29. Kaufman RM, Dinh A, Cohn CS, Fung MK, Gorlin J, Melanson S, et al. Electronic patient identification for sample labeling reduces wrong blood in tube errors. Transfusion. 2019;59:972–80.
30. Pagliaro P, Turdo R, Capuzzo E. Patients' positive identification systems. Blood Transfus. 2009;7:313–8.
31. Howanitz PJ, Renner SW, Walsh MK. Continuous wristband monitoring over 2 years decreases identification errors: a College of American Pathologists Q-tracks study. Arch Pathol Lab Med. 2002;126:809–15.
32. Ohsaka A, Kobayashi M, Abe K. Causes of failure of a barcode-based pretransfusion check at the bedside: experience in a university hospital. Transfus Med. 2008;18:216–22.
33. Ford E, Edelman N, Somers L, Shrewsbury D, Lopez Levy M, van Marwijk H, et al. Barriers and facilitators to the adoption of electronic clinical decision support systems: a qualitative interview study with UKgeneral practitioners. BMC Med Inform Decis Mak. 2021;21:193.
34. Van Dort BA, Zheng WY, Sundar V, Baysari MT. Optimizing clinical decision support alerts in electronic medical records: a systematic review of reported strategies adopted by hospitals. J Am Med Inform Assoc. 2021;28:177–83.
35. PHB B-M. SHOT conference report 2016: serious hazards of transfusion – human factors continue to cause most transfusion-related incidents. Transfus Med. 2016;26:401–5.
36. Jimenez A. E-learning supports EHR implementations. In addition to meaningful use, we need to define meaningful training. Health Manag Technol. 2010;31:22–3.
37. Murphy MF, Staves J, Davies A, Fraser E, Parker R, Cripps B, et al. How do we approach a major change program using the example of the development, evaluation, and implementation of an electronic transfusion management system. Transfusion. 2009;49:829–37.
38. Randhawa GK, Shachak A, Courtney KL, Kushniruk A. Evaluating a post-implementation electronic medical record training intervention for diabetes management in primary care. BMJ Health Care Inform. 2019;26:e100086.
39. Janssen A, Donnelly C, Elder E, Pathmanathan N, Shaw T. Electronic medical record implementation in tertiary care: factors influencing adoption of an electronic medical record in a cancer centre. BMC Health Serv Res. 2021;21:23.

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REVIEW ARTICLE

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Impact of disasters on blood donation rates and blood safety: A systematic review and meta-analysis

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Abstract

Background and Objectives: Timely and adequate access to safe blood forms an integral part of universal health coverage, but it may be compromised by natural or man-made disasters. This systematic review provides an overview of the best available scientific evidence on the impact of disasters on blood donation rates and safety outcomes.

Materials and Methods: Five databases (The Cochrane Library, MEDLINE, Embase, Web of Science and CINAHL) were searched until 27 March 2020 for (un)controlled studies investigating the impact of disasters on blood donation rates and/or safety. Risk of bias and overall certainty of the evidence were assessed using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach.

Results: Eighteen observational studies were identified, providing very low certainty of evidence (due to high risk of bias, inconsistency and/or imprecision) on the impact of natural (12 studies) and man-made/technological (6 studies) disasters. The available evidence did not enable us to form any generalizable conclusions on the impact on blood donation rates. Meta-analyses could not detect any statistically significant changes in transfusion-transmissible infection (TTI) rates [hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV)-1/2, human T-lymphotropic virus I and II (HTLV-I/II) and syphilis] in donated blood after a disaster, either in first-time or repeat donors, although the evidence is very uncertain.

Conclusion: The very low certainty of evidence synthetized in this systematic review indicates that it is very uncertain whether there is an association between disaster occurrence and changes in TTI rates in donated blood. The currently available evidence did not allow us to draw generalizable conclusions on the impact of disasters on blood donation rates.

KEYWORDS
blood collection, blood safety, donor health, donor motivation, transfusion-transmissible infections

Highlights

- It is very uncertain whether there is an association between disaster occurrence and statistically significant changes in transfusion-transmissible infection rates [hepatitis B virus (HBV),

hepatitis C virus (HCV), human immunodeficiency virus (HIV)-1/2, human T-lymphotropic virus I and II (HTLV-I/II) and syphilis] in donated blood.
- The currently available evidence does not allow us to draw any generalizable conclusions on the impact of disasters on blood donation rates.

INTRODUCTION

Transfusion of blood and blood components helps save millions of lives each year. As a result, blood and blood components are included in the Model List of Essential Medicines of the World Health Organization (WHO), which is a list of medicines that need to be available in a functioning health system at all times, in appropriate dosage forms, of assured quality and at prices individuals and the community can afford [1]. Blood services have the important task of maintaining a sufficient and safe blood supply [2]. A voluntary non-remunerated donation system is the best way to maintain a continuous, sustainable and safe supply of blood [3]. To minimize the risk of transfusion-transmitted infections (TTIs), a rigorous donor selection process is in place. The WHO recommends deferring high-risk blood donors based on the results of general donor assessment, donor medical history and TTI risk assessment [2]. In addition, blood donors (or donations) should be screened for, at a minimum, the most common TTIs: human immunodeficiency virus (HIV)-1/2, hepatitis B virus (HBV), hepatitis C virus (HCV) and syphilis.

Blood services must be prepared to respond quickly to changes in the everyday world, such as the occurrence of disasters. The United Nations Office for Disaster Risk Reduction defines a disaster as 'a serious disruption of the functioning of a community or a society at any scale due to hazardous events interacting with conditions of exposure, vulnerability and capacity, leading to one or more of the following: human, material, economic and environmental losses and impacts' [4]. These events are actualizations of hazards, which can be natural (e.g. seismic, meteorological, biological), man-made (e.g. technological, inter-human relationships such as terrorist attacks or war) or mixed (e.g. health-related, deforestation or drought) [5].

When disaster strikes, the blood supply chain may be affected in different ways. Blood supply may be disrupted, for example, because of damaged donor centres or staffing issues, or reduced donor availability. Depending on the disaster type and magnitude, blood demand may increase as a result of higher blood transfusion requirements. Such a scenario could result in blood shortages. Conversely, altruistic responses of potential blood donors, often donating for the first time, may lead to unnecessarily high stocks of blood, which may need to be destroyed because of their limited shelf-life [6]. Blood services should therefore have emergency plans in place to alleviate the stress in case of a disaster. The needs for blood should be assessed, and appeals to the community should be made only if absolutely necessary [7, 8]. Ideally, regular protocols for donor deferral should be maintained in order to ensure blood (component) safety. However, in response to certain types of emergencies where the risk of failure to provide blood would result in greater adverse health outcomes than the risk of issuing (partially) unscreened blood, blood services and the relevant regulatory authorities, governments and stakeholders may choose to deviate from standard procedures [9].

Currently, there is no systematic overview of the literature on the impact of disasters on blood donation rates, and in particular the number of first-time donors. Furthermore, it is unclear whether a disaster would adversely affect the TTI rates. Therefore, we conducted a systematic review to answer the following question: 'In (candidate) blood donors (Population), does the occurrence of a disaster (Intervention/exposure) compared to no disaster (Comparison), affect blood donation rates and/or blood safety (Outcome)?'

MATERIALS AND METHODS

This systematic review was not prospectively registered, nor was a protocol prepared. It was carried out according to the pre-defined methodological standards of the Centre for Evidence-Based Practice [10]. Its reporting adheres to the PRISMA 2020 checklist (Table S1) [11].

Eligibility criteria

Study design and publication type

Studies using an experimental [randomized, quasi- or non-randomized controlled trials, (un)controlled before–after studies or (un)controlled interrupted time series] or observational design [cohort, case–control, (un)controlled before–after, cross-sectional studies and (un)controlled interrupted time series] were eligible for inclusion. Other designs including computational modelling studies, case reports/series, narrative reviews and non-original studies (e.g. editorials, book reviews, and commentaries) were excluded. Conference abstracts were included if the data were not covered by a peer-reviewed publication. Other non-peer-reviewed publications and letters to the editor were excluded.

Population

All (candidate) whole-blood/plasma/platelet donors visiting or contacting blood collection centres were eligible for inclusion, regardless of donor status (i.e. first-time or repeat donors) and the blood

donation system used (i.e. voluntary non-remunerated, family/replacement or remunerated).

Intervention

Studies were included if they investigated the impact of any natural disaster (including, but not limited to cyclonic storms, droughts, floods, avalanches, earthquakes, landslides, tsunamis, tidal waves, acid rain, volcanic eruption, wildfires, storms, hurricanes, typhoons, blizzards, cyclones, heat waves, cold waves, extreme weather, rodent or insect infestation), technological or man-made disaster (including, but not limited to work-place, transport, biological accidents, acts of terrorism, warfare, armed conflicts, displacement of populations, starvation and famine) [12]. Studies were included regardless of whether they were accompanied by an active call to donate or not to donate blood (components). Studies investigating outbreaks (including epidemics and pandemics) were excluded.

Comparison

Studies were included if they compared donation rates and/or safety outcomes during a disaster scenario to those during a non-disaster scenario (e.g. pre-disaster).

Outcome

Studies containing quantitative data on outcomes reflecting blood donation rates and/or blood safety were eligible for inclusion.

For blood donation rates, these included the units of blood donated and the number of blood donors showing up at the blood bank either spontaneously or in response to an active call. Signs of willingness to donate blood in response to an active call to donate or an active call not to donate blood immediately (number of new donor registrations, website visits, phone calls to the blood bank centres) were also included. The number of blood units transfused, blood transfusions performed and patients requiring blood transfusion were not of interest.

Blood safety outcomes eligible for inclusion were positive screening reactivity rates or confirmed infection rates for TTIs, including all bacterial infections by blood-borne bacteria and all viral infections transmissible through transfusion: hepatitis B virus (HBV; hepatitis B surface antigen [HBsAg] and/or anti-hepatitis B core antibody [anti-HBc]); hepatitis C virus (HCV; anti-HCV antibody); human immunodeficiency virus 1 and 2 (HIV-1/2; anti-HIV-1/2 antibodies, HIV p24 antigen); human T-lymphotropic virus I and II (HTLV-I/II; anti-HLTV-I/II antibodies) and syphilis (rapid plasma reagin [RPR] and/or syphilis antibody). Also, studies containing information on blood donor referral rates due to infectious diseases were included. The number of post-transfusion TTIs in transfused patients and positive screening reactivity for non-specific infectious disease markers (e.g. alanine aminotransferase) were not of interest.

Other selection criteria

No date restrictions were applied. Publications in any language were included, provided that an English abstract was available.

Data sources and searches

Five databases were searched from the date of inception up to 27 March 2020: The Cochrane Library (both The Cochrane Database of Systematic Reviews and The Cochrane Controlled Register of Controlled Trials), MEDLINE (using the PubMed interface), Embase (using the Embase.com interface), CINAHL and Web of Science. Search strings comprising index terms and free-text words in title or abstract were tailored to each specific database (Table S2). Furthermore, reference lists and the first 20 related citations in PubMed of the included records were scanned for additional studies.

Study selection and data collection

A team of two reviewers (JL + DO/Luke Delfosse) independently screened titles and abstracts and subsequently full texts guided by the eligibility criteria, using EPPI-Reviewer Web [13] and EndNote [14]. Discrepancies were resolved by discussion, and, where necessary, a third reviewer was consulted (EDB).

Data extraction was performed independently by two reviewers (JL + DO). For each study, the following data were extracted: study design, description of the population, intervention, comparison and outcome(s) of interest. In case of insufficient or ambiguous data, study authors were contacted if contact details were available.

Dichotomous outcome data were expressed as risk ratios (RRs) with a 95% confidence interval (CI). If possible, effect measures were calculated from raw data using Review Manager 5.4 [15].

Risk of bias and GRADE

For each individual study, the quality was appraised by two reviewers independently (JL + DO). Risk of bias was assessed using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) key criteria for observational study limitations ('Inappropriate eligibility criteria', 'Inappropriate methods for exposure variables', 'Not controlled for confounding', 'Incomplete or adequate follow-up', 'Other limitations') [16]. Discrepancies between both reviewers about individual assessments were resolved through discussion.

Next, GRADE was used to assess the overall certainty of the body of evidence for each outcome as 'high', 'moderate', 'low' or 'very low'. Observational studies receive an initial grade of 'low' and subsequently can be downgraded [based on the risk of bias, imprecision, inconsistency, indirectness and publication (i.e. non-reporting) bias] or upgraded (based on large effect, dose–response gradient and plausible confounding) [17].

Data synthesis

If at least two studies provided data on the same outcome within the same treatment comparison, and we did not suspect large heterogeneity in outcome definitions and measurements, random effects meta-analysis was performed using Review Manager 5.4 [15]. Heterogeneity was assessed through visual inspection of the forest plot and by using the X^2-test and I^2 statistic. To investigate whether TTI rates varied with donor status (i.e. first-time or repeat donors), subgroup analyses were performed. The threshold for statistical significance was set at 5%.

In case a meta-analysis was not possible (i.e. data were reported by only a single study) or warranted (i.e. heterogeneity in outcome definitions was observed or suspected), outcome data were presented in a single forest plot (without calculating a total effect size) as a visual aid for result interpretation. Statistical synthesis of these results was deemed inappropriate, and no statements about the consistency of effects across studies or outcomes were made to avoid unintentional vote counting [18].

RESULTS

Search results

Figure 1 shows the detailed PRISMA study selection flow diagram. The primary searches yielded 5621 records. After removal of duplicates, titles and abstracts of the remaining 3813 records were screened. After full-text screening and resolving disagreements, 18 records reporting on 18 unique studies were included.

Study characteristics

A concise overview of the included studies is provided in Table 1. Detailed information on their characteristics is listed in Table S3.

All the 18 included studies [8, 19–35] were observational in nature. Seventeen were uncontrolled before–after studies, whereas the 18th adopted a cross-sectional design [33]. Eleven studies were conducted in Asia (Iran [19, 25, 26, 31], China [23, 27, 29], Iraq/Afghanistan [33], Turkey [32], Taiwan [28] and Sri Lanka [8]). The remaining seven were conducted in North America (United States [21, 22, 30, 34], Haiti [20]), South America (Chile [35]) or Africa (Egypt [24]).

Of the 12 studies providing insight into the impact of natural disasters, the vast majority ($n = 10$) reported on earthquakes hitting Iran (2003 Bam earthquake [19, 26], the 2017 Kermanshah earthquake [25, 31]), China (2008 Sichuan earthquake [23, 29]), Haiti [20], Turkey (1999 Marmara earthquake [32]), Chile (2010 earthquake [35]) and the United States (1989 San Francisco Bay earthquake [21]). The other two studies provided data on the 2004 Indian Ocean earthquake and tsunami [8] and the 2017 typhoon cyclone number 8 warning in Hong Kong [27].

Six studies contained data on the effect of man-made or technological disasters, which included the 9/11 terrorist attacks [22, 34] and the 2013 Boston Marathon bombing [30], the 2011 three-day Egyptian Revolution [24], the 2014 Kaohsiung gas explosions in Taiwan [28] and deployment to combat in Iraq or Afghanistan [33].

Risk of bias and certainty of evidence

The risk of bias in the individual studies is presented in Figure 2. The majority of the studies (12/18) did not adequately control their findings for confounding factors such as logistic issues (e.g. the collapse of blood collection sites during earthquakes) and differences in population demographics (e.g. more first-time and female donors after a disaster). Four studies [21, 23, 29, 33] applied inappropriate eligibility criteria (e.g. comparing a 10-day period after disaster to a 6-month period before disaster), whereas 10 studies did not report sufficient information (mainly on population demographics) to make an appropriate judgement. Follow-up was judged complete and adequate in all but one study [35]. The methods used to measure exposure and outcome variables did not raise any cause for concern in any of the studies.

Based on the risk of bias assessment, the overall certainty of the body of evidence was downgraded by one level for each outcome of interest. For the outcome of proportion of first-time donors, the evidence was further downgraded by one level because of inconsistency due to high levels of unexplained heterogeneity. For the outcomes of HCV, HIV-1/2 and HTLV-I/II reactivity, the evidence was downgraded by one level because of imprecision due to the low number of events and wide 95% CIs around the effect estimates. The outcome of syphylis reactivity was downgraded by one level because of imprecision (wide 95% CIs around the effect estimates and lack of data) and by another level because of inconsistency. As a result, a very low certainty evidence level was assigned to all outcomes of interest, indicating that we are uncertain about these effect estimates.

Synthesis of results

Blood donation rates

Two studies provided effect estimates on the impact of disasters on blood donation rates. In a first study, daily donations displayed a statistically significant increase of 72.6% after the 2008 Sichuan earthquake [29]. In a second study, the mean number of donation attempts was significantly lower after the Boston Marathon bombing, compared to before, but only in the group of donors who had never received a transfusion themselves [30]. The other 14 studies with data on blood donation rates after natural [8, 19–21, 23, 26, 27, 31, 32, 35] or man-made disasters [22, 24, 28, 34] reported only absolute numbers of donations before and after the disaster. As the means and standard deviations were not available and could not be calculated, mean differences could not be estimated and statistical significance

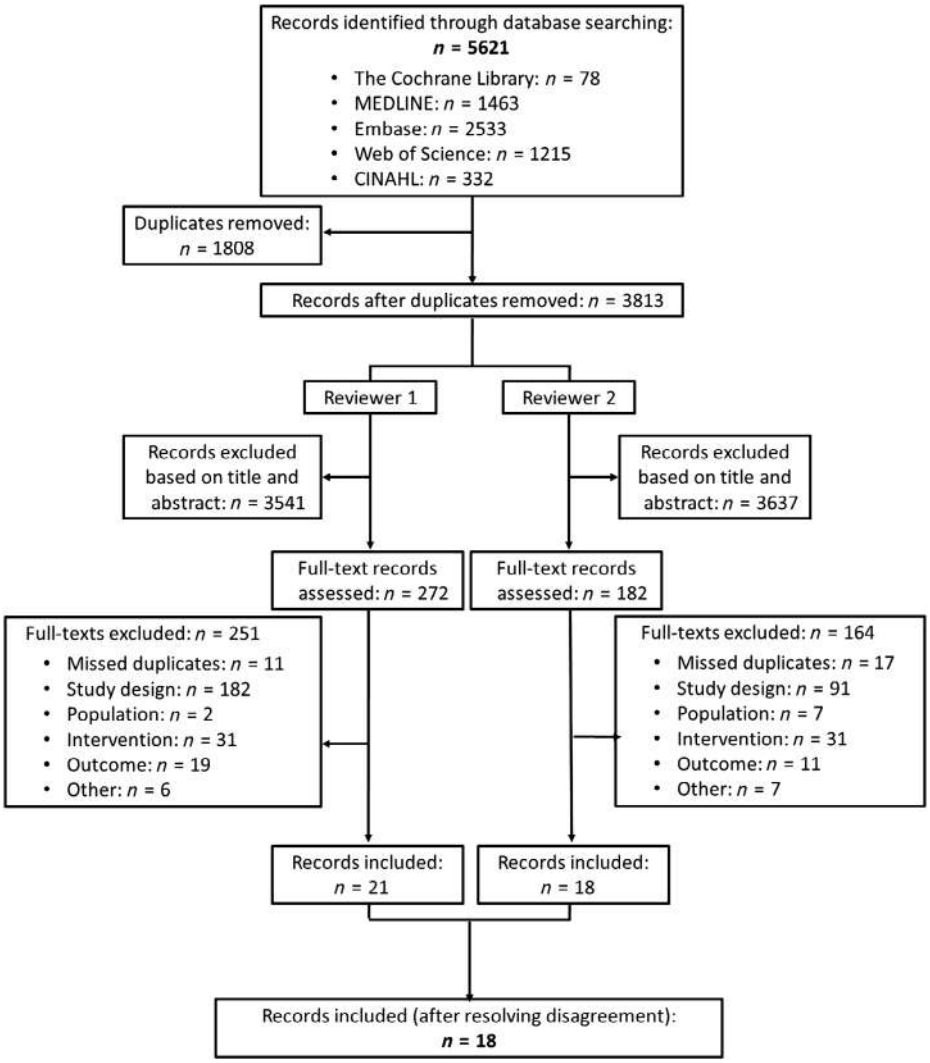

FIGURE 1 PRISMA study selection flow diagram

could not be judged. However, in all but three studies [8, 20, 27], the absolute number of donations after the disaster greatly exceeded the number before the disaster. The same was true for the study that provided data on plasma and platelet donation rates before and after the 2017 Kermanshah earthquake [25].

Owing to very high unexplained heterogeneity (further addressed in Discussion), a meta-analysis on the data of the effect of disasters on the proportion of first-time donors (i.e. the number of first-time donors divided by the total number of first-time and repeat donors) was not warranted. Therefore, the results are presented in Figure 3 and a narrative overview is provided in the paragraphs below.

A first study showed a statistically significantly higher proportion of first-time donors during the 10 days after the 1989 San Francisco Bay earthquake, compared to the 13-day period before the earthquake, both in the immediately affected area of San Francisco Bay and the unaffected area of Los Angeles/Orange Counties [21]. Two studies investigating the impact of the 2008 Sichuan earthquakes revealed a statistically significant increase in the proportion of first-time donors during the week after the earthquake, compared to the corresponding week in the following year [23], and in the proportion of daily donations made by first-time donors during the 6-day period after the earthquake, compared to the other 52 weeks of the same year [29]. Similarly, Kasraian et al. showed a statistically significantly higher proportion of first-time donors during the 3-day period following the 2003 Bam earthquake, compared to the corresponding 3 days in the previous month [26]. In the same way, there was a statistically significant increase in the proportion of first-time donors during the 16-day period following the 2017 Kermanshah earthquake, compared to the corresponding period of the previous year [31].

In contrast, during the 4-day period following the 1999 Marmara earthquake, the proportion of first-time donors was statistically significantly lower, compared to the corresponding 4-day period in the previous year [32]. According to Vásquez et al., there was no statistically significant increase in the proportion of first-time donors during the 5 days after the 2010 Chile earthquake, compared to the 5 days before the earthquake [35].

Following 9/11, there was a statistically significant increase in the proportion of first-time donors during the first, second, third and fourth week after the attacks, compared to the week prior to the

TABLE 1 Concise overview of the included studies

Author, year, country	Disaster	Outcome measure(s)
Observational before–after studies		
Abolghasemi, 2008, Iran [19]	2003 Bam earthquake	Daily average # donations
Björk, 2017, USA [20]	2010 Haiti earthquake	# whole-blood units collected
Busch, 1991, USA [21]	1989 San Francisco Bay earthquake	# donations collected Proportion of FTD HBsAg reactivity
Glynn, 2003, USA [22]	9/11 2001 terrorist attacks	# donations collected Proportion of FTD Weekly infectious disease marker prevalence (anti-HIV, anti-HCV, HBsAg) Weekly anti-HCV prevalence
Guo, 2012, USA [23]	2008 Sichuan earthquake	# donations collected Proportion of FTD HBsAg reactivity
Hussein, 2012, Egypt [24]	Egyptian Revolution	Daily average # donations Proportion of FTD
Jalali Far, 2018, Iran [25]	2017 Kermanshah earthquake	# plasma and platelet donations collected
Kasraian, 2010, Iran [26]	2003 Bam earthquake	# donations collected Proportion of FTD HBsAg reactivity
Kuruppu, 2010, Sri Lanka [8]	2004 tsunami	# donations collected
Leung, 2019, China [27]	2017 typhoon cyclone No. 8 warning	Blood donor attendance
Lin, 2015, Taiwan [28]	2014 Kaohsiung gas explosions	# donations collected Proportion of FTD
Liu, 2010, USA [29]	2008 Sichuan earthquake	Increase in daily donations Proportion of daily donations made by FTD Overall infectious disease marker reactivity (HBsAg, anti-HCV, anti-HIV-1/2, syphilis antibodies)
Rios, 2014, USA [30]	2013 Boston Marathon bombing	# donation attempts
Salah, 2018, Iran [31]	2017 Kermanshah earthquake	# donations collected Proportion of FTD Confirmed TTI seropositivity
Sönmezoglu, 2005, Turkey [32]	1999 Marmara earthquake	# donations collected Proportion of FTD HBsAg reactivity
Tran, 2010, USA [34]	9/11 2001 terrorist attacks	# donations collected Infectious disease deferral rates
Vásquez, 2011, Chile [35]	2010 Chile earthquake	# donations collected Proportion FTD
Observational cross-sectional studies		
Spinella, 2007, USA [33]	Deployment to combat in Iraq/Afghanistan	HBsAg reactivity Anti-HCV reactivity Anti-HIV reactivity Anti-HTLV-I/II reactivity

Note: '#' indicates 'number of'.
Abbreviation: FTD, first-time donors; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HTLV-I/II, human T-lymphotropic virus I and II.

attacks [22]. Similarly, there was a statistically significantly higher proportion of first-time donors during the 3-day Egyptian Revolution, compared to the month before [24]. Finally, Lin et al. showed a statistically significantly higher proportion of first-time donors during the first week after the 2014 Kaohsiung gas explosions, compared to the corresponding week in the previous year [28].

Blood safety outcomes

There is limited evidence showing a lack of association between the occurrence of a disaster and overall infectious disease marker reactivity. A meta-analysis combining data from four studies showed that disaster occurrence was not associated with a statistically significant

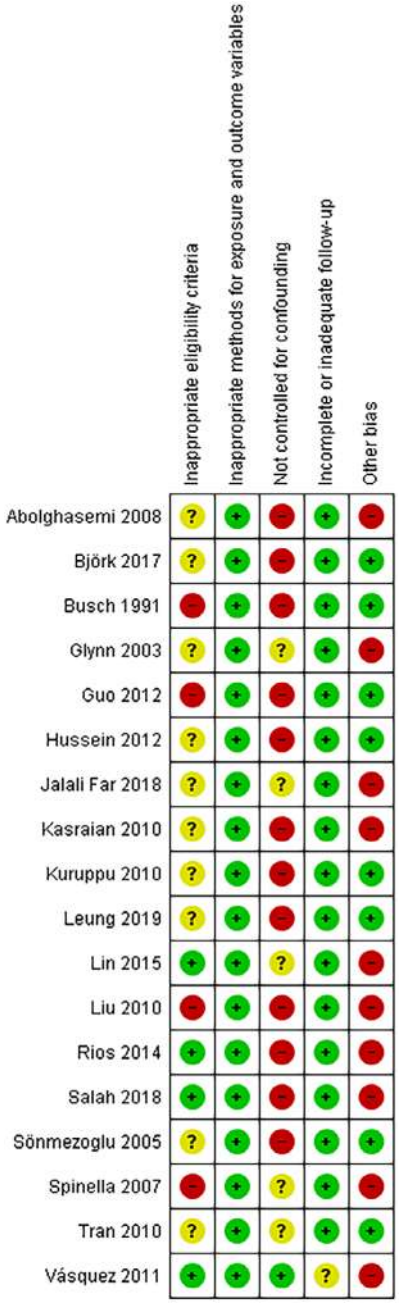

FIGURE 2 Risk of bias in the individual studies. Review authors' judgements about each risk of bias item for each included study. + Low risk of bias. ? Unclear risk of bias. - High risk of bias

increase in overall infectious disease marker reactivity (Figure S1) [23, 31, 32, 34]. In an additional study, the authors failed to report the number of blood donations investigated and reported only the prevalence rates of overall infectious disease marker reactivity (i.e. HBsAg, HCV, HIV-1/2 and syphilis) before and after the 2008 Sichuan earthquake [29]. Because of its lack of raw data, this study, in which a statistically significant increase in reactivity could not be demonstrated, was not included in the meta-analysis. Similarly, a study reporting the weekly infectious disease marker prevalence rates (i.e. HBsAg, HCV, HIV) could not demonstrate a statistically significant increase after 9/11 [22].

In the following paragraphs, the findings for the individual TTIs (HBV, HCV, HIV-1/2, HTLV-I/II and syphilis) are presented.

HBV. In a meta-analysis summarizing results of six studies, a statistically significant increase of HBsAg reactivity in case of a disaster could not be demonstrated (Figure 4a) [21, 23, 24, 26, 32, 33]. Similarly, in the 2008 Sichuan earthquake study, which was not included in the meta-analysis, a statistically significant increase of HBsAg reactivity could not be demonstrated [29]. Similarly, another study could not detect that the 1989 San Francisco Bay earthquake was associated with a statistically significant increase of anti-HBc reactivity in the unaffected area of Los Angeles/Orange Counties, although it did demonstrate a statistically significant increase in the immediately affected area of San Francisco Bay [21].

HCV. In a meta-analysis of five studies, a statistically significant increase of anti-HCV reactivity in case of a disaster could not be demonstrated (Figure 4b) [23, 24, 26, 32, 33]. In an additional study, which reported only the prevalence rates (therefore not included in the meta-analysis), a statistically significant increase of anti-HCV reactivity after the 2008 Sichuan earthquake could not be demonstrated [29]. Similarly, a study reporting weekly infectious disease marker prevalence rates could not demonstrate a statistically significant increase in anti-HCV reactivity after 9/11 [22].

HIV-1/2. In a meta-analysis combining data from six studies, a statistically significant increase of anti-HIV-1/2 reactivity in case of a disaster could not be demonstrated (Figure 4c) [21, 23, 24, 26, 32, 33]. In the 2008 Sichuan earthquake study, which reported only the prevalence rates (therefore not included in the meta-analysis), a statistically significant increase of anti-HIV-1/2 reactivity could not be demonstrated [29].

HTLV-I/II and syphilis. In a meta-analysis summarizing results of two studies, a statistically significant increase of anti-HTLV-I/II reactivity in case of a man-made disaster could not be demonstrated (Figure S2) [21, 33].

Similarly, in a meta-analysis of four studies, a statistically significant increase of RPR or anti-syphilis reactivity in case of a disaster could not be demonstrated (Figure 4d) [21, 23, 24, 32]. In an additional study, which reported only the prevalence rates (therefore not included in the meta-analysis), a statistically significant increase of anti-syphilis reactivity after the 2008 Sichuan earthquake could not be demonstrated [29].

To investigate whether TTI reactivity rates varied with donor status, three subgroups were created: (1) first-time donors only, (2) repeat donors only and (3) mixed donor status (first-time + repeat). None of the performed subgroup analyses revealed between-subgroup heterogeneity, indicating that TTI reactivity rates in first-time donors and repeat donors are not differentially impacted by the occurrence of a disaster.

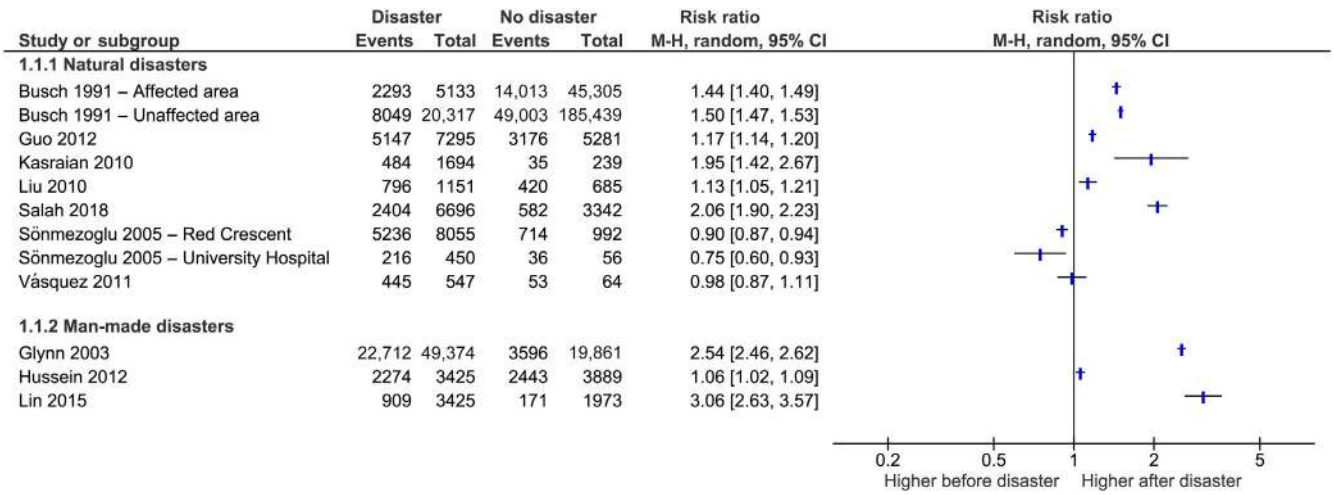

FIGURE 3 Forest plot on the proportion of first-time donors before and after disasters. The proportion of first-time donors presenting themselves to a blood bank in the aftermath of a disaster, compared to prior to the disaster. Events, number of first-time donors; Total, total number of donors

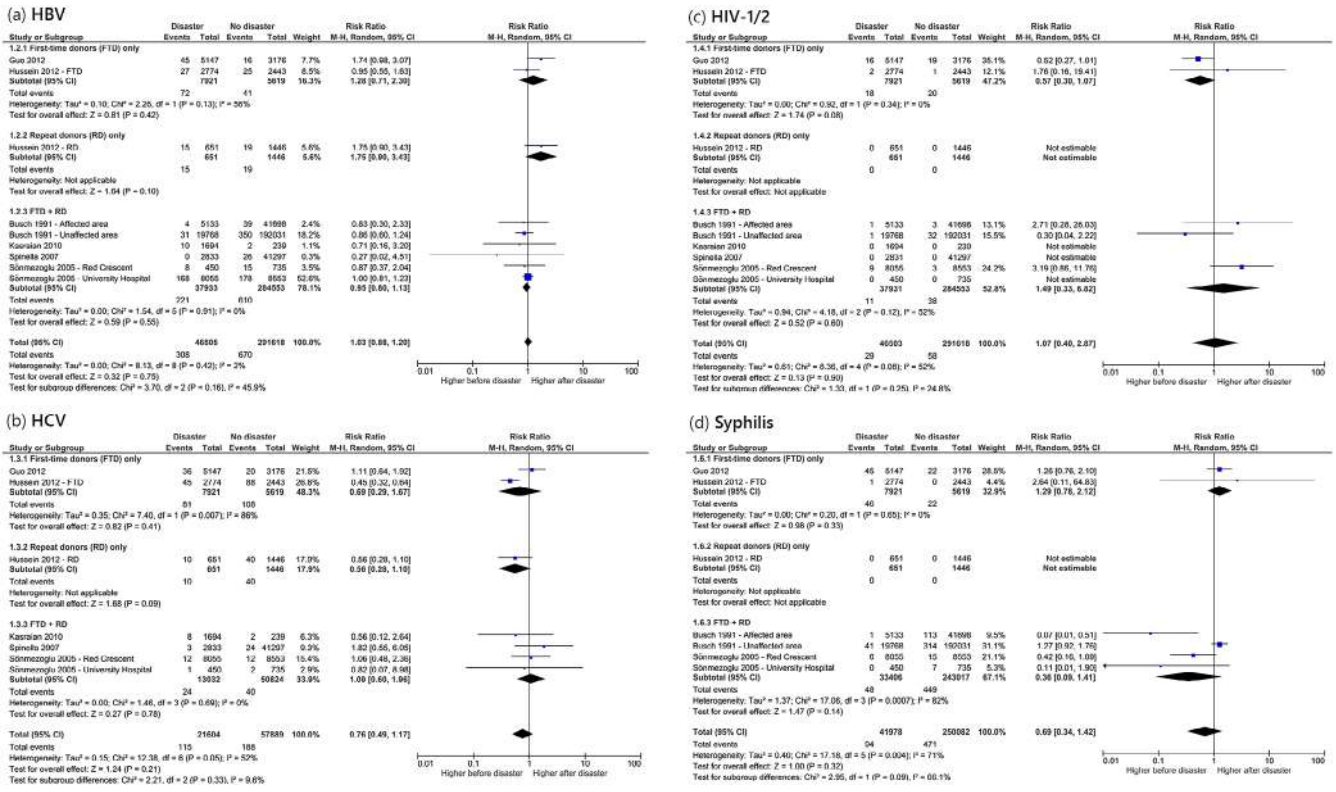

FIGURE 4 Meta-analyses on transfusion-transmissible infection marker reactivity rates before and after disasters. Meta-analysis on (a) HBV, (b) HCV, (c) HIV-1/2 and (d) syphilis marker reactivity rates in donated blood in the aftermath of a disaster, compared to prior to the disaster

DISCUSSION

This systematic review is the first to collect and synthetize the best available evidence on the impact of disasters on blood donation rates and blood safety outcomes, provided by 18 observational studies. Unfortunately, the available evidence did not enable us to form generalizable conclusions on the impact of disasters on blood donation rates. As for blood safety, meta-analyses could not detect any statistically significant changes in TTI rates (HBV, HCV, HIV-1/2, HTLV-I/II and syphilis) in donated blood after a disaster, either in first-time or

repeat donors, although the evidence is very uncertain (all very low certainty evidence).

Altruism had been identified by previous systematic reviews as an important motivator for blood donation [36–38]. Therefore, it is not surprising that in the vast majority of the included studies, disasters were accompanied by an increase in the number of people willing to donate blood. The contrasting decreases observed in four studies might be explained by the destructive effects of the 2010 Haiti earthquake [20] and the 2004 Indian Ocean earthquake and tsunami [8] on the blood collection centres, by the suspension of the Hong Kong blood transfusion services while the typhoon cyclone No. 8 warning was in effect [27], and by the fact that the studied group of donors was confined to repeat donors who had never received a blood transfusion themselves [30]. As multiple studies indicated that the number of donations collected largely surpassed the direct needs following the disaster [6, 19, 22, 24, 29, 32, 39], blood collection services should first assess the need for blood and determine the ability to meet the demand before appealing to the community and mobilizing additional personnel. Discarding unused expired blood units is not only costly for the blood collection services but also creates a negative public image [40], and may lead to a temporary donor shortage later on because of the deferral period after a previous donation. In addition, asking donors to leave and make an appointment later that week [24] can have a negative impact on the willingness to become a regular donor, as donors feel they have been denied the opportunity to help victims.

To investigate whether a disaster coincided with a higher influx of first-time donors, data on the proportion of first-time donors were evaluated. Owing to considerable variation in results and inconsistency in the direction of effect among the different studies, no meta-analysis could be performed. In exploring the causes of this heterogeneity, we considered multiple variables, including the disaster type (natural vs man-made), whether an active call for blood donation was made or not, the blood donation system applied in the corresponding country (voluntary non-remunerated vs remunerated vs family/replacement donation), study setting (high- vs low- and middle-income countries) and risk of bias (high vs low/unclear). None of these variables could be identified as the (suspected) cause of this variation. Possibly, differences in a number of variables that are nearly impossible to measure objectively and accurately, such as the impact level of a certain disaster on society, lie at the root of this variation.

Our review was able to synthetize the available evidence on the influence of disasters on blood safety outcomes. Our meta-analyses could not detect any statistically significant changes in TTI rates (HBV, HCV, HIV-1/2, HTLV-I/II and syphilis) in donated blood after a disaster, either in first-time nor repeat donors. However, caution is warranted, as the results remain uncertain. This does not mean that measures taken to ensure blood safety [e.g. identification of risk behaviour in (candidate) blood donors and performing laboratory testing of all donated blood] should no longer be respected in disaster settings. Stringent measures should always stay in place to maintain the safe collection and administration of blood (components), unless absolutely necessary to meet immediate needs [9]. Moreover, especially in low-income and lower middle-income countries, where only 80.3% and 82% of donations are screened following basic quality procedures [41], national blood policies and legislative frameworks covering blood (component) safety should be implemented.

The current systematic review has several strengths. By searching five relevant databases and adopting comprehensive selection criteria, the review has captured outcome data on blood donation rates and blood safety, both in the absence and presence of disasters.

Nevertheless, this review also has some important limitations. Firstly, we did not consult grey literature, such as research reports and data compilations. As publishing is often not the primary activity of blood services, additional relevant data might not have been captured. Secondly, owing to the observational character of the included studies, the evidence received an initial low certainty level by default. Thirdly, thanks to the stringent blood safety measures applied by the blood services, TTI seroprevalence rates in blood donors are low, thereby hindering the precision of the results for these outcomes, which is reflected in wide confidence intervals and consequently more uncertainty about the findings. Fourthly, because of substantial risk of bias in study design and execution, the overall certainty of the evidence was judged to be very low. In addition, publication bias could not be formally tested, as the meta-analyses performed involved only a limited number of studies. Therefore, the results of these analyses should be interpreted with caution.

In order to reach higher certainty evidence, further transparent and active reporting on the impact of disasters on blood supply and safety is warranted. At the moment, blood collection services seem to lack the reflex of publishing these readily available data in peer-reviewed publications or share these in publicly available reports or data repositories. In addition, the current studies have failed to provide clear and quantitative information on whether they looked at voluntary non-remunerated, family/replacement or remunerated donations (or a combination thereof). As many low-income and lower middle-income countries still heavily rely on family/replacement donations, or even on remunerated donations, which have been reported to be less safe than voluntary donations [3], it would be helpful to gather further evidence on the potentially differential impact of disaster occurrence across different donation systems.

On another note, in light of the global COVID-19 pandemic, future systematic review teams may wish to consider collecting evidence on the impact of (viral or other) outbreaks on blood supply and safety. This evidence may provide useful information to blood services and national health authorities on how to develop, implement and activate emergency response plans in dealing with future pandemic outbreaks.

In conclusion, the evidence synthetized in this systematic review indicates that it is very uncertain whether the occurrence of a disaster is associated with statistically significant changed rates of TTIs in donated blood. Conclusions on the impact of disasters on blood donation rates could not be made.

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CONFLICT OF INTEREST

J.L., D.O., E.V.d.B., E.D.B., V.C. and P.V. are employees of Belgian Red Cross-Flanders, which is responsible for supplying adequate quantities of safe blood (components) to hospitals in Flanders and Brussels on a continuous basis and is being paid for this activity by the Ministry of Social Affairs. E.S. has no conflict of interest.

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REFERENCES

1. World Health Organization. WHO model lists of essential medicines. [cited 2021 Aug 24]. Available from: https://www.who.int/groups/expert-committee-on-selection-and-use-of-essential-medicines/essential-medicines-lists
2. World Health Organization. Blood donor selection: guidelines on assessing donor suitability for blood donation. Geneva: WHO; 2012.
3. World Health Organization, International Federation of Red Cross Red Crescent Societies. Towards 100% voluntary blood donation: a global framework for action. Geneva: WHO; 2010.
4. United Nations Office for Disaster Risk Reduction. Disaster. [cited 2021 Apr 19]. Available from: https://www.undrr.org/terminology/disaster
5. Birnbaum ML, Daily EK, O'Rourke AP, Loretti A. Research and evaluations of the health aspects of disasters, part I: an overview. Prehosp Disaster Med. 2015;30:512–22.
6. Schmidt PJ. Blood and disaster—supply and demand. N Engl J Med. 2002;346:617–20.
7. The role of laboratories and blood banks in disaster situations. Washington, DC: Pan American Health Organization; 2001.
8. Kuruppu KK. Management of blood system in disasters. Biologicals. 2010;38:87–90.
9. GAP. Development of safe and sustainable national blood programmes manual. Perth, WA, Australia: Global Advisory Panel (GAP) on Corporate Governance and Risk Management of Blood Services in Red Cross and Red Crescent Societies; 2014.
10. CEBaP. Development of evidence-based guidelines and systematic reviews: methodological charter. [cited 2021 Jan 20]. Available from: https://www.cebap.org/storage/cebap/inf-methodology-charter-cebap.pdf
11. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6:e1000097.
12. International Federation of Red Cross Red Crescent Societies. Types of disasters: definition of hazard. [cited 2021 Jan 20]. Available from: https://www.ifrc.org/en/what-we-do/disaster-management/about-disasters/definition-of-hazard/
13. Thomas JBJ, Graziosi S. EPPI-reviewer 4: software for research synthesis. EPPI-Centre Software. London: Social Science Research Unit, UCL Institute of Education; 2010.
14. The Endnote Team. EndNote. Philadelphia: Clarivate; 2013.
15. Review manager (RevMan) [computer program]. Version 5.4. The Cochrane Collaboration, 2020.
16. Guyatt GH, Oxman AD, Vist G, Kunz R, Brozek J, Alonso-Coello P, et al. GRADE guidelines: 4. Rating the quality of evidence--study limitations (risk of bias). J Clin Epidemiol. 2011;64:407–15.
17. Atkins D, Best D, Briss PA, Eccles M, Falck-Ytter Y, Flottorp S, et al. Grading quality of evidence and strength of recommendations. BMJ. 2004;328:1490.
18. McKenzie J, Brennan S. Synthesizing and presenting findings using other methods [cited 2021 Jan 20]. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al., editors. Cochrane handbook for systematic reviews of interventions version 6,0. Chichester, UK: Cochrane; 2019. Available from: www.training.cochrane.org/handbook
19. Abolghasemi H, Radfar MH, Tabatabaee M, Hosseini-Divkolayee NS, Burkle FM Jr. Revisiting blood transfusion preparedness: experience from the Bam earthquake response. Prehosp Disaster Med. 2008;23:391–4.
20. Bjork A, Jean Baptiste AE, Noel E, Jean Charles NPD, Polo E, Pitman JP. Geographic distribution of blood collections in Haiti before and after the 2010 earthquake. ISBT Sci Ser. 2017;12:291–6.
21. Busch MP, Guiltinan A, Skettino S, Cordell R, Zeger G, Kleinman S. Safety of blood donations following a natural disaster. Transfusion. 1991;31:719–23.
22. Glynn SA, Busch MP, Schreiber GB, Murphy EL, Wright DJ, Tu Y, et al. Effect of a national disaster on blood supply and safety: the September 11 experience. JAMA. 2003;289:2246–53.
23. Guo N, Wang J, Ness P, Yao F, Bi X, Li J, et al. First-time donors responding to a national disaster may be an untapped resource for the blood centre. Vox Sang. 2012;102:338–44.
24. Hussein E, Teruya J. Evaluation of blood supply operation and infectious disease markers in blood donors during the Egyptian revolution. Transfusion. 2012;52:2321–8.
25. Jalali Far M, Salah A, Shirmohammadi Esfeh M, Khossravi A, Mavali M, Ehtiati M. Blood donation profile in Kermansah earthquake valuable experience for learning to donor recruitment in fixed and mobile collection centers (P-067). Vox Sang. 2018;113(S1):105.
26. Kasraian L. National disasters in Iran and blood donation: Bam earthquake experience. Iran Red Crescent Med J. 2010;12:316–8.
27. Leung K, Lee CK, Lau EHY, Lau CW, Wu JT. Assessing the impact of respiratory infections and weather conditions on donor attendance and blood inventory in Hong Kong. Vox Sang. 2019;114:137–44.
28. Lin KTYC, Tseng SB, Hung CM. Data analysis of blood donors post 2014 Kaohsiung gas explosions (P-046). Vox Sang. 2015;109:39.
29. Liu J, Huang Y, Wang J, Bi X, Li J, Lu Y, et al. Impact of the May 12, 2008, earthquake on blood donations across five Chinese blood centers. Transfusion. 2010;50:1972–9.
30. Rios J, Crumpton A, Khandaker M. Does being or knowing a blood transfusion recipient influence the frequency of blood donation attempts before and after a local act of terrorism? (SP126). Transfusion. 2014;54(S2):106A.
31. Salah A, Jalalifar M, Khosravi A, Shirmohammadi Esfeh M, Negravi S. How earthquake can affect blood donation; Kermanshah experience disaster management (P-056). Vox Sang. 2018;113:101.
32. Sonmezoglu M, Kocak N, Oncul O, Ozbayburtlu S, Hepgul Z, Kosan E, et al. Effects of a major earthquake on blood donor types and infectious diseases marker rates. Transfus Med. 2005;15:93–7.

33. Spinella PC, Perkins JG, Grathwohl KW, Repine T, Beekley AC, Sebesta J, et al. Risks associated with fresh whole blood and red blood cell transfusions in a combat support hospital. Crit Care Med. 2007;35:2576–81.
34. Tran S, Lewalski EA, Dwyre DM, Hagar Y, Beckett L, Janatpour KA, et al. Does donating blood for the first time during a national emergency create a better commitment to donating again? Vox Sang. 2010;98:e219–24.
35. Vasquez M, Maldonado M, Tagle F, Leon S, Soto A, Mena A, et al. Blood supply during disasters: the experience of Chile in 2010. Rev Panam Salud Publica. 2011;29:365–70.
36. Carver A, Chell K, Davison TE, Masser BM. What motivates men to donate blood? A systematic review of the evidence. Vox Sang. 2018; 113:205–19.
37. Irving AH, Harris A, Petrie D, Mortimer D, Ghijben P, Higgins A, et al. A systematic review and network meta-analysis of incentive- and non-incentive-based interventions for increasing blood donations. Vox Sang. 2020;115:275–87.
38. Piersma TW, Bekkers R, Klinkenberg EF, De Kort W, Merz EM. Individual, contextual and network characteristics of blood donors and non-donors: a systematic review of recent literature. Blood Transfus. 2017;15:382–97.
39. Hess JR, Thomas MJ. Blood use in war and disaster: lessons from the past century. Transfusion. 2003;43:1622–33.
40. Heinrich J, USGA Office. Public health: maintaining an adequate blood supply is key to emergency preparedness: U.S. General Accounting Office; 2002.
41. World Health Organization. Blood safety and availability. [cited 2021 Aug 24]. Available from: https://www.who.int/news-room/fact-sheets/detail/blood-safety-and-availability.

SUPPORTING INFORMATION

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

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REVIEW ARTICLE

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Plasma procurement and plasma product safety in light of the COVID-19 pandemic from the perspective of the plasma industry

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Abstract

This review, written from the perspective of the plasma industry, discusses plasma procurement and plasma product safety in light of the COVID-19 pandemic. The COVID-19 pandemic impacted the whole world and, therefore, not unexpectedly, the pharmaceutical industry too. In spite of this, the plasma protein industry has continued to provide life saving therapies to critically ill patients. Moreover, companies have collected COVID convalescent plasma (CP) to support development of investigational therapies, for example, hyperimmune globulins to potentially treat SARS-CoV-2 infection, and collaborated with those collecting COVID CP for direct transfusion, which has been made available under emergency use in the United States. For plasma that is fractionated to become a therapy, general knowledge of coronaviruses and numerous new studies on the structure and function of SARS-CoV-2 provide reassurance that existing industry precautions, including donor selection, as well as virus inactivation and removal steps during the manufacturing process are sufficient to maintain the high standards of virus safety of plasma products. The pandemic also revealed the vulnerability and inadequacy of the current plasma ecosystem. There is a need for more plasma to be collected around the world to meet the growing need for safe and efficacious plasma-derived therapies. This requires outdated regulatory and policy restrictions to be realigned with current scientific evidence. More countries around the world should be in a position to contribute to global supply of plasma so that patients with life-threatening conditions - and often no alternative therapeutic solutions - have better access to care.

KEYWORDS
COVID-19, pathogen safety, plasma for fractionation, plasma products, SARS-CoV-2

Highlights

- COVID-19 pandemic reemphasized the need for more plasma to be collected around the world to meet the growing need for safe and efficacious plasma-derived therapies.
- Although SARS-CoV-2 RNA was occasionally detectable in serum samples, the risk of transmission of infectivity by blood and blood products is considered negligible.

- Standard precautions for donor selection as well as virus inactivation and removal steps during the manufacturing process were sufficient to maintain the high standards of safety of plasma products, even during the COVID-19 pandemic.

INTRODUCTION

The production of plasma products depends on a diligently planned, complex system of willing donors, donation facilities, global manufacturing networks and pharmaceutical product distribution channels, which is largely robust under normal circumstances. However, since the advent of the coronavirus disease pandemic in late 2019 (COVID-19), the situation has been challenged. From early 2020, the pandemic significantly impacted the balanced and well-developed system of plasma procurement in ways not previously experienced, further hindering the industry's ability to keep pace with growing patient demand for these life-changing therapies. Challenges included the disruption of donor networks and blood/plasma donation, as well as supply chain networks, leading to reduced supply of plasma and plasma therapies. Local and global travel and trade restrictions hampered the acquisition of source plasma and reduced the available number of blood donations from which recovered plasma is obtained. In turn, this impacted pharmaceutical manufacturing and distribution by interfering with global supply channels of raw materials and devices, and affected the delivery of final drug products to the patients [1–3]. In parallel, global demand for plasma products has continued to grow across approved indications as a result of broader access in more countries and more patients with rare diseases being diagnosed. The notion that convalescent plasma (CP) and hyperimmune globulin products could be viable treatment options for COVID-19 patients has further increased the demand for plasma [4–6]. Moreover, with only a small percentage of the 3000+ proteins [7] circulating in plasma being used therapeutically today, research is currently under way to unlock the untapped potential in plasma to support treatment for many diseases such as rheumatoid arthritis, sickle cell anaemia and age-related macular degeneration [8–10]. Cumulatively, this is creating additional strain on sourcing this scarce life-changing resource.

In the early 1980s, recipients of biopharmaceuticals derived from human plasma were devastated by widespread infections with the human immunodeficiency virus (HIV) and hepatitis C virus (HCV), which were transmitted through plasma-derived concentrates sourced from virus-carrying donors. Recognition of this serious problem led to major changes in procedures for donor screening and testing and the validation of new process steps embedded into the manufacturing processes of plasma derivatives to remove or inactivate blood contaminants such as viruses and even prion agents [11]. The occurrence of new infectious and transmissible agents always alerts recipients, regulators, prescribers and manufacturers of blood products. This is what happened with SARS-CoV-2: The potential for transmission by transfusion and, therefore, blood derivatives prompted serious considerations towards the safety of blood and plasma products.

In this review, we assess the impact of the COVID-19 pandemic, and particularly the causing pathogenic SARS-CoV-2 on procurement of plasma for fractionation from the plasma industry perspective and focus specifically on sustaining supply of safe and efficacious plasma-derived medicines. Information was collected by literature search in PubMed® (https://pubmed.ncbi.nlm.nih.gov/; cut-off date, 10 January 2022), web search and personal communication of the authors who are connected to the specialist network of the plasma industries.

CORONAVIRINAE AND SARS-CoV-2

The severe acute respiratory syndrome (SARS) called COVID-19 is induced by SARS-CoV-2, which belongs to the group of β-coronaviruses. The virus has a single-stranded genome of about 26–32 kb (+ssRNA), which is the largest known genome size of an RNA virus. Coronaviruses belong to the order Nidovirales, family Coronaviridae and the subfamily Coronavirinae, which consists of α-, β-, γ- and δ-coronaviruses [12]. Coronavirus was first isolated from chicken in 1937. Before the SARS outbreak in February 2003, coronavirus was not considered highly pathogenic to humans [13]. Since 2002, three zoonotic outbreaks have been caused by β-coronaviruses: SARS-CoV in 2003 [14], MERS-CoV in 2012 [15] and the latest outbreak of SARS-CoV-2 at the end of 2019 [16]. There is ongoing debate about the origin of SARS-CoV-2 and a final assessment has not been made, but it seems likely that SARS-CoV-2 is a new evolutionary branch of coronaviruses originating from bats [17]. Despite their genomic differences, coronaviruses share a common structure. Human coronaviruses contain phosphorylated nucleocapsid (N) protein with genomic RNA as core enveloped by phospholipid bilayers to form spherical or pleomorphic particles of 80–120 nm size and outer surface-projected spike (S) proteins [18].

SARS-CoV-2 is a respiratory virus and primarily infects the airways. There is no reported evidence for the transmission of respiratory viruses, including the influenza viruses and the coronaviruses responsible for SARS (SARS-CoV) and Middle East respiratory syndrome (MERS) (MERS-CoV), by blood and blood components including plasma and plasma-derived medicinal products (PDMPs) [19–21].

BLOOD AND PLASMA DONATIONS

Testing of blood donations for the absence of infectious agents plays a vital role in providing safe blood for transfusion. Blood services are constantly on alert for the reported detection of emerging pathogens that may impact the safety of the blood supply [22]. When there is a risk that an infectious agent may be transmitted by blood transfusion,

this may trigger the implementation of additional screening of donors and testing of blood donations. The best example in this regard was the introduction of HIV testing in the 1980s. Other examples of potential risks to the safety of the blood supply occurred with the expanded geographic range of pathogens, including the West Nile virus (WNV), Zika virus and babesiosis.

To mitigate the impact of these emerging pathogens on the safety of the blood supply in a timely and effective manner, an effective interaction between all stakeholders, that is, blood services, regulatory authorities, public health institutions and industry, has been established. An example of this was the epidemic occurrence of the WNV, which first appeared in the United States in 1999 and has since spread across the entire country, resulting in thousands of cases of disease. By 2002, it was clear that the virus could be transmitted by blood transfusion, and by the middle of 2003, essentially all blood donations were being tested for WNV [23]. Subsequently, the resulting plasma products like immune globulins were tested for neutralizing antibody titers in plasma-derived intravenous immune globulin released in the United States during 2003–2008. Antibody titers correlated closely with the cumulative incidence of past WNV infection in blood- and plasma donors, with the lots released in 2008 indicating a seroprevalence of 1% [24]. Similarly, upon the occurrence of the corona pandemic, the discussion around testing of blood and blood derivatives for SARS-CoV-2 started early in 2020 [25].

While there was much uncertainty at the beginning of the pandemic, as it had been unclear whether SARS-CoV-2 could be transmitted from those with pre-symptomatic or asymptomatic infection, some tangible data have become available as of now. In May 2020, researchers from China found no evidence of SARS-CoV-2 RNA in the blood of donors in a multi-centre study in the province of Hubei [26]. They examined 98,342 blood donations including 87,095 whole-blood donations and 11,247 platelet donations by individual or minipool testing with the commercially available SARS-CoV-2 real-time RT-PCR assay from PerkinElmer (SYM-BIO LifeScience, Suzhou, China). All donations were negative for SARS-CoV-2 RNA over an observation period of 12 weeks. With a similar set-up, samples from 17,995 minipools of 6 or 16 donations corresponding to approximately 258,000 donations were tested for viral RNA (vRNA) in the United States from March to September 2020 [27]. In this study, a research-use-only transcription-mediated amplification (TMA) assay was used. Reactive results were confirmed using an alternate target region TMA assay. To estimate the viral load of reactive minipools, those were tested by TMA after serial dilution. Additionally, testing for anti-SARS-CoV-2 antibodies and infectivity was performed. Three confirmed reactive minipools from 16 donations were identified, which resulted in an estimated prevalence of vRNA reactive donations of 1.16/100,000 (95% CI 0.40–3.42). The vRNA-reactive samples were non-reactive for antibody. The estimated viral loads of the presumed single positive donations within each minipool ranged from <1000 to <4000 copies/ml. Most importantly though, for all these TMA-positive samples, no infectivity was observed in inoculated permissive cell cultures. At this point, it remains unclear why the study performed in China did not find any vRNA in blood donors while in the United States, vRNA could be detected in a few minipools. Most relevant, though, is the fact that a positive TMA signal did not predict the presence of infectivity.

Diagnosis of SARS-CoV-2 infections has largely been based on RT-PCR tests from nose or throat swabs resembling the viral load in the upper respiratory tract [28]. However, detection of vRNA has also been reported in blood, serum and plasma [29, 30]. A study in the United Kingdom aimed to determine whether PCR-positive blood samples could pose an infection risk by investigating the frequency and determinants of vRNA detection in blood using 424 samples collected from acutely infected and convalescent patients infected with SARS-CoV-2 [31]. The study group also attempted virus isolation from a subset of RNA-positive samples to determine whether RNA detection could be a marker of infectious virus. The results of this study reported that among the PCR-positive samples, cycle threshold (ct) values were high (range 33.5–44.8), suggesting low vRNA copy numbers. PCR-positive sera inoculated into SARS-CoV-2-susceptible cell culture did not produce any cytopathic effect or yield an increase in detectable SARS-CoV-2 RNA. The authors concluded that vRNA was detectable at low viral loads in a minority of serum samples collected in acute infection but was not associated with infectious SARS-CoV-2.

These studies and similar other observations resulted in considerations around blood and plasma donor deferral criteria for COVID-19 patients. The US FDA published several points that responsible physicians who evaluate prospective donors for blood establishments may wish to consider. Among these, the agency suggested individuals diagnosed with COVID-19 or who are suspected of having COVID-19, and who had symptomatic disease, as well as individuals who had a positive diagnostic test for SARS-CoV-2 but never developed symptoms, should refrain from donating blood for at least 10 days after complete resolution of symptoms or 10 days following the date of the positive test result, respectively [20].

PLASMA FOR FRACTIONATION

Plasma for fractionation can be obtained as surplus plasma separated from whole blood (recovered plasma), plasma intended or repurposed for fractionation collected by apheresis concurrently with a cellular product (in some regions called concurrent plasma) and as plasma solely intended for fractionation (source plasma). The assessment of blood and plasma donor suitability and deferral, where appropriate, aims to exclude donations from individuals at risk of transfusion-transmissible infection. All blood components should be obtained from healthy voluntary donors who are carefully selected using a systematic and validated process comprising review of the donor's health assessment and social behaviour history assessed through a donor questionnaire, as well as a medical examination. Current guidelines require a clearly defined list of permanent or temporary deferral criteria used for potential donors. However, standards for donor selection have always differed for the different types of plasma resulting from the circumstances of collection, which is particularly

relevant for newly discovered pathogens. Emerging infections that may influence donor and patient safety should be monitored and may necessitate the revision and modification of donor selection criteria. Donor acceptance and deferral criteria and blood screening procedures need to be balanced to provide optimal safety for both donors and recipients while at the same time ensuring an adequate supply of blood products [32, 33].

Early in the corona pandemic, Chinese authors discussed evidence and understanding of the transmission of SARS-CoV-2 through blood products, and also pathogen inactivation methods on coronaviruses. Although coronaviruses usually infect the upper or lower respiratory tract, the presence of vRNA in plasma or serum is possible, although infectivity has never been detected. Therefore, the risk of transmission of coronaviruses through the transfusion of blood products is theoretical. Given that many asymptomatic infections are found among COVID-19 cases, donor selection would fail under the current circumstances. So far, donation testing for plasma has not been implemented. Testing has not even been required for blood donations, that is, directly transfused components without any virus reduction before application to recipients. Low amounts of vRNA were only rarely found in blood donations (~1:100,000) and did not result in transfusion transmission. No infectivity was observed in inoculated permissive cell cultures, indicating no risk of transmission of the infection by blood components including plasma [26].

In low- and middle-income countries, an increase of blood donations from voluntary, unpaid donors to cover the unmet demand for red blood cell concentrates was observed because of global efforts [34, 35]. However, there is a continuous but slow decline in demand for red blood cells in high-income countries, and with the economic developments across the world, the decline in blood collection will likely continue [36]. Consequently, the amount of available recovered plasma used for plasma fractionation appears to decline. Simultaneously with the rise of the first wave of infections of the corona pandemic, a substantial decrease in the blood supply was observed. For example, in the Hubei province in China, the number of donations was 86% lower in February 2020, dropping from 34,059 to 4778. In some cities in the province, the reduction could reach 90% or even 95% [26]. Until April 2020, the number of donations gradually recovered but did not reach the numbers of the corresponding period of the earlier years. Similar observations were reported from all parts of the world but with varied numbers [37–39]. This caused a dramatic call to action by the International Foundation of Patient Blood Management (IFPBM) and the Society for the Advancement of Blood Management (SABM) Work Group, urging regional and national shortage plans worldwide and, more vitally, dissemination of knowledge and immediate implementation of patient blood management to optimize medical and surgical patient outcomes by clinically managing and preserving a patient's own blood [40]. Along that line, the UK National Blood Transfusion Committee provided a framework and triage tool to guide the allocation of blood for patients with massive haemorrhage during severe blood shortage. The goal of this document was to provide blood transfusions in an ethical, fair and transparent manner to ensure that the greatest number of life-years were saved [41].

Reduced or constant availability of recovered plasma before the corona pandemic was compensated by a steady increase of source plasma collections, which had improved by a factor of 3.4 from 2000 to 2017 [42]. Plasma supply and demand are held in fine balance. If demand exceeds supply, or in cases of disruptions in the supply chain, shortages of plasma-derived products can occur [43]. In the past, shortages of plasma-derived coagulation factors and albumin concentrates have altered medical practice. In recent years, intravenous immunoglobulin has been in short supply [44, 45], with the corona pandemic introducing additional stress on plasma supply and creating constraints across geographies ([46] and personal communication from plasma industry representatives).

At the same time, in absence of specific therapies, PDMPs have been proposed for the treatment of COVID-19, thereby further increasing plasma demand. Transfusions of CP have been used and are under investigation in approximately 200 studies worldwide with mixed results [47]. Some preliminary observations of treatment with CP therapy resulted in a clinically relevant increased risk of severe adverse events, prompting uncertainty whether CP would be beneficial for people admitted to hospital with COVID-19 [48]. In contrast, other studies have shown that CP could provide a safe and efficacious therapy, improving outcomes in severe SARS-CoV2 infection (e.g., [49]). A recent systematic review and meta-analysis of randomized clinical trials investigated the association between CP treatment and mortality and concluded that CP treatment of patients with COVID-19 did not reduce all-cause mortality [50]. Commonly, all studies have shown that most CP donations had high neutralizing antibody titers. Pre-testing of donations by ELISA which correlated to the neutralization titre with a certain threshold could be used to eliminate lower titre units, thus enabling an adequate pooling strategy of CP to level out variations of antibody titres and quality in the therapeutic units [51].

NEW PLASMA PRODUCTS AND SUPPLY

As plasma concentrates are the preferable alternative to whole plasma for safety, efficacy and convenience reasons, hyperimmune globulins (H-Igs) derived from CP have been produced for research use in clinical trials. Several attempts have been made around the world to produce such H-Igs, and studies on their use have been conducted in different centres. One global initiative was the CoVIg-19 Plasma Alliance, which comprised global and regional plasma product manufacturers who combined knowledge, resources and existing infrastructure to accelerate the collection of CP to produce a non-branded H-Ig. In parallel, two other plasma producers initiated similar development programmes independently. The four resulting investigational H-Ig products were clinically tested for safety, tolerability and efficacy in adult hospitalized patients at the onset of clinical progression of COVID-19 under the guidance of the National Institute of Allergy and Infectious Diseases (NIAID), one of the National Institutes of Health (NIH) (ClinicalTrials.gov Identifier: NCT04546581).

Regrettably, the trial did not meet its endpoint to show meaningful improvement in the clinical status of hospitalized adult COVID-19 patients through treatment with an H-Ig when given with standard of care including remdesivir [52]. Importantly, this outcome should not be interpreted as negating the value of plasma in approved indications. The particular study design did not prove a clinical benefit of an H-Ig for this targeted COVID-19 patient population in a hospitalized setting. However, it has significantly contributed to scientific understanding and mirrors mixed experience with CP [53]. The antibody treatment approach has thus far demonstrated efficacy at an earlier stage, namely pre-hospitalization, of the disease through trials with monoclonal antibodies. This was not something that was so apparent when the clinical trial was designed and warrants more research for better understanding [54–57].

The plasma industry's response to the pandemic has forced new ways of thinking and greater collaboration, bringing into sharp focus what must change if we are to improve access to essential plasma-derived therapies for people with rare and complex diseases who often have few alternative treatment options, regardless of the pandemic.

PLASMA PRODUCTS

The COVID-19 pandemic has further exposed the gap between plasma supply and demand, as well as the fragility of a system that is dependent on a scarce resource and heavily reliant on plasma donations from relatively few countries.

Throughout the pandemic, we have seen challenges to plasma donation across the industry, fluctuating in line with restrictions on the movement of people and travel. In spite of significant industry-led investment in new donation and production facilities, as well as major awareness campaigns, industry donation volumes are still lagging pre-pandemic levels. Ultimately, this shortfall is impacting the supply of plasma-derived therapies, particularly immunoglobulins, those plasma-derived therapies that are in highest demand and for which demand grows year on year. This risks potentially compromising patient care. Many of those patients with life-long, life-threatening conditions who rely on immunoglobulins—and who are more vulnerable during a pandemic—have no alternative treatment option. Hundreds of donations are required to keep just one patient on treatment for one year and it takes several months to make these therapies, from donation through to delivery to the patient [58]. There is a lack of global infrastructure for collecting and fractionating plasma across the world and highly restrictive regulatory and legislative frameworks are in place based on limited understanding of the unique profile of plasma and the extent of its potential therapeutic value. Currently, source plasma collection for manufacturing plasma-derived medications is limited to very few countries, predominantly the United States, because of these constraints. For example, the plasma volume collected in Europe meets only approximately 63% of the European clinical need for PDMPs, with the rest being imported from the United States. Only six countries in Europe account for about 80% of all plasma donations to be used for fractionation in Europe to manufacture PDMPs (Germany, France, Italy, Austria, Czechia and Hungary). However, since Italy, France and Spain use their collections exclusively for their own domestic clinical needs, only four countries (Austria, Czechia, Germany and Hungary) actually contribute more than 55% of the total plasma collected in Europe for use in manufacturing PDMPs [59]. Moreover, many of the policies and regulations that impede increased supply have not kept pace with current scientific understanding and should be revisited. The solution to increased availability lies in active collaboration between scientific researchers, the industry, policymakers and regulatory authorities to foster environments that encourage source plasma donation and support sustainable supply solutions. To this end, plasma fractionators are engaging broadly across the blood and plasma industries, with peers, researchers, professional associations, patient organizations, regulators and governments, to emphasize scientific developments and the critical importance of plasma sourcing for ensuring continuous supply of therapies for patients with chronic and complex conditions. Sustainable solutions include establishing or reviewing regulations to better encourage and incentivize donation as well as better global regulations, allowing collection and manufacture of plasma in a global network. Illustrative examples of changes in the scientific, societal, and regulatory environment of plasma collection can be found in the recently updated US guidance for reducing the risk of HIV transmission by blood and blood products [60]. As described in the document's background, the acquired immune deficiency syndrome (AIDS) was first identified in men who have sex with men (MSM). When it was recognized that AIDS could be transmitted through the transfusion of plasma-derived clotting factor concentrates, it was decided to indefinitely defer MSM from donating blood or plasma. Based on the implementation of pathogen inactivation and removal procedures for products manufactured from pooled plasma from the 1980s [61] and nucleic acid testing for HIV, HBV and HCV [62], it was concluded in 2015 that the indefinite deferral could be changed to a 12-month deferral. In 2021, with additional data on the effectiveness of the measures accumulated, the MSM deferral period was further shortened to three months, acknowledging though that it may not be possible to implement the change for all plasma collected in the United States, as it may be destined for fractionation elsewhere and thus different regulations may apply. Scientific advances have led to similar changes for other blood-transmissible infectious-agent risk factors, such as receipt of tattooing and piercing. In summary, regulations that are not harmonized across major geographies and not fully consistent with current science may still limit effective plasma collection. The ability to expedite the CoVIg-19 programme (explained above) in just one year was made possible only by temporary regulatory exemptions across the plasma value chain, which were granted by regulators and governments in the United States and European Union, in view of the urgent need and the available supporting scientific evidence. These included rapid approval of protocols to collect CP and to pool it for manufacture, regulatory exceptions to allow for faster processing and manufacture, for example, reduced inventory hold period, and facilitated import/export between the United States and the European

Union. CoVIg-19 serves as an important illustration of how the current plasma landscape in many countries cannot support rapid response with a potential plasma-derived therapy solution in times of crisis and, more importantly, limits access to potential life-saving and life-sustaining therapies for people with rare diseases. It is why the world relies so heavily on the United States to meet the growing need for plasma and plasma-derived therapies. This situation is likely not sustainable. It is hoped that heightened awareness of plasma-derived therapies and the positive engagement there has been with regulators and governments will help foster recognition and support for the urgent need to update laws and regulations supported by science that govern the donation and processing of human plasma for the production of these essential medicines. This will be vital towards ensuring sustainable patient access to safe and efficacious therapies for which demand is expected to grow for at least the next decade while new alternative synthetic solutions are being investigated to address the full gamut of approved indications.

PATHOGEN SAFETY

Safety of plasma-derived products relies on three complementary measures: (1) donor selection; (2) testing individual plasma donations and mini-pools and manufacturing pools using immunological and nucleic acid amplification technologies (NAT) and (3) manufacturing processes that include specific virus inactivation and removal steps. This is known as the Safety Tripod (Figure 1). The Safety Tripod effectively addressed safety concerns of plasma products, and today plasma products feature significant virus safety margins. The concept has proven so successful that it is now equally applied to and codified for cell-based biotechnology manufacturing platforms and increasingly

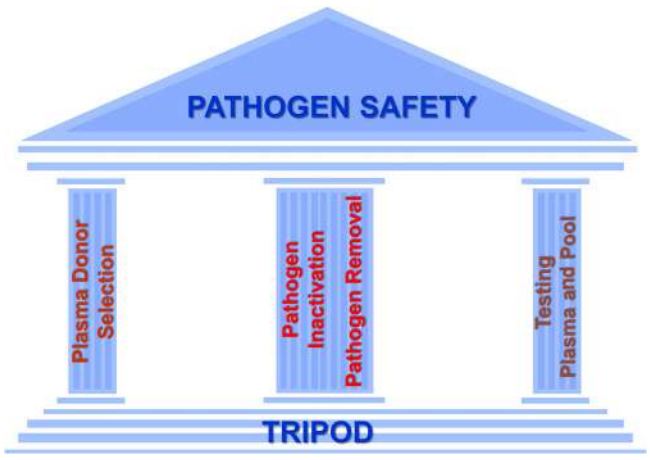

FIGURE 1 The so-called Safety Tripod best describes the safety of plasma-derived products relying on three complementary barriers: (1) selection of donors and plasma sources; (2) testing of individual plasma donations, and mini-pools and manufacturing pools using immunological and nucleic acid amplification technologies (NAT) and (3) manufacturing processes that include dedicated virus inactivation and removal steps

so for advanced therapy medicinal products, such as cell and gene therapies [63]. What does the Safety Tripod mean for SARS-CoV-2? Selection of lower-risk donors would be virtually impossible but also not necessary, as we have learned that blood donations and therefore also plasma would not transmit SARS-CoV-2. Consequently, also testing would not be required and would also not be helpful, as even the presence of SARS-CoV-2 vRNA would not result in infectious blood. Nevertheless, blood and plasma donor deferral criteria for individuals having recovered from COVID-19 have been applied as described above.

Coronaviruses are large lipid-enveloped viruses. Despite differences in their antigens, the main structural features regarding the size and lipid layer are conserved among all coronaviruses, meaning that their biochemical and physical properties are quite similar. Current regulations for plasma products require the use of at least two effective orthogonal virus reduction steps to eliminate infectious agents. The leading viral inactivation/removal technologies used today are solvent/detergent (S/D) treatment, heat treatments and nanofiltration. Given their envelope of phospholipid bilayers, coronaviruses are subject to effective inactivation by S/D treatment. A virus size of the spherical or pleomorphic particles of 80–120 nm size provides effective removal by nanofiltration with <35-nm virus-reduction nanofilter membranes.

To our knowledge, virus reduction validation studies for dedicated virus inactivation or removal steps for plasma products have not been performed with SARS-CoV-2. Nevertheless, the current situation with SARS-CoV-2 is comparable with the situation observed with other newly emerging pathogens. One recent example was the Zika virus (ZIKV), which caused large outbreaks in the Americas in 2015 and 2016, resulting in an increase in travel-associated cases in US states, which also raised concerns around the potential for ZIKV transmission via blood products. Before systematic virus inactivation and removal studies during the manufacture of PDMPs had been performed, risk assessments were made building on similarities between viruses, the so-called model virus concept. In the case of SARS-CoV-2, we do know its structure and function. As explained, the relatively large size and lipid envelope make SARS-CoV-2 highly susceptible to steps with virus inactivation and removal capacity used during the manufacturing processes, such as S/D [64], low-pH incubation, caprylate, pasteurization [65] or dry-heat treatments [66], nanofiltration or fractionation processes and others [67]. The effectiveness of these processes has been demonstrated on other lipid-enveloped model viruses that are quite similar to SARS-CoV-2, for example, human coronavirus 229E and OC43, SARS-CoV, and porcine coronavirus TGEV [68, 69]. In a recent publication, an array of effective coronavirus reduction steps during the manufacture of PDMPs was evaluated and discussed [70]. The authors concluded that, together with earlier reports that SARS-CoV and TGEV are effectively inactivated by pasteurization and standard S/D treatment conditions, these studies provide further evidence that various low-pH incubation and non-standard S/D treatment steps are also effective at inactivating coronaviruses, which, taken together with other safety measures, provide assurance of a high margin of virus safety against SARS-CoV-2 for

PDMPs. Thus, the Plasma Protein Therapeutics Association (PPTA) issued a statement in February 2020 - updated in April 2020 - that the SARS-CoV-2 outbreak is not a concern for the safety of plasma protein therapies manufactured by PPTA member companies [21].

DISCUSSION AND SUMMARY

Building on the historical use of plasma in earlier pandemics, the corona pandemic has substantially increased public awareness of the therapeutic use of plasma, although broader understanding of its use remains low. Use of CP and the potential for H-Igs made from CP to treat those at risk of serious complications from COVID-19 have been widely recognized, and this has drawn attention to the importance of blood and plasma as the source of life-saving human therapies.

Data obtained by in-depth investigations of the SARS-CoV-2 virus and derived from studies with highly similar viruses such as the human coronavirus 229E and OC43, SARS-CoV and porcine coronavirus TGEV confirmed that SARS-CoV-2 is not an issue for the safety of plasma derivatives. Several studies have suggested that infectivity will not be found in plasma donated by those individuals undergoing routine selection based on their health status.

There is a need for more plasma to be collected around the world to meet the growing need for safe and efficacious plasma-derived therapies—and this was evident even before the pandemic [71]. As an example, demand for immunoglobulin products is anticipated to grow steadily over the next few years, with an expected increase of 33% from 2017 to 2025. A key factor behind this growth is the growth in the use of immunoglobulins to treat secondary immunodeficiencies, or even cancer [72]. An increase in collections requires outdated regulatory and policy restrictions to be realigned with current scientific evidence. Specifically, several measures originally implemented with the intention to enhance the virus safety margins of PDMPs have now become redundant. This is largely a result of significantly enhanced and fully validated virus inactivation and removal processes that have been embedded into manufacturing processes, as well as advancements in the science of testing for infectious agents. The continued implementation of these measures limits collection of plasma for fractionation. By addressing this, as well as over-reliance on the few countries with regulatory frameworks conducive to effective plasma collection and shifting towards broader country contribution to global plasma supply, we can create a more sustainable plasma landscape. This means more patients around the world with life-threatening conditions—and often no therapeutic alternative solution— would be able to count on more reliable supply and, therefore, be more confident of access and continuity of care.

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CONFLICT OF INTEREST

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REFERENCES

1. Callaway Kim K, Tadrous M, Kane-Gill SL, Barbash IJ, Rothenberger S, Suda KJ. Changes in purchases for intensive care medicines during the COVID-19 pandemic: a global time series study. Chest. 2021;160:2123–34.
2. Kuo S, Ou HT, Wang CJ. Managing medication supply chains: lessons learned from Taiwan during the COVID-19 pandemic and preparedness planning for the future. J Am Pharm Assoc. 2003;2021:e12–5.
3. Schiroli D, Merolle L, Molinari G, Di Bartolomeo E, Seligardi D, Canovi L, et al. The impact of COVID-19 outbreak on the Transfusion Medicine Unit of a Northern Italy Hospital and Cancer Centre. Vox Sang. 2021;117:235–42.
4. Wendel S, Land K, Devine DV, Daly J, Bazin R, Tiberghien P, et al. Lessons learned in the collection of convalescent plasma during the COVID-19 pandemic. Vox Sang. 2021;116:872–9.
5. Russell WA, Grebe E, Custer B. Factors driving availability of COVID-19 convalescent plasma: insights from a demand, production, and supply model. Transfusion. 2021;61:1370–6.
6. Kaplon H, Reichert JM. Antibodies to watch in 2021. MAbs. 2021; 13:1860476.
7. Schwenk JM, Omenn GS, Sun Z, Campbell DS, Baker MS, Overall CM, et al. The human plasma proteome draft of 2017: building on the human plasma PeptideAtlas from mass spectrometry and complementary assays. J Proteome Res. 2017;16:4299–310.
8. Song S. Alpha-1 antitrypsin therapy for autoimmune disorders. Chronic Obstr Pulm Dis. 2018;5:289–301.
9. Brittain JE, Parise LV. Cytokines and plasma factors in sickle cell disease. Curr Opin Hematol. 2007;14:438–43.
10. Limoli PG, Vingolo EM, Limoli C, Scalinci SZ, Nebbioso M. Regenerative therapy by suprachoroidal cell autograft in dry age-related macular degeneration: preliminary in vivo report. J Vis Exp. 2018;132: 56469.
11. Kreil TR. Building blocks of the viral safety margins of industrial plasma products. Ann Blood. 2018;3:14.
12. Woo PC, Lau SK, Lam CS, Lau CC, Tsang AK, Lau JH, et al. Discovery of seven novel mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavirus. J Virol. 2012;86:3995–4008.
13. Fouchier RA, Kuiken T, Schutten M, van Amerongen G, van Doornum GJ, van den Hoogen BG, et al. Aetiology: Koch's postulates fulfilled for SARS virus. Nature. 2003;423:240.
14. Kuiken T, Fouchier RA, Schutten M, Rimmelzwaan GF, van Amerongen G, van Riel D, et al. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. Lancet. 2003; 362:263–70.
15. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med. 2012;367:1814–20.

16. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395:497–506.
17. Rabaan AA, Al-Ahmed SH, Haque S, Sah R, Tiwari R, Malik YS, et al. SARS-CoV-2, SARS-CoV, and MERS-COV: a comparative overview. Infez Med. 2020;28:174–84.
18. Kirtipal N, Bharadwaj S, Kang SG. From SARS to SARS-CoV-2, insights on structure, pathogenicity and immunity aspects of pandemic human coronaviruses. Infect Genet Evol. 2020;85:104502.
19. Transfusion News. Risk of Transfusion Transmission of SARS-CoV-2 Is Only Theoretical. 2020 Mar 04 [cited 2021 Oct 21]. Available from: https://transfusionnews.com/2020/03/04/risk-of-transfusion-transmission-of-sars-cov-2-is-only-theoretical/
20. US Food & Drug Administration. Updated Information for Blood Establishments Regarding the COVID-19 Pandemic and Blood Donation. Bethesda. 2022 Jan 11 [cited 2022 Jan 11]. Available from: https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/updated-information-blood-establishments-regarding-covid-19-pandemic-and-blood-donation
21. Plasma Protein Therapeutics Association (PPTA). New Coronavirus (SARS-CoV-2) and the Safety Margins of Plasma Protein Therapies. 2020 Apr 03 [cited 2021 Oct 21]. Available from: https://www.pptaglobal.org/media-and-information/ppta-statements/1055-2019-novel-coronavirus-2019-ncov-and-plasma-protein-therapies
22. Roman L, Armstrong B, Smart E. Donation testing and transfusion transmissible infections. ISBT Sci Ser. 2020;15:192–206.
23. Stramer SL, Fang CT, Foster GA, Wagner AG, Brodsky JP, Dodd RY. West Nile virus among blood donors in the United States, 2003 and 2004. N Engl J Med. 2005;353:451–9.
24. Planitzer CB, Modrof J, Yu MY, Kreil TR. West Nile virus infection in plasma of blood and plasma donors, United States. Emerg Infect Dis. 2009;15:1668–70.
25. Dodd RY, Stramer SL. COVID-19 and blood safety: help with a dilemma. Transfus Med Rev. 2020;34:73–4.
26. Chang L, Yan Y, Zhao L, Hu G, Deng L, Su D, et al. No evidence of SARS-CoV-2 RNA among blood donors: a multicenter study in Hubei, China. Transfusion. 2020;60:2038–46.
27. Bakkour S, Saá P, Groves JA, Montalvo L, Di Germanio C, Best SM, et al. Minipool testing for SARS-CoV-2 RNA in United States blood donors. Transfusion. 2021;61:2384–91.
28. Tang YW, Schmitz JE, Persing DH, Stratton CW. Laboratory diagnosis of COVID-19: current issues and challenges. J Clin Microbiol. 2020;58:e00512-20.
29. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA. 2020;323:1843–4.
30. Zheng S, Fan J, Yu F, Feng B, Lou B, Zou Q, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. BMJ. 2020;369:m1443.
31. Andersson MI, Arancibia-Carcamo CV, Auckland K, Baillie JK, Barnes E, Beneke T, et al. SARS-CoV-2 RNA detected in blood products from patients with COVID-19 is not associated with infectious virus. Wellcome Open Res. 2020;5:181.
32. Custer B, Johnson ES, Sullivan SD, Hazlet TK, Ramsey SD, Murphy EL, et al. Community blood supply model: development of a new model to assess the safety, sufficiency, and cost of the blood supply. Med Decis Making. 2005;25:571–82.
33. Seifried E, Klueter H, Weidmann C, Staudenmaier T, Schrezenmeier H, Henschler R, et al. How much blood is needed? Vox Sang. 2011;100:10–21.
34. Report of the Fifth General Meeting of the Global Collaboration for Blood Safety, Geneva, 10-12 November, 2004. Available from: https://www.who.int/bloodsafety/GCBS_Report_NOV_2004.pdf
35. World Health Organization. Home/Newsroom/Fact sheets/Detail/Blood safety and availability [cited 2020 June 10]. Available from: https://www.who.int/news-room/fact-sheets/detail/blood-safety-and-availability
36. Jones JM, Sapiano MRP, Savinkina AA, Haass KA, Baker ML, Henry RA, et al. Slowing decline in blood collection and transfusion in the United States - 2017. Transfusion. 2020;60:S1–9.
37. Pagano MB, Hess JR, Tsang HC, Staley E, Gernsheimer T, Sen N, et al. Prepare to adapt: blood supply and transfusion support during the first 2 weeks of the 2019 novel coronavirus (COVID-19) pandemic affecting Washington State. Transfusion. 2020;60:908–11.
38. Grandone E, Mastroianno M, Caroli A, Ostuni A. Blood supply and transfusion support in southern Italy: findings during the first four weeks of the SARS-CoV-2 pandemic. Blood Transfus. 2020;18:230–2.
39. Kumar S, Azim D, Nasim S, Hashmi SH. Dwindling blood reserves: an ominous downside of COVID-19 pandemic. Transfus Apher Sci. 2020;59:102818.
40. Shander A, Goobie SM, Warner MA, Aapro M, Bisbe E, Perez-Calatayud AA, et al. Essential role of patient blood management in a pandemic: a call for action. Anesth Analg. 2020;131:74–85.
41. Doughty H, Green L, Callum J, Murphy MF. Triage tool for the rationing of blood for massively bleeding patients during a severe national blood shortage: guidance from the National Blood Transfusion Committee. Br J Haematol. 2020;191:340–6.
42. Robert P. An analysis of the impact of international transfers of plasma on the availability of immunoglobulin therapies. Fall, 2019 [cited 2021 Oct 21]. Available from: https://www.pptaglobal.org/images/source/2019/Fall/An_Analysis_of_the_Impact_of_International_Transfers_of_Plasma_on_the_Availability_of_Immunoglobulin_Therapies.pdf
43. Hartmann J, Klein HG. Supply and demand for plasma-derived medicinal products - a critical reassessment amid the COVID-19 pandemic. Transfusion. 2020;60:2748–52.
44. Bayry J, Kazatchkine MD, Kaveri SV. Shortage of human intravenous immunoglobulin--reasons and possible solutions. Nat Clin Pract Neurol. 2007;3:120–1.
45. Hartmann J. Google Trends as an early indicator for shortages of intravenous immunoglobulin (IVIG). Transfusion. 2020;60:1656–7.
46. The United Kingdom Primary Immunodeficiency Network (UKPIN) Official Release. 2021 Nov 5. Available from: http://www.ukpin.org.uk/
47. Brown BL, McCullough J. Treatment for emerging viruses: convalescent plasma and COVID-19. Transfus Apher Sci. 2020;59:102790.
48. Chai KL, Valk SJ, Piechotta V, Kimber C, Monsef I, Doree C, et al. Convalescent plasma or hyperimmune immunoglobulin for people with COVID-19: a living systematic review. Cochrane Database Syst Rev. 2020;10:CD013600.
49. Fodor E, Müller V, Iványi Z, Berki T, Kuten Pella O, Hornyák I, et al. Early transfusion of convalescent plasma improves the clinical outcome in severe SARS-CoV2 infection. Infect Dis Ther. 2022;11:293–304.
50. Axfors C, Janiaud P, Schmitt AM, Van't Hooft J, Smith ER, Haber NA, et al. Association between convalescent plasma treatment and mortality in COVID-19: a collaborative systematic review and meta-analysis of randomized clinical trials. BMC Infect Dis. 2021;21:1170.
51. Jungbauer C, Weseslindtner L, Weidner L, Gänsdorfer S, Farcet MR, Gschaider-Reichhart E, et al. Characterization of 100 sequential SARS-CoV-2 convalescent plasma donations. Transfusion. 2021;61:12–6.
52. ITAC (INSIGHT 013) Study Group. Hyperimmune immunoglobulin for hospitalised patients with COVID-19 (ITAC): a double-blind, placebo-controlled, phase 3, randomised trial. Lancet. 2022;399:530–40.

53. Hartmann J, Bloch EM, Burnouf T. Experience with COVID-19 convalescent plasma provides vital guidance to future pandemics. Transfusion. 2022 Jan 30. https://doi.org/10.1111/trf.16810. Epub ahead of print.
54. Chen P, Nirula A, Heller B, Gottlieb RL, Boscia J, Morris J, et al. SARS-CoV-2 neutralizing antibody LY-CoV555 in outpatients with Covid-19. N Engl J Med. 2021;384:229–37.
55. Gottlieb RL, Nirula A, Chen P, Boscia J, Heller B, Morris J, et al. Effect of bamlanivimab as monotherapy or in combination with etesevimab on viral load in patients with mild to moderate COVID-19: a randomized clinical trial. JAMA. 2021;325:632–44.
56. Weinreich DM, Sivapalasingam S, Norton T, Ali S, Gao H, Bhore R, et al. REGN-COV2, a neutralizing antibody cocktail, in outpatients with Covid-19. N Engl J Med. 2021;384:238–51.
57. So-Osman C, Valk SJ. High-dose immunoglobulins from convalescent donors for patients hospitalised with COVID-19. Lancet. 2022;399:497–9.
58. Plasma Protein Therapeutics Association (PPTA). Advocacy Toolkit [cited 2021 Oct 21]. Available from: https://www.pptaglobal.org/advocacy/advocacy-toolkit
59. Kluszczynski T, Rohr S, Ernst R. Key economic and value considerations for plasma-derived medicinal products (PDMPS) in Europe [cited 2020 Mar 5]. Available from: https://www.vintura.com/wp-content/uploads/2020/03/White-paper-key-economic-and-value-considerations-for-plasma-derived-medicinal-products-PDMPs-in-Europe_Vintura-and-PPTA.pdf
60. Guidance for Industry: Revised Recommendations for Reducing the Risk of Human Immunodeficiency Virus Transmission by Blood and Blood Products. August 2020. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/revised-recommendations-reducing-risk-human-immunodeficiency-virus-transmission-blood-and-blood
61. Klamroth R, Gröner A, Simon TL. Pathogen inactivation and removal methods for plasma-derived clotting factor concentrates. Transfusion. 2014;54:1406–17.
62. Custer B, Kessler D, Vahidnia F, Leparc G, Krysztof DE, Shaz B, et al. NHLBI Retrovirus Epidemiology Donor Study-II (REDS-II). Risk factors for retrovirus and hepatitis virus infections in accepted blood donors. Transfusion. 2015;55:1098–107.
63. Barone PW, Wiebe ME, Leung JC, Hussein ITM, Keumurian FJ, Bouressa J, et al. Viral contamination in biologic manufacture and implications for emerging therapies. Nat Biotechnol. 2020;38:563–72.
64. Rabenau HF, Biesert L, Schmidt T, Bauer G, Cinatl J, Doerr HW. SARS-coronavirus (SARS-CoV) and the safety of a solvent/detergent (S/D) treated immunoglobulin preparation. Biologicals. 2005;33:95–9.
65. Gröner A, Broumis C, Fang R, Nowak T, Popp B, Schäfer W, et al. Effective inactivation of a wide range of viruses by pasteurization. Transfusion. 2018;58:41–51.
66. Yunoki M, Urayama T, Yamamoto I, Abe S, Ikuta K. Heat sensitivity of a SARS-associated coronavirus introduced into plasma products. Vox Sang. 2004;87:302–3.
67. Keil SD, Bowen R, Marschner S. Inactivation of Middle East respiratory syndrome coronavirus (MERS-CoV) in plasma products using a riboflavin-based and ultraviolet light-based photochemical treatment. Transfusion. 2016;56:2948–52.
68. Lamarre A, Talbot PJ. Effect of pH and temperature on the infectivity of human coronavirus 229E. Can J Microbiol. 1989;35:972–4.
69. Bucknall RA, King LM, Kapikian AZ, Chanock RM. Studies with human coronaviruses. II. Some properties of strains 229E and OC43. Proc Soc Exp Biol Med. 1972;139:722–7.
70. Schräder T, Koch J, Ross R, Schäfer W, Keiner B, Roth NJ, et al. Effective coronavirus reduction by various production steps during the manufacture of plasma-derived medicinal products. Transfusion. 2020;60:1334–5.
71. The Economist. Blood Money. May 2018. Available from: https://www.economist.com/leaders/2018/05/12/lift-bans-on-paying-for-human-blood-plasma.
72. Marketing Research Bureau. Forecast of the Global Immunoglobulin (IgG) Market 2025. Available from: https://marketingresearchbureau.com/forecast-of-the-global-immunoglobulin-igg-market-2023-2/

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REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

Stepwise access to safe plasma proteins in resource-constrained countries: Local production and pathways to fractionation—Report of an International Society of Blood Transfusion Workshop

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Abstract

Background and Objectives: Actions are needed to improve access to safe plasma-derived medicinal products (PDMPs) in low- and middle-income countries (LMICs).

Materials and Methods: The International Society of Blood Transfusion (ISBT) Working Party for Global Blood Safety organized an on-line workshop during 21–23 September 2021 to advance access to safe plasma proteins in resource-constrained countries, consistent with recent World Health Organization (WHO) guidance documents.

Results: The meeting drew attention to the considerable unmet needs for access to essential PDMPs in LMICs, in particular coagulation factors and immunoglobulins, and stepwise actions to address these deficits. First, improved access to safe plasma protein therapies requires blood component separation with prevention of wastage of recovered plasma. Quality and safety of collected blood and plasma must be assured so that plasma in excess of transfusion needs can be processed into safe plasma proteins. Second, local production of safe plasma proteins can be implemented using available technologies to locally obtain pathogen-reduced plasma and prepare pathogen-reduced cryoprecipitate and immunoglobulins from small plasma pools. Third, when a sufficient, stable volume of quality-assured plasma is available (approximately 50,000 L/year), contract or toll fractionation by a foreign plasma fractionator can expand the supply of PDMPs. Fourth, when the national infrastructure supports high-technology industrial production and stable volumes of quality plasma reach at least 200,000 L/year, technology transfer for domestic fractionation can be considered.

Conclusion: Action is needed including commitments of the organizations that made the workshop possible (WHO, ISBT, World Federation of Haemophilia [WFH], Plasma Protein Therapeutics Association [PPTA], International Plasma Fractionation Association [IPFA], International Patient Organization of Primary Immunodeficiencies [IPOPI] and International Federation of Blood Donor Organizations [FIODS]).

KEYWORDS
haemophilia, immunodeficiency, low- and middle-income countries, plasma, proteins

Highlights

- Patients' access to plasma-derived medicinal proteins (PDMPs) essential for their treatment is still poor or absent in low- and middle-income countries (LMICs) due to the unavailability or unaffordability of the products.
- Stepwise technological actions to process domestic quality plasma can advance the access to safe plasma protein products in LMIC, consistent with recent WHO guidance documents.
- Practical pilot projects to address deficits in PDMPs in LMIC are now planned; implementation will require the continued commitments of the international organizations that contributed to the workshop and the support from technology and equipment suppliers.

INTRODUCTION

A 3-day on-line workshop was organized by the International Society of Blood Transfusion (ISBT) Working Party on Global Blood Safety (WP GBS) during 21–23 September 2021 to discuss 'Stepwise Access to Safe Plasma Proteins in Resource-Constrained Countries'. Delegates from 84 countries attended the workshop, which gathered major stakeholders in the plasma product field, including representatives from the World Health Organization (WHO), patient organizations, blood donor organizations, plasma fractionation organizations and technology and equipment suppliers.

Data indicate that unlike in high-income countries, in most low- and middle-income countries (LMICs) patients' access to plasma-derived medicinal proteins (PDMPs) essential for their treatment is still poor or absent due to unavailability or unaffordability of the products. To identify and facilitate new avenues for the supply of plasma protein products, in 2017 the WP GBS published recommendations on pathogen-reduced cryoprecipitate and on plasma for fractionation. More recently, the WHO launched an 'Action framework to advance universal access to safe, effective and quality-assured blood products 2020–2023' under which it published 'Guidance on increasing supplies of plasma-derived medicinal products in low- and middle-income countries through fractionation of domestic plasma' [1] and 'Guidance on centralization of blood donation testing and processing' [2].

The aim of the WP GBS workshop was to 'identify pragmatic technical options for stepwise access to safe plasma protein therapies in resource-constrained countries to support implementation of these WHO guidance documents'.

UNMET NEEDS FOR PDMPs IN LMICs

Significant imbalances exist in global plasma collection, with 65% from the United States, and the need for more regionally balanced collection towards global PDMP sufficiency. To reach 'Treatment for All' as an ultimate goal, additional innovative strategies and actions are necessary. Patients with bleeding disorders (haemophilia A and B, von Willebrand disease and other rare haemorrhagic diseases) and a variety of immunodeficiencies suffer from similar problems: underdiagnoses of patients and short supplies of safe and effective therapeutic products. Inherited bleeding disorders (IBDs) are present worldwide, but in LMICs only 8% of those are identified because of lack of awareness, inadequate diagnoses and unavailability of treatment products. A striking imbalance between the developed and developing world exists, with 82% of total factor VIII (FVIII) products being used in the Americas and Europe, which comprise only 29% of the global population. In the World Federation of Hemophilia (WFH) Global Survey 2019, the mean FVIII usage reported per capita was 6.01 IU (international units) in high-income countries versus 0.06 IU in LMICs. This gap reflects that many patients are not diagnosed and suffer from early-life haemorrhage or do not have access to treatment including from barriers of cost, leading to the high morbidity and mortality among haemophilia patients in LMICs. The WFH has taken steps to mitigate this unsatisfactory supply situation, which globally affects 70%–80% of haemophilia patients with no improvement in the past 20 years. WFH activities in LMICs aim to improve the level of knowledge through capacity building and data collection, enhance advocacy capacities of different stakeholders and facilitate access to care under a Humanitarian Aid Program (HAP). The HAP provides consistent and predictable access to PDMPs in LMICs through product donations by industry, representing a fine example of solidarity with patients. Making use of optimal treatment paradigms, the HAP provides evidence of the benefits that can motivate governments to support the purchase or production of clotting factor concentrates (CFCs). However, as HAP donations cover less than 1% of global CFC consumption, this programme alone will not resolve shortages in LMICs.

To prevent serious infections, patients with primary immune deficiencies (PIDs) require life-long immunoglobulin (Ig) replacement therapy with individualized product selection and dosages. Access to Igs in the world varies widely, with about 50% of the worldwide Ig consumed in North America alone. The International Patient Organization for Primary Immunodeficiencies (IPOPI) estimates that 80% of PID patients worldwide do not have access to appropriate therapies. Significant disparities exist in PID diagnosis rates and patient access to Ig therapies between regions. Prevalences of diseases differ between regions. Based on a conservatively estimated PID prevalence of 1/1200–1/5000, at least 1.4 million people live with PID worldwide, most of whom require Ig therapy. In 2018, 211 tons of Ig was used worldwide (up from 19.7 tons in 1992), whereas at least 305 tons of Ig are needed to cover PID patient needs only. Demand for Ig is growing at 6%–8% yearly across a broad range of indications, in particular for secondary

immunodeficiencies, and is not forecasted to decrease for years to come. Improved global PDMP sufficiency with better Ig access in LMICs requires collaboration guided by patient needs, donor care, safety of PDMPs, and a better understanding of patient-centred Ig therapies. To overcome existing product shortages, the IPOPI aims to increase the availability of high-quality plasma for fractionation, by improving good manufacturing practices (GMPs) in blood and plasma collection and processing and preventing plasma wastage.

OPTIONS FOR THE FRACTIONATION OF DOMESTIC PLASMA

World Health Assembly Resolutions 58.13 (2005) and 63.12 (2010) urge countries to ensure adequate availability of safe quality blood, blood components and PDMPs. In particular, several PDMPs, including clotting factors and Igs, are included in the WHO Model List of Essential Medicines. Therefore, ensuring a safe, secure, sufficient and ethically obtained supply of essential PDMPs in LMICs is regarded as an important public health responsibility. Plasma collected to improve the sufficiency of PDMPs should meet quality requirements for fractionation. However, a substantial volume of recovered plasma, a valuable source of clotting factors and having, in some LMIC countries, a high content in immunoglobulins, is discarded because quality requirements for industrial fractionation are not met. This wastage is due to deficiencies, which include a fragmented blood collection system, absence of regulations to assure plasma quality and safety, deficiencies in testing, and inadequate infrastructure for plasma freezing, storage and transport. Corrective actions are urgently needed, as the steady increase in whole-blood collection in LMICs to provide for red blood cells may increase the volume of wasted plasma. Thus, the WHO published a high-level guidance document entitled 'Increasing supplies of PDMPs in LMICs through fractionation of domestic plasma'. The guidance provides a strategic framework to assist member states in increasing the volume of quality plasma suitable for fractionation and understanding stepwise approaches for providing safe plasma protein products by local small-scale processing and through industrial fractionation. This guidance is complementary to WHO guidance on centralizing blood donation testing and processing, which is intended to assist member states in deciding whether to centralize blood donation testing and processing. Such centralization can help LMICs gradually increase the availability of quality plasma for fractionation and access to safe plasma protein fractions including PDMPs.

The possibility for fractionation of plasma into PDMPs depends on assurances that the plasma meets international quality standards. Measures are needed to correct non-conformities in quality management systems and/or deficiencies in GMPs as required by plasma fractionators. Key steps and changes can be implemented by blood services to improve the quality of their plasma. A strong commitment from government agencies and adequate funding for GMP implementation are needed. Improvements need to be made throughout the manufacturing chain, from donor selection to shipping of frozen plasma and fractionation. Plasmapheresis as a source of additional plasma for further processing into plasma fractions can be introduced after GMP compliance is established in blood establishments.

Contract or toll plasma fractionation through an agreement with an established plasma fractionator is a pragmatic and feasible way to obtain PDMPs from domestic plasma. Domestic plasma recovered from whole blood or obtained by plasmapheresis is sent to a fractionator to obtain PDMPs under different contractual agreements. Domestic plasma should comply with mandatory quality requirements from the plasma fractionator and relevant regulatory authorities. This requires implementation of quality management systems for blood, blood components and plasma collection, resulting in a win-win situation for both blood collection and plasma fractionation organizations. With consistent volumes of quality plasma sufficient to be industrially fractionated (estimated to be 50,000 L/year), the decision to initiate contract manufacturing can be made, involving all stakeholders (blood establishments, local authorities, fractionators, the ministry of health and dedicated funding organizations if needed). The PDMPs manufactured through contract fractionation abroad should obtain a license by local or regional competent authorities supervising the country. A quality agreement between blood establishment organizations and the fractionator is a building block that defines all quality, technical and contractual conditions. Such quality management systems can be implemented at the regional level to increase quality plasma volume, perform contract manufacturing and contribute to the availability of PDMPs in the world.

Understanding the complexity of the plasma fractionation industry would help make optimal technical and financial decisions. Plasma fractionation is a complex technology with benefits and limitations. The original process based on ethanol precipitation developed 75 years ago to fractionate albumin is still the core technology today. Countries interested in building a fractionation plant to improve PDMP supplies should consider the WHO guidance recommendations to ensure success and sustainability. Economic considerations, sometimes underestimated, include costs of complex engineering, heavy capital investments, and, typically, technology transfer with a long-term endeavour. For a domestic facility, a minimum targeted annual volume of 200,000 L, ideally expandable to 300,000 L of plasma, is needed.

Thailand is one example where a domestic plasma fractionation programme was implemented to resolve issues of variable costs and insufficient supplies of imported PDMPs and address inequitable access to treatment for patients. The country was running a small-scale plasma fractionation facility, and in 2011 it decided on an industrial plasma fractionation plant to meet increasing local demands for FVIII, intravenous Ig (IVIG), and albumin. In 2013, a technology transfer agreement was signed with Green Cross Corporation (GCC). Construction of the plasma fractionation facility began, followed by technology transfer, qualification, validation and production batch trials. Clinical studies of locally made PDMPs were conducted in university hospitals. The first licensed products were released in 2016, which showed a quality comparable to that of imported products. Critical factors in their success included royal and government support, good diplomatic relationships between Thailand and South Korea, fruitful cooperation among various local health

organizations, the availability of local technical expertise and experienced staff, and a reliable domestic plasma supply. Building such a domestic facility is very costly and requires complex technology transfers. Thus, careful assessment and feasibility studies are needed.

The Hemoderivatives Laboratory (HL) of the National University of Cordoba (UNC), Argentina, was established in 1965 to fractionate plasma from Argentina. In-house plasma fractionation technologies were developed, allowing HL to produce a range of PDMPs. Domestic blood centres supply plasma and receive PDMPs in return as well as equipment and supplies for enhanced blood/plasma quality collection. In addition, HL signed plasma fractionation agreements with Uruguay, Chile and Paraguay. A percentage of the produced PDMPs is returned to the plasma suppliers, while HL keeps excess products to compensate for the fractionation cost, thus improving the availability of PDMPs in Argentina. Insufficient political will for plasma fractionation and insufficient plasma quality initially impeded the development of the plasma fractionation programme. Implementation of the Plasma Quality Assurance Program by HL, which focused on compliant plasma collection procedures and GMP implementation, and better organization of the national blood collection system were vital to the eventual success.

Thus, both sufficient quality and volume of plasma are needed for industrial-scale processing. However, many LMICs struggle to make enough safe blood available, while for small countries the minimum plasma volume needed for a plasma fractionation programme is out of reach, justifying the need to identify pragmatic solutions to increase PDMP supplies at the national or regional level.

OPTIONS TO IMPROVE THE ACCESS TO SAFE PLASMA PROTEIN PRODUCTS IN LMICs

The International Federation of Blood Donor Organizations (FIODS) emphasizes the fundamental role of safe blood and plasma donors in every blood programme. Up-to-date strategies for recruitment and retention of safe blood and plasma donors include "social marketing" with culturally sensitive promotion of donations that focuses on donors' natural desire to enhance their standing in their own social networks. Thus, the safety and security of the blood supply and voluntary unpaid donations should be priorities, targeting low-risk populations, promoting blood and plasma donation as part of a healthy lifestyle, and protecting donors' health and rights. Blood donor organizations and associations can play an important role, making a significant contribution to their respective national blood organizations and healthcare systems.

The French Blood Establishment (EFS) reported on the successful use of quarantine/release as an alternative to pathogen reduction (PR) to ensure the safety of plasma for transfusion. Fully tested plasma from whole blood or apheresis is held in quarantine for at least 60 days and released for transfusion on days 61–160 of storage based on a subsequent donation with negative infectious disease test results. Plasma quarantine/release mitigates the infectious risk associated with a donation in an infectious 'window period' for tested agents. However, the practice is feasible only if a large proportion of donors returns for donation during the defined period. The efficiency of plasma quarantine/release depends on multiple factors, including the ability to identify and engage with donors willing to undergo subsequent donations. A robust infrastructure capable of dealing with large volumes of cryopreserved plasma should be available, as well as a robust system of product information and traceability. The sensitivity of donation screening tests (which governs the necessary duration of the quarantine) and the incidence of transfusion-relevant pathogens in donors are relevant elements to consider. Rules and procedures for disposition (i.e., fractionation or destruction) of plasma for which quarantine/release was unsuccessful should be defined. Plasma quarantine in LMICs might not be easily implementable as donor return rates are generally low.

Pathogen reduction (PR) is an option to improve the safety of plasma. Several companies presented their respective PR technologies for blood components. Intercept/Cerus uses amotosalen + ultraviolet A (UVA) to treat plasma (and platelets); at the workshop, feasibility was also claimed for PR of cryoprecipitates and cryo-poor plasma. Mirasol/TerumoBCT uses riboflavin + UVB/A spectra to treat plasma (and platelets); at the workshop, PR of cryoprecipitate was also described. Theraflex/Macopharma uses methylene blue + UVC to treat plasma and cryoprecipitate. VIPS/VIPS uses solvent-detergent (SD) for mini-pool PR of plasma and cryoprecipitate and caprylic acid for mini-pool purification and PR of Ig. At this time, only VIPS offers a PR method for the preparation of Ig. All presenting manufacturers emphasized the safety and efficacy of their technologies. Each of the technologies offers highly significant inactivation of major enveloped viruses relevant to blood safety (human immunodeficiency virus [HIV], hepatitis B virus [HBV] and HCV) but lesser reduction of non-enveloped viruses (i.e., parvovirus B19). Chemical characteristics of treated plasma and cryoprecipitate conform to internationally recognized standards. The companies claim that implementation of their technologies is reasonably quick, with training of staff being critical.

In Jeddah, Saudi Arabia, coronavirus disease 2019 (COVID-19) convalescent plasma (CCP) was successfully prepared using plasmapheresis and Intercept PR. Challenges encountered during implementation of CCP production included regulatory approval for the production of a novel pharmaceutical, labelling of the CCP product, implementation of a plasmapheresis system, operator training, on-site validation of the plasmapheresis system, adjustments of the PR process and on-site validation of the overall process. Additional difficulties specific to the COVID-19 pandemic included scarce resources (supplies, budgets and human resources) and limited availability of sets for plasmapheresis and PR (due to interrupted distribution because of flight cancellations and supply shortages).

Experience from Cairo, Egypt, demonstrated that Mini Pool Plasma Fractionation developed with CE-marked medical devices can enable high-end blood transfusion centres in LMICs (like the national blood transfusion centres) to produce safe alternatives for CFCs and IVIG at affordable costs. Specifically, in Cairo, production of PR cryoprecipitates and Ig was implemented using VIPS technologies to address unmet needs for plasma protein products of patients with

haemophilia A and immune deficiencies. The technology enables production of protein concentrates on a scale that can be adapted to blood transfusion centres in LMICs. To make anti-haemophilic factor, a series of CE-marked sterile medical devices is used to generate approximately 400-ml mini-pools of cryoprecipitate, which are subjected to PR using SD and filtration (F) to produce safe coagulation factors as cryo-SD/F. The product contains concentrated FVIII, von Willebrand factor (VWF), fibrinogen and factor XIII (FXIII). Remarkably, one-third of all FVIII used in Egypt is being produced as cryo-SD/F. A comparable approach was developed to produce safe IVIG fractions. The production process, which models scaled-down fractionation, differs from that for cryo-SD/F but is similar in principle: pooling of plasma, precipitation to get the cryo-supernatant, PR, Ig purification (with caprylic acid), several filtration steps, and conditioning of safe Ig to treat patients with immunodeficiencies. Products have demonstrated both clinical safety and efficacy in the absence of significant adverse reactions. At the same time, recovered plasma is utilized, thus reducing wastage of this valuable resource. All VIPS products are CE-marked as medical devices and comply with local blood bank regulations.

LMICs should implement PR on plasma, cryoprecipitate and cryo-supernatant as part of local production of safe plasma protein products when industrially manufactured clotting PDMPs are unavailable or unaffordable. To render plasma proteins virus-safe, complementary measures are needed, including selection of low-risk blood/plasma donors, testing for specific blood-transmissible agents, and use of validated technologies for virus inactivation/removal. Robust methods for virus inactivation/removal such as SD treatment, pasteurization, dry heat and (nano)filtration have been successfully applied against relevant blood-transmitted viruses such as HIV, HBV and HCV. While there is no single inactivation/removal method that reliably clears all kinds of viruses, a combination of methods offers a high degree of safety against a wide range of potential contaminants that may include unknown or unexpected viruses. Available methods for virus inactivation/removal vary with respect to their range of 'viral kill' as well as to their critical process parameters. Considering that individual inactivation steps could be overloaded by highly viremic donations, it may be preferable to apply virus inactivation on pooled/homogeneous plasma pools rather than to individually treat units for virus inactivation. Nucleic acid testing (NAT) of donations might not be required when the implemented pathogen/virus reduction treatment has demonstrated robustness against viruses of concern.

Regulatory approval of medical devices for pathogen inactivation of plasma from the design phase to clinical use is complex. Objective evidence related to the performance, safety, and efficacy (PS&E) must be provided to a notified body (NB), and the national regulatory authority (NRA). When chemicals are used for pathogen inactivation, the device may be certified either as an integrated set including the chemicals or separately. Certification typically takes several years and is increasingly costly and knowledge-intensive, thus, unfortunately, limiting the availability of medical devices for pathogen inactivation in resource-constrained countries.

SCALE-UP FOR DOMESTIC PROCESSING OF PLASMA: TECHNOLOGICAL OPTIONS, EQUIPMENT AND OVERSIGHT

Small-scale processing technologies in single-use devices and equipment from industry suppliers may help fill technical gaps in locally supplying safe plasma proteins. Validated protein purification and virus reduction methods adapted to small-scale plasma processing fit a stepwise approach for preparing virus-safe plasma products such as FVIII/VWF/fibrinogen, and Ig. This ramp-up phase can help familiarize local stakeholders and the workforce with specific requirements of plasma processing.

Industry suppliers representing Sartorius, Merck Millipore and Asahi Kasei Medical presented examples of pragmatic, scalable single-use technologies for various types of chromatographic processing, filtration, tangential flow filtration and virus removal by nanofiltration. Single-use processing can increase the flexibility and efficiency, lower contamination risk, avoid heavy capital investment, and facilitate process implementation and scale-up for domestic plasma processing, including for purified virus-inactivated Igs and cryoprecipitate. Suppliers indicated that they have technical support teams dedicated to LMICs to assist process implementation and performance optimization.

Within a short time frame, Intas Pharmaceutical, India, implemented a dedicated purification process of severe acute respiratory syndrome coronavirus (SARS CoV)-2 Ig from CCP collected by local blood banks. This process included steps for virus inactivation and removal. A concrete example of the production of plasma-derived Igs from small plasma pools was given from the angle of the manufacture of antivenom polyclonal IVIGs in Costa Rica. Antivenom manufacture uses GMP-compliant processing methods analogous to those used in the human plasma fractionation industry to purify Igs. The experience developed by several LMICs in the fractionation of antivenom Igs illustrates the feasibility of domestic production of plasma-derived biologicals that may provide a roadmap for the supply of human plasma fractions in LMICs.

THE WAY FORWARD

The workshop concluded with a presentation of models for technical assistance and technology transfer followed by a panel discussion which focused on identifying practical next steps.

Consistent with WHO guidance documents [1], access to plasma protein products can be approached stepwise (Table 1). First, and most fundamental, is to ensure the quality of blood and plasma collection and processing. Second, as plasma is increasingly generated from component separation of whole blood, it becomes important to eliminate wastage of plasma that is not utilized for transfusions. Local production of small-scale virus-inactivated cryoprecipitate and Igs can be considered using available technologies. Third, as more stable, quality-assured plasma becomes available (approximately 50,000 L/year), collaboration with a plasma fractionator through contract or toll fractionation becomes possible to expand the supply of plasma protein products. Subsequent technology transfer

TABLE 1 Stepwise access to safe plasma protein products in resource-constrained countries where commercial concentrates are unavailable or unaffordable

Step	Volume of quality plasma (L)	Safety measures
1	<50,000	• Quarantine or holding period of plasma donations • Validated pathogen reduction of plasma, cryoprecipitate and cryo-poor plasma
2	<50,000	• Validated preparation of mini-pools of virus-inactivated cryoprecipitate or immunoglobulins • Small-pool, scalable, plasma fractionation
3a	≥50,000	• Consider contract/toll fractionation by a licensed fractionator abroad
3b	≥200,000	• Consider domestic or regional plasma fractionation

for the development of domestic fractionation is an option when stable volumes of quality plasma reach a level of 200,000–300,000 L/year and there is a national infrastructure to support high-technology industrial production. Establishment of a national fractionation plant may eventually lead to collaboration with other countries of the region to provide contract or toll fractionation, enabling economies of scale for a national fractionation plant while expanding access to industrially prepared plasma protein concentrates across the region.

A key issue in advancing access to plasma protein products is justification for promoting plasma production when collections of whole blood are insufficient to meet patient needs for transfusion. A major barrier in LMICs exists from the under-diagnosis of rare disorders, since authorities face difficulties prioritizing budgetary needs of very small patient populations. Awareness should be raised of public health decision makers through scientific-evidence-based advocacy about the importance of plasma collection to care for patients whose lives depend on plasma protein products. The WHO could play a larger role in advancing this message globally. For example, World Blood Donor Day could be re-invented as World Blood and Plasma Donor Day. Advocacy for plasma quality also advances the quality and safety of blood components for transfusions and is linked to promotion of a safe and stable blood donor base. Raising awareness of the need for plasma proteins helps to increase the willingness of donors to donate.

Wastage of plasma and failure to generate plasma and cryoprecipitate by component separation from whole blood are missed opportunities and should be supported in countries where treatment with PDMPs or safe plasma components is lacking. A first step is improving blood collection systems to meet transfusion needs while promoting the quality of component separation according to WHO guidance such that plasma ultimately can fulfil the requirements for fractionation. Short of fractionation, with the production of quality plasma, various patient needs can be met through local production of small-scale plasma protein products. For example, cryoprecipitate has proven value in treating peripartum haemorrhaging and massive bleeding in trauma cases, and can also be used to treat patients with haemophilia A and von Willebrand disease. Ensuring viral safety of cryoprecipitate is paramount to expanding its use. In general, scientific evidence of safety and efficacy is central to advocating for the local production of small-scale plasma protein products. Depending on the product, with tests in place to prevent transfusion-transmitted infections and methods of PR, for example, SD treatment, it might be possible in some settings to obviate the need for NAT to ensure viral safety. Local patient organizations can support investments in locally generated treatments.

Technology suppliers have the ability and willingness to provide technical assistance starting at the early stages of plasma preparation and throughout the 'plasma value chain' from donation to fractionation. Technical assistance at the level of plasma preparation is relatively easy and can include training and help with implementation of quality standards and controls; equipment set-up, validation, and maintenance; and material procurement. Assistance at this level can facilitate operation of blood establishments under GMPs and preparation of plasma that meets regulatory standards while also improving the quality and safety of labile blood components for transfusion.

When preparation of quality plasma is managed under a nationally coordinated and well-regulated blood system, contract fractionation becomes an efficient option for access to industrially manufactured products. The partnering fractionator can take steps to ensure that locally generated plasma fulfils all requirements for quality based on GMPs through audits and inspections by both the fractionator and relevant regulatory authorities. This process of assessments concurrently provides valuable domestic learning on how plasma collection and preparation should be done. Limitations of contract fractionation include the likelihood of a narrow range of products, dependence on a third party with importation of finished products, and costs.

Creation of a national or regional fractionation plant can be considered when there is certainty to provide >200,000–300,000 L/year of quality-assured plasma as needed to enable economies of scale. Such plants may eventually provide contract fractionation services to neighbouring countries in the region. Building a fractionation plant is a long-term enterprise of 5–10 years taking at least 3–4 years from planning to operation. Owing to the skills, time and effort required, as well as the current regulatory requirements of technology transfer, technology licensing from an established fractionator is preferred over building up a system from scratch, potentially by expansion of a prior relationship with a contract fractionator. Additionally, there must be the necessary political, public health, economic and social infrastructure and commitments. Clinical studies are also needed to support product registration with competent regulatory authorities.

International plasma fractionators are generally interested in collaboration with LMICs to establish contract fractionation and are willing to provide developmental assistance. In general, it is futile to pursue contract fractionation when the country lacks sufficient blood

collection to satisfy transfusion needs. Under the Achilles Project, the WHO focused on providing assistance to Indonesia and South Africa as individual countries with large populations that could generate large volumes of plasma recovered from whole blood. The approach was comprehensive, including restructuring of the blood supply system, for example, through centralization of some functions, advancement of blood regulation, and implementation of quality systems at blood establishments. However, fractionators can accept pooling of plasma volumes from multiple countries in a region when the countries have common quality standards and the regulators mutually agree on acceptance criteria for plasma and final products.

The way forward from the guidance provided in the foundational WHO documents and the knowledge shared during the workshop is to launch practical pilot projects based on a stepwise approach in line with recent WHO guidance and available plasma resources (Table 1) [1]. In a country with severe shortages of plasma proteins, a pilot project for local production of viral-inactivated cryoprecipitate is an option. Other countries may additionally benefit from small-scale production of Igs. Pilot projects for the local production of small-scale plasma protein products can simultaneously be pursued in multiple countries with consideration of local conditions and overall lessons learned. Successful pilot programmes can lead to expansion through replication of strategies that have worked. In yet other countries, pilot projects for plasma fractionation comparable to the Achilles Project in Indonesia and South Africa may be feasible. To make these pilot projects possible, the local situation should be supportive. First, the pilot project needs a person who is trusted in the local environment and has good connections with key local and international stakeholders. Support from stakeholder organizations is needed, including the scientific and medical communities. In all countries, including high-income countries, development of fractionation is a gradual and stepwise process. Hence, the appropriate time to begin considering small-scale local plasma processing and/or contract fractionation is when a country identifies its need for PDMPs.

In conclusion, practical solutions are needed to resolve problems in LMICs where patients with haemophilia and PIDs are not diagnosed and plasma protein products are unavailable or unaffordable. There are many hurdles, but action is urgently needed. The continued collaboration of organizations that made the workshop possible (WHO, ISBT, WFH, IPOPI and FIODS) can be a great help in supporting stepwise improvements for patients who depend on treatments with plasma proteins. At all stages of such an effort, government support is mandatory and crucial.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. WHO Guidance on increasing supplies of plasma-derived medicinal products in low- and middle-income countries through fractionation of domestic plasma [cited 2021 Dec 24]. Available from: https://apps.who.int/iris/handle/10665/340171
2. WHO Guidance on centralization of blood donation testing and processing [cited 2021 Dec 24]. Available from: https://www.who.int/publications-detail-redirect/9789240020825

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ORIGINAL ARTICLE

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Survey of blood centre readiness regarding removal of DEHP from blood bag sets: The BEST Collaborative Study

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Abstract

Background and Objectives: Di(2-ethylhexyl) phthalate (DEHP) must be removed from blood bag sets in Europe by 27 May 2025. DEHP is known to interact with the red blood cell (RBC) membrane, resulting in reduced haemolysis and thus prolonging shelf-life. Current non-DEHP alternatives result in increased haemolysis requiring reconsideration of the RBC shelf-life. Although the immediate impact of eliminating DEHP is to the European community, the non-DEHP movement could affect blood bag set availability globally. The purpose of this survey is to understand blood centre readiness regarding the transition to non-DEHP blood collection and storage systems.

Materials and Methods: A 24-question on-line survey was completed by members of the Biomedical Excellence for Safer Transfusion Collaborative research network.

Results: Responses were obtained from 16 blood collection or processing institutions. A majority of respondents (12/16) indicated that both shelf-life and haemolysis were equally important in selecting non-DEHP blood bag sets. Six respondents would accept a lower RBC product shelf-life compared to current practice. Respondents were not clear on the best non-DEHP vinyl material or RBC storage solution. Three European blood centres indicated they have developed non-DEHP transition plans. One challenge identified regarding the transition to non-DEHP is the extensive validation testing that will be required.

Conclusion: Blood centres in Europe are concerned with meeting the sunset date for DEHP, considering that limited non-DEHP blood bag and RBC storage solutions are currently available. Banning DEHP in Europe, which may have global ramifications, represents a major challenge not yet fully understood by the transfusion medicine community.

KEYWORDS
blood storage, DEHP, plasticizer

Highlights

- Blood centres in Europe are concerned with meeting the sunset date for removal of DEHP from use.
- Currently there is no clarity regarding the best combination of non-DEHP vinyl material and RBC storage solution.
- Extensive validation testing of all blood products represents a major challenge to blood centres during the transition to non-DEHP.

INTRODUCTION

The reproductive toxicity and possible endocrine disrupting activity of phthalates have been debated for years [1–3]. Consequently, phthalates have been banned from industrial applications such as cosmetics, foods and toys by the European Union [1–3]. Although phthalates, including di(2-ethylhexyl) phthalate (DEHP), were identified as a substances of very high concern, they were granted exception for use in medical devices [1, 4]. The European Medical Device Regulations (EU MDR 2017/745) limits phthalate use only when there is an acceptable risk–benefit assessment [5]. Recently, the European Chemicals Agency (ECHA) has proposed to the European Commission to terminate the medical device exemption for DEHP [6]. As a result, DEHP must be removed from blood bag sets in Europe, with an effective sunset date of 27 May 2025 [6]. There is minimal interest in non-DEHP blood bag sets in other world areas, with the exception of South Korea.

DEHP is known to interact with the red blood cell (RBC) membrane, enhancing osmotic stability and reducing haemolysis during storage, which facilitates an extended RBC shelf-life [7, 8]. A longer shelf-life helps blood centres to manage inventories especially during shortage periods when collections are low (e.g., holidays). Conversely, shelf-life can be shortened to provide RBC products with lower haemolysis. Current non-DEHP alternatives result in increased haemolysis during blood storage, requiring reconsideration of the RBC shelf-life [9–12]. In order to mitigate this impact, research is moving towards 'alternative combinations' of new plasticizers with new RBC storage solutions [12, 13]. Nevertheless, RBC shelf-life may be shortened to ensure acceptable level of haemolysis with the understanding that haemolysis and RBC quality will be compromised compared to current combinations of DEHP blood bag sets and RBC storage solutions.

Transition to non-DEHP blood bag sets used in the collection and storage of blood will impact blood establishment processes and blood product validation. Lack of preparedness for the sunset date may impact the availability of blood bag sets and consequently the availability of blood for transfusion. Although the immediate impact of removing DEHP from medical devices and all other industrial applications is currently only mandated for the European community, the non-DEHP movement could affect availability of products globally as DEHP or DEHP-plasticized materials could become scarce and/or expensive worldwide. The purpose of this survey is to understand blood centres' expectations and requirements regarding the transition to non-DEHP blood bag sets.

METHODS

An on-line survey was distributed to the Biomedical Excellence for Safer Transfusion (BEST) Collaborative research network, a scientific group of professionals from transfusion medicine, blood centres, and blood bag set manufacturers working in the field of blood transfusion [14]. The survey was tested by two blood bank professionals prior to distribution to optimize questions and response options. The survey consisted of 24 questions categorized as current state, future state, validation and/or implementation and expected manufacturing support. Some questions were multiple choice, whereas others were free text.

The survey was open from 5 February to 2 May 2021. The survey was distributed to BEST Collaborative members who then forward the survey to appropriate blood centre partners; therefore, the total number of individuals who received the survey link is unknown. Responses were reviewed to ensure that only one response was received per blood collection or blood processing organization. No duplicate responses were received. Data from the survey were compiled in Microsoft Excel 365.

RESULTS

In total 16 responses were obtained representing 16 unique blood collection or blood processing institutions: 9 from Europe, 3 from Asia-Pacific and 4 from North America. Survey responses originated from nine different countries. Table 1 summarizes the blood bag set manufacturers, apheresis devices and RBC storage solutions in current use for each survey participant.

When asked about the level of awareness at blood collection or processing institutions regarding the transition to non-DEHP, 4 of the 16 respondents said that non-DEHP is a priority at their organization; all 4 of these responses were from blood centres in Europe. Three respondents indicated a high awareness level and six a moderate awareness level. Three respondents indicated poor awareness of non-DEHP: one in Europe, one in Asia-Pacific and one in North America.

Survey participants were asked to report their current RBC shelf-life and the lowest acceptable RBC shelf-life (Figure 1). The longest reported shelf-life was 49 days and the shortest was 21 days. The typical RBC shelf-life reported in Europe was 35 days (seven of nine centres). Six of the 16 respondents would accept a lower shelf-life compared to current practice, 3 of which were in Europe and 3 in North America (Figure 1). Of the six respondents accepting a shorter shelf-life, five would shorten shelf-life by 7 days and one by 14 days (Figure 1). Only three respondents would accept a shelf-life below 35 days.

Table 2 includes current haemolysis levels as measured at the end of shelf-life by quality control testing and future acceptable haemolysis levels reported by the survey participants. The mean haemolysis reported for all the respondents is around 0.3% in top-and-bottom blood bag sets and a slightly higher (0.45%) for top-and-top blood bag sets. These values suggest that there is a slight margin of increase in haemolysis without having an impact on process compliance or necessity to reduce the shelf-life.

One respondent in Europe represents a blood processing facility (e.g., RBC freezing, storage, thawing) not a blood collection facility, and hence was not included in Table 2. This centre reported a mean haemolysis level of 0.5% ± 0.3% after deglycerolization and

TABLE 1 Blood bag set manufacturers and RBC storage solutions in current use by survey participants

Centre	World area	Whole blood collections sets for preparation of RBCs	RBC storage solution for whole-blood-derived RBCs	Apheresis devices used to collect RBCs	RBC storage solution for apheresis RBCs
1	Europe	Macopharma Top and Top Macopharma Top and Bottom	SAGM	No	
2	Europe	Blood processing not blood collection site		No	
3	Europe	Macopharma Top and Top Macopharma Top and Bottom	SAGM	No	
4	Europe	Macopharma Top and Top Fresenius Top and Bottom	PAGGS-M	No	
5	Europe	Macopharma Top and Bottom	SAGM	Not reported	
6	Europe	Macopharma Top and Top Macopharma Top and Bottom	SAGM	No	
7	Europe	Macopharma Quadruple Bag Macopharma Quintuple Bag Top and Bottom Fresenius Quintuple Bag Top and Bottom	SAGM	Terumo Trima Accel	SAGM
8	Europe	Fresenius Kabi CompoFlow Top and Bottom	SAGM	No	
9	Europe	Macopharma Top and Top Macopharma Top and Bottom	SAGM	No	
10	Asia-Pacific	Terumo Top and Bottom Terumo Top and Bottom JMS Top and Bottom	SAGM	Not reported	SAGM
11	Asia-Pacific	Macopharma Top and Top Macopharma Top and Bottom Macopharma WB Collection without AC	SAGM	No	
12	Asia-Pacific	Kawasumi ABQ Kawasumi ABQ Kawasumi ABQ Terumo Top and Top JMS Top and Top	MAP	No	
13	North America	Macopharma Top and Top Macopharma Top and Bottom	SAGM	No	
14	North America	Haemonetics Leucotrap WB System Heamonetics Leucotrap RC System Terumo Reveos LR Set	AS-3	Terumo Trima Accel	AS-3
15	North America	Fresenius-Kabi Bio-Flex Red Cell Filter Fenwal BPU with BioFlex RBC Filter	AS-1	Fresenius Kabi Alyx Fresenius Kabi Amicus	AS-3
16	North America	Fresenius-Kabi Bio-Flex Red Cell Filter Fresenius-Kabi Bio-Flex Red Cell Filter Haemonetics RC2D CP2D/AS-3 Haemonetics CPDA-1	AS-1, AS-3	Terumo Trima Accel Fresenius Kabi Alyx	AS-1, AS-3

Note: RBC storage solutions: AS-1, additive solution-1; AS-3, additive solution-3; MAP, mannitol-adenine-phosphate; PAGGS-M, phosphate, adenine, glucose, guanosine, saline and mannitol; SAGM, saline adenine glucose and mannitol [15]. Anticoagulant (AC): CPD, citrate phosphate dextrose; CP2D, CPD with high-dextrose; CPDA-1, CPD with adenine; CPDA-2, CPD with adenine and extra dextrose [15].

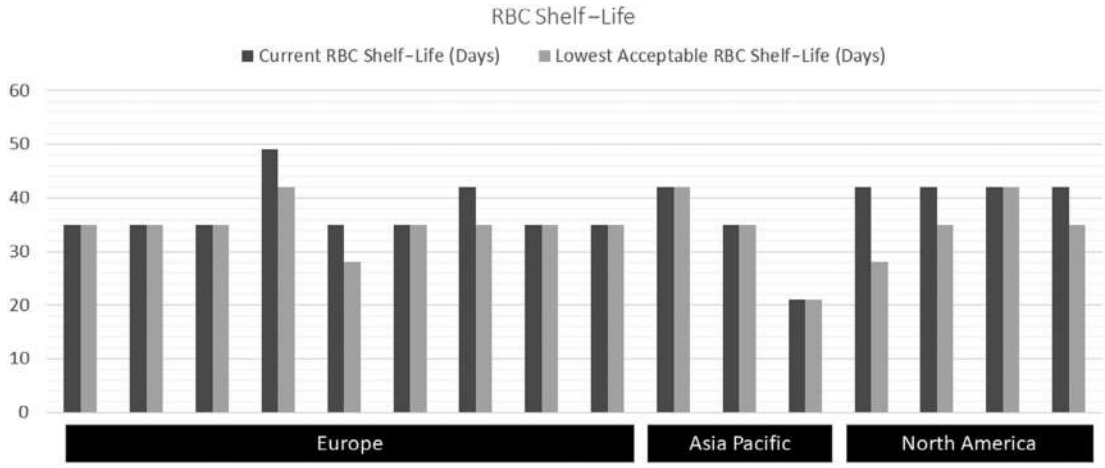

FIGURE 1 Survey participants were asked to report (■) the current shelf-life of RBCs at their institutions and (■) the lowest RBC shelf-life that their institutions would be willing to accept

TABLE 2 Current haemolysis levels measured at end of RBC shelf-life by quality control testing and highest acceptable average haemolysis level for future non-DEHP systems

Centre	World area	Blood bag sets	Mean haemolysis (%)	Standard deviation	Sample size (*N*)	Highest acceptable mean haemolysis at end of shelf-life for non-DEHP system
1	Europe	Not specified	0.3	0.2	141	0.50%
3	Europe	Not specified	0.304	0.21	612	<0.80%
4	Europe	Not specified	0.2	0.4	5000	0.40%–0.50%
5	Europe	Top and Bottom	0.01	0.002	14	0.8% in 90% of tested units.
6	Europe	Top and Top	0.23	0.13	100	0.80%
		Top and Bottom	0.18	0.32	98	
7	Europe	Whole Blood Filtration	0.46	0.33	382	0.53%
		Top and Bottom	0.34	0.28	738	
8	Europe	Top and Bottom	0.3	0.25	194	0.3% ± 0.25%
9	Europe	Top and Top	0.55	0.6	83	0.5% to meet 75% <0.8% OR 0.3% to meet 90% <0.8%
		Top and Bottom	0.27	0.46	107	
10	Asia-Pacific	Top and Bottom	0.31	0.19	120	0.80%
11	Asia-Pacific	Not specified	0.23	0.18	2169	<0.80%
13	North America	Top and Top	0.3	0.15	960	0.40%
		Top and Bottom	0.2	0.12	6180	
14	North America	Top and Top - WB System	0.22	0.01	1662	0.38%
		Top and Top - RC System	0.19	0.01	1621	
		Whole Blood Automation	0.29	0.01	328	

13–15 days storage in AS-3 (1066 units tested). One blood centre in Asia-Pacific and two in North America do not routinely measure haemolysis and were excluded from Table 2.

When asked which is more important to the blood centres, that is, longer shelf-life or lower haemolysis, 12 of the 16 respondents said both are equally important, 2 responded lower haemolysis, two responded longer shelf-life. Higher haemolysis can result in failed quality control testing. However, a lower shelf-life jeopardizes blood product availability.

Survey participants were asked if their institutions are currently using non-DEHP bags for blood collection, processing, or RBC storage, and only three institutions responded in the affirmative. One

blood centre responded using BTHC as a platelet storage bag. One blood centre responded with respect to platelets, using the TACSI automated blood processing system and the INTERCEPT blood system, which are non-DEHP. One blood centre in Europe is using non-DEHP RBC storage bags for paediatrics but the blood collection system contains DEHP.

The survey asked to identify what is the best non-DEHP vinyl material for use in blood bags from the following choices: di(isononyl) cyclohexane-1,2-dicarboxylate (DINCH), tris (2-ethylhexyl)trimellitate, di-2-ethylhexyl-terephtalate (DEHT), n-butyryl-trihexyl-citrate (BTHC), other, do not know. Nine of the respondents indicated that they did not know. Three chose DEHT, two chose DINCH and two chose BTHC. The survey also asked, 'which RBC storage solution would you choose assuming all solutions are available in your country'. Nine respondents chose PAGGS-M from the following choices: AS-1, AS-3, AS-5, AS-7, SAGM, PAGGS-M, other, do not know. One chose AS-1, two chose AS-3 and one chose 'other' (MAPS). Several respondents commented that PAGGS-M was considered a better storage solution, which is already approved for in use in Germany. Two respondents commented that AS-7 is interchangeable with PAGGS-M but not currently available. This trend towards PAGGS-M may have been influenced by the fact that it is approved and used in Germany with limited use in the Netherlands and Switzerland, and approval is in process in France.

The survey included the question, 'How important is harmonization (selection of same non-DEHP vinyl and RBC storage solution) between different manufacturers?' Twelve participants chose 'important but not critical', two chose 'very important', and one chose 'not important'. Respondents commented that harmonization would allow an international collaborative approach to validation testing, which would reduce the testing burden on individual blood centres. Data could be collected at multiple centres and pooled to meet regulatory requirements. One survey participant responded that they are required to have two blood bag suppliers for emergency and disaster planning.

Only 2 of 16 respondents would maintain a dual inventory for RBC products with different shelf-lives. The primary reason for the resistance to dual inventory is because it would be too difficult to manage by blood centres and hospitals. One respondent would consider dual inventories if it maximizes the RBC supply, and one respondent indicated that their organization already manages RBCs with different shelf-lives.

Regarding validation requirements, 13 of 16 of respondents selected that a national agency would define requirements for validation of non-DEHP systems and new RBC storage solutions. Two respondents selected validation requirements to be defined at the local blood centre and one respondent selected validation requirements to be defined at the international governing level. If the level of effort required for validation of a new non-DEHP system is significant, 9 of the 16 respondents would be extremely likely or likely to choose a sole source provider or manufacturer, 5 would be unlikely or extremely unlikely, while 2 did not know. The decision to go sole source or carry more than one non-DEHP blood bag set will be made by local blood centres (four respondents), regional organizations (three respondents), national agencies (six respondents), in collaboration with other countries (one respondent), and do not know (one respondent).

Only three blood centres participating in the survey have developed transition plans to move to non-DEHP blood bags sets. All these three blood centres are in Europe. The transition plans are currently in the draft stage.

The survey included an open-ended question regarding the biggest challenges with the transition to non-DEHP blood bag sets. Several blood centres responded that non-DEHP systems will require validation of all three blood products (platelets, plasma and RBC) the scale of which is 'daunting'. Because of the challenges with haemolysis of non-DEHP systems, suppliers of blood collection and processing systems have not released commercial options for blood centres to evaluate in order to choose the best option. There is also concern that initial validation testing by the blood bag manufacturers and blood centres will focus on routine blood component storage and will not include testing of secondary processing steps such as irradiation, freezing, thawing, pathogen inactivation, deglycerolization and washing.

The survey also included questions about data reporting and the level of support expected by blood centres of blood bag set manufacturers. The most common data requested for RBCs was percent haemolysis, but three respondents would like to see the full panel of in vitro quality parameters (e.g., ATP, 2,3-DPG, Na^+, K^+, pH) and there was one comment about potential regulatory requirement for clinical studies. The most common data requested for platelets was pH, but five respondents would like to see additional in vitro quality parameters (e.g., platelet count, platelet activation markers, morphology) and there was one request for radiolabel recovery and survival studies. The most common data requested for plasma was FVIII followed by fibrinogen, with two respondents interested in additional factor levels. No respondents indicated the need for clinical studies for plasma. All respondents would like to see the blood bag manufacturers share data regarding quality of blood products with non-DEHP blood sets. Fourteen of 15 respondents would also like to see data regarding vinyl material and bag integrity during routine use, storage and freeze/thaw conditions.

DISCUSSION

To date, DEHP alternatives have not shown the same level of RBC quality during storage. Adoption of non-DEHP blood bag sets will likely result in higher RBC haemolysis or shorter shelf-life. As the absolute levels of haemolysis in an RBC product will depend on the RBC preparation methods (e.g., top-and-top, top-and-bottom systems), the RBC storage solution and the intended shelf-life, this must be considered when comparing data across blood centres [16, 17]. In addition, haemolysis data are technique- and time-dependent, making it difficult to compare or combine haemolysis levels between blood centres that are likely using different sampling and measuring techniques [18]. Discrepancies in how haemolysis is measured raises questions about the forthcoming evaluations of non-DEHP systems and acceptance by the different jurisdictions around the world.

It is anticipated that non-DEHP systems will result in an increase in haemolysis measured in stored RBCs [9]. Current regulatory agencies require that the haemolysis at the end of storage does not exceed 0.8% in 90% of units tested (Council of Europe) [19] or does not exceed 1% in 95% of units with 95% confidence (US Food and Drug Administration) [17]. Current levels of haemolysis might allow for a slight increase in average haemolysis (e.g., from 0.3% to 0.5%) without fear of failing quality control testing (Table 2). However, if the mean haemolysis increases significantly, there is higher risk of failing quality control requirements when taking into consideration the standard deviation of haemolysis measurements due to donor and product manufacturing factors [16]. The majority of respondents (10/16) were unwilling to accept a lower shelf-life compared to current practice (Figure 1). Shorter shelf-life may impact blood availability, especially when blood collections are low during summer months and holidays. Shorter shelf-life will also impact the availability of rare blood types.

When asked if blood centres are currently using non-DEHP bags for blood collection, processing, or RBC storage, only three institutions responded 'yes' even though some blood storage bags in current use are non-DEHP. In retrospect, the question was poorly worded and should have specified WB collection versus RBC storage versus platelet storage. Nevertheless, the response to this question demonstrates that, in general, there is still a misunderstanding in transfusion medicine about non-DEHP blood collection and storage systems. For example, BTHC platelet storage bags are non-DEHP but they are connected to collection sets that contain DEHP. In order for a blood product to qualify as non-DEHP, the entire blood fluid pathway must be manufactured from non-DEHP materials. The ECHA mandates that DEHP must be removed from the entire system including bags, tubing, connectors, and so on.

Respondents were not clear on the best non-DEHP vinyl material and RBC storage solution. Responses may have been influenced by studies showing that haemolysis levels with DEHT or DINCH in combination with PAGGS-M were similar to those with DEHP [10–12]. Larsson et al. reported haemolysis values less than the regulatory threshold of 0.8% for DEHT blood bags with PAGGS-M storage solution; however, these values were still higher compared to the values for DEHP blood bags with SAGM storage solution throughout the 49-day RBC storage period [10]. Similarly, Graminske et al. reported higher haemolysis levels for DEHT with PAGGS-M compared to DEHP with AS-1 at the end of the 42-day storage period [11]. Both these studies were pool-and-split studies, which likely contributed to the lower measured haemolysis levels [10, 11]. An increase in the overall haemolysis poses a risk to meeting regulatory statistical requirements. Lagerberg et al. reported haemolysis levels for (i) DINCH with PAGGS-M and (ii) DINCH with AS-3 comparable to those of DEHP with SAGM at 42 days of storage [12]. Dabay et al. demonstrated the equivalence in a paired pool-and-split study where DINCH blood bags with AS-7 RBC storage solution were compared with DEHP blood bags with AS-1 [13]. In this study, RBC haemolysis at 42 days of storage without periodic mixing was equivalent between the two study arms. The influence of RBC storage solutions is significant as a means of meeting haemolysis requirements while maintaining the current conventional storage period with non-DEHP blood bag sets.

Removal of DEHP from the entire blood bag set requires validation of RBC, platelet, and plasma quality by both the blood bag set manufacturers and the blood centres. One survey participant commented that the validation required to transition to non-DEHP for all three blood products is daunting. More than half of the respondents would be extremely likely or likely to choose a sole source provider or manufacturer. However, a sole source challenges business continuity plans at the blood centre and country level if no alternative is available in case a major issue arises with an individual supplier. Only three blood centres in Europe who participated in the survey have made progress in terms of validation and transition plans. These blood centres may emerge as leaders, and the lagging blood centres may follow in their pathway in terms of validation testing and requirements.

Owing to the magnitude of the change, there is a higher level of expectation regarding data sharing by blood bag set manufacturers regarding blood product quality and vinyl material integrity. There are also concerns about testing secondary processing of blood products, such as irradiation, washing, pathogen inactivation and deglycerolization. These manipulations will require some level of validation with non-DEHP materials, and it is not clear who is responsible for this testing.

Survey participants represented blood collection and blood processing facilities. One limitation of this survey was the lack of representation from healthcare providers using the final blood product. It would be important to conduct a survey of the end users of blood products to understand their perspective on the impending transition to non-DEHP. Another limitation of the survey is that it did not address the cost, cost of the blood bag sets, as well as cost of testing and validation studies by blood centres who are either government or nonprofit organizations.

In general, blood centres in Europe are concerned with meeting the 2025 sunset date, as there are no clear alternatives to DEHP that perform as well as current DEHP blood bag sets. New non-DEHP blood bag sets require the CE Mark, which will be a long process especially if the new EU Medical Device Regulations (EU MDR 2017/745) up-classify blood bag sets from Class IIb to Class III [20]. The CE Mark is followed by the tender and procurement process, which can take 6–12 months during which blood centres can begin their validation activities. The transition to non-DEHP in Europe may also have broader global implications. With respect to blood collection, it may be cost-prohibitive for manufacturers to maintain two separate inventories (DEHP and non-DEHP) such that all countries will eventually migrate to non-DEHP products.

Polyvinyl chloride with DEHP has been used for over 50 years and has proven to be a reliable and robust technology allowing major improvements in blood component quality, safety, storage and transport compared to the previously used glass bottles. Current environmental concerns are mainly focused on the life cycle of DEHP and its potential health hazard to donors and recipients. This European effort is pushing transfusion medicine into a necessary change towards safer plasticizers with a lower environmental impact. Banning DEHP, the silent champion of RBC storage, represents a major challenge to the industry which, as this study shows, is not yet fully understood by

the transfusion medicine community. Nevertheless, the time has come for manufacturers, blood suppliers and competent authorities to implement non-DEHP blood bag sets and work together to share the burden.

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CONFLICT OF INTEREST

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REFERENCES

1. SCHEER (Scientific Committee on Health, Environmental and Emerging Risks). Guidelines on the benefit-risk assessment of the presence of phthalates in certain medical devices covering phthalates which are carcinogenic, mutagenic, toxic to reproduction (CMR) or have endocrine-disrupting (ED) properties. Final version adopted at SCHEER plenary on 2019 June 18.
2. Shaz BH, Grima K, Hillyer CD. 2-(Diethylhexyl)phthalate in blood bags: is this a public health issue? Transfusion. 2011;51:2510–7.
3. Sampson J, de Kort D. DEHP-plasticised PVC: relevance to blood services. Transfus Med. 2011;21:73–83.
4. ECHA (European Chemicals Agency). Opinion of the Member State Committee on ECHA's draft recommendation for the first amendment of existing entries in Annex XIV of the REACH Regulation. Final 2019 June 26.
5. Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC.
6. Council of European Union. Annex to the Commission Regulation (EU) amending Annex XIV to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) [cited 2021 Aug 24]. Available from: https://data.consilium.europa.eu/doc/document/ST-6893-2021-ADD-1/en/pdf.
7. Rock G, Tocchi M, Ganz PR, Tackaberry ES. Incorporation of plasticizer into red cells during storage. Transfusion. 1984;24:493–8.
8. AuBuchon JP, Estep TN, Davey RJ. The effect of the plasticizer di-2-ethylhexyl phthalate on the survival of stored RBCs. Blood. 1988;71:448–52.
9. Prowse CV, de Korte D, Hess JR, van der Meer PF, Biomedical Excellence for Safer Transfusion (BEST) Collaborative. Commercially available blood storage containers. Vox Sang. 2014;106:1–13.
10. Larsson L, Sandgren P, Ohlsson S, Derving J, Friis-Christensen T, Daggert F, et al. Non-phthalate plasticizer DEHT preserves adequate blood component quality during storage in PVC blood bags. Vox Sang. 2021;116:60–70.
11. Graminske S, Puca K, Schmidt A, Brooks S, Boerner A, Heldke S, et al. In vitro evaluation of di(2-ethylhexyl) terephthalate–plasticized polyvinyl chloride blood bags for red blood cell storage in AS-1 and PAGGSM additive solutions. Transfusion. 2018;58:1100–7.
12. Lagerberg JW, Gouwerok E, Vlaar R, Go M, de Korte D. In vitro evaluation of the quality of blood products collected and stored in systems completely free of di(2-ethylhexyl)phthalate-plasticized materials. Transfusion. 2015;55:522–31.
13. Dabay M, Kline L, Zia M, Kandler R. Evaluation of SOLX® red blood cells stored in DINCH plasticized PVC container. Transfusion. 2012;52:SP56.
14. The Biomedical Excellence for Safer Transfusion (BEST) Collaborative. http://www.bestcollaborative.org. Accessed 2 Aug 2021.
15. Hess JR. An update on solutions for red cell storage. Vox Sang. 2016;91:13–9.
16. Jordan A, Chen D, Yi Q-L, Kanias T, Gladwin M, Acker J. Assessing the influence of component processing and donor characteristics on quality of red cell concentrates using quality control data. Vox Sang. 2016;111:8–15.
17. Hess JR, Sparrow RL, van der Meer PF, Acker JP, Cardigan RA, Devine DV. Red blood cell hemolysis during blood bank storage: using national quality management data to answer basic scientific questions. Transfusion. 2009;49:2599–603.
18. Almizraq RJ, Yi Q-L, Acker JP, Biomedical Excellence for Safer Transfusion (BEST) Collaborative. Impact of technical and assay variation on reporting of hemolysis in stored red blood cell products. Clin Chim Acta. 2017;468:90–7.
19. European Directorate for the Quality of Medicines and HealthCare (EDQM). Guide to the preparation, use and quality assurance of blood components. 20th ed. FRANCE: European Directorate for the Quality of Medicines and HealthCare (EDQM); 2020.
20. The Blood Transfusion Alliance [cited 2021 Aug 23]. Available from: https://bloodtransfusionassociation.org/publications/post/potential-up-classification-of-blood-bag-sets-to-class-iii

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ORIGINAL ARTICLE

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Tangential flow filtration facilitated washing of human red blood cells: A proof-of-concept study

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Abstract

Background and Objectives: Red blood cell (RBC) units in hypothermic storage degrade over time, commonly known as the RBC storage lesion. These older RBC units can cause adverse clinical effects when transfused, as older RBCs in the unit lyse and release cell-free haemoglobin (Hb), a potent vasodilator that can elicit vasoconstriction, systemic hypertension and oxidative tissue injury after transfusion. In this study, we examined a novel method of washing ex vivo stored single RBC units to remove accumulated cellular waste, specifically cell-free Hb, using tangential flow filtration (TFF) driven by a centrifugal pump.

Materials and Methods: The TFF RBC washing system was run under hypothermic conditions at 4°C, at a constant system volume with 0.9 wt% saline as the wash solution. The RBC washing process was conducted on 10 separate RBC units. For this proof-of-concept study, RBC units were expired at the time of washing (60–70 days old). Cell-free Hb was quantified by UV–visible absorbance spectroscopy and analysed via the Winterbourn equations. Pre- and post-wash RBC samples were analysed by Hemox Analyser, Coulter counter and Brookfield rheometer. The RBC volume fraction in solution was measured throughout the wash process by standard haematocrit (HCT) analysis.

Results: No substantial decrease in the HCT was observed during the TFF RBC washing process. However, there was a significant decrease in RBC concentration in the first half of the TFF RBC wash process, with no significant change in RBC concentration during the second half of the TFF cell wash process with an 87% overall cell recovery compared with the total number of cells before initiation of cell washing. Utilization of the extinction coefficients and characteristic peaks of each Hb species potentially present in solution was quantified by Winterbourn analysis on retentate and permeate samples for each diacycle to quantify Hb concentration during the washing process. Significant cell-free Hb reduction was observed within the first four diacycles with a starting cell-free Hb concentration in the RBC unit of 0.105 mM, which plateaus to a constant Hb concentration of 0.01 mM or a total extracellular Hb mass of 0.2 g in the resultant washed unit. The oxygen equilibrium curve showed a significant decrease in P_{50} between the initial and final RBC sample

Shuwei Lu and Megan Allyn contributed equally to the work.

cell wash with an initial P_{50} of 15.6 ± 1.8 mm Hg and a final P_{50} of 14 ± 1.62 mm Hg. Cooperativity increased after washing from an initial Hill coefficient of 2.37 ± 0.19 compared with a final value of 2.52 ± 0.12.

Conclusion: Overall, this study investigated the proof-of-concept use of TFF for washing single RBC units with an emphasis on the removal of cell-free Hb from the unit. Compared with traditional cell washing procedures, the designed system was able to more efficiently remove extracellular Hb but resulted in longer wash times. For a more complete investigation of the TFF RBC washing process, further work should be done to investigate the effects of RBC unit storage after washing. The designed system is lightweight and transportable with the ability to maintain sterility between uses, providing a potential option for bedside ex vivo transfusion in clinical applications.

KEYWORDS
diafiltration, haemoglobin, haemolysis, RBC washing, red blood cell, tangential flow filtration

Highlights

- The use of TFF to wash RBCs can significantly decrease the cell-free Hb concentration in RBC units.
- Cell washing using TFF did not negatively affect the RBC population. RBCs before and after washing exhibit basically the same oxygen equilibrium curve.
- The shear stress between RBCs and hollow fibre inner wall does not increase haemolysis during washing, and the total RBC concentration remains constant throughout the washing process.

INTRODUCTION

Red blood cells (RBCs) degrade during ex vivo storage and lead to the accumulation of toxic haemolysis by-products in the unit such as haemoglobin (Hb) during the maximum 42-day storage period set by the US Food and Drug Administration (FDA) [1, 2]. Upon transfusion, cell-free Hb in the stored RBC unit can extravasate from the blood volume into the tissue space, where it scavenges nitric oxide, a potent vasodilator and elicits vasoconstriction and systemic hypertension within the patient [3]. Additionally, tissue extravasation of cell-free Hb leads to tissue deposition of iron and inevitably leads to oxidative tissue injury [4].

Therefore, in light of the accumulation of haemolysis by-products during ex vivo RBC storage, RBC washing is often employed to remove accumulated waste products within an RBC unit prior to transfusion to mitigate any potential side effects [5, 6]. Many commercially available technologies are clinically employed to wash stored RBC units prior to transfusion [7, 8].

Manual washing of single RBC units is an attractive approach due to its low cost, but it is laborious, limited in processing volume by the available centrifuge cup size and exposes the unit to a high risk of bacterial contamination [5, 9]. In contrast, automated RBC unit washing systems are most commonly used in clinical settings to remove toxic by-products, one example is the COBE 2991 cell processor (Terumo, Somerset, NJ) [7, 10]. The COBE 2991 is an open cell processing system that utilizes centrifugation to facilitate separation based on differences in blood component density and can effectively reduce proinflammatory markers, restoring overall RBC quality near the end of the unit's ex vivo shelf life [9, 10]. Unfortunately, levels of haemolysis have been shown to rapidly increase after washing with the COBE 2991 and often surpass prewashed levels before the 24-h transfusion window is reached [9]. Additional work investigating the ability of the COBE 2991 to wash 40- to 42-day stored RBC units showed that after washing, the COBE 2991 is unable to provide significant reduction in total cell-free Hb after the washing process, Hb being a toxic by-product of the storage lesion [8]. Regarding this limitation, it is clear that there is an urgent need for an innovative, easy-to-use RBC washing system that addresses the current pitfalls of both manual and automated washing systems.

Considering the plethora of centrifugation-based RBC washing systems in existence, there has been substantially less research into the use of tangential flow filtration (TFF) for RBC unit washing, with no commercially available system on the market. TFF utilizes a porous hollow fibre or flat sheet membrane to enable continuous flow purification. Molecules larger than the pore size cut-off of the membrane are retained on the membrane and in the system, while molecules smaller than the pore size cut-off permeate through the membrane and are removed from the system. The use of TFF techniques on

whole blood currently utilize gravity-driven separation, and are primarily focused on the separation of whole blood into plasma and RBC fractions [11, 12]. Compared with centrifugal separation, TFF systems can process a wide range of RBC concentrate volumes, allows for easy storage solution exchange and has the ability to maintain sterility via the use of autoclavable materials and a closed-loop system. Additionally, the currently designed TFF system is lightweight and easily transportable, with the system as a whole (system vessel, pump, tubing and hollow fibre filter) weighing less than 2 kg. One study used TFF to wash RBCs in diafiltration mode, and concentrated a cryopreserved RBC unit, but resulted in significant RBC lysis (most likely due to use of a peristaltic pump), and focused on investigating the rheological properties of the washed unit [13]. Alternatively, in this study, we explored implementation of a novel TFF system using a low shear stress inducing centrifugal pump to separate stored RBCs from their primary haemolysis by-product Hb. This proof-of-concept study developed a system that effectively washes RBC units as demonstrated by the successful removal of cell-free Hb and shows negligible process-induced haemolysis, providing a viable alternative to current manual and automated RBC washing systems. Because this is a proof-of-concept study, we evaluated TFF-facilitated RBC washing effectiveness using outdated stored human RBCs and saline as the wash solution to investigate the absolute worst-case scenario of RBC unit quality. Future studies will focus on washing unexpired stored RBC units, using FDA-approved storage solutions as the wash solution, and expanding the analysis of analytes beyond cell-free Hb.

MATERIALS AND METHODS

Materials

Sodium chloride (NaCl), sodium hydroxide (NaOH) and 0.2-μm Titan3 sterile filters were purchased from Fisher Scientific (Waltham, MA). Hollow fibre TFF modules (S02-E65U-07N, modified polyethersulfone membrane, 0.65-μm pore size, composed of 110 individual hollow fibres, 0.75 mm internal diameter and 520 cm^2 total surface area) were purchased from Repligen (Rancho Dominguez, CA). A biocompatible centrifugal pump (PuraLev i30SU) that exposes cells to low shear stresses was purchased from Levitronix (Framingham, MA). A minicentrifuge (50-090-100, working speed 6000 rpm, max speed 6600 rpm) from Fisher Scientific was used to separate RBCs from the wash solution. Expired leuko-reduced packed human RBCs (RBC units, 60–70 days old, stored in AS-1) were generously donated by the Transfusion Services of the Wexner Medical Center at The Ohio State University, Columbus, Ohio. The RBC units used in this study were expired and deidentified and thus required no ethics committee approval.

RBC washing

The TFF-facilitated RBC washing process was performed on individual stored RBC units expired past the FDA-regulated 42-day storage period. All RBC units were stored and washed at 4°C in a chromatography refrigerator. A single RBC unit was transferred to a 1-L Nalgene container by opening and transferring the RBC unit in a sterile biosafety cabinet. The haematocrit (HCT) in the total system volume (which includes the combined fluid volume in the TFF filter, lines and retentate vessel) was standardized to 45% with 0.9 wt% saline to decrease variability in the starting HCT between RBC units and was selected as an appropriate average between male and female HCT values. Prior to washing, single RBC units were mixed by gentle inversion to yield a homogenous cell suspension. An initial sample of the RBC unit was taken to establish baseline conditions prior to washing. Figure 1 shows the general schematic of the TFF-facilitated RBC washing system. Exactly 0.9 wt% saline solution was diafiltered into the retentate vessel to maintain a constant system volume. The sample port in the RBC retentate loop was used to take retentate samples. The retentate line was connected to a centrifugal pump from the reservoir, which operated at a constant flow rate of 1000 ml/min and directed RBCs through the bottom of the TFF filter against gravity, with RBCs being retained in the retentate, while cell debris, proteins and other molecules smaller than 0.65 μm passing into the permeate. The permeate line enters a cell waste container with samples collected directly from the permeate line.

RBCs in the retentate vessel were first acclimated to the system components via circulation for 2 min with the permeate line closed. This ensured proper mixing of the RBCs in the system before starting the constant volume diafiltration cell washing process. During the acclimatization period, an initial 0× diacycle sample was taken to confirm the HCT of 45% was successfully achieved before initiating the diafiltration process. The total system volume was used to determine the volume per diacycle (i.e., one complete system exchange volume) and was measured by collecting permeate leaving the system. Retentate and permeate samples were taken at the end of each diacycle and stored at 4°C for analysis. RBC units were washed with standard 0.9 wt% saline washing solution for the entirety of the process and were not stored ex vivo after the washing process was completed. Instead, newly washed RBCs were utilized for haemoglobin purification based on published procedure. A total of 10 diacycles were completed per RBC wash for each individual RBC unit, with a total of 10 individual RBC units being subjected to the TFF RBC washing process. The scope of this work focuses primarily on establishing the feasibility of washing expired RBC units as a proof of concept of the TFF-facilitated RBC washing approach; however, preliminary results from a limited study of TFF-facilitated RBC washing of unexpired units is included in Supporting information section.

HCT analysis

The HCT was determined by injecting 65 μl of each retentate sample, including an initial sample from the RBC unit, into a mylar-wrapped 75-mm capillary tube (Drummond, Broomall, PA) followed by centrifugation in a Sorvall Legend Micro 17 Microcentrifuge (Fisher Scientific) for 5 min to pellet the RBCs. After centrifugation, the capillary tubes

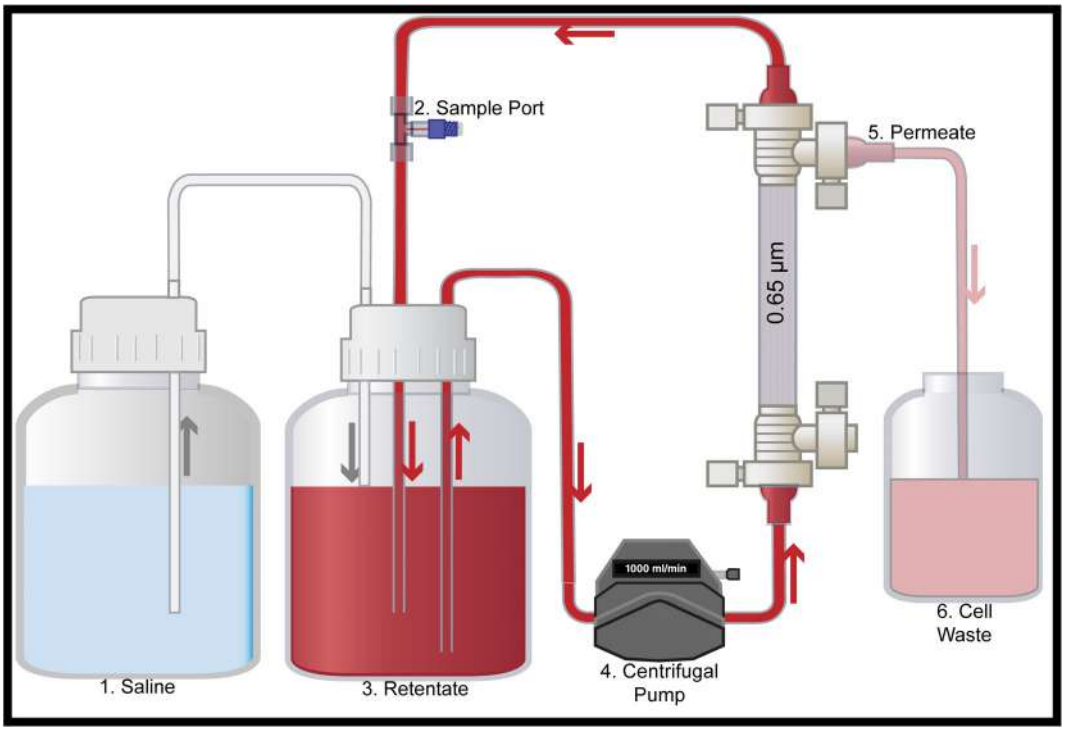

FIGURE 1 Process flow diagram for the red blood cell (RBC) washing process. Exactly 10 single RBC units were processed using the tangential flow filtration (TFF) RBC washing system. (1) Reservoir containing 0.9 wt% saline; (2) sample port used for retentate sampling; (3) retentate vessel, 0.65-µm TFF filter used to wash RBCs; (4) centrifugal pump; (5) permeate waste from the process (contains species <0.65 µm in size) and (6) cell waste. Arrows indicate the direction of flow

were quantified using a standardized HCT graph to obtain the HCT of RBCs in the retentate.

Total Hb quantification

The cell-free Hb concentration was quantified via UV–visible absorbance spectrometry on a diode-array spectrophotometer HP 8452A (Olis, Bogart, GA). Retentate supernatants were isolated via centrifugation using a minicentrifuge (Fisher Scientific) at 6000 rpm for 2 min to pellet the RBCs and analysed after separation. Processed retentate and permeate samples were sterile filtered through a 0.2-µm Titan3 filter (Fisher Scientific) for UV–visible spectral analysis. Sterile filtration was employed to reduce light scattering during optical measurements to only quantify cell-free Hb. The Winterbourn equations were used to determine the total concentration of cell-free Hb in the permeate and retentate samples and further used for the cell-free Hb mass balance [14]. Quantification using UV–visible spectral analysis examines the absorbance of the various Hb oxidation species that could be present in a sample. Using the characteristic absorbance peaks—oxyhaemoglobin at 577 nm, methaemoglobin at 630 nm and haemichrome at 560 nm—and the defined extinction coefficients of each species at each wavelength previously described by Winterbourn, the quantity of each different species can be found and used to find the total Hb of the sample. The equations used for each species quantification are given as follows:

$$[\text{Oxyhaemoglobin}] = 119_{A577} - 39_{A630} - 89_{A560} \quad (1)$$

$$[\text{Methaemoglobin}] = 28_{A577} + 307_{A630} - 55_{A560} \quad (2)$$

$$[\text{Hemichrome}] = -133_{A577} - 114_{A630} + 233_{A560} \quad (3)$$

Oxygen equilibrium of RBCs

Oxygen equilibrium curves (OECs) for RBCs pre- and post-wash were measured using a Hemox Analyser (TCS Scientific Corp., New Hope, PA) operated at 37 ± 0.1°C. RBC samples were diluted into 5 ml of Hemox buffer (pH 7.4) with 20-µl additive A, 20-µl additive B and 20-µl anti-foaming agent (TCS Scientific). Data obtained from the Hemox Analyser were fit to the Hill equation using an Igor (Wavemetrics, Portland, OR) script to regress the oxygen affinity (P_{50}, pO_2 at which half of the Hb is saturated with oxygen) and Hill coefficient (*n*, cooperativity of O_2 binding to Hb) [15].

RBC viscosity

A Brookfield DV3T rheometer with a CP-40 spindle (Brookfield, Middleboro, MA) was used to measure the viscosity of RBC samples at 37°C and a shear rate of 160 s^{-1} [16, 17].

RBC cell count

Cell counts for retentate samples were measured using a Multisizer 4e Coulter counter (Beckman Life Sciences, Indianapolis, IN). RBC samples were diluted ×100 prior to addition of 100 μl of the diluted cells into 20 ml of filtered Isoton solution (Beckman Life Sciences) prior to Coulter counter analysis.

Data analysis

Results are reported as the mean ± standard deviation. RStudio (version 1.3.1093; RStudio Inc., Boston, MA) was used to analyse all data. A one-way analysis of variance (ANOVA) was utilized along with Tukey honestly significant difference (HSD) post-test for data analysis. *T*-tests were used for P_{50} and *n* initial and final comparisons. A two-tailed *p*-value <0.05 was considered statistically significant.

RESULTS

Time for each diacycle remained constant

The average time per diacycle remained constant at ~10 min throughout the RBC washing process for a total of 10 diacycles (Figure 2a). The entire process takes 100 min to wash one RBC unit (10 diacycles) or 40 min to remove the majority of cell-free Hb (four diacycles). The lesser washing time (40 min) necessary to remove the majority of cell-free Hb in the unit is triple the average 14-min washing time for processing a single unexpired RBC unit using the COBE 2991 [8]. There was no significant difference in the time per diacycle during the washing process ($p = 0.999$, NS) (Figure 2a). The residence time of RBCs in the retentate reservoir varied slightly due to the variance in the volume of each RBC unit, but on average, the system volume was ~350 ml. Based on the system volume and the pump volumetric flow rate, the residence time was calculated to be 0.4 min (i.e., time for the system volume to complete one circuit in the TFF system).

HCT remains constant throughout TFF processing

RBCs from a single RBC unit (initial) were standardized to 45% HCT (0×) in the system from an initial HCT of ~65% (Figure 2b). The effect of each diacycle on the HCT in the system was analysed using a one-way ANOVA from 0× to 10× diacycle. The HCT did not change significantly throughout the course of the RBC washing process ($p = 0.124$, NS).

RBC concentration remains constant across diacycles

The concentration of RBCs in the retentate vessel was measured throughout the RBC washing process (Figure 2c). The RBCs were measured at a diameter of 4.4 μm, which corresponds to the approximate spherical diameter of RBCs measured via Coulter counter analysis. The initial RBC concentration is significantly higher than the 0×

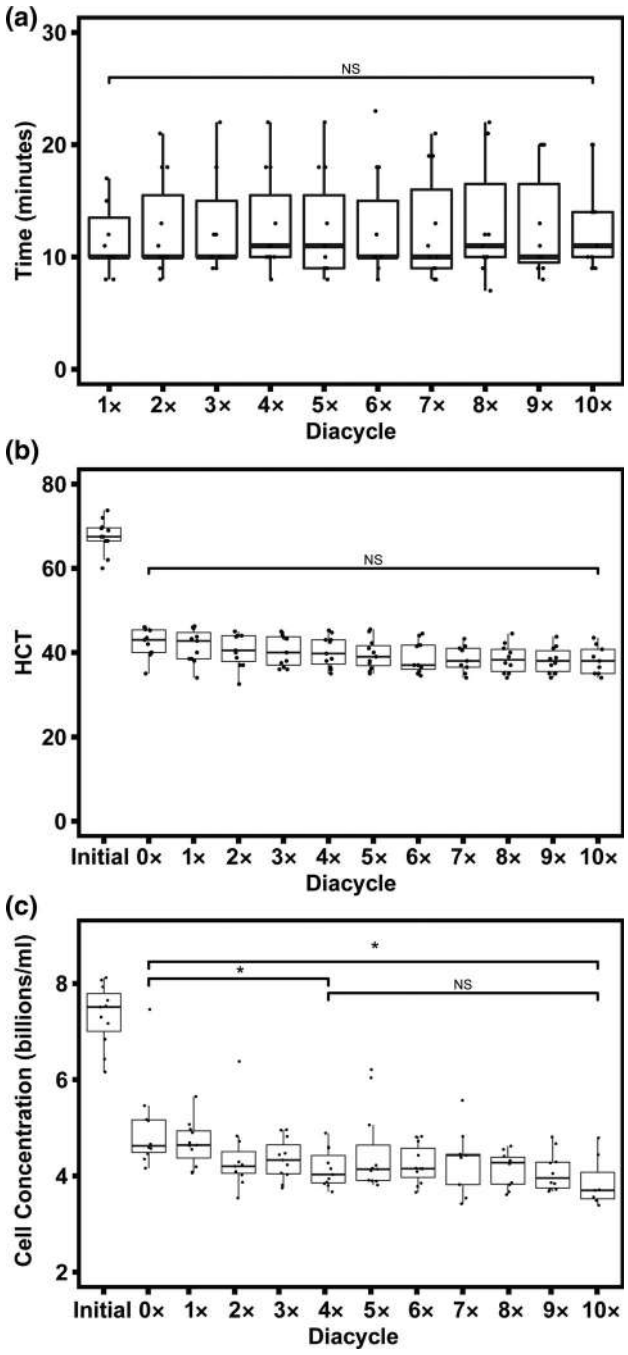

FIGURE 2 Time per diacycle during the red blood cell (RBC) washing process did not vary significantly between diacycles ($p = 0.999$, NS) (a). Haematocrit (HCT) was standardized to 45% at the 0× diacycle and did not decrease significantly over the course of washing ($p = 0.124$, NS) (b). Cell count within the tangential flow filtration (TFF) system was measured across diacycles (c). An analysis of variance for cell count change from the 0× diacycle to 10× diacycle was significant ($p = 0.0107$, *), with additional significance found between the 0× diacycle to the 4× diacycle ($p = 0.031$, *). Exactly 10 single RBC units were processed using the TFF RBC washing system

diacycle, due to standardization to 45% HCT. The initial RBC concentration measured directly from RBC units was ~7.510 ± 0.37 billion cells/ml and decreased to 4.625 ± 0.35 billion cells/ml at the 0× diacycle after standardizing the HCT to 45%. These RBC concentration values are similar to values in the literature [18]. RBC concentration differences between diacycles were analysed using one-way ANOVA comparing the entire RBC wash process from 0× to 10× diacycle and were found to show a significant decrease in cell concentration over the entire TFF process (p = 0.0107, *). The RBC concentration decreased significantly from 4.625 ± 0.35 billion cells/ml at 0× to 4.030 ± 0.27 billion cells/ml at the 4× diacycle (p = 0.031, *) corresponding to 87% cell recovery at the end of the wash process, with no significant cell loss after the 4× diacycle (p = 0.356, NS). This suggests haemolysis of cells with significantly compromised cell membranes occurs early in the TFF washing process and then tapers off as the process continues.

Hb concentration in the retentate and permeate decreases over multiple washing diacycles

The retentate cell-free Hb concentration is shown in Figure 3a. There is, on average, 0.105 mM of cell-free Hb within the RBC unit before processing. After wash, the cell-free Hb concentration decreases to ~0.0157 mM (at the end of the 10× diacycle). The TFF RBC washing process significantly reduces the cell-free Hb concentration after four diacycles (p = 2.8E-7, ***) and remains constant from 4× to 10× (p = 0.458, NS) diacycles. A Tukey HSD test was performed within the 0× to 4× diacycles and found significance between the 0× to 1×, 2×, 3× and 4× diacycles (p = 0.001, 2.9E-5, 1.6E-6 and 7E-7, respectively). No significance was observed between 1× and 4× diacycles. This suggests that the majority of cell-free Hb was removed at the beginning of the TFF wash process.

The permeate cell-free Hb concentration is shown in Figure 3b. Significance was found within the 1× to 10× diacycle dataset (p = 6.2E-15, ***). The overall dataset was then split into two subsets from 1× to 4× diacycle and from 4× to 10× diacycle, with significance found within the 1× to 4× diacycle dataset (p = 1.6E-5, ***). A Tukey HSD test was performed within the dataset from the 1× to 4× diacycle and found that there was a significant difference between the 1× diacycle and the 2×, 3× and 4× diacycles (p = 0.006, 0.0001 and 3.2E-5, respectively). No significance was found between the other diacycles. The US FDA considers stored RBC units with a haemolysis level less than 1% to be safe for transfusion. Exactly 1% haemolysis is roughly equivalent to a cell-free Hb concentration of 0.01 mM in the unit, which is lower than the final cell-free Hb concentration achieved in this study of ~0.0157 mM observed post-wash [19]. One must, however, remember that the RBC units in this study were outdated (60–70 days old), which likely contributed to the higher final cell-free Hb concentration compared with literature values for washing non-expired RBC units [20]. Again, expired units were used in this proof-of-concept study, as a worst-case scenario for RBC unit quality, to demonstrate the feasibility of washing RBCs using this novel TFF RBC washing process. Future studies will examine the effectiveness of washing unexpired RBC units via TFF.

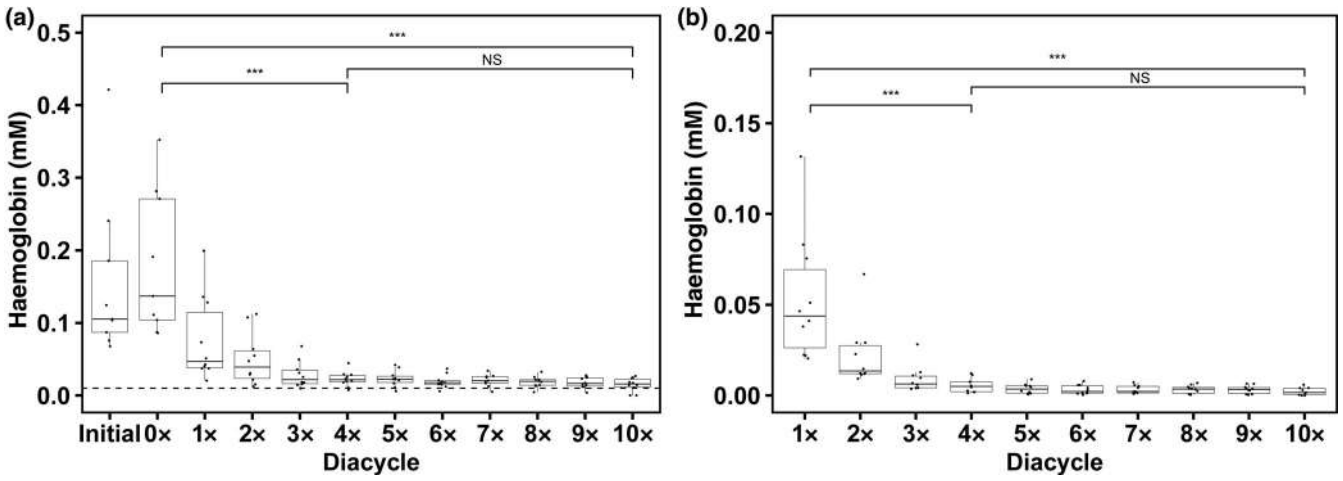

FIGURE 3 Retentate cell-free haemoglobin (Hb) concentration (a) over 10 diacycles of red blood cell (RBC) washing using tangential flow filtration (TFF). Exactly 10 single RBC units were processed using the TFF RBC washing system. An analysis of variance (ANOVA) test was performed on the data subsets 0× to 10× diacycles, 0× to 4× diacycles and 4× to 10× diacycles (α = 0.05, H_o = haemoglobin [Hb] concentration is independent of the diacycle). Significance was found within the 0× to 10× diacycles (p = 8E-16, ***), and 0× to 4× diacycles (p = 2.8E-7, ***) subgroups. Significance was found between the 0× diacycle and 1×, 2×, 3× and 4× diacycles using Tukey honestly significant difference (HSD) (p = 0.001, 2.9E-5, 1.6E-6 and 7E-7, respectively). No significance was found within the 4× to 10× diacycles subgroup (p = 0.458, NS). The dotted line indicates 1% haemolysis. Permeate cell-free Hb concentration (b) over 10 diacycles of RBC washing using TFF. An ANOVA test was performed on the data subsets 1× to 10× diacycles, 1× to 4× diacycles and 4× to 10× diacycles (α = 0.05, H_o = Hb concentration is independent of the diacycle). There was significance within the data for 1× to 10× diacycles (p = 6.2E-15, ***) and for 1× to 4× diacycles (p = 1.6E-5, ***). Within the 1× to 4× diacycle subgroup, significant differences were found between the 1× diacycle and 2×, 3× and 4× diacycles using Tukey HSD (p = 0.006, 0.0001 and 3.2E-5, respectively). No significance was found within the 4× to 10× diacycle (p = 0.151, NS)

TABLE 1 Cell-free haemoglobin (Hb) overall mass balance

Diacycle	Retentate cell-free Hb (g)	Permeate cell-free Hb (g)
Initial	2.060 ± 1.539	
0×	2.196 ± 1.191	
1×	1.052 ± 0.834	1.648 ± 1.100
2×	0.618 ± 0.347	0.678 ± 0.547
3×	0.363 ± 0.187	0.273 ± 0.227
4×	0.296 ± 0.106	0.173 ± 0.130
5×	0.291 ± 0.106	0.114 ± 0.089
6×	0.245 ± 0.084	0.097 ± 0.081
7×	0.256 ± 0.070	0.100 ± 0.071
8×	0.229 ± 0.066	0.099 ± 0.069
9×	0.218 ± 0.056	0.099 ± 0.068
10×	0.211 ± 0.043	0.094 ± 0.069
Total cell-free Hb removed: 1.849 g.		

Total cell-free Hb mass balance

The total cell-free Hb for each diacycle was quantified in order to perform an overall cell-free Hb mass balance and provides a complete understanding of the fate of the cell-free Hb removed via the TFF washing process. The mass of cell-free Hb for retentate and permeate samples was averaged for all 10 replicates (Table 1). The initial mass of cell-free Hb in individual RBC units is, on average, 2.06 g with a Hb concentration of 0.105 mM, which corresponds to a haemolysis level of ~10%. After the completion of the first diacycle, the cell-free Hb in the retentate is ~1.05 g, indicating that ~50% of the extracellular Hb has been removed at this stage. Cell-free Hb continues to be removed from the retentate for all subsequent diacycles. The system reached peak performance at approximately four diacycles, reaching a haemolysis level of 2.2 ± 1%, and decreased slightly with the additional six diacycles. The percent haemolysis at four diacycles for unexpired RBC units was decreased to 1.3 ± 0.6% from an initial value of 4.2 ± 2.1% haemolysis (Supporting information). Therefore, the TFF system is effective at removing cell-free Hb, but more optimization is necessary to increase the washing speed and facilitate greater removal of cell-free Hb to match current accepted practices in RBC washing.

Additionally, a cell-free Hb mass balance analysis on the permeate samples shows significant Hb removal at the start of the diafiltration process (Table 1). The 1× diacycle is the first diacycle with permeate flow and removes the majority of cell-free Hb. The total mass of cell-free Hb continually decreases in the permeate as washing proceeds, supporting the theory that the TFF system is not inducing additional shear stress on the RBCs to cause lysis beyond what is needed to enable separation of cell-free Hb from the remaining RBCs in the retentate.

RBC mechanical quantification

The viscosity of RBCs in unprocessed RBC units and final post-wash RBCs (10× diacycle) was measured to be 9.252 ± 1.477 cP and 3.928 ± 1.766 cP, respectively. A significant change in RBC viscosity was observed due to the initial dilution of the RBC unit to 45% HCT, followed by removal of cell debris, proteins and smaller molecules. The final washed RBC concentrate viscosity was higher than fresh RBCs (2.9 cP at 160 s^{-1} and 37°C) and is indicative of the advanced age of the RBC units used in this current study [17]. This viscosity is, however, a significant improvement from the aged RBC unit's initial viscosity of 9.252 cP.

At low shear rates, blood behaves as a Casson fluid and is shear thinning, whereas at shear rates above 100 s^{-1}, it behaves as a Newtonian fluid [21]. The following equation was used to calculate the shear stress on the inner wall of the TFF hollow fibre lumen with the assumption that the RBC suspension behaves as a Newtonian fluid above a shear rate of 100 s^{-1} and does not require additional analysis based on the Casson fluid model. In Equation (4), P_o is the pressure at the inlet and P_L is the pressure at the outlet of an individual hollow fibre in the TFF cartridge. R is the inner radius of the hollow fibre and L is the effective length of each hollow fibre.

$$\tau_w = \frac{(P_o - P_L)R}{2L}. \quad (4)$$

The shear rate value was extrapolated to 3670 s^{-1} from manufacturer-provided values of 4000 s^{-1} at a flow rate of 1.09 L/min. The pressure drop within the TFF system from the inlet to the outlet of the hollow fibre cartridge was measured at an average value of 2 psig over 10 diacycles. From this value, we calculated the shear stress to be 12.9 Pa, which is not significantly higher than physiological conditions, and significantly lower than haemolytic shear stress levels of ~400 Pa [22, 23]. By exposing the aged RBC unit to significant shear stress prior to transfusion, RBCs with weakened cell membranes are lysed and removed from the system. From the applied shear stress, we obtained the theoretical viscosity of 3.5 cP for the washed RBC suspension, which corroborates our experimentally measured viscosity using rheometry.

Oxygen equilibrium measurements

To confirm that TFF-facilitated RBC washing does not negatively affect the oxygen delivery characteristics of RBCs, the OEC of RBCs pre- and post-wash was measured. The OEC of RBCs directly from the unprocessed RBC unit (initial) and after the 10× diacycle (final) is shown in Figure 4a. The left shift of the curve after washing is indicative of the higher oxygen affinity of washed RBCs versus unwashed RBCs.

The OEC provides key details about the ability of the Hb encapsulated in the RBC to bind and release oxygen, which is represented by the regressed P_{50} and n. A direct comparison between P_{50} values of the initial unwashed RBCs and the final washed RBCs shows that the P_{50} decreased from an initial value of 15.6 ± 1.8 mm Hg to 14 ± 1.62 mm Hg post-wash ($p = 0.0493$, *) (Figure 4b). The Hill coefficient (n) comparison between the initial unwashed RBCs and final washed RBCs shows an increase ($p = 0.0497$, *) from

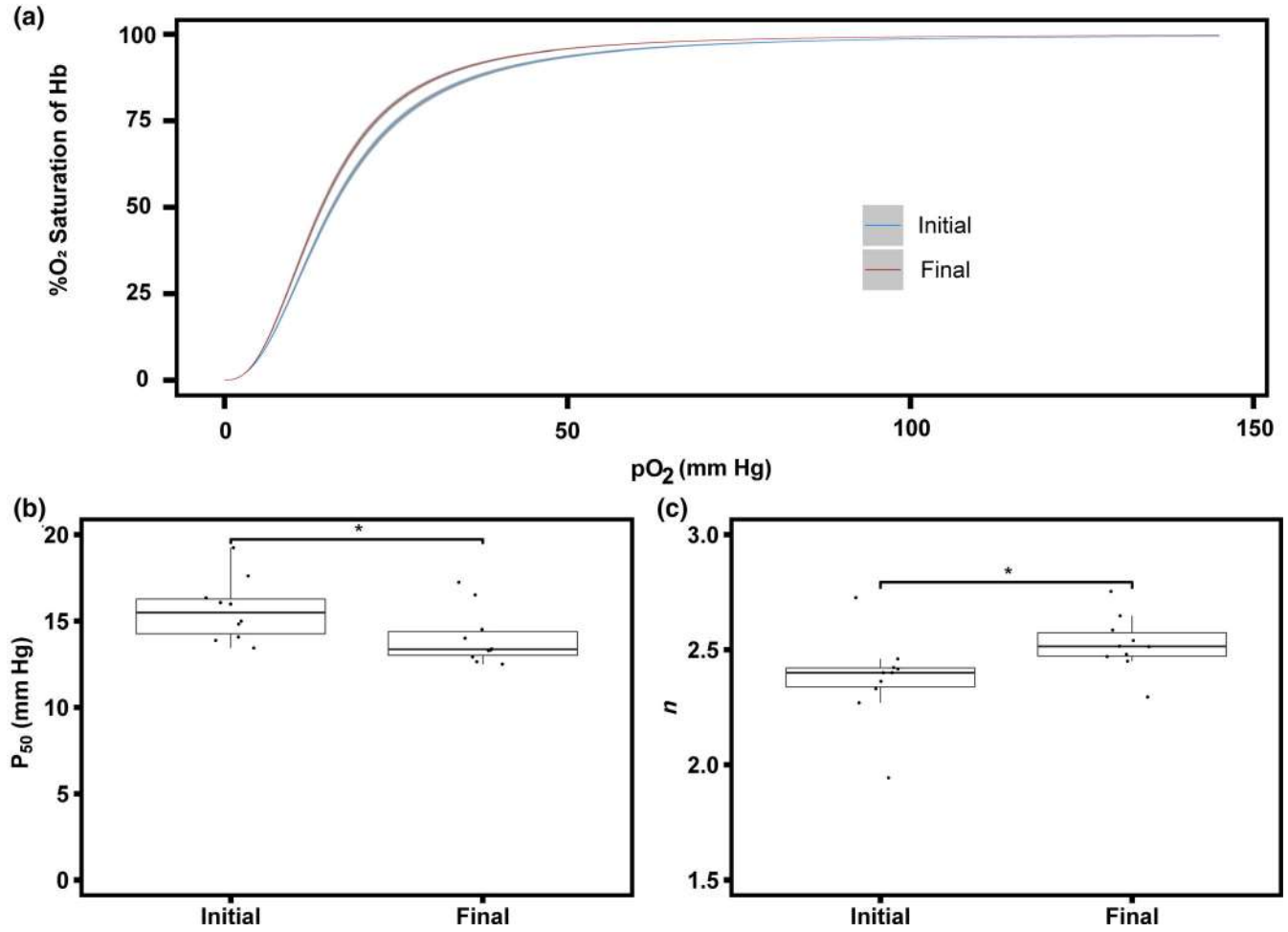

FIGURE 4 Oxygen equilibrium curve (OEC) of both the initial red blood cell (RBC) unit and 10× dicycle sample (a). Exactly 10 single RBC units were processed using the tangential flow filtration (TFF) RBC washing system. The OEC of the initial RBC unit is shown in blue with a dark grey 95% CI. The OEC of the final 10× sample is shown in red with a dark grey 95% CI. P_{50} values (b) of the initial sample from the RBC unit and the final sample (10× diacycle) after the TFF wash process ($p = 0.0493$, *). Hill coefficient (n) (c) of the initial sample from the RBC unit and the final sample (10× diacycle) after the TFF wash process ($p = 0.0493$, *)

2.37 ± 0.19 to 2.52 ± 0.12 (Figure 4c). 2,3-Bisphosphoglycerate, the allosteric effector, that decreases Hb-binding affinity to oxygen by stabilizing the tense quaternary state of Hb is significantly depleted during ex vivo RBC storage, which shifts the OEC to the left, increasing the oxygen affinity of the RBCs [24]. A left shift in the OEC suggests increased oxygen affinity and tighter binding of oxygen by Hb [25].

DISCUSSION

In conclusion, a novel RBC washing technique utilizing TFF for removing haemolysis by-products in a single RBC unit was proposed, with the primary goal of this study to determine the effectiveness of the system in removing extracellular cell-free Hb without inducing further cellular damage. Quantification and characterization of pre- and post-wash RBC units was centred around the presence of cell-free Hb due to equipment availability at The Ohio State University and does not include the full extent of analytical methods or analytes that could be used to characterize RBC washing effectiveness. While there was no direct comparison between TFF and manual or automated RBC washing systems, comparisons could be drawn between the results from this study to results in the literature. A recent review of RBC washing technology focusing on manual washing and open and closed automated washing systems compares the removal of immunogenic components within RBC units and the resulting long-term storage impacts [26]. Compared with manual and automatic systems, the proposed TFF system uses more wash solution volume, but shows improved removal of Hb and comparable RBC recovery. The wash time is longer for the TFF process in part due to the increased wash volume. Results show that successful removal of free Hb plateaus after four diacycles, allowing for the potential for optimization of the number of diacycles beyond the standard 10 that was utilized. A major shortcoming of the current study is the limited information about long-term storage after the TFF wash process. This proof-of-concept study was focused on an immediate comparison between the RBC unit pre- and post-wash

and aimed to only verify that the TFF process can remove the majority of cell-free Hb from expired RBC units. Preliminary data, presented in Supporting information section, show the effectiveness of the TFF RBC washing system in removing cell-free Hb from unexpired units and presents similar results. To provide a more complete comparison between RBC washing by the TFF system and traditional washing procedures, further work characterizing more analytes post-wash is necessary along with optimization of the pump flow rate and back-pressure regulation for potential process time improvements. Despite the need for an expanded investigation into post-wash storage of RBC units, the designed system has the potential to revolutionize RBC washing systems in part due to its small physical footprint and sterilizable components. This allows the system to easily be transported for potential bedside ex vivo RBC washing and with the implementation of sterile conditions, could allow for direct transfusion of the post-wash RBCs to mitigate increases in potentially immunogenic by-products derived from ex vivo storage.

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S.L. and M.A. contributed equally to this project, ran red blood cell washing processes and analysed process data. S.L. and M.A. both wrote and edited the manuscript. M.W. provided instrumentation resources and helped in meeting project goals. A.F.P. and J.J.C. provided lab resources and funding, designed experiments, analysed data, advised S.L., M.A. and M.W. and edited the manuscript.

CONFLICT OF INTEREST

There are no conflicts of interest.

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REFERENCES

1. Food and Drug Administration (FDA). CFR - code of Federal Regulations Title 21. 2019. Vol. 21. Available from: Www.Fda.Gov
2. Hess JR, Sparrow RL, Van Der Meer PF, Acker JP, Cardigan RA, Devine DV. Red blood cell hemolysis during blood bank storage: using national quality management data to answer basic scientific questions. Transfusion. 2009;49:2599–603.
3. Buehler PW, Alayash AI. Toxicities of hemoglobin solutions: in search of in-vitro and in-vivo model systems. Transfusion. 2004;44:1516–30.
4. Smith A, McCulloh RJ. Hemopexin and haptoglobin: allies against heme toxicity from hemoglobin not contenders. Front Physiol. 2015;6:187.
5. Cholette JM, Henrichs KF, Alfieris GM, Powers KS, Phipps R, Spinelli SL, et al. Washing red blood cells and platelets transfused in cardiac surgery reduces postoperative inflammation and number of transfusions. Pediatr Crit Care Med. 2012;13:290–9.
6. Stapley R, Rodriguez C, Oh JY, Honavar J, Brandon A, Wagener BM, et al. Red blood cell washing, nitrite therapy, and antiheme therapies prevent stored red blood cell toxicity after trauma-hemorrhage. Free Radic Biol Med. 2015;85:207–18.
7. Hansen A, Yi QL, Acker JP. Quality of red blood cells washed using the ACP 215 cell processor: assessment of optimal pre- and post-wash storage times and conditions. Transfusion. 2013;53:1772–9.
8. Bennett-Guerrero E, Kirby BS, Zhu H, Herman AE, Bandarenko N, McMahon TJ. Randomized study of washing 40-to 42-day-stored red blood cells. Transfusion. 2014;54:2544–52.
9. Lu M, Lezzar DL, Vörös E, Shevkoplyas SS. Traditional and emerging technologies for washing and volume reducing blood products. J Blood Med. 2019;10:37–46.
10. O'Leary MF, Szklarski P, Klein TM, Young PP. Hemolysis of red blood cells after cell washing with different automated technologies: clinical implications in a neonatal cardiac surgery population. Transfusion. 2011;51:955–60.
11. Johnson L, Kwok M, Marks DC. Preparation of red blood cell concentrates and plasma units from whole blood held overnight using a hollow-fibre separation system. Transfus Med. 2015;25:13–9.
12. Brune T, Hannemann-Pohl K, Nißle K, Ecker N, Garritsen H. Quality, stability, and safety data of packed red cells and plasma processed by gravity separation using a new fully integrated hollow-fibre filter device. Adv Hematol. 2009;2009:1–6.
13. Castino F, Wickramasinghe SR. Washing frozen red blood cell concentrates using hollow fibres. J Membr Sci. 1996;110:169–80.
14. Winterbourn CC. Oxidative reactions of hemoglobin. Methods Enzymol. 1990;186:265–72.
15. Hill AV. The possible effects of the aggregation of the molecules of hæmoglobin on its dissociation curves. J Physiol. 1910;40:i–vii.
16. Cabrales P, Martini J, Intaglietta M, Tsai AG. Blood viscosity maintains microvascular conditions during normovolemic anemia independent of blood oxygen-carrying capacity. Am J Physiol Heart Circ Physiol. 2006;291:581–90.
17. Cabrales P, Tsai AG, Intaglietta M. Is resuscitation from hemorrhagic shock limited by blood oxygen-carrying capacity or blood viscosity? Shock. 2007;27:380–9.
18. Yoon C, Noh S, Lee JC, Ko SH, Ahn W, Kim HC. Influence of the washing program on the blood processing performance of a continuous autotransfusion device. J Artif Organs. 2014;17:118–22.
19. Ni J, Zhu W, Wang Y, Wei X, Li J, Peng L, et al. A reference chart for clinical biochemical tests of hemolyzed serum samples. J Clin Lab Anal. 2021;35:1–7.
20. Sowemimo-Coker SO. Red blood cell hemolysis during processing. Transfus Med Rev. 2002;16:46–60.
21. Fournier RL. Basic transport phenomena in biomedical engineering. 2nd ed. New York, NY: Taylor & Francis; 2007. p. 450.
22. Koutsiaris AG, Tachmitzi SV, Batis N. Wall shear stress quantification in the human conjunctival pre-capillary arterioles in vivo. Microvasc Res. 2013;85:34–9.
23. Paul R, Apel J, Klaus S, Schügner F, Schwindke P, Reul H. Shear stress related blood damage in laminar Couette flow. Artif Organs. 2003;27:517–29.
24. Valtis DJ, Kennedy AC. Defective gas-transport function of stored red blood-cells. Lancet. 1954;263:119–25.
25. Hamilton C, Steinlechner B, Gruber E, Simon P, Wollenek G. The oxygen dissociation curve: quantifying the shift. Perfusion. 2004;19:141–4.
26. Cardigan R, New HV, Tinegate H, Thomas S. Washed red cells: theory and practice. Vox Sang. 2020;115:606–16.

SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Comparison of the programmed freezer method and deep freezer method in the manufacturing of frozen red blood cell products

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Abstract

Background and Objectives: Frozen-thawed red blood cells (FTRCs) are useful blood components to patients with rare blood phenotypes. However, frozen red blood cells (FRCs) sometimes cause significant haemolysis after thawing due to the freeze/thaw process. In this study, we aimed to focus on the former process and reduce process-related haemolysis.

Materials and Methods: Five-day-old red blood cells (RBCs) (5D) or 9-week-old RBCs (9 W) were glycerolized, pooled and split into two aliquots. RBCs were frozen using either the programmed freezer (PF) method or the deep freezer (DF) method. After 4–8 weeks, the FRCs were thawed and washed. In vitro characteristics were compared between the PF and DF methods. Nine week were used as a starting material for FTRCs with the assumption that they can mimic disqualified FTRCs with respect to Hb recovery.

Results: The PF method resulted in a significantly higher Hb recovery rate than the DF method (5D: 85.9 ± 2.1 vs. $81.1\% \pm 3.5\%$, $p < 0.001$) (9 W: 56.8 ± 4.0 vs. $52.4\% \pm 3.5\%$, $p < 0.001$). Both 5D and 9W-derived FTRCs immediately after preparation prepared by the PF method were more resistible to haemolysis than those prepared by the DF method. On the other hand, there were no significant differences between PF and DF methods in Adenosine 5′-triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG).

Conclusion: The PF method was more suitable for RBC freezing than the DF method in terms of Hb recovery in FTRCs. Although it was only 4%–5%, the improvement in the Hb recovery rate will contribute to a more stable supply.

KEYWORDS
deep freezer, freeze-thawing, haemoglobin recovery, haemolysis, programmed freezer

Highlights

- The programmed freezer (PF) method improved the Hb recovery rate by approximately 4%–5% compared to the deep freezer method.
- The FTRCs prepared by the PF method can be expected to further the blood transfusion effect compared to the deep freezer method.

INTRODUCTION

Red blood cell (RBC) products with rare blood phenotypes can be prepared from registered rare blood donors if the pool size of such donors is adequately large [1]. However, arranging for registered donors to donate blood on demand is not easy with such a small donor pool. Therefore, frozen RBC (FRC) products are often used for transfusion in patients with rare blood phenotypes [2].

Upon receipt of an order, FRC products are thawed, washed and then shipped as frozen-thawed red blood cells (FTRCs). The method for preparing FTRCs varies from country to country. According to the Minimum Requirements for Biological Products in Japan, FRCs from RBCs should be prepared within 5 days after the blood collection and stored at less than −65°C. The storage life of FRCs is defined as up to 10 years. The standards for total Hb content also vary by country. For example, in Japan, there are no standards for haemolysis but there is a visual inspection and a haemoglobin (Hb) content test where 28 g total Hb weight or more was required until August 2020, and 24 g or more thereafter, when FTRCs are prepared from 400 ml of whole blood. In our centre, 18.6% (29 out of 156) of all FTRCs prepared between April 2012 and March 2020 did not meet the standards for total Hb content. Notably, the Hb recovery rate was 69.7% ± 9.0% (54.2%–89.0%) in the acceptable products, while the disqualified ones represented a lower Hb recovery of 44.9% ± 8.2% (20.8%–53.2%). Thus, newer technical advances for FTRC preparation to obtain higher Hb recovery may be critical to achieve a stable supply and optimal clinical effects of FTRCs.

To guarantee higher Hb recovery, erythrocyte membrane damage, one of the main causes of haemolysis, should be reduced as much as possible during the freeze/thaw/wash process. Currently, two types of freezing methods with different freezing rates are used to freeze FRCs, namely, slow freezing and rapid freezing methods [3–5]. The slow freezing method is presently used in many countries, including Japan, because it allows glycerolized RBCs to be frozen and stored in a <−65°C deep freezer (DF). By contrast, the rapid freezing method requires liquid nitrogen and the FRCs are stored at <−150°C.

The increase in osmotic pressure due to the concentration of intracellular electrolytes that occurs during freezing is thought to be the most significant cause of haemolysis [6–8]. At the cellular level, to suppress haemolysis, it is important to rapidly pass the freezing point that concentration of intracellular electrolytes occurs by rapid freezing and to minimize the increase in intracellular osmolarity. At the product level, Kobayashi et al. [9] reported that making ice crystals homogeneous and fine is possible by quick freezing after reducing the temperature difference between the central and surface parts of the sample by precooling. Thus, the rapid freezing method may reduce membrane damage; however, this method is not adopted in many countries because it requires liquid nitrogen.

In this study, we aimed to develop an alternative freezing method using a programmed freezer (PF) by regulating the freezing speed with agitation to allow uniform temperature distribution in the RBC products. The proposed method comprises of two freezing steps with agitation. In the first step (precooling step), glycerolized RBCs were gradually cooled down close to the freezing point with agitation to limit the temperature difference between the central and peripheral parts of the bag. In the second step (rapid freezing step), the glycerolized RBCs were quickly frozen so that both the central and peripheral parts of the bag went through the freezing point rapidly, mitigating the temperature difference between them. The target temperature was −120°C to avoid damage to the bag due to freezing. We compared the characteristics of the FTRCs frozen using the new method to those frozen using the well-known, slow-freezing method with 5-day (5D)-old (standard) RBCs and 9-week (9-W)-old (old) RBCs. We hypothesized that the FTRCs derived from old RBCs may mimic disqualified products with respect to low Hb recovery as starting materials for FTRCs [10, 11].

MATERIALS AND METHODS

Source of RBCs, preparation of RBC products and research ethics

Whole blood (400 ± 46 ml) was collected in polyvinyl chloride bags containing 56 ml citrate phosphate dextrose solution and was leuko-depleted by in-line filtration. The leuko-depleted whole blood was centrifuged at 4780g, for 12 min at 22°C with a centrifuge (9942; Kubota Shoji, Ltd., Tokyo, Japan) and the plasma was removed using an automatic separation device (AC-215; Termo BCT, Ltd., Tokyo, Japan). The resultant erythrocyte sediment was suspended in 95 ml of mannitol–adenine–phosphate (MAP) solution [12]. The volume range of RBCs was 220–320 ml. The RBC-MAP products were irradiated with 15 Gy of x-rays (MBR-1530A-TW; Hitachi Healthcare Systems, Ltd., Tokyo, Japan) and then stored at 4°C (MPR-415R; Panasonic, Ltd., Osaka, Japan). The 5D-old RBCs were used as standard RBCs, and 9-W-old RBCs were used as old RBCs.

This study was approved by the institutional review board of the Ethics Committee of the Japanese Red Cross Society, Blood Service Headquarters (ethical review number: 2019-015).

Glycerolization

RBCs were incubated at room temperature for 3 h and 100 ml of 60% glycerol solution (SF-60; Fuso Pharmaceutical Industries, Ltd., Osaka, Japan) was slowly added over 2 min. The mixture was stored at room temperature for 5 min and a further 300 ml of 60% glycerol solution was added. The final concentration of glycerol in the RBCs was calculated to be 33%–39%, depending on the RBC contents of the original whole blood. These manufacturing processes were performed in a closed system using a glycerolization kit (225j; Haemonetics, Inc., Boston, Massachusetts) and ACP215 (Haemonetics, Inc., Massachusetts). The glycerolized RBCs were centrifuged at 3250g, for 10 min at room temperature, and the supernatant comprising of the MAP solution and excess was removed manually using an extractor to yield a glycerolized RBC unit with a Hct of 80% ± 5%.

DF method and freezing point of glycerolized RBCs

In the DF method, the target freezing temperature of glycerolized RBCs was set at −80°C using a freezer (MDF-594; Panasonic, Ltd., Osaka, Japan). The freezing point of glycerolized RBCs was measured by monitoring the blood bag (average volume: 240 ml) using a thermo-recorder (TR-81; T&D, Ltd., Nagano, Japan) to monitor the internal transition temperature of the products.

PF method

The CM-21 (Taiyo Nippon Sanso, Ltd., Tokyo, Japan) is a PF that can control not only the freezing speed but also the agitation speed. At the first step of freezing, the target temperature of the PF was set at −20°C (rate = −40°C/min), approximately 5°C higher than the freezing point of the glycerolized RBCs, and the duration time were set to 30 min. For the second step of freezing, the glycerolized RBCs were quickly frozen until −120°C (rate = −33.3°C/min), and the duration time were set to 15 min. The agitator on the PF agitates at 120 rpm for both steps (Table 1). In addition, the internal transition temperature of the glycerolized RBCs was measured.

Preservation, thawing and washing

In the preservation process, the glycerolized RBCs were stored in a freezer (MDF-594) at −80°C for 4 to 8 weeks in both methods. After preservation, thawing and subsequent washing of the products were performed according to Meryman's method [8, 13] (three-component washing method) adopted by Japanese Red Cross Society using ACP215. Pairs of RBCs frozen using the DF or PF method were simultaneously thawed and subjected to the following processes. The FRCs were thawed in a 37°C water bath (personal-11; Taitec, Ltd., Saitama, Japan) and then washed using a washing kit (235j; Haemonetics, Inc., Massachusetts, CA) with 100 ml of 8% sodium chloride, followed by 450 ml of 1.6% sodium chloride and finally, with 1 L of 0.8% sodium chloride and 0.2% dextrose solution (Fuso Pharmaceutical Industries, Osaka, Japan). The three-component washing method is shown in Figure 1. After washing, they were centrifuged at 2690g, for 12 min at 22°C and the RBC pellets were resuspended in 95 ml of MAP solution.

TABLE 1 Programmed freezer protocol

	Step 1 Precooling		Step 2 Rapid freezing	
Target temperature (°C)	−20		−120	
Time (min)	1	29	3	12
Target cooling rate (°C/min)	−40.0	0[a]	−33.3	0[a]

[a]Maintained at target temperature.

Criteria for appropriate storage period as 'old RBCs'

Three bags of RBC products with the same ABO blood type were pooled and split to obtain three equivalent RBC units. Each RBC unit was stored at 4°C for 6, 8 or 10 weeks, and was then glycerolized, frozen using DF, thawed after 4–8 weeks and washed. Hb recovery was calculated as follows: Hb recovery rate (%) = FTRC Hb (g/unit)/glycerolized RBC Hb (g/unit) × 100. 'Old RBCs' were defined as RBCs showing an Hb recovery rate of less than 50%.

Study workflow

A schematic diagram of the workflow is shown in Figure 2. Two types of RBCs were prepared: one was standard RBCs that had been stored at 4°C for 5 days and the other was old RBCs that were stored at the same temperature but consequently showed an Hb recovery of less than 50% after freezing and thawing processes. Two products of standard RBCs were glycerolized, pooled and divided into 2 units/aliquots. One was frozen using the PF method, while the other was frozen using the DF method. The old RBCs were processed similarly. After 4–8 weeks, pairs of RBCs frozen using the DF or PF method were thawed and washed simultaneously. Finally, in vitro tests were performed, and the results were compared between the PF and DF methods.

In vitro tests

Test samples

The following samples were used for in vitro tests: [I] glycerolized RBCs after pooling and splitting but just before freezing, [II] glycerolized RBCs after freezing and thawing, [III] FTRCs immediately after preparation (Day 1) and [IV] FTRCs after storage at 4°C for 4 days (Day 4).

In vitro characteristics of the products

Product supernatants were obtained by centrifugation at 1710g for 10 min at 4°C twice to measure either Hb or K^+ concentrations. Hb concentration was quantified using the leukocrystal violet method [14, 15] as described previously, and K^+ concentration was measured at 22°C on a blood gas and electrolyte analyser (Cobas b 221; Roche Diagnostics, Ltd., Mannheim, Germany).

Weight was measured using an electronic balance (GX2000R; A&D Co., Tokyo, Japan), and volume was calculated from the product weight by taking their specific gravity (glycerolized RBCs: 1.11, FTRCs: 1.06). Hb and haematocrit (Hct) were determined using an automated blood cell counter (xs-1000i; Sysmex Co., Kobe, Japan). Hb conversion per unit is as follows: Hb (g/unit) = Hb (g/dL) × volume (dL/unit). Haemolysis was calculated as [(100-Hct) × supernatant Hb concentration (g/L) / total Hb concentration (g/L)]. The osmotic fragility of RBCs, measured by the procedure of Parpart [16], was defined as the

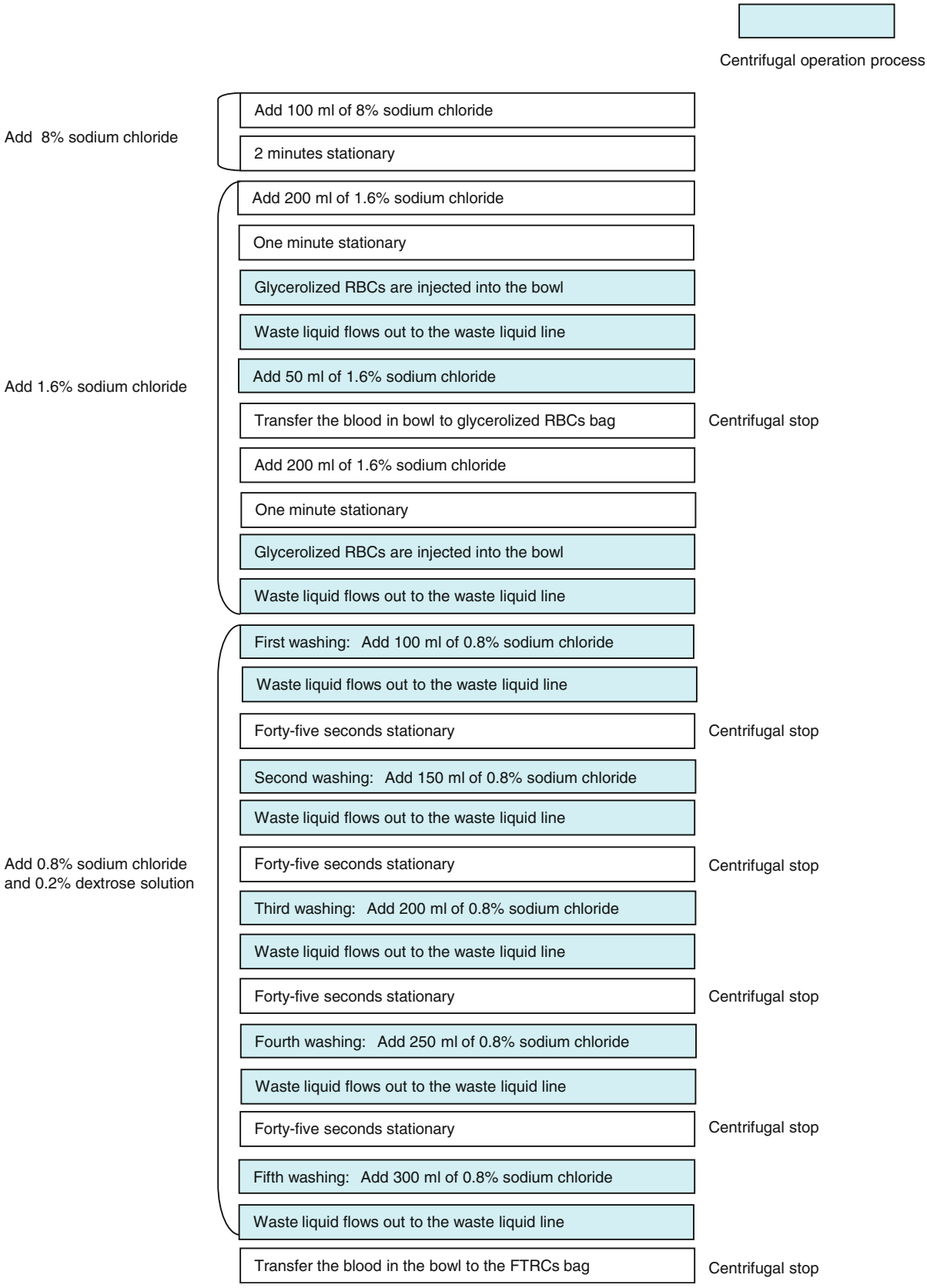

FIGURE 1 Three-component washing method protocol. FTRC, frozen-thawed red blood cell; RBC, red blood cell

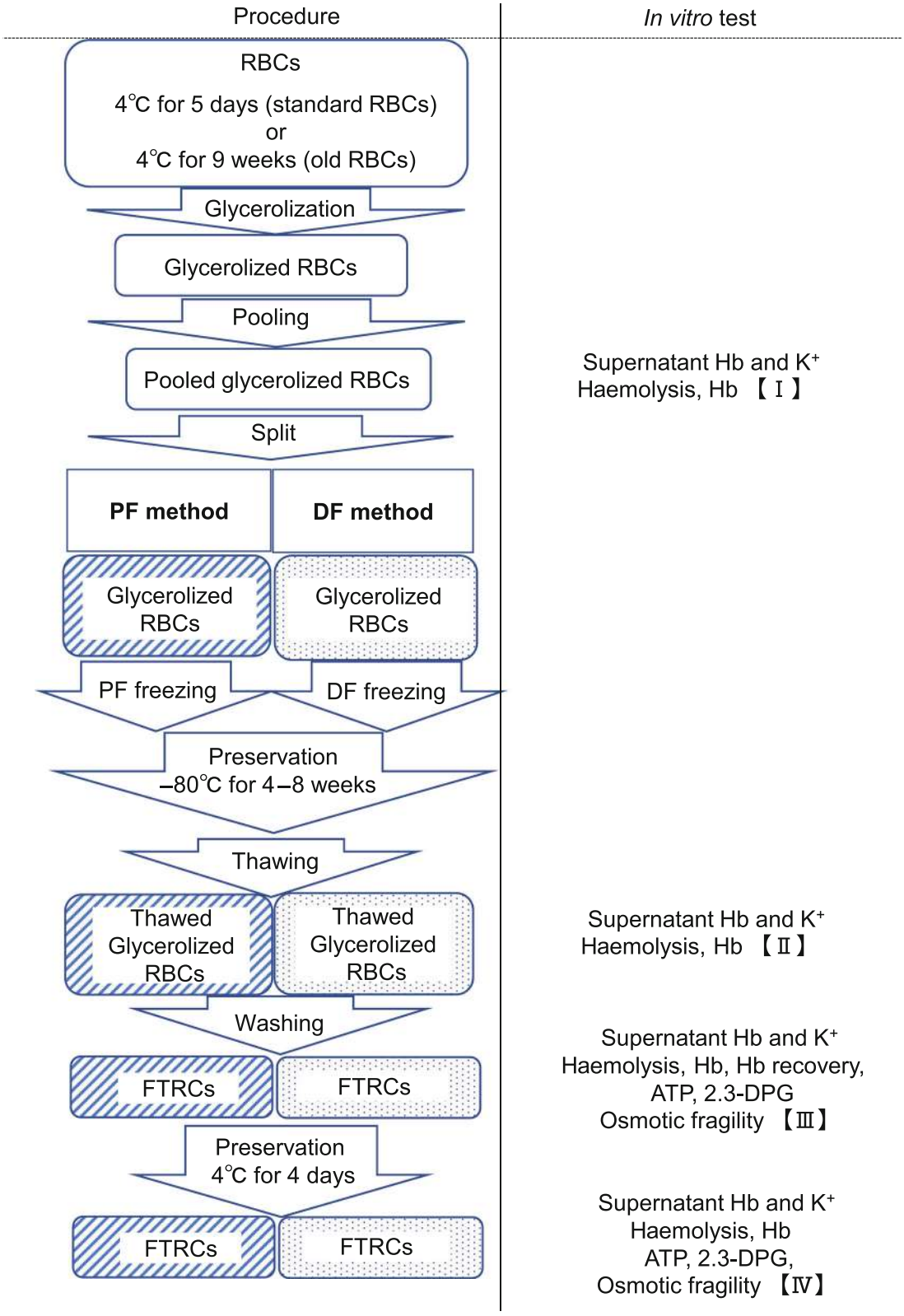

FIGURE 2 Study workflow. Red blood cell (RBC) products were stored at 4°C for either 5 days or 9 weeks. Two 5D-old products were glycerolized, pooled and into 2 units/aliquots. One was frozen using the programmed freezer (PF) method, and the other was frozen using the deep freezer (DF) method. 9 W-old RBCs were processed similarly. After 4–8 weeks, they were thawed and washed. In vitro tests were conducted on glycerolized RBCs [I] just before freezing and [II] immediately after thawing and on frozen-thawed red blood cells (FTRCs) [III] immediately after preparation and [IV] after storage at 4°C for 4 days

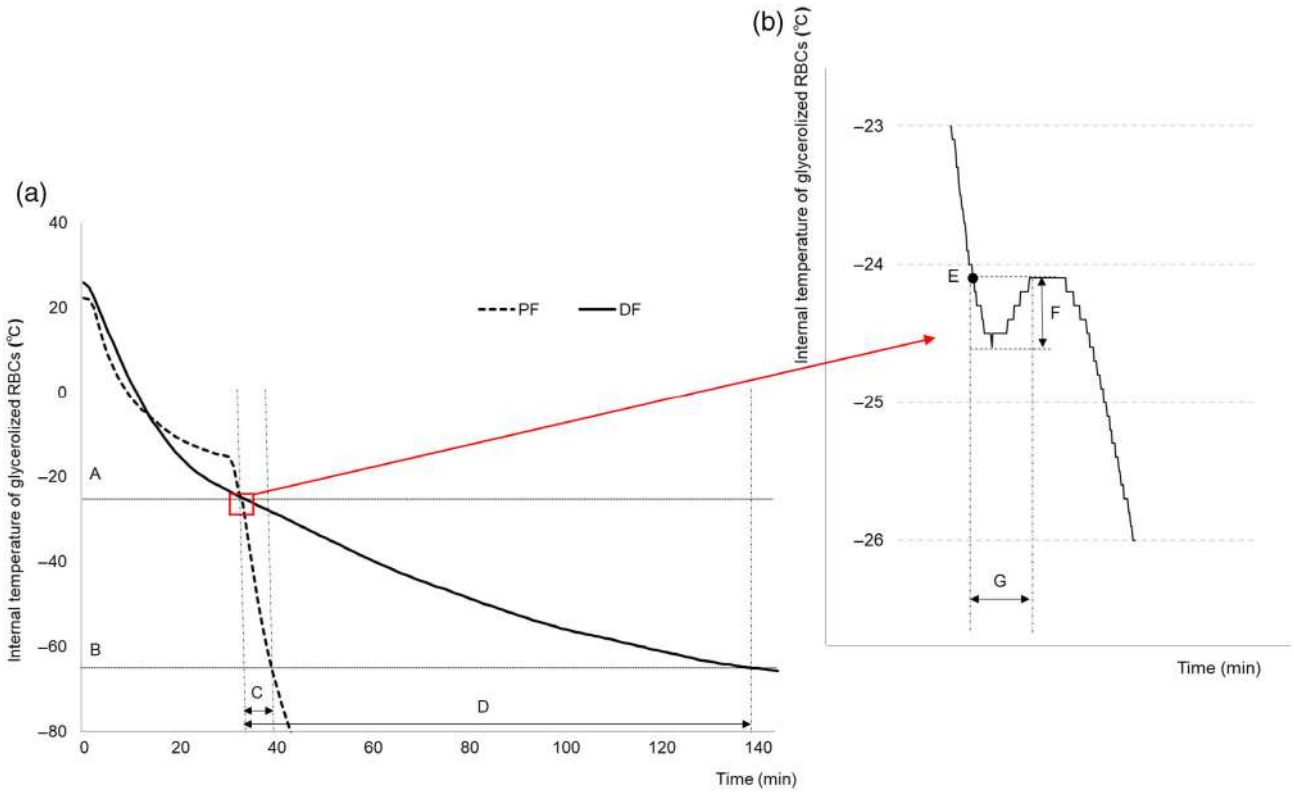

FIGURE 3 Difference in internal temperature transition on glycerolized red blood cells (RBCs) between the programmed freezer (PF) and deep freezer (DF) methods. The internal temperature transition on glycerolized RBC products that were cooled by either the PF (dotted line) or DF (solid line) method is shown in (a). The cooling temperature curve near the freezing point is shown in (b). The horizontal lines A and B indicate the freezing point (−24.1°C) and preservation temperature (−65.0°C), respectively. Points C and D indicate the freezing time in the PF method (6 min) and DF method (107 min). Points E and F indicate the freezing point and supercooling, respectively. Point G indicates the time of supercooling, which was 106 s

concentration of sodium chloride to induce 10%, 30% and 50% haemolysis. Adenosine 5′-triphosphate (ATP) and 2,3-diphosphoglycerate (DPG) were measured with commercial kits using enzymatic methods, Lucifer 250-plus kit (Kikkoman, Ltd., Chiba, Japan), and Roche diagnostic kit (Roche Diagnostics, Ltd., Mannheim, Germany) respectively, according to the manufacturer's instructions.

Statistical analyses

Results are expressed as mean ± standard deviation. Statistical differences between the PF and DF methods were analysed using a two-sided paired *t*-test. Statistical analysis was performed using GraphPad Prism6 software (GraphPad, Inc., La Jolla). The significance level was set at $p < 0.01$.

RESULTS

Freezing point and internal temperature transition of glycerolized RBCs

The internal temperature of glycerolized RBCs frozen using the DF method was monitored (solid line, Figure 3a). The cooling temperature curve near the freezing point is magnified in Figure 3b, which shows a small transient increase in temperature (point F). We presumed that point E (−24.1°C) was the freezing point. Based on this observation, the PF protocol was established so that in the first step, the PF was set at −20°C, which is approximately 4°C above the freezing point, for 30 min. In the second step, the PF was set to quickly freeze the products at −120°C (Table 1). The internal temperature curve of glycerolized RBCs frozen using the PF method is shown as a dotted line in Figure 3a. The times required to cool from the freezing point (−24.1°C) to FRC preservation temperature (−65.0°C) were 6 min in the PF method (point C) and 107 min in the DF method (point D). Points E and F indicate the freezing point and supercooling, respectively. Point G indicates the time of supercooling, which was 106 s.

Higher Hb recovery rate achieved using the PF method than using the DF method on standard RBCs

The results were summarized in Table 2. The Hb and K^+ concentrations in the supernatants and haemolysis rates of thawed glycerolized RBCs in the PF method were significantly lower than those in the DF method. No significant difference was found between the PF and DF methods in total Hb content before freezing and immediately after

TABLE 2 Comparison of characteristics between programmed freezer versus deep freezer method using standard RBCs

			Glycerolized RBCs			FTRCs on Day 1		FTRCs on Day 4	
		Freezing method	Just before freezing [I]	Just after thawing before washing [II]	*p* value	Immediately after preparation [III]	*p* value	After storage at 4°C for 4 days [IV]	*p* value
Supernatant	Hb (mg/dl)	PF	95 ± 43	1232 ± 347	<0.01	39 ± 8	0.42	66 ± 11	0.15
		DF		2413 ± 988		42 ± 9		61 ± 9	
	K^+ (mEq/L)	PF	13.0 ± 2.1	20.3 ± 3.0	<0.01	1.6 ± 0.6	0.32	16.3 ± 1.9	0.01
		DF		25.8 ± 5.1		1.7 ± 0.3		18.0 ± 1.9	
	Haemolysis (%)	PF	0.07 ± 0.03	0.9 ± 0.3	<0.01	0.11 ± 0.02	0.18	0.18 ± 0.03	0.58
		DF		1.7 ± 0.7		0.12 ± 0.02		0.18 ± 0.03	
Whole bag	Hb (g/unit)	PF	51.9 ± 3.3	51.7 ± 3.3	0.11	41.3 ± 3.2	<0.001	41.3 ± 3.2	<0.001
		DF		51.8 ± 3.3		39.2 ± 3.4		39.3 ± 3.4	
	10% haemolysis point (% NaCl)	PF	NT	NT		0.57 ± 0.02	<0.01	0.54 ± 0.02	0.57
		DF				0.58 ± 0.02		0.53 ± 0.06	
	30% haemolysis point (% NaCl)	PF	NT	NT		0.51 ± 0.02	<0.01	0.49 ± 0.02	0.94
		DF				0.52 ± 0.02		0.49 ± 0.02	
	50% haemolysis point (% NaCl)	PF	NT	NT		0.49 ± 0.01	<0.01	0.46 ± 0.02	0.42
		DF				0.49 ± 0.01		0.46 ± 0.02	
	ATP (μmol/g Hb)	PF	NT	NT		5.3 ± 0.5	0.41	4.9 ± 0.4	0.28
		DF				5.3 ± 0.4		4.8 ± 0.4	
	2,3-DPG (μmol/g Hb)	PF	NT	NT		4.8 ± 2.7	0.55	1.6 ± 1.2	0.50
		DF				4.5 ± 2.1		1.5 ± 1.0	

Note: Data are shown as mean ± SD (N = 10). Statistical difference between PF versus DF freezing method was with paired *t*-test.
Abbreviations: DF, deep freezer; NT, not tested; PF, programmed freezer.

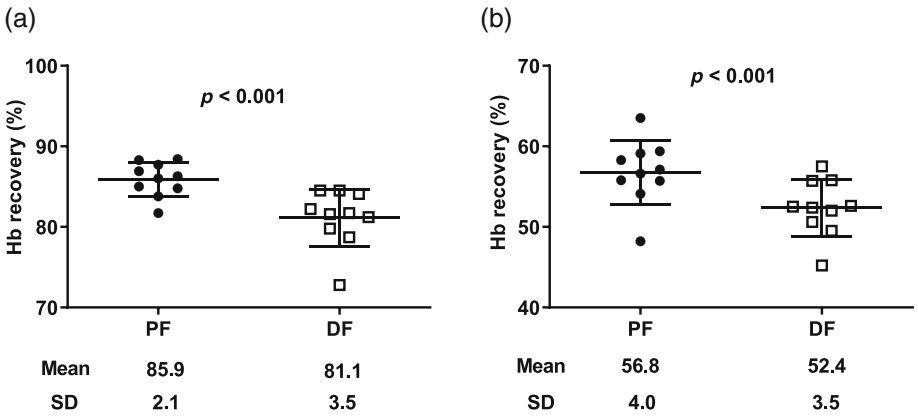

FIGURE 4 Comparison of Hb recovery between the programmed freezer (PF) and deep freezer (DF) methods. The Hb recovery rate on standard RBCs (a) and old RBCs (b) were shown. All results were presented as mean ± SD (N = 10). The paired *t*-test was used to determine statistical significance

thawing in glycerolized RBCs. However, the total Hb content in FTRCs decreased due to the removal of damaged RBCs that were haemolysed during the washing process. Because the total Hb reduction in the PF method was less than that in the DF method, the PF method (85.9% ± 2.1%) yielded a significantly higher Hb recovery rate than the DF method (81.1% ± 3.5%) (Figure 4a). FTRCs on Day 1 prepared by the DF method were more susceptible to haemolysis than those prepared by the PF method. Significant difference was not noted between the PF and DF methods in ATP and 2.3-DPG contained in FTRCs.

Higher Hb recovery rate achieved using the PF method than using the DF method on old RBCs

To prepare old RBCs, we stored RBCs for 6, 8, or 10 weeks, froze them using the DF method, and measured temporal changes in the Hb recovery rate. As a result, Hb recovery rates decreased over time to 58%, 55% and 44%, respectively. Because an Hb recovery rate of 50% could be obtained after 9 weeks, we used 9 W-old RBCs as 'old RBCs.'

TABLE 3 Comparison of characteristics between programmed freezer versus deep freezer method using old RBCs

			Glycerolized RBCs			FTRCs on Day 1		FTRCs on Day 4	
		Freezing method	Just before freezing 【I】	Just after thawing before washing 【II】	*p* value	Immediately after preparation 【III】	*p* value	After storage at 4°C for 4 days 【IV】	*p* value
Supernatant	Hb (mg/dL)	PF	511 ± 157	2740 ± 503	<0.01	117 ± 19	0.13	176 ± 29	0.54
		DF		5649 ± 2066		129 ± 23		183 ± 43	
	K^+ (mEq/L)	PF	22.7 ± 0.6	30.2 ± 1.2	<0.001	0.7 ± 0.2	0.66	5.4 ± 1.0	0.33
		DF		35.3 ± 2.2		0.7 ± 0.2		5.1 ± 0.6	
	Haemolysis (%)	PF	0.44 ± 0.17	2.3 ± 0.5	<0.001	0.45 ± 0.08	0.02	0.68 ± 0.13	0.09
		DF		4.7 ± 1.4		0.55 ± 0.13		0.77 ± 0.21	
Whole bag	Hb (g/unit)	PF	50.2 ± 1.4	50.1 ± 1.6	0.07	26.7 ± 2.5	<0.001	26.7 ± 2.5	<0.001
		DF		50.2 ± 1.6		24.7 ± 2.1		24.7 ± 2.2	
	10% haemolysis point (% NaCl)	PF	NT	NT		0.67 ± 0.02	<0.001	0.67 ± 0.03	0.03
		DF				0.71 ± 0.01		0.70 ± 0.02	
	30% haemolysis point (% NaCl)	PF	NT	NT		0.54 ± 0.03	<0.01	0.54 ± 0.02	<0.01
		DF				0.57 ± 0.02		0.56 ± 0.02	
	50% haemolysis point (% NaCl)	PF	NT	NT		0.48 ± 0.01	<0.01	0.47 ± 0.02	0.02
		DF				0.49 ± 0.01		0.48 ± 0.02	
	ATP (μmol/g Hb)	PF	NT	NT		1.0 ± 0.2	0.55	0.8 ± 0.2	0.71
		DF				1.0 ± 0.2		0.8 ± 0.2	
	2,3-DPG (μmol/g Hb)	PF	NT	NT		0.1 ± 0.2[a]	0.72	0.1 ± 0.2[a]	0.54
		DF				0.0 ± 0.1[a]		0.0 ± 0.1[a]	

Note: Data are shown as mean ± standard deviation (N = 10). Statistical difference between PF versus DF freezing method was with paired *t*-test.
Abbreviations: DF, deep freezer; NT, not tested; PF, programmed freezer.
[a]Nine out of ten samples were below the detection limit of 0.02 μmol.

The results were summarized in Table 3. The Hb and K^+ concentrations in the supernatants and haemolysis rates of thawed glycerolized old RBCs were significantly lower in the PF than in the DF method. The total Hb/unit in the PF method in FTRCs was higher than that in the DF method. The Hb recovery rate in the PF method (56.8% ± 4.0%) was significantly higher than that in the DF method (52.4% ± 3.5%) (Figure 4b). FTRCs prepared by the DF method were more susceptible to haemolysis than those prepared by the PF method. No significant difference was found between the PF and DF methods in the ATP contained in FTRCs. The 2,3-DPG was below the detection limit of 0.02 μmol in 9 out of 10 samples in old RBC-derived FTRCs regardless of the freezing method because it might have been depleted quickly in stored red cells [17].

DISCUSSION

In this study, we explored the possible utility of our PF method as a new freezing method for rare RBCs, particularly focusing on Hb recovery after freeze-thawing procedure, by comparing the in vitro properties prepared by the current method (DF method) or the new method (PF method). Results showed that the PF method improved the Hb recovery rate by approximately 4%–5% compared to the DF method; this was the case not only for standard RBCs but also for old RBCs.

The PF method has two techniques to ensure uniform product temperature, the designated two-step temperature control and agitation. The internal temperature of glycerolized RBCs in the latter half of the first step dropped more slowly in the PF method than in the DF method (Figure 3a). On the contrary, the time required to be cooled from the freezing point (−24.1°C) to the FRC preservation temperature (−65.0°C) was much shorter in the PF method (6 min) than in the DF method (107 min). This unique temperature change in the PF method may have reduced the temperature difference between the central and surface parts of the products [8], making ice crystals more homogeneous and finer.

Valeri et al. reported that the Hb recovery rate after 14 years in FRCs produced by the slow freezing method was 80% ± 7% [18]. They washed the FRCs after thawing with Valeri's method (two-component washing method). Lagerberg et al. also reported the Hb recovery rate of 81% ± 5% in FRCs washed by the Valeri's method after 2 weeks of freezing with slow freezing [19]. These results were similar to ours.

Preparation of FTRCs in Japan as in other countries use the slow freezing methods, but there are also manufacturing processes unique to Japan. RBCs as a raw material are irradiated with x-rays before freezing, thawed FRCs are washed by the Meryman's method, the MAP solution is used as a preservation solution. Turner et al. reported that the K^+ concentration in FTRCs at 24 h after production varied

depending on the raw material, but also reported that Hb recovery did not depend on irradiation timings [20]. Although we irradiated RBCs before cryopreservation unlike other countries, a previous study showed that irradiation timing did not affect Hb recovery. Tamura et al. compared two washing methods, the Meryman's and Valeri methods, and found no significant difference between the two methods in percent haemolysis 24 h after washing [21]. They also examined the effect of two additive solutions, the AS-3 and MAP solutions, on the haemolysis rate of FTRCs. With the exception of mannitol for preventing haemolysis, the components of the MAP solution are almost the same as those of additives used in the United States and Europe such as the AS-3 solution [22, 23]. FTRCs stored for 7 days in MAP solution showed similar haemolysis inhibitory effects to the additives used in the United States and Europe. These results suggest the possibility that the manufacturing process unique to Japan is equivalent to those performed in other countries.

The PF method increased the Hb recovery rates in old or damaged RBC-derived FTRCs by 4.4% (Figure 4b) and in standard RBC-derived FTRCs by 4.8% (Figure 4a), respectively, compared to the current method. Applying the former result to the disqualified FTRCs in the past 8 years at our institution (29 out of 156), as many as approximately 34% (10 in 29 units) of the disqualified ones would have satisfied the shipping standard. Moreover, these results, indicating a small but significant difference, also suggest the increased Hb content in the qualified FTRCs prepared by the PF method, by which further blood transfusion effect can be expected.

On the other hand, this study has four limitations. First, we used old RBCs as starting materials which could be an alternative starting material for "real-world" FRCs that eventually resulted in disqualified FTRCs. Although the Hb recovery rate of the old RBC-derived FTRCs was as low as that of "real-world" disqualified FTRCs, our hypothesis could still be wrong. In the future, we would like to explore the possibility of evaluating the PF method using RBCs of rare blood phenotypes, such as McLeod phenotypes [24], the standard of which are difficult to meet for FTRCs by the conventional method. Second, it remains to be clarified how much of each of the two factors, temperature control and agitation, contributed to the improvement of FTRC quality in the PF method. Third, we could not elucidate the biological basis of why the rates of Hb recovery improvement of old and standard RBCs were comparable when frozen by the PF method, despite the membrane vulnerability observed more clearly in old RBCs than in standard RBCs. Lastly, there may be some difficulties to introduce the unfamiliar PF method in a blood product factory, except through having a cord blood bank.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to this manuscript.

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REFERENCES

1. Red Cell Immunogenetics and Blood Group Terminology ISBT Working Party. Cited 2021. Available from https://www.isbtweb.org/
2. Lecak J, Scott K, Young C, Hannon J, Acker JP. Evaluation of red blood cells stored at −80°C in excess of 10 years. Transfusion. 2004; 44:1306–13.
3. Meryman HT, Hornblower M. A method for freezing and washing red blood cells using a high glycerol concentration. Transfusion. 1972;12:145–56.
4. Valeri CR. Simplification of the methods for adding and removing glycerol during freeze-preservation of human red blood cells with the high or low glycerol methods: biochemical modification prior to freezing. Transfusion. 1975;15:195–218.
5. Krijnen HW, de Wit JJ, Kuivenhoven AC, Loos JA, Prins HK. Glycerol treated human red cells frozen with liquid nitrogen. Vox Sang. 1964; 9:559–72.
6. Hess JR. Red cell freezing and its impact on the supply chain. Transfus Med. 2004;14:1–8.
7. Lovelock JE. The hemolysis of human red blood-cells by freezing and thawing. Biochim Biophys Acta. 1953;10:414–26.
8. Meryman HT. Red cell freezing by the American National Red Cross. Am J Med Technol. 1975;41:265–82.
9. Kobayashi R, Kanesaka N, Watanabe M, Suzuki T. Effect of the breaking temperature of supercooling on ice characteristics and drip loss of foods in supercooled freezing method (in Japanese). Trans Jpn Soc Refrig Air Condition Eng. 2014;31:297–303.
10. Holovati JL, Wong KA, Webster JM, Acker JP. The effects of cryopreservation on red blood cell microvesiculation, phosphatidylserine externalization, and CD47 expression. Transfusion. 2008;48: 1658–68.
11. Gelderman MP, Vostal JG. Rejuvenation improves roller pump–induced physical stress resistance of fresh and stored red blood cells. Transfusion. 2011;51:1096–104.
12. Yang P, Zhou J, Kang Y, Gong L, Zhang J, Yu J, et al. Mannitol-adenine-phosphate: a novel solution for intraoperative blood salvage. Transfusion. 2014;54:1146–52.
13. Hornblower M, Meryman HT. Relative efficiency and interchangeability of Huggins and American red Cross red cell freezing procedures. Transfusion. 1977;17:417–24.
14. Kikuchi G, Kurita R, Ogasawara K, Isa K, Tsuneyama H, Nakamura Y, et al. Application of immortalized human erythroid progenitor cell line in serologic tests to detect red blood cell alloantibodies. Transfusion. 2018;58:2675–82.
15. Takayanagi M, Yashiro T. Development of a new colorimetric determination of hemoglobin in plasma. Jpn J Clin Chem. 1985;14: 247–52.
16. Parpart AK, Lorenz PB, Parpart ER, Gregg JR, Chase AM. The osmotic resistance (fragility) of human red cells. J Clin Invest. 1947;26: 636–40.

17. Hogma CF. Preparation and preservation of red cells. Vox Sang. 1998;74:177–87.
18. Valeri CR, Srey R, Tilahun D, Ragno G. The in vitro quality of red blood cells frozen with 40 percent (wt/vol) glycerol at - 80°C for 14 years, deglycerolized with the Heamonetics ACP215, and stored at 4°C in additive solution-1 or additive solution-3 for up to 3 weeks. Transfusion. 2004;44:990–5.
19. Lagerberg JWM, Lange R T-d, De Korte D, Verhoeven AJ. Altered processing of thawed red cells to improve the in vitro quality during postthaw storage at 4°C. Transfusion. 2007;47:2242–9.
20. Turner TR, Lautner L, Hill A, Howell A, Skeate R, Acker JP. Evaluating the quality of red blood cell concentrates irradiated before or after cryopreservation. Transfusion. 2020;60:26–9.
21. Tamura S, Akino M, Sato M, Homma C, Yamamoto S, Kato T, et al. Extended storage of frozen thawed red cells following deglycerolization with an automated cell processor ACP215 and storage in additive solutions (in Japanese). Jpn J Trans Ther. 2009; 55:508–15.
22. Cicha I, Suzuki Y, Tateishi N, Shiba M, Muraoka M, Tadokoro K, et al. Gamma-ray-irradiated red blood cells stored in mannitol-adenine-phosphate medium: rheological evaluation and susceptibility to oxidative stress. Vox Sang. 2003;79:75–82.
23. Hansen AL, Kurach JDR, Turner TR, Jenkins C, Busch MP, Norris PJ, et al. The effect of processing method on the in vitro characteristics of red blood cell products. Vox Sang. 2015;108:350–8.
24. Kuypers FA, van Linde-Sibenius TM, Roelofsen B, Op den Kamp JA, Tanner MJ, Anstee DJ. The phospholipid organisation in the membranes McLeod and Leach phenotype erythrocytes. FEBS Lett. 1985; 184:20–4.

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

International Society of Blood Transfusion survey of experiences of blood banks and transfusion services during the COVID-19 pandemic

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Abstract

Background and Objectives: The coronavirus disease 2019 (COVID-19) pandemic has impacted blood systems worldwide. Challenges included maintaining blood supplies and initiating the collection and use of COVID-19 convalescent plasma (CCP). Sharing information on the challenges can help improve blood collection and utilization.

Materials and Methods: A survey questionnaire was distributed to International Society of Blood Transfusion members in 95 countries. We recorded respondents' demographic information, impacts on the blood supply, CCP collection and use, transfusion demands and operational challenges.

Results: Eighty-two responses from 42 countries, including 24 low- and middle-income countries, were analysed. Participants worked in national (26.8%) and regional (26.8%) blood establishments and hospital-based (42.7%) institutions. CCP collection and transfusion were reported by 63% and 36.6% of respondents, respectively. Decreases in blood donations occurred in 70.6% of collecting facilities. Despite safety measures and recruitment strategies, donor fear and refusal of institutions to host blood drives were major contributing factors. Almost half of respondents working at transfusion medicine services were from large hospitals with over 10,000 red cell transfusions per year, and 76.8% of those hospitals experienced blood shortages. Practices varied in accepting donors for blood or CCP donations after a history of COVID-19 infection, CCP transfusion, or

vaccination. Operational challenges included loss of staff, increased workloads and delays in reagent supplies. Almost half of the institutions modified their disaster plans during the pandemic.

Conclusion: The challenges faced by blood systems during the COVID-19 pandemic highlight the need for guidance, harmonization, and strengthening of the preparedness and the capacity of blood systems against future infectious threats.

KEYWORDS
blood supply, convalescent plasma, COVID-19, pandemic, transfusion

Highlights

- This article summarizes the impact of the coronavirus disease 2019 (COVID-19) pandemic on the blood systems worldwide including blood supply challenges, transfusion demand, convalescent plasma collection and use, and operational challenges.
- To the best of our knowledge, this is the largest survey conducted to assess the impact of the COVID-19 pandemic on blood establishments and transfusion medicine services worldwide.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has created challenges for many blood establishments (BEs) and transfusion medicine services (TMS) worldwide. African [1], Asian [2], Eastern Mediterranean [3] and some Western countries [4–6] reported blood supply challenges. They faced decreased rates of donations [1, 3], public fear of infection when donating blood [7] and decreased numbers of, or outright cancellations of, blood drives [3] due to lockdown measures. In addition, unpredictable clinical demands for blood, linked to cancellations of elective surgeries and non-urgent interventions, were described, while blood demand for emergency situations and patients on chronic transfusion support remained relatively unchanged [3, 6]. Some centres reported lower blood supply in the early stages of the pandemic, compensated by decreased blood demand for elective surgeries and other medical procedures [8].

Many institutions collected COVID-19 convalescent plasma (CCP) and/or used it for passive immunotherapy on a compassionate basis or for clinical trials. Guidelines on the collection and use of CCP in high-income and low- and middle-income countries (LMICs) have been published [9–13]. During the period covered by the survey, the World Health Organization (WHO) developed interim guidances, which underwent updates throughout the pandemic, for maintaining a safe and adequate blood supply and applying COVID-19-related donor deferral criteria, temporary deferral after vaccination and CCP usage [14, 15].

Early in the pandemic, the International Society of Blood Transfusion (ISBT) established a multidisciplinary working group, comprised of international transfusion experts, to review the impacts of COVID-19 on blood systems and existing practices on CCP collection and use from donor, product and patient perspectives [11, 16–18]. This survey aims to assess the impact of COVID-19 pandemic on BEs and TMS around the world to summarize the experiences and provide guidance to prepare for future pandemics.

MATERIALS AND METHODS

A web-based survey was designed using Survey Monkey and piloted for content validity and avoidance of ambiguity by the working group. The survey, written in English, was distributed by the ISBT central office to 1481 ISBT members from 95 countries spanning all WHO regions. Respondents provided consent through completing the survey. Data were collected between 25 May and 9 July 2021.

The survey contained 77 questions covering demographics ($n = 13$), and questions tailored to BEs ($n = 33$) and TMS ($n = 31$) and others addressing blood supply challenges ($n = 10$) and demand ($n = 11$), and CCP collection ($n = 23$) and transfusions ($n = 15$). All respondents were invited to complete five questions on the operational challenges faced and to list recommendations for future pandemics. Descriptive statistics were performed, and the reported variables are expressed in numbers and percentages.

RESULTS

Ninety-three ISBT members participated. In total, 82 responses from 42 countries across all WHO regions, including 24 LMICs [19], were included in the analysis after excluding participants with <75% completion of the survey (Figure 1; Table S1). At the time of completion of this survey, most respondents indicated that their countries were either in the second wave of COVID-19 with a decreasing number of cases or in the third wave with either decreasing or increasing number of cases.

FIGURE 1 Geographical distribution and demographics of survey respondents ($n = 82$, from 42 countries)

Respondents worked in national ($n = 22$, 26.8%), regional ($n = 22$, 26.8%) and hospital-based ($n = 35$, 42.7%) institutions. These included respondents who worked in institutions involved in blood donations ($n = 69$, 84.1%) and TMS ($n = 57$, 69.5%). Most institutions involved in donation activities annually collected ≥10,000 units ($n = 45$, 65.2%) of blood. A majority of respondents working in TMS were from large hospitals offering medical and surgical services with >500 beds ($n = 36$, 63.2%) and ≥10,000 red blood cell (RBC) transfusions per year ($n = 32$, 56.1%).

Impacts of COVID-19 on blood collection and supply

Almost two-thirds of respondents from BEs and hospital-based blood services indicated a decrease in donations during the pandemic, while 23.5% reported fluctuations (Table S2). Blood donation drives were either cancelled or decreased in two-thirds of institutions, primarily due to increased COVID-19 cases and lockdowns. Several institutions reported blood donors' fear of visiting hospital-based blood banks. Other stated reasons were donor deferrals due to infections or quarantine requirements.

Some respondents described declines during the lockdown period in the first wave, while in a few countries, the number of donors decreased at the start of the pandemic but improved over the following months. One respondent reported decreased donations at the start of the pandemic due to increasing imported cases with a lockdown, followed by a temporary recovery before a drop due to surging numbers of COVID-19 cases. A respondent from a blood collector in the United States described a sharp decline in blood donations when COVID-19 was declared a national emergency and stay-at-home orders were mandated in multiple states. Free donor testing for antibodies against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was initiated, and donations steadily increased thereafter. Other respondents shared similar experiences, unveiling a decline in blood donations in the first and third waves of the pandemic, with decreased demand for elective surgeries and transplants, followed by an increase over the usual demand thereafter. In contrast, in Italy, donations increased in the early stages of the pandemic as a result of calls for blood donors.

COVID-19 infection-related deferrals

At the time of undertaking this survey, most institutions were applying a 14- (50.7%) or 28-day (38.8%) deferral period for donors with a history of COVID-19 infection after full recovery/resolution of symptoms (Table S2). In one Nigerian institution, testing for COVID-19 was needed before a blood donation. Policies with regard to donor deferral after a CCP transfusion varied. Over one-quarter of respondents (27.7%) indicated a 12-month temporary deferral, and 7.7% respondents reported permanent deferral (from the Netherlands, Malaysia, Congo, Kenya and one center in Saudi Arabia), while 13.8% were awaiting a policy decision. In Germany, a 4-month deferral period was applied.

SARS-CoV-2 vaccine-related deferrals

A wide variety of SARS-CoV-2 vaccines were available in the responding countries, with the commonest being Oxford/AstraZeneca

and Pfizer/BioNTech. Others included Moderna, Sinovac-CoronaVac, Johnson & Johnson, Sputnik V, Sinopharm, Covaxin and Verocell (Table S2).

Post-vaccination deferrals for blood donations varied, with a majority implementing 14- (33.8%) or 7-day (20.6%) deferrals. A few reported a 48-h deferral for mRNA or non-replicative vector-based vaccines ($n = 4$), or a 72-h deferral period after vector-based or inactivated vaccines ($n = 2$). Elsewhere, the practice varied depending on the vaccine type, including no or 7 days deferral post mRNA, 7 days up to 4 weeks deferral post vector-based vaccines, 48 or 72-h deferral for inactivated virus vaccines and 1 year deferral if the donor was enrolled in vaccine-related clinical trials.

In the United States, individuals who received a live-attenuated viral vaccine had to refrain from donating blood for 14 days, but no such vaccines were authorized in that country. In Singapore, individuals receiving mRNA or inactivated vaccines were deferred by the national BE for 3 days post vaccination if free from side effects, for 1 week after resolution of localized pain at the injection site and for 4 weeks after resolution of a fever, generalized muscle aches, rashes, or lymph node swelling. The deferral period for vector-based or live-attenuated vaccines was 28 days.

Safety measures to protect blood donors and staff

Safety measures were implemented to protect donors and staff in BEs and hospital-based blood services (Table S2). Almost one-third of BEs performed CCP collection procedures in a room separate from standard donations. Other measures included screening donors for signs and symptoms of COVID-19 and a relevant travel history, frequent disinfection of equipment and donation chairs and minimizing snack consumption post-donation. In addition, social distancing was applied, as well as installation of an electronic appointment system, extending working hours, increasing the number of donation sites and setting up collection areas outside hospital-based blood services.

Challenges in donor recruitment and strategies utilized

The most common challenges were donor fear of contracting COVID-19 at blood donation facilities, refusal of partner institutions to host blood drives and lockdowns (Table S2). Staff concerns from handling blood donors were reported by 40% of respondents. Other reported concerns included the inability to conduct donation drives due to a lack of resources/mobile units, closure of public venues (such as shopping centres) and the cancellation of gatherings by community organizations where blood drives are usually conducted. There were staff and donor losses due to COVID-19 infection or exposure, and staff fears of job security.

Strategies to recruit donors and reduce blood shortages encompassed increased blood donation promotion via text messages, phone calls and key societal influencers. Hospital employees were encouraged to donate blood. Use of social media and collaboration with voluntary organizations were done to recruit donors and conduct donation drives. Other solutions included donor transportation and the issuance of travel passes to help donors reach donation sites during lockdowns. The use of virtual blood drives to recruit donors by inviting individuals and supporters to promote donations at any of its fixed donation sites was reported [20]. Another operation allocated funds to retain workers and provided supplies including thermometers, personal protective equipment (PPE) and hand-held equipment at blood drives.

COVID-19 convalescent plasma collection

Fifty-two institutions collected CCP during the pandemic (Table S3). Most institutions accepted first-time apheresis donors, and 40.4% accepted family/relative donations, which accounted for more than one-third of CCP donors in five institutions in LMICs. Almost two-thirds of respondents (66.7%) indicated that their institutions could meet CCP demands. In the Netherlands, there were long waiting lists for CCP donations. In the United States, as more of the general population began to get vaccinated and increasing questions about CCP efficacy appeared in the literature, hospital demand declined and the need for CCP donations substantially decreased.

Almost two-thirds of the institutions used a specific consent form for CCP donation. This included details on CCP donations, testing to be performed, donation by apheresis and trial/research use. At U.S. centres, the consent form specified that high-titre donations, as defined by the Food and Drug Administration (FDA), and non-reactivity for relevant transfusion-transmitted infections qualified the plasma to be labelled as CCP under an FDA-approved Expanded Access protocol.

Most respondents accepted donors recovered from COVID-19 infection for CCP donations after at least 14 ($n = 29$, 49.2%) or 28 ($n = 18$, 30.5%) days after recovery. With the majority mandating a history of natural COVID-19 infection, 27 institutions accepted vaccinated donors. CCP donors were accepted post vaccination with a variable deferral period ranging from none ($n = 4$) to 7 ($n = 4$) and 14 ($n = 14$) days. The period of deferral varied based on the type of vaccine in three institutions, while two remained undecided. For vaccines, the FDA required prior symptomatic COVID-19 infection, documented by a swab polymerase chain reaction test for 14 days to 6 months after the last symptoms before a CCP donation [21].

Six institutions from three countries accepted vaccinated donors to donate CCP for fractionation if they had a history of COVID-19 infection. In a blood service in Canada, plasma collection for fractionation paralleled that for transfusions. Six respondents reported competition for CCP collection from plasma fractionators. Two reported that this worked in favour of their institutions and helped them to acquire additional apheresis machines. Twenty-one respondents indicated a lack of a domestic fractionation programme.

Most institutions collected CCP by apheresis. Three performed only whole-blood (WB) collection, and 14 simultaneously conducted WB and apheresis collection. In 10 institutions, WB donations contributed to >50% of the CCP source (Argentina, North Macedonia, Iran, Indonesia, Israel, Nepal, Italy, Sweden, and two in India). Of those countries that performed WB collection, all but three reported that RBCs or platelet units derived from WB convalescent donations were used for standard transfusions.

Most institutions utilized in-house-generated labels for the CCP, while six used the standard ISBT label and 16 uses specific ISBT label for CCP. Two institutions used a nationally developed label. CCP was kept frozen for up to 1 year in 83% of those institutions. Plans for the utilization of excess CCP were variable, with the commonest being to ship it for regular fractionation (27.1%).

Blood inventory and demand

Three-quarters of the 56 TMS experienced blood shortages during the COVID-19 pandemic, and in 35 (62.5%), the shortage correlated with the number of cases reported in their countries (Table S4). Most of these respondents reported <10% red cell wastage (86%). While RBC demands fluctuated in almost half of these institutions, platelet demands were variable. Shortages affected a wide range of patients including those with underlying haemoglobinopathies and with haematological malignancies and transplant patients. Two respondents from the Middle East indicated that the demand did not change since most transfusions in their institutions were for patients with haemoglobinopathies and haematological malignancies. While some respondents indicated that the blood demand had decreased because of cancellations of surgeries, for others the major procedures could not be conducted owing to blood shortages. One country in Africa reported that challenges in meeting transfusion demands led to rescheduling of surgeries.

Different measures were applied to meet transfusion demands. The most common strategies were to cancel elective surgeries/procedures (90.5%) and maximize the use of alternatives to RBC transfusions (50%). Respondents indicated the usefulness of maintaining effective communication with clinical teams and BEs and applying patient blood management. In addition, respondents described screening transfusion requests, extending the shelf life of platelets to 7 days after bacterial testing, splitting platelet units and issuing RBC units up to 2- weeks-old to patients with haemoglobinopathies and to neonates.

COVID-19 convalescent plasma transfusions

Thirty TMS transfused CCP to patients with SARS-CoV-2 infection, including five that transfused CCP into paediatric patients (Table S5). More than half of institutions transfused CCP as part of national

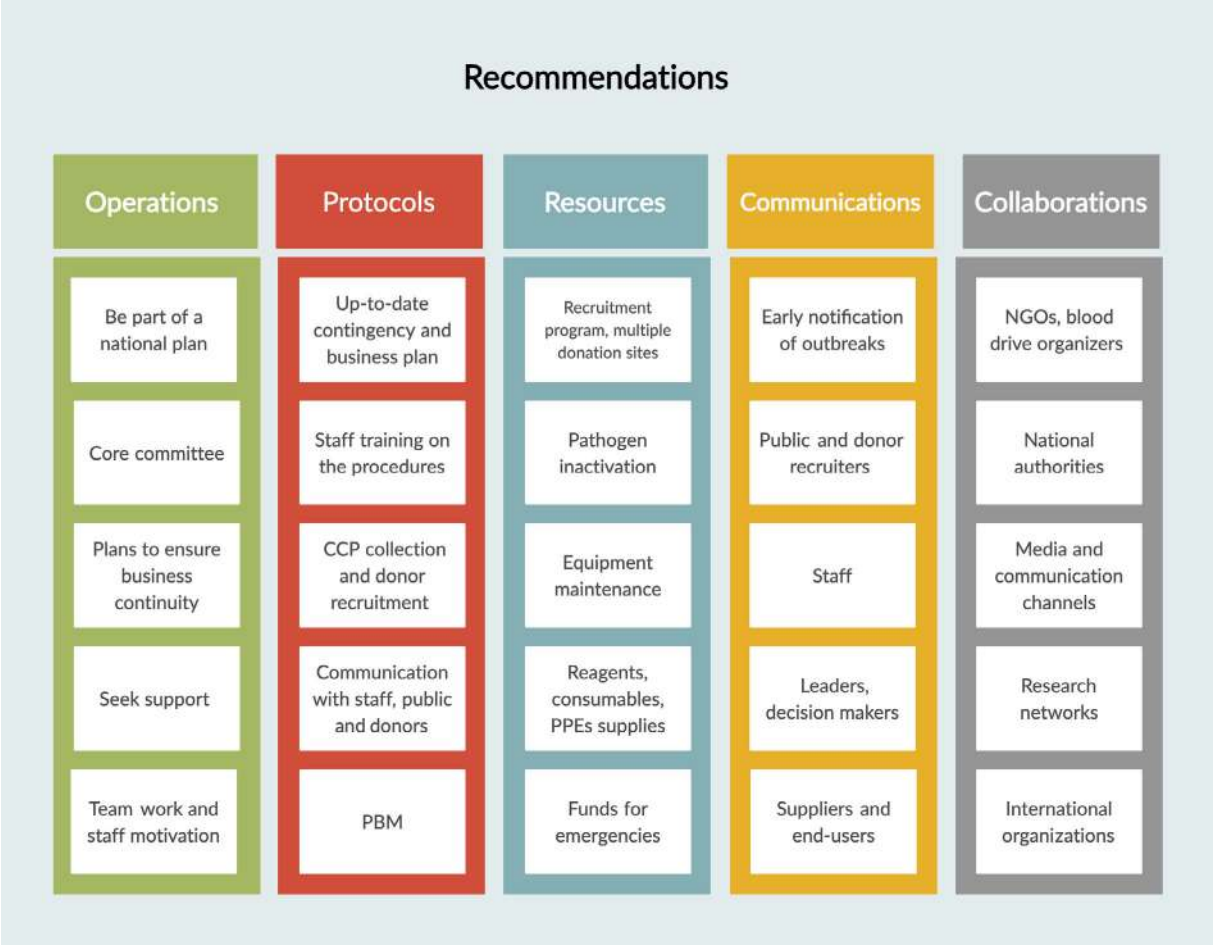

FIGURE 2 Operational recommendations for blood establishments and transfusion services to prepare for future viral threats/pandemics. CCP, COVID-19 convalescent plasma; PBM, patient blood management; PPE, personal protective equipment; NGO, non-governmental organization

programmes (60%) and for both clinical trials and compassionate/emergency use. Most institutions enrolled in clinical trials used CCP therapeutically for hospitalized patients. Only three institutions were enrolled in clinical trials assessing CCP use in a pre-hospital setting, and only one as a prophylactic measure for high-risk exposure. The vast majority of institutions utilizing CCP on a compassionate basis used a specific consent form for treated patients. More than half of the respondents indicated having sufficient CCP supply.

The protocol for unit selection—if more than one dose of CCP was given—ranged from random selection to selecting units from the same donor (Table S5). ABO-matched CCP was used by 56.7% of the respondents. The ISBT haemovigilance definition was used by 46.7% of institutions when reporting adverse reactions to transfused CCP [22]. Most institutions reported to the blood bank, national haemovigilance committee and/or a blood transfusion/safety committee.

Operational challenges

The commonest operational challenges faced during the pandemic were staff losses due to sickness or quarantine and increased workloads (Table S6). More than one-quarter of respondents reported staff deployment to other services such as to support a virology laboratory. One-third of respondents reported a lack of PPE. Other challenges included delays in reagent supplies and instrument maintenance, and the need for team segregation for business continuity planning, resulting in longer working hours and higher workloads in blood processing and testing laboratories. A respondent from one LMIC described a lack of support for blood systems in the country.

Challenges upon initiating a CCP collection and transfusion programme included cost/funding, setting up clinical trials, and obtaining institutional review board approvals. One respondent from Africa described challenges with the lack of sufficiently qualified physicians to initiate clinical trials and slow initial patient enrollment. Another respondent described donor recruitment as the main challenge, as donors were unwilling to come to the hospital to donate CCP or had transportation difficulties due to lockdowns. Two respondents indicated that collecting CCP on a voluntary, non-remunerated basis without proof of its benefits for hospitalized patients was extremely difficult. Challenges with the demand exceeding supply at certain stages of the pandemic were described by respondents from India and Portugal. Other challenges included donor recruitment and eligibility determination, managing human and testing resources, apheresis equipment and required logistics.

For 47 institutions, contingency/disaster plans were effective in meeting transfusion demands. However, almost one-half of the respondents indicated a change in disaster plans, such as adding a section of operating plans during a pandemic. Others indicated updates on safety/infection control measures, staff management and redeployment, extending working hours, and management of inventories of blood and critical reagents. Specific changes in relationships with BEs included donations by appointment only, defining donor workflow and modifying eligibility criteria as per national protocols.

Respondents shared the lessons learned and recommendations to help in preventing/managing blood shortages, meeting transfusion demands during a pandemic, establishing a CCP programme and overcoming operational challenges (Table S7; Figure 2).

DISCUSSION

To the best of our knowledge, this is the largest survey conducted to assess the impact of the COVID-19 pandemic on BEs and TMS worldwide. This survey highlights some specific challenges faced during different waves of the pandemic and the mitigation steps undertaken.

National measures to contend with the pandemic had negative impacts on the blood supply in most BEs, with the demand fluctuating in half of the TMS. Challenges included public fear, transport restrictions and staff shortages. These specifically threatened transfusion support to certain patient populations in some institutions, in line with previous reports [3, 23–25]. Although cancellations of surgeries were reported to decrease blood demand in the early stages of the pandemic [8, 26], most of the participants reported blood shortages. This could be explained by the proportion of participants in our survey from LMICs. The fragility of blood supply systems in LMICs likely makes it particularly challenging for blood banks in those countries to meet ongoing demands. In the early stages of the pandemic, the WHO recommended that centres be prepared to move quickly in response to changes in managing demands for blood and blood products while mitigating potential risks to staff and donors from exposure to SARS-CoV-2 [15]. With universal impacts on the blood supply and donation rates, there is a need for a global message to the public and national authorities on the continuing need for blood donors.

The need to initiate CCP donation and transfusion trials and programmes has added extra challenges. The survey reveals wide variations in donor eligibilities, donation methods, types of donors enrolled in CCP donation and methodologies used in obtaining consent. While the majority reported meeting CCP demands, certain local challenges were highlighted such as competition with other parties for CCP collection, lack of standardization in labelling CCP units, variations in the shelf life of frozen units and plans to utilize excess units. Common challenges of funding and the lack of test facilities should be resolved in preparation for future pandemics.

We report variation in certain practices, such as donor deferral post-recovery from COVID-19 or for recipients of CCP transfusions, and deferral after receiving COVID-19 vaccines for blood and CCP donations. While there was guidance from different organizations such as the WHO [14], ISBT [9, 11] and Association for the Advancement of Blood & Biotherapies [12], there was variability in practice, which may be ascribed to slightly diverging recommendations in those guidelines over time, local policies and regulations, resources, facilities and expertise. This highlights the need for harmonization of international guidelines and updating them as scientifically based knowledge and circumstances (such as vaccinations) evolve throughout the pandemic. A harmonized reporting scheme for CCP reactions is also required.

This survey also highlights the wide range of operational challenges encountered during the pandemic. The lack of PPEs in one-third of institutions reflects that experienced in other sectors of healthcare systems worldwide, especially in the early stages of the pandemic [27, 28]. A lack of previous experience with pandemics resulted in one-half of the institutions changing their contingency/disaster plans, which illustrates the need that essential plans be developed for pandemic preparedness, preferably in line with national policy responses.

One limitation of this cross-sectional survey is that it captured data only at a single moment in time. Hence, the survey results do not reflect the trend or the magnitude of the blood shortages and challenges observed beyond the period assessed. This limitation is particularly relevant considering the variable degree of virus spread between different countries worldwide. In addition, relying on voluntary participants may have introduced a selection bias. Furthermore, a non-response bias could arise, as the set of people willing to participate may differ from those who did not respond. Finally, the survey was provided only in English, which may have hindered non-English-speaking respondents. However, to the best of our knowledge, this is the most extensive assessment of the impact of the COVID-19 pandemic on BEs and TMS worldwide. The participation from all WHO regions provides a broad comparative snapshot of this impact.

In conclusion, the COVID-19 pandemic imposed challenges on BEs and TMS worldwide. The information gathered through this survey and recommendations can be used to guide policymakers and governmental and non-governmental organizations to strengthen the capacities of national blood transfusion systems, enhance risk mitigation and develop preparedness plans for future pandemics.

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A.Z.R. acquired and analysed the data and wrote the first draft of the manuscript; T.B. initiated the research idea, supervised the research, and reviewed and edited the manuscript; A.Z.R., T.B., E.M.W., D.V.D., A.O., T.O.A., R.G., E.M.B., K.D.B., M.G., V.L., NRL, A.L.A., C.K.L., E.H. and C.S.O. designed the survey. The remaining authors participated in the survey and provided detailed responses. All authors reviewed the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Loua A, Kasilo OMJ, Nikiema JB, Sougou AS, Kniazkov S, Annan EA. Impact of the COVID-19 pandemic on blood supply and demand in the WHO African Region. Vox Sang. 2021;116:774–84.
2. Choudhury N, Mathur A, Smit Sibinga CT, AATM. COVID-19 pandemic–blood supply challenges and approaches in AATM member countries. ISBT Sci Ser. 2020;15:353–61.
3. Al-Riyami AZ, Abdella YE, Badawi MA, Panchatcharam SM, Ghaleb Y, Maghsudlu M, et al. The impact of COVID-19 pandemic on blood supplies and transfusion services in Eastern Mediterranean Region. Transfus Clin et Biol. 2021;28:16–24.
4. Franchini M, Farrugia A, Velati C, Zanetti A, Romanò L, Grazzini G, et al. The impact of the SARS-CoV-2 outbreak on the safety and availability of blood transfusions in Italy. Vox Sang. 2020;115:603–5.
5. Pagano MB, Hess JR, Tsang HC, Staley E, Gernsheimer T, Sen N, et al. Prepare to adapt: blood supply and transfusion support during the first 2weeks of the 2019 Novel Coronavirus (COVID-19) pandemic affecting Washington State. Transfusion. 2020;60:908–11.
6. Pagano MB, Cataife G, Fertrin KY, Gernsheimer T, Hess JR, Staley E, et al. Blood use and transfusion needs at a large health care system in Washington state during the SARS-CoV-2 pandemic. Transfusion. 2020;60:2859–66.
7. Wang Y, Han W, Pan L, Wang C, Liu Y, Hu W, et al. Impact of COVID-19 on blood centres in Zhejiang province China. Vox Sang. 2020;115:502–6.
8. Stanworth SJ, New HV, Apelseth TO, Brunskill S, Cardigan R, Doree C, et al. Effects of the COVID-19 pandemic on supply and use of blood for transfusion. Lancet Haematol. 2020;7:e756–e64.
9. Bloch EM, Goel R, Wendel S, Burnouf T, Al-Riyami AZ, Ang AL, et al. Guidance for the procurement of COVID-19 convalescent plasma: differences between high-and low-middle-income countries. Vox Sang. 2021;116:18–35.
10. Epstein J, Burnouf T. Points to consider in the preparation and transfusion of COVID-19 convalescent plasma. Vox Sang. 2020;115:485–7.
11. Al-Riyami AZ, Schäfer R, van den Berg K, Bloch EM, Estcourt LJ, Goel R, et al. Clinical use of convalescent plasma in the covid-19 pandemic: a transfusion-focussed gap analysis with recommendations for future research priorities. Vox Sang. 2021;116:88–98.
12. Cohn CS, Estcourt L, Grossman BJ, Pagano MB, Allen ES, Bloch EM, et al. COVID-19 convalescent plasma: interim recommendations from the AABB. Transfusion. 2021;61:1313–23.
13. Epstein J, Smid WM, Wendel S, Somuah D, Burnouf T. Use of COVID-19 convalescent plasma in low- and middle-income countries: a call for ethical principles and the assurance of quality and safety. Vox Sang. 2021;116:13–4.
14. WHO Interim Guidance. Maintaining a safe and adequate blood supply and collecting convalescent plasma in the context of the COVID-

19 pandemic Interim guidance. Geneva: World Health Organization 2021. [cited 2021 Dec 13]. Available from: https://apps.who.int/iris/bitstream/handle/10665/339793/WHO-2019-nCoV-BloodSupply-2021.1-eng.pdf?sequence=1
15. WHO Interim Guidance on maintaining a safe and adequate blood supply during the coronavirus disease 2019 (COVID-19) pandemic and on the collection of COVID-19 convalescent plasma. Geneva: World Health Organization; 2020 [cited 2021 Dec 13]. Available from: https://apps.who.int/iris/bitstream/handle/10665/333182/WHO-2019-nCoV-BloodSupply-2020.2-eng.pdf?sequence=1&isAllowed=y
16. Al-Riyami AZ, Burnouf T, Yazer M, Triulzi D, Kumas LT, Sagdur L, et al. International forum on the collection and use of COVID-19 convalescent plasma: responses. Vox Sang. 2021;116:e71–e120.
17. Al-Riyami AZ, Burnouf T, Yazer M, Triulzi D, Kumas LT, Sagdur L, et al. International forum on the collection and use of COVID-19 convalescent plasma: protocols, challenges and lessons learned: summary. Vox Sang. 2021;116:1117–35.
18. Wendel S, Land K, Devine DV, Daly J, Bazin R, Tiberghien P, et al. Lessons learned in the collection of convalescent plasma during the COVID-19 pandemic. Vox Sang. 2021;116:872–9.
19. World Bank Country and Leading Groups. The World Bank, 2021 [cited 2021 Nov 11]. Available from: https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups
20. Vitalant. Virtual blood drive. 2021. Available from: https://vitalant.org/virtual-blood-drive
21. Investigational COVID-19 convalescent plasma-Guidance for Industry. Food and Drug Administration, 2021 [cited 2021 Oct 23]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/investigational-covid-19-convalescent-plasma
22. Haemovigilance ISBT Working Party. Proposed Standard Definitions for surveillance of non infectious adverse transfusion reactions [cited 2021 Nov 11]. Available from: https://www.isbtweb.org/working-parties/haemovigilance
23. Hossain MS, Raheem E, Siddiqee MH. The forgotten people with thalassemia in the time of COVID-19: South Asian perspective. Orphanet J Rare Dis. 2020;15:1–2.
24. Yadav U, Pal R. Challenging times for children with transfusion-dependent thalassemia amid the COVID-19 pandemic. Indian Pediatr. 2020;57:478.
25. de Sanctis V, Canatan D, Corrons JLV, Karimi M, Daar S, Kattamis C, et al. Preliminary data on COVID-19 in patients with hemoglobinopathies: a multicentre ICET-a study. Mediterr J Hematol Infect Dis. 2020;12:e2020046.
26. Lozano M, Yazer M, Jackson B, Pagano M, Rahimi-Levene N, Peer V, et al. Vox Sanguinis international forum on hospital transfusion services' response to COVID-19. Vox Sang. 2020;115:e1–e17.
27. WHO. Shortage of personal protective equipment endangering healthcare workers worldwide. 2020. [cited 2021 Dec 13]. Available from: https://www.who.int/news/item/03-03-2020-shortage-of-personal-protective-equipment-endangering-health-workersworldwide#:~:text=The%20World%20Health%20Organization%20has,coronavirus%20and%20other%20infectious%20diseases
28. Burki T. Global shortage of personal protective equipment. Lancet Infect Dis. 2020;20:785–6.

SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Modelling haemoglobin incremental loss on chronic red blood cell transfusions

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Abstract

Background and Objectives: Understanding the impact of red blood cell (RBC) lifespan, initial RBC removal, and transfusion intervals on patient haemoglobin (Hb) levels and total iron exposure is not accessible for chronic transfusion scenarios. This article introduces the first model to help clinicians optimize chronic transfusion intervals to minimize transfusion frequency.

Materials and Methods: Hb levels and iron exposure from multiple transfusions were calculated from Weibull residual lifespan distributions, the fraction effete RBC removed within 24-h (X_e) and the nominal Hb increment. Two-unit transfusions of RBCs initiated at patient [Hb] = 7 g/dl were modelled for different RBC lifespans and transfusion intervals from 18 to 90 days, and X_e from 0.1 to 0.5.

Results: Increased X_e requires shorter transfusion intervals to achieve steady-state [Hb] of 9 g/dl as follows: 30 days between transfusions at $X_e = 0.5$, 36 days at $X_e = 0.4$, 42 days at $X_e = 0.3$, 48 days at $X_e = 0.2$ and 54 days at $X_e = 0.1$. The same transfusion interval/X_e pairs result in a steady-state [Hb] = 8 g/dl when the RBC lifespan was halved. By reducing transfused RBC increment loss from 30% to 10%, annual transfusions were decreased by 22% with iron addition decreased by 24%. Acute dosing of iron occurs at the higher values of X_e on the day after a transfusion event.

Conclusion: Systematic trends in fractional Hb incremental loss X_e have been modelled and have a significant and calculatable impact on transfusion intervals and associated introduction of iron.

KEYWORDS
haemoglobin, increment, model, RBC, transfusion

Highlights

- A new model was introduced for evaluating chronic RBC transfusions.
- RBC usage and introduced iron are strongly correlated with haemoglobin incremental loss.

INTRODUCTION

Haematological disorders such as sickle-cell disease, beta-thalassemia and myelodysplastic syndromes require careful, long-term management of red blood cell (RBC) transfusions including RBC type and number of units required to achieve targeted patient haemoglobin (Hb) levels. Chronic transfusions are administered to increase the oxygen-carrying capacity or as part of an exchange transfusion programme to mitigate anaemia or morbidity associated with haemoglobinopathy. The frequency of patient transfusions can occur

as often as every 15 days [1] and is dependent upon many factors including disease type, stage of disease, effectiveness of available drug therapies, current clinical best practices, etc. In many cases, these chronic transfusions are needed throughout the lifetime of the individual patient.

Chronic RBC transfusions, part of a supportive care regimen to treat anaemia, can be the major source of iron overload in many RBC disorders [2]. Recurring transfusions will introduce iron at concentrations that can surpass the binding capacity of transferrin resulting in non-transferrin bound iron (NTBI). NTBI represents a potentially toxic species due to its ability to create reactive oxygen species (ROS) that can lead to a variety of organ/cellular damage [3]. Patients on chronic transfusion gain on average about 0.5 mg/kg/day of iron and can rapidly become iron overloaded [4, 5]. In addition, transfusion of RBCs with longer storage times has been associated with increases in extravascular haemolysis, saturated serum transferrin, elevated NTBI [6] and increased morbidity following acute transfusion [7].

Another challenge of chronic RBC transfusions is the variation in the incremental proportion of transfused RBCs that remain recirculating 24 h after transfusion. The so-called incremental haemoglobin (Hb_{incr}) measured as the difference between pre- and post-transfusion Hb levels is known to be highly variable and can be significantly lower than the nominal Hb_{incr} amount of 1 g/L per unit [8–11]. Patient-dependent factors with respect to RBC loss include febrile patients showing 50% RBC loss within 24 h [12]. Patients undergoing haematopoietic stem cell transplantation also had a broad range of RBC loss between 29% and 71% [13]. Immunological factors influencing transfused RBC effectiveness continues to be an active area of research [14, 15]. The average Hb_{incr} was reported reduced by 50% for older stored blood compared to relatively 'fresh' blood [16] with more modest reductions also reported [17, 18]. While studies have shown no significant impact of blood age on patient outcomes [19–23], these acute care settings were primarily measuring mortality outcomes with typically less than three transfused units. This acute patient population is not representative of chronically transfused patients with haematological disorders that have unique, longer-term outcome criteria. The complex cross-section between (a) the diverse, clinically relevant chronically transfused patient populations, (b) non-uniform transfusion practices [24], and (c) the breadth of factors influencing variation in transfusion efficacy require a more quantitative approach towards improving outcomes in chronic transfusion settings.

A variety of predictive RBC lifespan models and measurements have been thoroughly reviewed in the literature [25]. A breadth of lifespan-based indirect response models has also been successfully applied [26]. Measurement of alveolar carbon monoxide has been an effective indirect measurement of RBC destruction and associated lifespans [27]. Almost all the direct models have been developed based on fitting kinetic data from labels based on ^{51}Cr, biotin, ^{15}N or ^{14}C and the models are fit starting 24 h after the transfusion. Thus, these models do not address the Hb loss within the first day. In addition, most of these RBC lifespan models are based on a single transfusion (to multiple recipients) where a labelled aliquot is given to a healthy recipient which is not necessarily representative of the critical patient populations. None of the RBC lifespan models have been extended to multiple transfusions, account for Hb_{incr}, or quantitatively address iron dosing variation.

The purpose of this article is to extend and augment an RBC lifespan model across multiple transfusions to evaluate transient Hb levels over longer timeframes (4–12 months). This RBC transfusion model needs to provide clinicians with a quantitative framework that accounts for loss within 24 h of transfusion, consider varying transfusion intervals, different RBC lifespans and impact on iron overload.

MATERIALS AND METHODS

A full review and summary of RBC lifespan models have been presented elsewhere [25], and this work utilizes the Weibull lifespan distribution model to evaluate transfusion dynamics including initial RBC loss and quantifying introduced iron specific to transfused RBC degradation. The Weibull model was chosen due to its well-established fit of RBC lifespan, and it was the best distribution for a finite RBC lifespan (closest approach to zero survival probability at max lifespan). Starting with a single cohort where all the RBCs are of the same age born at time $t = 0$, the full RBC survival function for the Weibull lifespan distribution is represented by:

$$\overline{W}_{\text{full}}(t;\alpha,\beta) = e^{-\left(\frac{t}{\beta}\right)^{\alpha}}, \tag{1}$$

where α and β (both >0) are referred to as the shape and scale parameters, respectively, and t is the time in days. The associated probability density function (PDF) of the full Weibull distribution, denoted by $w(t; \alpha, \beta)$, is represented by:

$$w(t;\alpha,\beta) = \frac{\alpha}{\beta}\left(\frac{t}{\beta}\right)^{\alpha-1} e^{-\left(\frac{t}{\beta}\right)^{\alpha}}. \tag{2}$$

The Weibull model has many useful, descriptive values of the Weibull lifespan distribution that are easily calculated. The mean lifespan value for the full Weibull lifespan distribution, $\mu_{W,\text{full}}$, is given by:

$$\mu_{W,\text{full}} = \beta\Gamma\left(1+\frac{1}{\alpha}\right), \tag{3}$$

$$\Gamma(x) = \int_0^{\infty} t^{x-1}e^{-t}dt, \tag{4}$$

where Γ is the Euler gamma function. The standard deviation, σ, of the full Weibull distribution is given by:

$$\sigma = \sqrt{\beta^2\left[\Gamma\left(1+\frac{2}{\alpha}\right) - \left(\Gamma\left(1+\frac{1}{\alpha}\right)\right)^2\right]}. \tag{5}$$

The maximum lifespan can be approximated by the 95th percentile of the full distribution, τ_{95}:

$$\text{Max lifespan}\tau_{95} = \beta(\ln(20))^{1/\alpha}. \quad (6)$$

In the case of transfusions, donor RBCs have a random distribution of ages ($t = 0$ to max lifespan) and therefore are referred to as a random sample. The Weibull residual lifespan survival function is the appropriate model for determining the remaining time that a random, transfused RBC population remains in circulation before 'death'. Per the Shrestha review article, the Weibull residual lifespan survival function can be simplified and expressed in terms of the Gamma lifespan distribution as follows:

$$\overline{W}_{\text{res}}(t;\alpha,\beta) = \overline{G}(t^{\alpha};1/\alpha,\beta^{\alpha}). \quad (7)$$

Substituting the transformed Weibull t, α and β parameters into the Gamma function yields the key residual lifespan curve utilized in all subsequent transfusion calculations. This ideal random RBC residual lifespan was also simulated as described previously [28]. In all simulations presented here, one unit of label is assumed to bind to one RBC. The mean residual lifespan, μ_{res}, of the Weibull distribution can be represented by either expression below.

$$\mu_{\text{res}} = \frac{\beta\Gamma(2/\alpha)}{\Gamma(1/\alpha)}, \quad (8)$$

$$\mu_{\text{res}} = \frac{\sigma^2+\mu^2}{2\mu}. \quad (9)$$

The residual lifespan of transfused RBCs is well-known to have significant initial loss (within 24 h of transfusion) followed by a linear decrease well-fit by the Weibull residual lifespan distribution. Therefore, the time-dependent concentration of haemoglobin, Hb(t), needs to account for both initial loss and residual lifespan of transfused RBCs through the derived equation:

$$\text{Hb}(t) = \text{Hb}_{\text{incr}} \times (1 - X_e) \times \overline{W}_{\text{res}}(t;\alpha,\beta), \quad (10)$$

where Hb_{incr} is the nominal increment of haemoglobin (g/dl), X_e is the fraction of effete RBCs eliminated within the first 24 h post-transfusion, and $\overline{W}_{\text{res}}$ is the residual Weibull lifespan distribution. The nominal Hb_{incr} is taken as 1 g/dl per unit of transfused blood in the calculations performed here. A 5-parameter, flexible Weibull distribution for RBC lifespan introduced by Korell [28] was also evaluated for modelling initial loss plus residual lifespan, but the simplicity and fit of the 3-parameter approach were best-suited for the transfusion dynamics being modelled here.

The fraction of eliminated RBCs, X_e, can be expressed as follows:

$$X_e = 1 - \frac{\Delta\text{Hb}_{\text{meas}}}{\text{Hb}_{\text{incr}}}, \quad (11)$$

where $\Delta\text{Hb}_{\text{meas}}$ is the actual, measured change in haemoglobin 24 h after transfusion. With this expression, an estimate for the relative standard deviation for X_e can be calculated using literature values for variation of $\Delta\text{Hb}_{\text{meas}}$ (~10%) [6] and Hb_{incr} (~12%) [18]. This results in a relative standard deviation for X_e of 16%.

Finally, the total iron introduced per transfused RBC unit was set equal to 225 mg. Therefore, the cumulative amount of iron attributable solely to transfused RBC 'death' is given by:

$$\text{Fe(total)} = \text{Fe(initial)} + \text{Fe(time)} = 225 \times \text{Hb}_i \times X_e + 225 \times \Delta\text{Hb}(t). \quad (12)$$

The initial iron release in the first 24 h is determined by X_e and then subsequently by the change in Hb over time, t (in days), characterized by $\overline{W}_{\text{res}}$. Multiple transfusion events would simply sum Equation (12) across each individual transfusion event timeline to calculate the total iron introduced. The steady-state iron eliminated (estimated 1.5 mg/day) by the body was omitted to focus on transfused RBC impact. Other sources of iron input (i.e., diet) were likewise not included.

All calculations were performed using Microsoft Excel for Microsoft 365 (Version 2105) utilizing built-in functionality for Weibull and Gamma distributions and Euler's gamma function. Two unique RBC lifespans were modelled for comparison in transfusion analyses. The previously reported RBC full lifespan, $\mu_{\text{full}} = 115.6$ days, was reproduced here using Weibull α and β parameters (shape and scale) equal to 5.38 and 125.38, respectively. This modelled full lifespan is consistent with a healthy, normal RBC cohort. The second Weibull function targeted a 50% reduction in $\mu_{\text{full}} = 58.1$ days using $\alpha = 5.38$ and $\beta = 63$. The related PDF and residual lifespan distributions were also calculated and visualized for comparative purposes. It is the residual lifespan distributions that are utilized for the transfusion calculations as they represent the randomly labelled, diversified 'ages' of transfused RBCs. The initial Hb level triggering the first transfusion was chosen to be 7 g/dl with a nominal 2-unit RBC transfusion ($\text{Hb}_{\text{incr}} = 2$ g/dl) that target a steady-state [Hb] range between 8 and 9 g/dl consistent with current clinician practices [24, 29].

RESULTS

The RBC lifespan distribution was modelled by two separate Weibull functions for comparative purposes (Table 1). The single cohort, RBC full lifespan, $\mu_{\text{full}} = 115.6$ days, was reproduced here using Weibull α and β parameters (shape and scale) equal to 5.38 and 125.38, respectively [25]. The mean residual lifespan, μ_{res}, of randomly labelled, healthy RBCs is 60.5 days. The second Weibull function ($\alpha = 5.38$, $\beta = 63$) yielded $\mu_{\text{full}} = 58.1$ days and $\mu_{\text{res}} = 30.4$ days for the decreased RBC lifespan. The two different RBC residual lifespan distributions will be referred to as the 60- and 30-day residual survival curves referring to their respective μ_{res} values. The Weibull PDF, full lifespan distribution and residual lifespan distribution are all plotted together for the two comparative cases in Figure 1. Comparing RBC residual distributions (used for transfusion calculations), the magnitude of the negative linear slope of the survival fraction is about a factor of two greater for the 30-day μ_{res} relative to the 60-day μ_{res} as

TABLE 1 Weibull α and β parameters and associated outputs for red blood cell lifespans

α	β	μ_{full} (days)	σ-SD (days)	τ_{95}, max LS (days)	μ_{res}[a] (days)
5.38	125.38	115.6	24.8	153.7	60.5
5.38	63	58.1	12.4	77.3	30.4

Abbreviation: LS, lifespan.

[a]The residual lifespans, μ_{res}, are approximated to 60 and 30 days in text and figures for clarity.

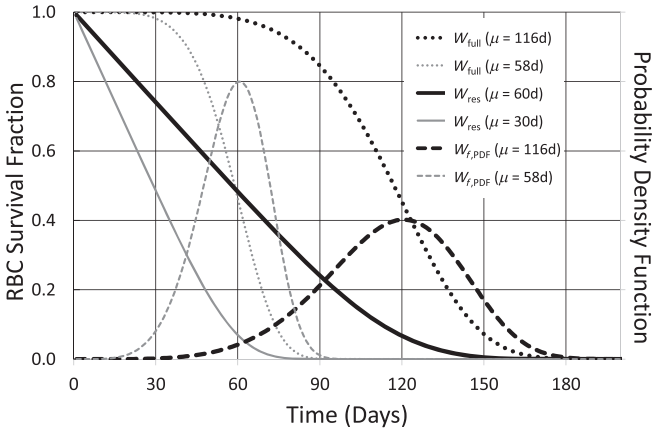

FIGURE 1 The Weibull full lifespan distribution (W_{full}), its associated probability density function ($W_{f,PDF}$) and the Weibull residual lifespan distribution (W_{res}) are plotted for Weibull parameters $\alpha = 5.38$ with $\beta = 63$ and $\beta = 125.38$ corresponding to mean residual lifespans (μ_{res}) of 30 and 60 days, respectively. RBC, red blood cell

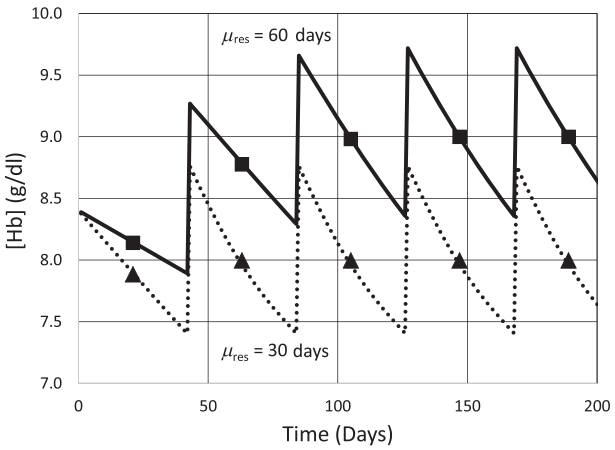

FIGURE 2 The haemoglobin concentration, [Hb], is plotted against the number of days since the first transfusion on day 0. The initial [Hb] = 7 g/dl, nominal Hb_{incr} = 2 g/dl, transfusion interval = 42 days and $X_e = 0.3$. [Hb] values are compared for the residual Weibull distributions where μ_{res} = 60 days and μ_{res} = 30 days. The mean [Hb] values between transfusions are denoted by filled squares and triangles, respectively, and are unchanged by subsequent transfusions at steady state

expected. The linear portion of the RBC residual survival curves are maintained for around 45 and 90 days, respectively, before tapering asymptotically towards zero.

Using Equation (10), the Hb levels after multiple RBC transfusions over 200 days for the two lifespan distributions are depicted in Figure 2. The transfusion conditions were chosen to represent an intermediate level of Hb loss within 24 h represented by $X_e = 0.3$, with Hb_{incr} = 2 g/dl given every 42 days starting on day 0 with an initial [Hb] = 7 g/dl. The sawtooth pattern results from the RBC introduction with the transfusion event and subsequent residual RBC lifespan decay. At 42-day intervals, the Hb decay portion remains linear for the 60-day RBC residual lifespan but appears to taper slightly at the end of the decay portion for the 30-day lifespan. It requires three transfusions to reach a steady-state mean [Hb] ~ 9 g/dl for 60-day lifespan. The transfusions modelled by 30-day residual lifespan appear to reach a steady-state mean [Hb] ~ 8 g/dl after just two transfusions. Therefore, the lower RBC lifespan results in lower achievable [Hb] at steady state but reaches steady state with fewer transfusions.

RBC lifespan results were compared across a range of X_e and transfusion intervals as shown in Figure 3. X_e and transfusion intervals were varied to obtain mean [Hb] values at steady state of 9 or 8 g/dl for the 60- and 30-day RBC lifespans, respectively. For each RBC lifespan, the lower X_e allows for a longer transfusion interval to achieve the same [Hb] at steady state (Figure 3). The transfusion

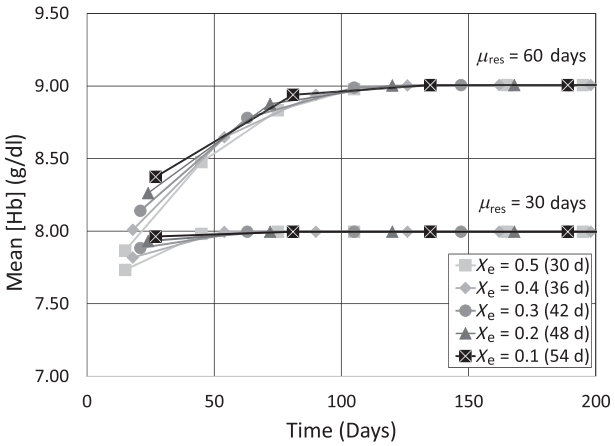

FIGURE 3 The mean [Hb] is plotted against the number of days since the first transfusion on day 0 for a paired series of X_e and transfusion intervals. The initial [Hb] = 7 g/dl, nominal Hb_{incr} = 2 g/dl, and transfusion intervals are selected for each X_e to yield equivalent steady-state mean [Hb] = 9.0 g/dl and [Hb] = 8.0 g/dl for μ_{res} = 60 days and μ_{res} = 30 days, respectively

intervals range from 30 days ($X_e = 0.5$) to 54 days ($X_e = 0.1$), and the X_e range was chosen based on relevant literature derived from clinical data. There is a broad range of steady-state [Hb] attainable by simply

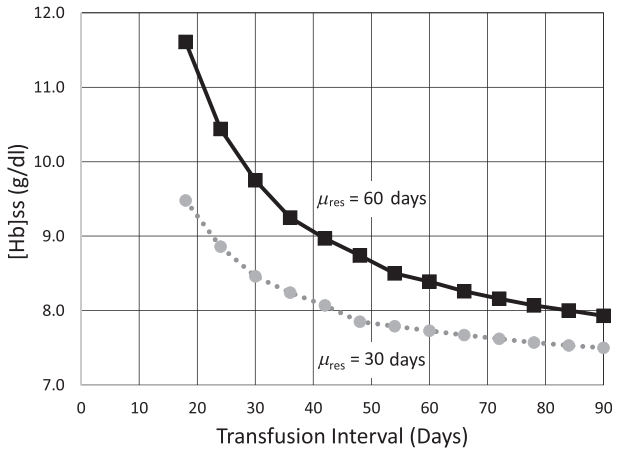

FIGURE 4 The mean [Hb] achieved at steady state is plotted as a function of the transfusion interval in days for two Weibull residual lifespan distributions with $\mu_{res} = 60$ days and $\mu_{res} = 30$ days. In this comparative graph, initial [Hb] = 7 g/dl, nominal $Hb_{incr} = 2$ g/dl and $X_e = 0.3$

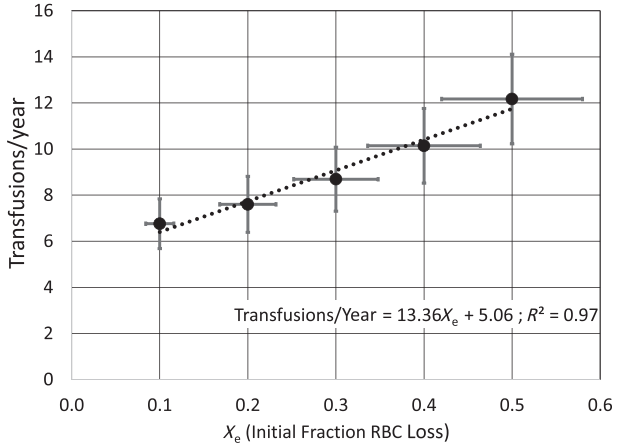

FIGURE 5 The transfusions per year is plotted as a function of X_e where [Hb] = 7 g/dl, nominal $Hb_{incr} = 2$ g/dl. The transfusion intervals of 30, 36, 42, 48 and 54 days correspond to X_e values of 0.5, 0.4, 0.3, 0.2 and 0.1, respectively. The results are identical for red blood cell (RBC) lifespan distributions $\mu_{res} = 60$ days and $\mu_{res} = 30$ days and are reasonably fit by a line to define a useful relationship. The error bars represent a relative standard deviation of 16% (discussed further in results)

varying the transfusion interval. Figure 4 depicts the range of mean [Hb] at steady state obtained using 18- to 90-day transfusion intervals when $X_e = 0.3$, $Hb_{incr} = 2$ g/dl and an initial [Hb] = 7 g/dl. While the number of transfusions and total days to reach steady state vary, all intervals examined achieved steady state by 120 and 60 days for the 60- and 30-day residual RBC lifespans, respectively. The graphs show a gradual tapering and decreasing differences at longer transfusion intervals.

For both 60- and 30-day RBC residual lifespan data, one can construct a relationship between X_e and the number of transfusions per year as shown in Figure 5. The results show that the X_e range 0.1–0.5

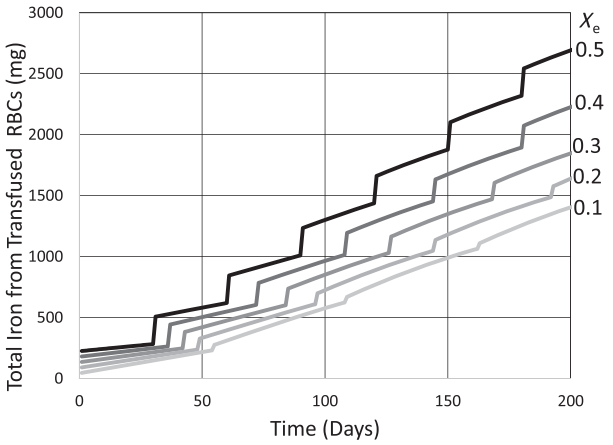

FIGURE 6 The total iron delivered from the addition and degradation of transfused red blood cells (RBCs) is plotted over 200 days of transfusions with the first transfusion on day 0. The initial [Hb] = 7 g/dl, nominal $Hb_{incr} = 2$ g/dl, $\mu_{res} = 60$ days and X_e values 0.1, 0.2, 0.3, 0.4 and 0.5 are associated with transfusion intervals of 54, 48, 42, 36 and 30 days, respectively

produces an annual transfusion frequency between 7 and 12. The 16% estimated relative error is meant to help put the model within the context of known transfusion variability as described in the Materials and Methods section. The biggest difference between 60- and 30-day lifespans when modelling transfusions is the steady-state [Hb] achieved (9 and 8 g/dl, respectively). The lower X_e is desirable for minimizing transfusion frequency and the differences appear statistically and practically significant.

Finally, the impact of X_e and RBC transfusions on the total iron introduced through transfusions was examined. Figure 6 shows the 60-day RBC lifespan model using $Hb_{incr} = 2$ g/dl given on day 0 with varying transfusion intervals dictated by X_e. The higher X_e results in more frequent transfusions with 'acute' iron exposure events that occur within 24 h of each transfusion. By example, for $X_e = 0.5$ with 30-day transfusion intervals, over 50% of the iron (1575 mg) is delivered in just seven individual days. The seventh transfusion is on day 180, and on day 181 the total iron delivered by transfused RBC degradation is just over 2500 mg. The introduced iron would only be slightly higher for the 30-day RBC residual lifespan case due to the accelerated RBC decay curve which keeps the circulating, steady-state [Hb] 1 g/dl lower than the 60-day RBC lifespan. A summary of all the key [Hb] and iron results are shown in Table 2.

DISCUSSION

Individual RBC lifespan models have historically ignored RBC loss in the first 24 h due to high variability and a poor fit with the linear decay of RBCs typically observed after 24 h. This gap has been addressed herein using the factor X_e (fraction effete RBCs lost in first 24 h) in modelling transfusions. While an individual healthy RBC lifespan is known to be ~116 days, it is the mean residual RBC

TABLE 2 Transfusion data for two unique red blood cell residual lifespans modelled by Weibull distributions

$\mu_{W,res}$ (days)	X_e	$[Hb]_{SS}$[a] (g/dl)	Interval (days)	Transfusions/year	Total iron at 365 days (mg)
60.5	0.1	9.0	54	6.8	2567
60.5	0.2	9.0	48	7.6	2955
60.5	0.3	9.0	42	8.7	3385
60.5	0.4	9.0	36	10.1	3864
60.5	0.5	9.0	30	12.2	4715
30.4	0.1	8.0	54	6.8	2636
30.4	0.2	8.0	48	7.6	3076
30.4	0.3	8.0	42	8.7	3518
30.4	0.4	8.0	36	10.1	3968
30.4	0.5	8.0	30	12.2	4845

[a]Steady-state values of 9 g/dl and 8 g/dl match the 30-day transfusion interval results when $X_e = 0.5$.

lifespan of a diverse-aged RBC population that is most relevant for modelling transfused RBC units because each unit is captured as a diverse-aged population from the donor. The model reported here reveals both the significant impact short-term RBC loss and the reduction in mean residual lifespan has on achievable [Hb] steady-state chronic transfusions. A lower amplitude of Hb fluctuations for MDS patients has been previously correlated to improved quality of life [30], and both the RBC half-life and X_e have an impact on maintaining a desired threshold Hb level.

The mean residual lifespan of transfused RBCs has been previously proposed as a measure of quality [31] where the impact of RBC residual lifespan was elucidated across two patient populations: sickle cell disease with much shorter RBC lifespans and diabetes mellitus with highly variable RBC lifespans. The model presented here delivers a tool for considering *both* RBC lifespan and actual Hb_{incr} simultaneously and quantitatively as measures of quality and/or effectiveness. These parameters vary independently and should be accounted for when deciding upon the most appropriate transfusion interval. In more clinical examples, neocyte transfusions have been shown to extend the transfusion interval and require less overall blood relative to standard RBCs in chronic transfusion settings [32–35]. If the actual Hb_{incr} and a neocyte lifespan were known, one could better elucidate the key drivers of the highly significant 20% decrease in overall blood usage. These represent real opportunities for gaining insights. In the future, donor, recipient, immunological factors, and RBC processing variables should be evaluated with respect to both X_e and RBC lifespan to elucidate a more complete mapping of factors impacting chronic transfusion outcomes.

A strength of this model is the reliance on objectively measurable inputs and the production of clinically relevant outputs of patient haematocrit and iron dose. The well-known Weibull RBC lifespan model was utilized here but substituting gamma or lognormal RBC lifespan functions would have yielded similar results due to their equivalent, linear RBC survival curve at the transfusion time intervals examined [25]. Independent measures of patient RBC lifespan through alveolar carbon monoxide measurements may improve data fits relative to literature or population estimates of RBC lifespan. Identifying clinical data with baseline Hb and Hb_{incr} over multiple transfusions of representative patient populations would help validate this model.

An inherent weakness of this model is that, while increasing patient haematocrit is necessary to increase oxygen-carrying capacity, it may not be sufficient. The current model reported here has been directed towards actual Hb_{incr} (without clinical complications such as red cell loss due to bleeding and assuming an equilibrium between patient intrinsic RBC production and elimination); it does not explicitly model changes in oxygen delivery capacity. Haemodynamic functionality (HF) and RBC P50 are examples of two characteristics to be considered when describing increased oxygen delivery capacity. HF [36] of transfused describes the ability of RBC to effectively interact with the microvasculature. Once in the microvasculature, the RBC need to be able to effectively deliver oxygen [37]. Future work could apply the current framework to HF or p50 depending upon the specific measurements available or to illustrate changes in microvascular perfusion or oxygen delivering capacity.

The model also suggests a new approach for reducing transfusion-related iron overload. For transfusions where unavoidable loss of greater than 20% Hb occurs within 24 h ($X_e \geq 0.2$), it seems reasonable that providing chelation therapies concurrently with transfusion may mitigate acute iron exposure. Aggressively treating these periodic 'spikes' in iron during the immediate aftermath of a transfusion event may provide a route to prevent long term iron accumulation. In addition, matching the timing of chelation treatment to the acute dose of free iron due to transfusion may mitigate the potential ROS that are produced from excess NTBI, thus mitigating oxidative damage. Iron homeostasis comprises numerous protein-regulated pathways and modelling the ultimate disposition of iron influx from RBC transfusions is beyond the scope of this work.

This model allows clinicians to simultaneously solve for X_e and RBC lifespan over multiple transfusions. If the residual RBC lifespan is known, then X_e can theoretically be determined from single Hb measurements per transfusion interval. More preferred from a diagnostic viewpoint is a Hb measurement just prior to transfusion then again 24–48 h after transfusion. Protocols that document individual variation in transfusion efficiencies over multiple transfusions would

enhance understanding while minimizing X_e, minimizing iron exposure and maximizing transfusion intervals.

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CONFLICT OF INTEREST

PC is a consultant for Hemanext and AD has no conflict of interest.

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REFERENCES

1. Gamberini MR, Fortini M, Stievano A, Calori E, Riontino MV, Ceccherelli G, et al. Impact of the preparation method of red cell concentrates on transfusion indices in thalassemia patients: a randomized crossover clinical trial. Transfusion. 2021;61:1729–39.
2. Vinchi F, Hell S, Platzbecker U. Controversies on the consequences of iron overload and chelation in MDS. Hemasphere. 2020;4: e357-1–16.
3. Brissot P, Ropert M, Lan CL, Loréal O. Non-transferrin bound iron: a key role in iron overload and iron toxicity. Biochim Biophys Acta. 2012;1820:403–10.
4. Porter JB. Practical management of iron overload. Br J Haematol. 2001;115:239–52.
5. Coates TD. Physiology and pathophysiology of iron in hemoglobin-associated diseases. Free Radic Biol Med. 2014;72:23–40.
6. Rapido F, Brittenham GM, Bandyopadhyay S, Carpia FL, L'Acqua C, McMahon DJ, et al. Prolonged red cell storage before transfusion increases extravascular hemolysis. J Clin Invest. 2016;127:375–82.
7. Ozment CP, Turi JL. Iron overload following red blood cell transfusion and its impact on disease severity. Biochim Biophys Acta. 2009; 1790:694–701.
8. Karafin MS, Bruhn R, Roubinian NH, Chowdhury D, Qu L, Snyder EL, et al. The impact of recipient factors on the lower-than-expected hemoglobin increment in transfused outpatients with hematologic diseases. Transfusion. 2019;59:2544–50.
9. Peyrony O, Gamelon D, Brune R, Chauvin A, Ghazali DA, Yordanov Y, et al. Red blood cell transfusion in the emergency department: an observational cross-sectional multicenter study. J Clin Med. 2021;10:2475.
10. Reikvam H, Prowse C, Roddie H, Heddle NM, Hervig T, BEST collaborative. A pilot study of the possibility and the feasibility of haemoglobin dosing with red blood cells transfusion. Vox Sang. 2010;99:71–6.
11. Roubinian NH, Plimier C, Woo JP, Lee C, Bruhn R, Liu VX, et al. Effect of donor, component, and recipient characteristics on hemoglobin increments following red blood cell transfusion. Blood. 2019; 134:1003–13.
12. Wendelbo Ø, Opheim EN, Hervig T, Lunde THF, Bruserud Ø, Mollnes TE, et al. Cytokine profiling and post-transfusion haemoglobin increment in patients with haematological diseases. Vox Sang. 2018;113:657–68.
13. Zhang X, Xiao Y, Ran Q, Liu Y, Duan Q, Duan H, et al. Clinical observation of factors in the efficacy of blood component transfusion in patients following hematopoietic stem cell transplantation. PLoS One. 2012;7:e36912.
14. Maitta RW. Immunologic concepts in transfusion medicine; 2020.p. 81–95. Amsterdam, Netherlands: Elsevier.
15. Youssef LA, Spitalnik SL. Transfusion-related immunomodulation: a reappraisal. Curr Opin Hematol. 2017;24:551–7.
16. Hunsicker O, Hessler K, Krannich A, Boemke W, Braicu I, Sehouli J, et al. Duration of storage influences the hemoglobin rising effect of red blood cells in patients undergoing major abdominal surgery. Transfusion. 2018;58:1870–80.
17. Rydén J, Clements M, Hellström-Lindberg E, Höglund P, Edgren G. A longer duration of red blood cell storage is associated with a lower hemoglobin increase after blood transfusion: a cohort study. Transfusion. 2019;59:1945–52.
18. Luten M, Roerdinkholder-Stoelwinder B, Schaap NPM, Grip WJD, Bos HJ, Bosman GJCGM. Survival of red blood cells after transfusion: a comparison between red cells concentrates of different storage periods. Transfusion. 2008;48:1478–85.
19. Lacroix J, Hébert PC, Fergusson DA, Tinmouth A, Cook DJ, Marshall JC, et al. Age of transfused blood in critically ill adults. N Engl J Med. 2015;372:1410–8.
20. McQuilten ZK, French CJ, Nichol A, Higgins A, Cooper DJ. Effect of age of red cells for transfusion on patient outcomes: a systematic review and meta-analysis. Transfus Med Rev. 2018;32:77–88.
21. Fergusson DA, Hébert P, Hogan DL, LeBel L, Rouvinez-Bouali N, Smyth JA, et al. Effect of fresh red blood cell transfusions on clinical outcomes in premature, very low-birth-weight infants: the ARIPI randomized trial. JAMA. 2012;308:1443–51.
22. Heddle NM, Cook RJ, Arnold DM, Liu Y, Barty R, Crowther MA, et al. Effect of short-term vs. long-term blood storage on mortality after transfusion. N Engl J Med. 2016;375:1937–45.
23. Steiner ME, Ness PM, Assmann SF, Triulzi DJ, Sloan SR, Delaney M, et al. Effects of red-cell storage duration on patients undergoing cardiac surgery. N Engl J Med. 2015;372:1419–29.
24. Goss C, Giardina P, Degtyaryova D, Kleinert D, Sheth S, Cushing M. Red blood cell transfusions for thalassemia: results of a survey assessing current practice and proposal of evidence-based guidelines. Transfusion. 2014;54:1773–81.
25. Shrestha RP, Horowitz J, Hollot CV, Germain MJ, Widness JA, Mock DM, et al. Models for the red blood cell lifespan. J Pharmacokinet Pharmacodyn. 2016;43:259–74.
26. Krzyzanski W, Ruixo JJP. Lifespan based indirect response models. J Pharmacokinet Pharmacodyn. 2012;39:109–23.
27. Zhang HD, Ma YJ, Liu QF, Ye TZ, Meng FY, Zhou YW, et al. Human erythrocyte lifespan measured by Levitt's CO breath test with newly developed automatic instrument. J Breath Res. 2018;12:36003.
28. Korell J, Coulter CV, Duffull SB. Evaluation of red blood cell labelling methods based on a statistical model for red blood cell survival. J Theor Biol. 2011;291:88–98.
29. Mueller MM, Remoortel HV, Meybohm P, Aranko K, Aubron C, Burger R, et al. Patient blood management. JAMA. 2019;321: 983–97.
30. Caocci G, Baccoli R, Ledda A, Littera R, Nasa GL. A mathematical model for the evaluation of amplitude of hemoglobin fluctuations in elderly anemic patients affected by myelodysplastic syndromes: correlation with quality of life and fatigue. Leukemia Res. 2007;31: 249–52.
31. Kuruvilla DJ, Nalbant D, Widness JA, Veng-Pedersen P. Mean remaining life span: a new clinically relevant parameter to assess the quality of transfused red blood cells. Transfusion. 2014;54: 2724–9.
32. Kumbhakar D. Transfusion of neocytes concentrate/pooled neocytes and conventional packed red blood cells in beta-thalassemia major patients: a comparative study. J Evol Med Dent Sci. 2016;5: 4285–90.
33. Collins AF, Gonçalves-Dias C, Haddad S, Talbot R, Herst R, Tyler BJ, et al. Comparison of a transfusion preparation of newly formed red

cells and standard washed red cell transfusions in patients with homozygous β-thalassemia. Transfusion. 1994;34:517–20.
34. Sharma DC, Rai S, Agarwal N, Sao S, Gaur A, Sapra R. Transfusion of neocytes concentrate/pooled neocytes in β-thalassemic patients. Indian J Hematol Blood Transfus. 2008;24:173–7.
35. Spanos T, Ladis V, Palamidou F, Papassotiriou I, Banagi A, Premetis E, et al. The impact of neocyte transfusion in the management of thalassaemia. Vox Sang. 1996;70:217–23.
36. Barshtein G, Arbell D, Yedgar S. Hemodynamic functionality of transfused red blood cells in the microcirculation of blood recipients. Front Physiol. 2018;9:41.
37. Srinivasan AJ, Morkane C, Martin DS, Welsby IJ. Should modulation of p50 be a therapeutic target in the critically ill? Expert Rev Hematol. 2017;10:1–10.

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Baseline assessment findings of the Africa Society for Blood Transfusion Step-Wise Accreditation Programme in 10 sub-Saharan African countries, 2016–2018

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Abstract

Background and Objectives: The accreditation of blood services promotes continuous quality improvement in blood and transfusion services. The Africa Society for Blood Transfusion (AfSBT) conducted 20 baseline assessments of National Blood Transfusion Services (NBTS) or blood banks as part of the Step-Wise Accreditation Programme (SWAP) in 10 sub-Saharan African (SSA) countries from 2016 to 2018. This paper aims to elucidate the process and findings of the baseline assessments.

Materials and Methods: This is a descriptive study of 20 baseline assessments of NBTS. Eleven sections of the AfSBT assessment were reviewed, and 48 out of 68 standards and 356 out of 466 criteria were assessed. Each standard was assigned a value of 1 if it was fully achieved, 0.5 if partially achieved and 0 if not achieved. We defined average section scores >75% as having 'met AfSBT Standards', ≤25% as not meeting standards, 26%–50% as needs major improvement, and 51%–75% as needs some improvement and >75% as meets standards.

Results: The AfSBT SWAP standards were met in 4 out of the 11 sections: donor management, blood collection, component production and compatibility testing. Three sections were determined to need some improvement (quality system; handling, transport and storage and testing of donated blood), and three sections were determined to need major improvement (haemovigilance, blood administration and national blood service accreditation). One section (receipt, ordering, and issuing of blood) did not meet standards.

Conclusion: Despite improvements in the quality of blood services in SSA over the past two decades, governments may consider the importance of prioritizing investments in NBTS, ensuring these institutions meet international accreditation standards that are aligned with safe blood transfusion services.

KEYWORDS
accreditation, AfSBT, blood transfusion systems

Highlights

- Understanding of the AfSBT SWAP assessments provides an opportunity for NBTS in SSA to improve and achieve accreditation.
- Findings from the AfSBT SWAP assessments highlights key areas for improvement of National Blood Transfusion Services in 10 SSA countries.
- Standards such as haemovigilance, blood administration, national blood service accreditation, and the receipt, ordering of blood would benefit from much improvement.

INTRODUCTION

Blood transfusions can be a critical and lifesaving intervention, especially in low-resource settings where conditions requiring transfusions, such as malaria, and post-partum haemorrhage, are often associated with high mortality and morbidity [1]. National Blood Transfusion Services (NBTS) are an integral component of resilient healthcare systems [2–4]. An efficient NBTS, that provides safe and adequate blood is a fundamental component for any healthcare delivery system and is critical for establishing safe, adequate and high-quality blood and transfusion services [5]. In 1975, the World Health Assembly first highlighted the global need for adequate safe blood, which has led to prioritization globally and at the level of national health systems [6]. However, of the estimated 112.5 million units of blood collected in 2013, approximately 5.6 million units were collected in the World Health Organization (WHO) Africa region which makes up 16% of the global population, accounting for only about 4% of global donations [6].

In 1994, the WHO regional committee for the Regional Office for Africa (AFRO) expressed concern that, out of 44 countries in the Africa region, only 10 had the appropriate policies and systems in place to support the safety of blood transfusion in their respective countries [7]. In an effort to address these concerns, the WHO proceeded to adopt the Resolution AFR/RC44/R12 (in 2001), which encouraged member countries 'to enact safe blood policies and mobilize resources for the development of the infrastructure of blood transfusion services in their countries' [7]. The WHO recommends that all activities that are associated with the process of blood collection, testing, processing, storage and distribution be coordinated at the national level through an effective organization and integrated blood supply networks [8, 9].

Accreditation is defined by Hindawi as 'a non-governmental, voluntary process whereby an agency or association grants public recognition to an organization for having met certain established standards' [10]. These established standards may be determined through either initial and periodic evaluations that involve submitting a self-evaluation report, site inspection or by a team of experts conducting an evaluation by an independent board or commission [10–12]. NBTS in Africa operate at widely different levels of development from high to more basic levels. Consequently, most African countries have found international blood transfusion standards and requirements too complex and expensive to adopt in resource-constrained settings [7].

To address the challenges in meeting accreditation standards, the Africa Society for Blood Transfusion (AfSBT), which was established in 1997, developed blood transfusion standards relevant to Africa [7]. The mission of the AfSBT is to, 'advocate for the highest ethical and professional standards, practices and skills in blood transfusion across the African continent, enabling safe, universally accessible and sustainable national blood programs in participating countries' [13].

The AfSBT Standards are evidence-based best practices in blood transfusion and were initially based on the WHO Aide Memoire for Blood Safety. The Standards for the AfSBT Step-Wise Accreditation Program (SWAP) were initially developed in 2013 by a sub-group of the Task Team for Accreditation established by the AfSBT with guidance from the American Association of Blood Banks. The primary goal of the Standards is to provide a benchmark that is achievable for the accreditation of blood banks and NBTS to maintain and improve the quality and safety of blood transfusion infrastructure, systems, and practices in Africa [10, 13]. Since AfSBT's establishment in 2013, over 20 countries have been engaged in the process of acquiring accreditation.

The AfSBT Standards are applicable to blood transfusion services or individual health facilities that perform the following blood processing functions: mobilization, recruitment, selection and screening of blood donors; collection of blood, processing of blood into blood products, testing of blood and blood products for group and transfusion transmissible infectious disease, pre-transfusion/compatibility testing; and the storage, handling, transportation and distribution of blood and products [13]. The AfSBT accreditation process is entirely voluntary and consists of using a set of standards which is made up of three progressively more uncompromising levels of compliance, required as follows: Step 1; meeting minimum (basic) level certification, Step 2: meeting intermediate level certification (the intermediate step includes progressively more detailed requirements and standards than basic but less than full requirements), and Step 3: full accreditation at international standard. The entire accreditation process is further comprised of a series of assessments conducted by external independent blood transfusion experts: baseline assessment, progress assessment, formal assessment, re-assessment, surveillance assessment and repeat assessments [13]. A compliance chart defines the evidence required to achieve compliance at each of the three steps. The baseline assessment is the tool used to determine compliance with requirements of the AfSBT Standards at the initiation of the accreditation process. Prior to any external assessments, a blood transfusion service utilizes a gap analysis to perform a self-assessment and determines the most appropriate step that best correlates with its performance concomitant with AfSBT

assistance. The AfSBT supports the country in conducting an initial baseline assessment and an action plan is developed to address the deficiencies in obtaining the standards, with established timelines for rectifying said deficiencies. Once the gaps have been addressed, follow-on assessments can be conducted so that certification at Steps 1 or 2 or full accreditation at Step 3 can be achieved. To receive full accreditation at an international standard, the blood service must comply with all the required criteria of the standards. The AfSBT accreditation is valid for 3 years.

This paper aimed to summarize and describe the findings from 20 AfSBT baseline assessments of the AfSBT and identify those sections, standards and required criteria that were hard to achieve by NBTS.

METHODS

We conducted a descriptive study of baseline assessments conducted by AfSBT at 20 blood transfusion centres (sites) supported by the NBTS in 10 SSA countries between July 2016 and December 2018. For this study, sites are defined as those blood centres that are authorized by the NBTS to conduct blood collection, testing, processing, storage and distribution; some countries had more than one site that was assessed.

The baseline assessments were conducted by an external team comprising of blood transfusion experts employed or hired by the AfSBT. The baseline assessments consist of the first step in the SWAP process for achieving accreditation. Baseline assessments were conducted using three modalities: interviews, direct observation and record review.

The baseline assessment consists of 12 sections that cover the whole blood transfusion process from vein to vein. The sections are made up of 68 standards, which are sub-divided into 466 individual required criteria. In this paper, section 12 of the standards (Plasma Provided for Fractionation) and its standards (14) were excluded from the analysis across all 20 assessments as this function is considered 'elective' and was not assessed uniformly at all sites. This section is also not considered a minimum requirement for a well-organized and safe blood transfusion service by the WHO (Aide Memoire) [9, 11, 12].

TABLE 1 African Society for Blood Transfusion Accreditation Standards — Section, required criteria, priority indicators and standards

Section ($N = 12$)		Standards ($N = 68$)	Number of standards assessed ($N = 48$)	Required criteria ($N = 466$)	Priority criteria assessed ($N = 356$)
I	Quality system[a]	12	12	177	134
II	Blood Donor Management[b]	5	5	17	17
III	Collection of Blood from Donor[c]	7	7	37	37
IV	Handling, Transport and Storage[d]	4	4	17	17
V	Testing of Donated Blood[e]	3	3	22	22
VI	Blood Component Production[f]	3	1	30	24
VII	Receipt Ordering, Selection and Issuing of Blood and Blood Components[g]	4	4	28	28
VIII	Compatibility Testing[h]	4	2	39	29
IX	Haemovigilance and Clinical Interface[i]	4	4	16	16
X	Blood Administration[j]	4	2	18	13
XI	National Blood Service Accreditation[k]	4	4	19	19
XII	Requirements if plasma is provided for fractionation[l]	14	-	46	-

[a]The organization's structure, responsibilities, policies, procedures and resources established and approved by top management to achieve quality.
[b]Entails several key processes that together aim at providing for the proper number of donations and blood product needed.
[c]Process that includes recruitment, donor invitation, donor selection, donation procedures and donor retention.
[d]Procedures to ensure that blood and blood components are handled, stored and transported in a manner that prevents damage and meets specific requirements.
[e]Process for performing blood group serology and testing for infectious diseases carried out on donated specimens.
[f]Methods that ensure the quality and safety of blood components, including aliquots and pooled components.
[g]Procedures to check all incoming blood and blood components from another center against delivery document for number and group of components.
[h]Testing of each blood specimen from a potential recipient for ABO group, for Rhesus factor type and for clinically significant antibodies.
[i]Adverse events related to blood donation process are assessed, investigated and monitored.
[j]Procedures for administering blood and blood components.
[k]Requirements for a well-organized, nationally coordinated blood transfusion service to ensure availability of safe blood that is accredited.
[l]Excluded from analysis.

Data extraction

The authors used the WHO Aide Memoire which contains a checklist of key components that must be included in a well-organized NBTS to purposively identify 356 (356/466) criteria and 48 (48/54) standards, which represented the remaining 11 (11/12) sections for analysis (Table 1) [9, 14]. The number of standards assessed per section ranged from 1 to 12. The data from these 20 baseline

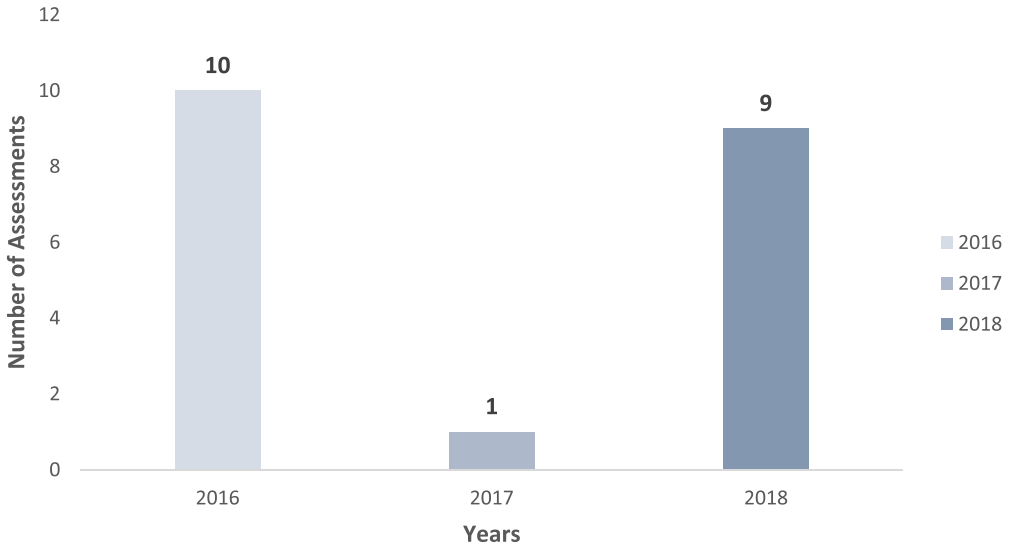

FIGURE 1 Number of AfSBT baseline assessments — 2016–2018. AfSBT, African Society for Blood Transfusion

TABLE 2 Average score of assessed standards within sections of the AfSBT[a] baseline assessment

Section		Score		Average percent (%)	Median percent (%)	Quartiles	
		Average	Maximum			1	3
I	Quality System[a]	8	12	66.7	69	61	72
II	Blood Donor Management[b]	3.9	5	78.0	80	68	90
III	Collection of Blood from Donor[c]	6.3	7	90.0	86	84	100
IV	Handling, Transport and Storage[d]	2.8	4	75.0	75	50	88
V	Testing of Donated Blood[e]	2	3	66.7	67	63	83
VI	Blood Component Production[f]	0.97	1	97.0	100	100	100
VII	Receipt Ordering, Selection and Issuing of Blood and Blood Components[g]	0.6	4	15.0	13	13	13
VIII	Compatibility Testing[h]	1.6	2	80.0	100	50	100
IX	Haemovigilance and Clinical Interface[i]	1.9	4	47.5	50	38	50
X	Blood Administration[j]	0.6	2	30.0	25	0	50
XI	National Blood Service Accreditation[k]	1.8	4	45.0	38	38	50
XII	Requirements if plasma is provided for fractionation[l]	-	-	-	-	-	-

Abbreviation: AfSBT, African Society for Blood Transfusion.

[a]The organization's structure, responsibilities, policies, procedures and resources established and approved by top management to achieve quality.

[b]Entails several key processes that together aim at providing for the proper number of donations and blood product needed.

[c]Process that includes recruitment, donor invitation, donor selection, donation procedures, and donor retention.

[d]Procedures to ensure that blood and blood components are handled, stored and transported in a manner that prevents damage and meets specific requirements.

[e]Process for performing blood group serology and testing for infectious diseases carried out on donated specimens.

[f]Methods that ensure the quality and safety of blood components, including aliquots and pooled components.

[g]Procedures to check all incoming blood and blood components from another center against delivery document for number and group of components.

[h]Testing of each blood specimen from a potential recipient for ABO group, Rhesus factor type and clinically significant antibodies.

[i]Adverse events related to blood donation process are assessed, investigated and monitored.

[j]Procedures for administering blood and blood components.

[k]Requirements for a well-organized, nationally coordinated blood transfusion service to ensure availability of safe blood that is accredited.

[l]Excluded from analysis.

assessments were extracted from the baseline assessments using a standardized structured questionnaire comprising the 356 criteria in Microsoft Excel.

Statistical analysis

Each of the 48 standards was assigned a value of 1 if it was achieved fully, 0.5 if achieved partially and 0 if not achieved [13]. For each site, standard scores were summed by section, and the mean, median and the first and third quartile scores for each section were calculated across the 20 baseline assessments. These summary measures were then divided by the maximum score available for each section (Table 1) and expressed as percentages of the total available score. For the purpose of this paper, we determined that sites that had section scores ≤25% as not meeting standards, >26%–50% as needs major improvement, >51%–75% as needs some improvement and >75% as meets standards [15].

Ethical review

Data analysed in this report were routinely collected for program monitoring, improvement and evaluation purposes only. This non-research activity was reviewed by centers for disease control and prevention (CDC) and was conducted consistent with applicable federal law and CDC policy.

RESULTS

Baseline assessments at 20 blood transfusion sites in 10 SSA countries were conducted over a period of 3 years (Figure 1). Ten baseline assessments were conducted in 2016, one in 2017 and nine in 2018. Due to funding constraints, only one baseline assessment was conducted in 2017. The 20 baseline assessments represent the NBTS of 10 PEPFAR-supported countries that engaged the AfSBT in the accreditation process. All sites managed by the NBTS in the 10 countries were assessed as part of the accreditation process, as such, some country's NBTS had multiple transfusion sites that participated in the analysis. All sites were located in urban centres, and none had achieved prior accreditation. However, for the purpose of this paper, we analysed the scores from each site individually.

Four sections (Blood Donor Management, Collection of Blood from Donors, Blood Component Production and Compatibility Testing) out of the 11 (Table 2) had mean and median percentages exceeding 75%. These four sections had high average scores indicating that these sections were performing well and had met the standards of

TABLE 3 Site achievement of standards by sections of the AfSBT baseline assessment

Section		Not meeting standard (<25%) *N* (%)	Needs major improvement (>26%–50%) *N* (%)	Needs some improvement (>51%–75%) *N* (%)	Meet standard (>75%) *N* (%)	Total *N*	Total %
I	Quality System[a]	0 (0)	2 (10)	13 (65)	5 (25)	20	100%
II	Blood Donor Management[b]	0 (0)	3 (15)	4 (20)	13 (65)	20	100%
III	Collection of Blood from Donor[c]	0 (0)	0 (0)	1 (5)	19 (95)	20	100%
IV	Handling, Transport and Storage[d]	1 (5)	5 (25)	3 (15)	11 (55)	20	100%
V	Testing of Donated Blood[e]	1 (5)	4 (20)	9 (45)	6 (30)	20	100%
VI	Blood Component Production[f]	0 (0)	1 (5)	0 (0)	19 (95)	20	100%
VII	Receipt Ordering, Selection and Issuing of Blood and Blood Components[g]	16 (80)	4 (20)	0 (0)	0 (0)	20	100%
VIII	Compatibility Testing[h]	0 (0)	6 (30)	0 (0)	14 (70)	20	100%
IX	Haemovigilance and Clinical Interface[i]	0 (0)	16 (80)	1 (5)	3 (15)	20	100%
X	Blood Administration[j]	10 (50)	9 (45)	0 (0)	1 (5)	20	100%
XI	National Blood Service Accreditation[k]	0 (0)	17 (85)	3 (15)	0 (0)	20	100%
XII	Requirements if plasma is provided for fractionation[l]	-	-	-	-	-	-

Abbreviation: AfSBT, African Society for Blood Transfusion.

[a]The organization's structure, responsibilities, policies, procedures and resources established and approved by top management to achieve quality.

[b]Entails several key processes that together aim at providing for the proper number of donations and blood product needed.

[c]Process that includes recruitment, donor invitation, donor selection, donation procedures, and donor retention.

[d]Procedures to ensure that blood and blood components are handled, stored and transported in a manner that prevents damage and meets specific requirements.

[e]Process for performing blood group serology and testing for infectious diseases carried out on donated specimens.

[f]Methods that ensure the quality and safety of blood components, including aliquots and pooled components.

[g]Procedures to check all incoming blood and blood components from another center against delivery document for number and group of components.

[h]Testing of each blood specimen from a potential recipient for ABO group, Rhesus factor type and for clinically significant antibodies.

[i]Adverse events related to blood donation process are assessed, investigated and monitored.

[j]Procedures for administering blood and blood components.

[k]Requirements for a well-organized, nationally coordinated blood transfusion service to ensure availability of safe blood that is accredited.

[l]Excluded from analysis.

that section (Table 2). Averages were as follows: blood component production (97%); collection of blood from donors (90%); compatibility testing (80%) and blood donor management (78%). Two sections (Blood Component Production and Collection of Blood from Donors) performed above (75%) at 19 out of 20 sites (Table 3).

Three sections (Quality Systems; Handling, Transportation, and Storage and Testing of Donated Blood) out of the 11 had mean and median percentages between >51% and 75%. These three sections had averages that indicated that these sections needed some improvement (i.e., >51%–75%; Table 2). Averages were as follows: handling, transport, and storage (75%); quality system (67.7%); and testing of donated blood (67.7%) (Table 2). One section (Quality System) performed above 51% at 18 out of 20 sites (Table 3).

Three sections (Haemovigilance and Clinical Interface, Blood Administration and National Blood Service Accreditation Requirements) out of the 11 had averages that indicated that these sections needed major improvement (i.e., >26%–50%; Table 2). Averages were as follows: haemovigilance and clinical interface (47%), blood administration (30%) and national blood service accreditation requirements (45%). Two sections (Haemovigilance and Clinical Interface and National Blood Accreditation Requirements) performed above 26% at 16 out of 20 and 17 out of 20 sites, respectively (Table 3).

One section (Receipt, Ordering, Selection, and Issuing of Blood and Blood Components) scored below 25% (considered not meeting the standards). This section performed below 25% at 16 out of 20 sites that completed the baseline assessment (Table 3).

DISCUSSION

Across the 10 countries and 20 sites that underwent the baseline assessment, which is the first stage of the AfSBT SWAP, standards were met in 4 out of the 11 sections. This is the first report of the baseline assessment findings of the AfSBT SWAP. Currently, there is a paucity of information on accreditation and quality standards of NBTS in SSA. Prior to the AfSBT SWAP, most international standards were considered inapplicable or too difficult to implement in low- and middle-income country (LMIC) settings [2, 16, 17]. Our findings emphasize the importance of accreditation standards to ensure the availability of safe and adequate blood supplies in SSA [12]. At the time of data collection and analysis, only four countries in SSA had achieved full accreditation while several others were at various stages of the SWAP process [3]. Of the 10 countries assessed, four ranked within the medium human development index (HDI) category, while the other six all ranked in the low HDI category, highlighting the importance of the AfSBT SWAP as a tool for low resource settings [4, 5].

Despite a good performance in four sections of the stepwise accreditation standards assessed by the AfSBT, essential components for providing safe blood to the public such as quality systems; testing of donated blood; receipt, ordering, and issuing of blood components; haemovigilance and NBTS accreditation, still require significant improvement.

Safe and available blood is particularly important for reducing maternal mortality, under-five mortality associated with malaria and trauma-associated mortality [18–21]. However, the availability of safe blood for use within the healthcare system depends upon routinely meeting high-quality standards established by AfSBT. Accreditation is considered an important approach for improving the quality of any NBTS. The AfSBT SWAP was developed to address the lack of applicable quality standards for the African context [7]. Since its establishment in 1997, and as of 2018, AfSBT has conducted 20 baseline assessments at 20 sites and in 10 countries across the continent. Regardless of the progress made in the field of blood safety in Africa by AfSBT, little has been published about the SWAP and its achievements [13, 16]. The International Organization for Standards (ISO) is the most common standard used by medical laboratories; however, this is often an expensive and a laborious process for LMIC. The more recent WHO-AFRO Stepwise Laboratory Quality Improvement Process Towards Accreditation, like the AfSBT SWAP, has been an attempt to make the process more achievable and affordable [22]. Findings from evaluations of laboratory programs highlight the importance of accreditation and its contribution towards a resilient healthcare system and quality improvement [23, 24]. The findings from this report are important for countries to learn from and to encourage the widespread use of the AfSBT SWAP standards across the respective NBTS on the African continent. It is important to highlight that the analysis conducted focuses on select priority standards and does reflect an in-depth review of all standards assessed by the AfSBT SWAP [9, 16].

The primary goal of an NBTS is to ascertain that there is a national supply of safe and appropriate blood products for the population in need at the right time and place [25, 26]. To ensure safe blood requires the NBTS to have a well-delineated system that prioritizes quality from vein to vein. To safeguard this process, a quality system which identifies a set of standards that the organization commits to achieving, is required [27]. There are two key elements to any quality system, which are technical standards (which define what needs to be achieved) and quality standards (that help determine how technical standards are met) [27, 28]. Technical standards (what needs to be achieved, e.g., NBTS organogram) are easier to achieve with multiple international and regional guidelines available, however, quality standards (e.g., minimum equipment requirements) require much more rigorous support and investments [27, 29, 30]. Among the AfSBT SWAP Standards, the quality system section consists of 12/68 standards and 177/466 required criteria, respectively. For this study, we assessed 134/177 required criteria within the quality section, based on WHO recommendations [9]. The quality standards help ensure that the NBTS provides safe and infection-free blood per international standards. Notably, the average baseline score for the quality system section of the AfSBT SWAP standards was only 66.7%, suggesting considerable room for the continuous quality improvement and need for countries to prioritize and invest in strengthening NBTS quality systems.

Behavioural screening of blood donors and pre-transfusion testing are some of the most important tasks undertaken by an NBTS. Behavioural screening includes the recruitment of low-risk donors and repeat donors, while screening out high-risk donors who are subsequently deferred [31, 32]. In 2016, the percentage of blood donations that were screened and found to be HIV positive in seven sub-Saharan African countries remained higher than the WHO target of <1% [3, 33, 34]. The WHO Global Database for Blood Safety (GDBS) 2016 report stated that in high-income countries, 99.6% of the donations were screened following basic quality-assured procedures, compared to 97% in upper middle-income countries, 81% in lower middle-income countries and only 66% in low-income countries [6]. The overall average score of 66.7% for the testing section of the AfSBT SWAP Standards supports GDBS findings underscoring the need to improve NBTS testing practices. These findings are significant given that prevalence of blood-borne diseases such as HIV, hepatitis B and C are highest in LMICs [34–36]. If infectious disease such as HIV, hepatitis, tuberculosis and malaria transmission in healthcare settings are to be successfully prevented, increased investments are needed to improve testing processes in NBTS and laboratories. The testing process can be broken down into three phases: the preanalytical, analytical and postanalytical phases [27]. Improvement in all three of these phases requires investments in training, equipment, systems, internal proficiency testing and appropriate external quality assurance schemes [37]. The Maputo Declaration calls for collaboration and coordination between host governments, donors and partners to ensure resilient and sustainable laboratory systems in LMICs, and such initiatives are also applicable to transfusion systems [38].

Within a transfusion system, the proper management of blood products and inventory of blood ensure that products are available as needed, and are suitable for blood transfusions for those patients who need it most while avoiding wastage of a valuable resource. Traceability from blood donor to recipient is of utmost importance for blood components. Findings from this assessment show that one of the most important aspects of the NBTS to strengthen is that of the receipt, ordering, selection, and issuing of blood and blood components (15%). The availability of well-structured systems and processes for the management and control of blood products produced and distributed by a transfusion centre is critical. Several studies and policy documents emphasize the need for inventory management systems that can track blood from the donor to that of the recipient [12, 27, 37, 39, 40]. Haemovigilance aims to detect adverse events associated with blood products, both with donors and recipients [17, 25, 41]. The findings from the baseline assessment further emphasize the lack of adequate haemovigilance and clinical interface (47.5%) and blood administration (30%). A similar finding was reported in 2013, which stated that 'only 13 out of 46 African countries had a national hemovigilance system' [36]. A successful and effective haemovigilance system requires reporting of set indicators, implementation, and the regular monitoring and evaluation of these systems [25, 42]. However, for these systems to be effective, the literature and best practices suggest the use of electronic blood safety systems that are connected to the hospitals and health centres that request and use blood [43].

This evaluation had several limitations. First, due to the length and complexity of the AfSBT SWAP Standards, we were not able to compare each individual criteria across the 20 different assessments. Second, these baseline assessments were undertaken at different points in time, at different sites and in different countries across SSA and, therefore, findings cannot be generalized to all countries. Third, the findings are from the initial baseline assessments conducted between 2016 and 2018. Since then, follow-up assessments have been conducted and several countries have progressed to achieve Step 1 or 2 certification or full accreditation at Step 3 [13]. More countries have joined the AfSBT accreditation process since the engagement of the 10 initial countries. As such, we cannot conclusively use these findings to represent transfusion services across the African continent. Finally, all assessments were not conducted by the same assessment team as such it is possible that there might have been some degree of assessor bias that could have resulted in some baseline assessments receiving higher evaluations versus others.

In conclusion, while the AfSBT SWAP provides countries with low resources a pathway and opportunity to accomplish international accreditation much needs to be done at the NBTS to achieve all the requirements for standards and accreditation. This system acknowledges the variability of SSA blood services, healthcare systems and resources, making allowance for different levels to be considered for certification and accreditation. Organizations such as WHO, PEPFAR and the Global Fund to Fight AIDS, Tuberculosis and Malaria have provided support and technical assistance to SSA countries in an effort to improve transfusion services. However, consideration for further investments may be instrumental to improving key areas such as quality systems, testing, inventory management and haemovigilance to ensure the provision of safe and adequate blood transfusion services. Accreditation of systems provides crucial evidence to users or purchasers of a service that appropriate standards are in place. The need for all NBTS in SSA to meet international standards is one which has been emphasized by WHO since the 1990s. However, the findings from the baseline assessments show that few blood banks, or NBTS, initially met the basic requirements necessary to move to the next step of the accreditation process without first making significant improvements and investments.

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CONFLICT OF INTEREST

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the funding agencies. The authors declare no conflict of interests.

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REFERENCES

1. Van HM, Th C, Sibinga S, Postma MJ. Health economics of blood transfusion safety – focus on sub-Saharan Africa. Biologicals. 2010; 38:53–8.
2. AfSBT. AfSBT-Step-Wise-Accreditation Overview. Africa Soc Blood Transfus; 2018:1. https://afsbt.org/accreditation-overview/. Accessed April 11, 2019.
3. Kanagasabai U, Chevalier MS, Drammeh B, Mili FD, Qualls ML. Trends and gaps in National Blood Transfusion Services - 14 Sub-Saharan African Countries, 2014-2016. Morb Mortal Wkly Rep. 2018;67:1392–6.
4. UNDP. The next frontier: human development and the Anthropocene; 2020.
5. UNDP. Human development reports. United Nations Development Programme. n.d. http://hdr.undp.org/en/countries/profiles/LBR (2015).
6. World Health Organisation. Global status report on blood safety and availability. Geneva: World Health Organization; 2016.
7. M'baya B. Development of a stepwise accreditation programme from the perspective of the Africa Society for Blood Transfusion. ISBT Sci Ser. 2014;9:68–71.
8. WHO. Blood Transfusion Safety. Univers Access to Safe Blood Transfus 2021. https://www.who.int/bloodsafety/universalbts/en/. Accessed 14 May 2021.
9. World Health Organization. Aide-memoire for national health programmes: blood safety. Geneva: WHO; 2012.
10. Tapko J-B, Tagny CT. The AfSBT Step-wise Accreditation program: improving the quality in the African blood services. Transfus Clin Biol. 2021;28:146–7.
11. WHO. Blood safety and availability. https://www.who.int/news-room/fact-sheets/detail/blood-safety-and-availability (2020). Accessed 8 Aug 2021.
12. WHO Action framework to advance universal access to safe, effective and quality-assured blood products 2020–2023. 2020.
13. Africa Society for Blood Transfusion. https://afsbt.org/ (n.d.). Accessed 15 May 2019.
14. World Health Organization. The clinical use of blood. Geneva: World Health Organization; 2003.
15. Haller DM, Meynard A, Pejic D, Sredic A, Huseinagic S, Courvoisier DS, et al. YFHS-WHO+ Questionnaire: validation of a measure of youth-friendly primary care services. J Adolesc Health. 2012;51:422–30.
16. AfSBT. Accreditation overview. https://afsbt.org/accreditation-overview/ (2018). Accessed 11 Apr 2019.
17. Weimer A, Tagny CT, Tapko JB, Gouws C, Tobian AAR, Ness PM, et al. Blood transfusion safety in sub-Saharan Africa: a literature review of changes and challenges in the 21st century. Transfusion. 2019;59:412–27.
18. Mpemba F, Kampo S, Zhang X. Towards 2015: post-partum haemorrhage in sub-Saharan Africa still on the rise. J Clin Nurs. 2015;23:774–83.
19. Owusu-Ofori A, Gadzo D, Bates I. Transfusion-transmitted malaria: donor prevalence of parasitaemia and a survey of healthcare workers knowledge and practices in a district hospital in Ghana. Malar J. 2016;15:1–7.
20. Bates I, Chapotera GK, Mckew S, Van Den BN. Maternal mortality in sub-Saharan Africa: the contribution of ineffective blood transfusion services. BJOG. 2008;115:1331–9.
21. Kralievits KE, Raykar NP, Greenberg SLM, Meara JG. The global blood supply: a literature review. Lancet (London, England). 2015; 385 Suppl 2:S28.
22. Abimiku A, Timperi R, Blattner W. Laboratory innovation towards quality program sustainability. Curr HIV/AIDS Rep. 2016;13:202–8.
23. Nkengasong JN, Yao K, Onyebujoh P. Laboratory medicine in low-income and middle-income countries: progress and challenges. Lancet. 2018;391:1873–5.
24. Bedford J, Farrar J, Ihekweazu C, Kang G, Koopmans M, Nkengasong J. A new twenty-first century science for effective epidemic response. Nature. 2019;575:130–6.
25. Ayob Y. Hemovigilance in developing countries. Biologicals. 2010; 38:91–6.
26. Blood Safety. Promoting safe and sustainable national blood systems. 2005.
27. Flanagan P. Aiming for quality and accreditation in the hospital blood bank. ISBT Sci Ser. 2017;12:46–50.
28. Armstrong VA. Quality assurance in blood banking: the basis for safety. ISBT Sci Ser. 2009;4:281–5.
29. Ifland L, Bloch EM, Pitman JP. Funding blood safety in the 21st century. Transfusion. 2018;58:105–12.
30. WHO. Guidelines for Establishment of Accreditation of Health Laboratories on Establishment of Accreditation of Health Laboratories. Geneva, Switzerland; 2007.
31. Bates I, Manyasi G, Lara AM. Reducing replacement donors in Sub-Saharan Africa: challenges and affordability. Transfus Med. 2007;17: 434–42.
32. Wakaria EN, Rombo CO, Oduor M, Kambale SM, Tilock K, Kimani D, et al. African journal of laboratory medicine. Afr J Lab Med. 2012; 6:7.
33. Chevalier MS, Kuehnert M, Basavaraju SV, Bjork A, Pitman JP. Progress toward strenthening National Blood Transfusion Services - 14 countries, 2011-2014. Morb Mortal Wkly Rep. 2016;12: 2011–4.
34. Tagny CT, Kouao MD, Touré H, Gargouri J, Fazul AS, Ouattara S, et al. Transfusion safety in francophone African countries: an analysis of strategies for the medical selection of blood donors. Transfusion. 2012;52:134–43.
35. Kaur P, Basu S. Transfusion-transmitted infections: existing and emerging pathogens. J Postgrad Med. 2005;51:146–51.
36. Tagny CT, Mbanya D, Tapko JB, Lefrère JJ. Blood safety in Sub-Saharan Africa: a multi-factorial problem. Transfusion. 2008;48: 1256–61.
37. Ifland L. Promoting national blood systems in developing countries. Curr Opin Hematol. 2014;21:497–502.
38. Nkengasong JN, Nsubuga P, Nwanyanwu O, Gershy-Damet GM, Roscigno G, Bulterys M, et al. Laboratory systems and services are critical in global health: time to end the neglect? Am J Clin Pathol. 2010;134:368–73.
39. World Health Organization Blood transfusion policy. Blood Transfus Saf. http://www.who.int/bloodsafety/transfusion_services/nat_blood_pol/en/ (2018). Accessed 16 Jan 2018.
40. Marwaha N. Whole blood and component use in resource poor settings. Biologicals. 2010;38:68–71.
41. Jain A, Kaur R. Hemovigilance and blood safety. Asian J Transfus Sci. 2012;6:137–8.
42. Benjamin RJ. Donor hemovigilance: safety as the first priority of blood donor management. USA. ISBT Sci Ser. 2010;5:206–11.
43. Hindawi S. Systems for accreditation in blood transfusion services. ISBT Sci Ser. 2009;4:14–7.

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ORIGINAL ARTICLE

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Breast milk contains red cell isohaemagglutinins: An observational study of 176 mothers

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Abstract

Background and Objectives: Maternal antibodies are transferred to the child, predominantly IgG, via the transplacental route, and mostly IgA through breast milk. Cases reported by us and others have shown the transfer of red cell allo-antibodies through breast milk. This study was conducted to assess the presence of isohaemagglutinins in breast milk, the range of titres, and the correlation between breast milk and maternal plasma titres.

Materials and Methods: A total of 176 mothers were recruited in this study. Breast milk was collected after sufficient feeding was established and within 2–5 days of delivery in a sterile container without any anticoagulant. Antibody screen, identification and titres were performed on maternal plasma as well as breast milk.

Results: Anti-A and anti-B in breast milk corresponding to their respective maternal blood groups were found in all the samples. This study has shown titres in the breast milk of anti-A and anti-B ranging from 2 to 1024 in both saline and Coombs phases. There was no association between plasma and breast milk titres, thus making it impossible to predict which mother may potentially transfer a larger amount of these haemagglutinins. Isotypes of anti-A and anti-B were evaluated in both plasma and breast milk of 11 samples, which showed predominantly IgG in 7 (63.63%) and predominantly IgA in 4 (36.36%) samples.

Conclusion: Our study demonstrates the presence of a wide range of titres for IgG antibodies of the ABO blood group system in breast milk. The clinical impact of this finding needs to be studied further, as it assumes great relevance in developing countries where anaemia continues to challenge young infants.

KEYWORDS
breast milk, HDFN, IgG antibodies, infantile anaemia, isohaemagglutinins

Highlights

- Anti-A, anti-B and allo-antibodies to red cell antigens are consistently present in breast milk.
- The isotypes of antibodies include IgG, IgM and IgA.
- No correlation was noted between titre of antibodies in maternal serum and those identified in breast milk.

Snehil Kumar and Jess Elizabeth Rasalam contributed equally to the study.

INTRODUCTION

Maternal antibodies are transferred to child, predominantly IgG via the transplacental route and mostly IgA through breast milk. These antibodies act as a source of humoral immunity to the developing immune response in the foetus and neonates. Emerging literature in murine models and humans has shown that red cell antibodies also can be transferred through breast milk [1–5]. We have reported two cases of red cell allo-antibodies (anti-KELL1 and anti-D) being transferred through breast milk. We had hypothesized that this transfer was through breast milk based on the history of exclusive breastfeeding in these children and identification of the antibodies implicated (namely, anti-KELL1 and anti-D) in maternal plasma, breast milk and the baby's plasma in both cases [6].

The above possibility of transfer of red cell antibodies through breast milk is also important in ABO-mismatched mother–baby pairs, as mothers with incompatible natural isohaemagglutinins (anti-A and anti-B) in their plasma could transfer these maternal antibodies through the period of breastfeeding and worsen physiological anaemia. If present as IgG antibodies, and in significant titres, it could impact the newborn and young infant. There is limited data regarding the presence of these naturally occurring isohaemagglutinins in breast milk. This study was therefore conducted to assess their presence and the range of titres (IgG and IgM) and whether any correlation exists between titres of these antibodies in breast milk and titres in maternal plasma in the immediate post-natal period.

MATERIALS AND METHODS

This observational study was performed in a tertiary referral institute, between June 2020 and November 2020, following approval from the institutional review board. We recruited 176 consecutive mothers who delivered in our institute and for whom the babies' blood group was determined as per routine institutional practice. Informed consent was obtained from all the participants. Venous blood samples were collected in 4-ml K2EDTA BD Vacutainer tubes. Plasma was separated by centrifugation at 3000 rpm for 3 min. Breast milk was collected after sufficient feeding was established and within 2–5 days of delivery in a sterile container without any anticoagulant. Fresh, uncentrifuged breast milk was used for testing.

Antibody screen, identification and titres (anti-A, anti-B and any allo-antibody identified) were performed on maternal plasma and breast milk. The saline and Coombs titres in plasma and breast milk for a sample is shown in Figure S1. No extra samples were collected from the newborn for the purpose of this study.

Methodology for testing in mothers

Blood grouping, antibody screen, identification and titres were performed using the column agglutination technique. Forward grouping was done using an anti-A/anti-B/anti-D Ortho Biovue System (Ortho-Clinical Diagnostics, UK) and DiaClon ABD (anti-A, anti-B and anti-DVI-, DiaMed GmbH). Reverse typing was performed using a Reverse Diluent Ortho Biovue System (Ortho-Clinical Diagnostics, UK). Red cell antibody screen and indirect antiglobulin test (IAT) were performed for all the recruited mothers using LISS/Coombs ID card (DiaMed GmbH). Identification of antibody, if present, was performed using 11-cell identification panel (ID-DiaPanel, DiaMed GmbH) in Coombs phase. The titre of an antibody was determined by testing the serial two-fold dilution of the plasma against selected red cells. For the Coombs phase titre, the plasma and milk were treated with dithiothreitol (DTT) (Sigma-Aldrich, USA) for inactivation of IgM antibodies. Rh-D negative cells were used to ensure that the anti-A and anti-B titres were accurate with no interference of existing anti-D. The allo-antibody titres were determined using "O" group cells, homozygous for the corresponding antigens.

The above procedures were performed following the manufacturers' guidelines. Standard operating procedure for blood grouping, antibody screen, identification and titres are provided in Supplementary files.

Eluate

To evaluate the isotypes of the anti-A and anti-B detected in both plasma and breast milk, an experiment was designed. "O" group plasma/breast milk was incubated with either A or B red blood cells (RBCs). After 30 min incubation at 37°C, the direct antiglobulin test (DAT) was performed. If DAT was positive, they were subjected to elution using a Bio-Rad, DiaCidel kit (DiaMed GmbH). Elution was performed as per the manufacturer's instruction. The eluates were tested against the corresponding cells to ascertain anti-A and anti-B specificity; subsequently, both eluates (from plasma and breast milk) were tested for immunoglobulin levels by immunoturbidimetry.

Statistical analysis

Categorical variables were summarized using counts and percentages. Quantitative variables were summarized using mean, standard deviation and medians with ranges. Pearson correlation (r) was estimated to find the correlation between titres of breast milk and plasma.

RESULTS

A total of 176 mothers who delivered during the period between June 2020 and November 2020 were included in this study. The mean age of the enrolled mothers was 26.5 $\pm$ 4.4 years. Maternal blood type was distributed as follows: 3.4% ($n = 6$) as A, 10.2% ($n = 18$) as B and 85.2% ($n = 150$) as O. One mother each had AB and Bombay blood group. Mean gestational age was 271 $\pm$ 10 days and the mean parity was 2.

Titres in plasma and breast milk

The presence of anti-A and anti-B in breast milk corresponding to the respective maternal blood groups was demonstrated in all the

samples. In breast milk, the median titre of anti-B for "A" and "O" blood group mothers was 2 (2–4) and 128 (2–1024) in saline phase and a titre of 2 and 32 (2–1024) in Coombs phase, respectively. Similarly, the median anti-A titres for "B" and "O" blood group mothers were 8 (2–128) and 64 (2–1024) in saline phase and 2 (2–32) and 16 (2–1024) in Coombs phase, respectively. The median titre, its ranges in plasma and breast milk and the distribution of titres in the study population are shown in Figure 1.

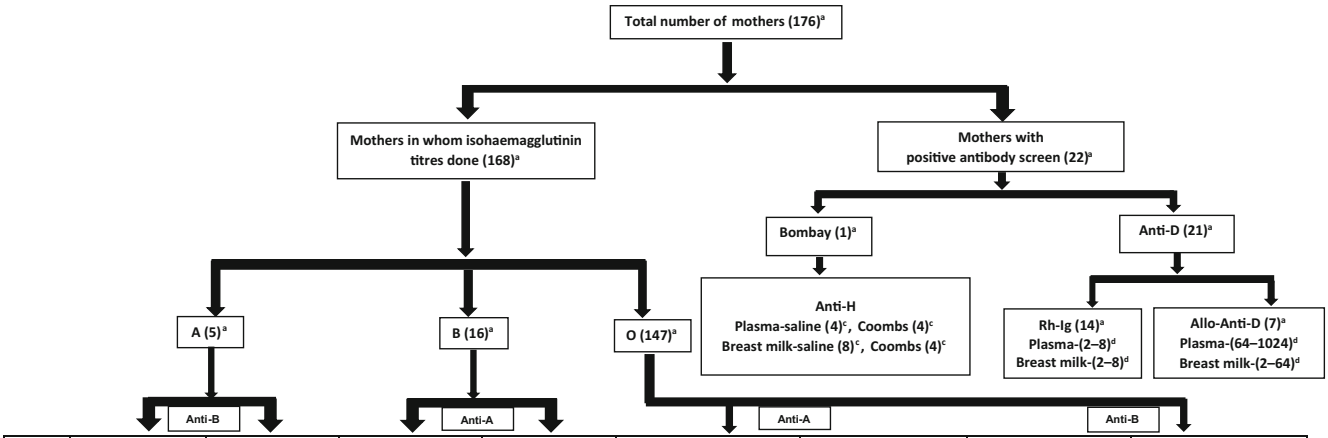
The maternal plasma titres of anti-A and anti-B were categorized as 2–16, 32–64, 128–256 and 512–1024. Ranges of breast milk titres in the respective category are shown in Table S1. A two-fold or higher difference between saline titres for anti-A in plasma and breast milk was seen in 37.4% ($n = 61$) mothers, whereas 21.5% ($n = 35$) mothers showed a similar difference in the Coombs phase with higher titres in breast milk than plasma. A similar comparison for anti-B titres showed 40.1% ($n = 61$) and 22.4% ($n = 34$) mothers in the saline and Coombs phase, respectively.

The relationships between titres of agglutinins in breast milk and those in maternal plasma are shown in Figure 2. In these diagrams, the titre is expressed in units of the number of serial dilutions and not in the actual dilution. A titre of 2 is denoted as 1; a titre of 4 as 2; and so on, and a titre of 1024 as 10. Negligible correlation was observed between the plasma titres and the breast milk titres (r-value of 0.2 for saline anti-A, anti-B as well as Coombs anti-A, anti-B).

Antibody screen was positive in 12% ($n = 21$) of enrolled mothers, excluding the mother with Bombay blood group. The antibodies identified were anti-D in 90.5% ($n = 19$), while anti-D along with anti-C was seen in 9.5% ($n = 2$) of mothers. Among these mothers with anti-D, allo-anti-D was present in 33.3% ($n = 7$) mothers, whereas 66.7% ($n = 14$) had received Rh immunoglobulin. The two mothers with anti-D and anti-C showed only the presence of anti-D in milk. Allo-anti-D titres ranged from 32 to1024 and from 2 to 64 in the plasma and milk of these mothers, respectively. Anti-D due to Rh immunoglobulin showed a titre ranging from 2 to 8 in their breast milk.

Immunoglobulin levels in eluates

Immunoglobulin levels in eluates, obtained following incubation of plasma and breast milk with corresponding red cells specific to the haemagglutinating antibody, were ascertained in a small subset of



Titer	Saline		Coombs		Saline		Coombs		Saline		Coombs		Saline		Coombs	
	Plasma	Breast milk	Plasma	Breast milk	Plasma	Breast milk	Plasma	Breast milk	Plasma	Breast milk	Plasma	Breast milk	Plasma	Breast milk	Plasma	Breast milk
2	1[b]	3	5	5	1	2	16	10	0	3	7	23	1	4	6	19
4	1	2	0	0	0	4	0	2	1	7	19	12	1	7	12	11
8	1	0	0	0	3	3	0	1	13	11	17	28	11	10	12	23
16	1	0	0	0	6	1	0	1	22	17	22	19	23	14	37	17
32	0	0	0	0	2	3	0	2	43	22	25	22	42	12	23	23
64	1	0	0	0	3	2	0	0	37	25	27	16	33	26	25	16
128	0	0	0	0	1	1	0	0	20	26	19	14	23	23	12	14
256	0	0	0	0	0	0	0	0	8	13	7	5	11	18	12	8
512	0	0	0	0	0	0	0	0	3	11	4	7	2	14	6	6
1024	0	0	0	0	0	0	0	0	0	12	0	1	0	19	2	10
Median	8 (2–64)[e]	2 (2–4)	2 (2)	2 (2)	16 (2–128)	8 (2–128)	2 (2)	2 (2–32)	32 (4–512)	64 (2–1024)	32 (2–512)	16 (2–1024)	32(2–512)	128 (2–1024)	32 (2–1024)	32 (2–1024)

a- Number of mothers are shown in brackets, **b-** The numbers show the number of mothers with the corresponding titre, **c-** Titres of anti-H in saline and Coombs phase. Only one sample was present for Bombay blood group, **d-** Range of titres in breast milk in Coombs phase, **e-** Range of isohaemagglutinin titres in plasma and breast milk in different blood groups and different phases.

FIGURE 1 Distribution of mothers with corresponding haemagglutinin titres in plasma and breast milk. A total of 176 mothers were enrolled in study. Isohaemagglutinin titres were done in 168 mothers, of whom 154 showed negative antibody screen while 22 showed positive antibody screen. Out of 22 mothers, 1 belonged to Bombay blood group, 14 had been administered Rh immunoglobulin and 7 were alloimmunized. Isohaemagglutinin titres were also carried out in mothers administered with Rh immunoglobulin ($n = 14$) using "O"-group RhD-negative cells. Median titre for anti-A in "B" group mothers was higher than anti-B in "A" group mother in breast milk. In the "O" group mothers, these titres were comparable and ranged from 2 to 1024 for anti-A and anti-B in both saline and Coombs phases. [a]Number of mothers are shown in brackets; [b]The numbers show the number of mothers with the corresponding titre, [c]Titres of anti-H in saline and Coombs phase. Only one sample was present for Bombay blood group. [d]Range of titres in breast milk in Coombs phase, [e]Range of isohaemagglutinin titres in plasma and breast milk in different blood groups and different phases

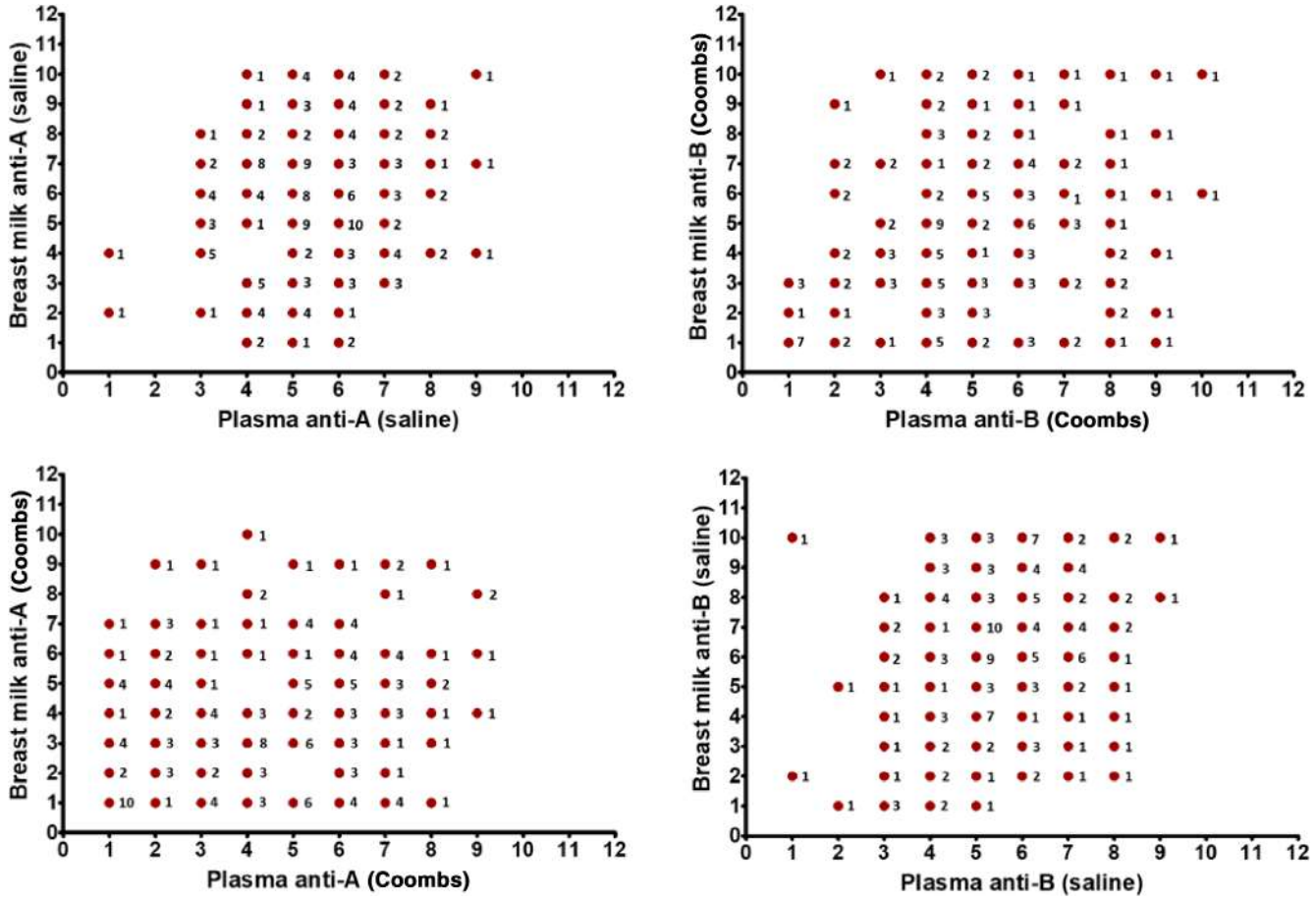


FIGURE 2 Correlation of titres of anti-A and anti-B between plasma and breast milk. The titres are expressed in units of the number of serial dilutions and not in the actual dilution (a titre of 2 is denoted as 1, a titre of 4 as 2, and so on, with a titre of 1024 as 10). Negligible correlation was observed between the plasma titres and the breast milk titres. Breast milk titres for samples with plasma titre range 2–16 were spread across 2–1024. The number with dots represents the number of mothers with corresponding titres

TABLE 1 Levels of immunoglobulins identified in plasma and breast milk eluates with corresponding titres of anti-A and anti-B

Sl. no.	Mother's blood group	Blood group of red cells incubated with breast milk	Plasma titres of corresponding anti-A/anti-B		Breast milk titres of corresponding anti-A/anti-B		Immunoglobulin levels in plasma eluates[a]			Immunoglobulin levels in breast milk eluates		
			Saline	Coombs	Saline	Coombs	IgG	IgM	IgA	IgG	IgM	IgA
1	O	A	128	256	128	32	–	–	–	11	0.8	1.3
2	O	A	32	256	64	32	–	–	–	12	0	2.8
3	O	A	32	32	128	32	4	0	5.2	4	0	5.2
4	O	A	16	32	512	128	3	0	5.5	17	4.7	5.4
5	O	B	64	64	1024	512	–	–	–	12	5.6	1
6	O	B	256	64	128	32	–	–	–	12	0	0.9
7	O	B	16	64	64	32	–	–	–	12	0.5	3.9
8	O	B	32	64	128	128	5	0	7	5	0	5.3
9	O	B	16	64	512	256	4	0	5.9	5	0	9.1
10	O	B	32	32	512	256	5	0	6.5	5	0.7	7.6
11	O	B	32	32	512	1024	13	0	3.3	12	0.8	42.6

[a]The immunoglobulin levels are measured in mg/dl.

samples using the method described previously. Samples from 11 "O" blood group mothers were tested. In six samples, eluates obtained from plasma and breast milk were tested, while for the remaining five, eluates of the breast milk alone was tested. The eluates were added to the corresponding red cells to exclude non-specific reactivity, as shown in Figure *S2*. Four samples were incubated with A cells, and remaining seven with B cells. The IgG levels in the milk eluate ranged from 4 to 17 mg/dl, IgM levels from 0 to 5.6 mg/dl and IgA levels from 0.9 to 42.6 mg/dl. The levels of immunoglobulin isotypes in eluates and the corresponding titres in plasma and breast milk are shown in Table 1.

DISCUSSION

Our study, involving the largest cohort to date, clearly demonstrates the presence of naturally occurring anti-A and anti-B in the breast milk of mothers included in this study. Limited literature, however, exists. Boorman et al. demonstrated the presence of anti-A and anti-B in breast milk; Li et al. and Leonard et al. demonstrated prolonged haemolytic disease of foetus and newborn in a neonate due to passive acquisition of allo-antibodies in the mother's breast milk [2, 3, 7].

Our study has shown titres in breast milk of anti-A and anti-B ranging from 2 to 1024 in both the saline and the Coombs phase. Rh immunoglobulin was administered in 14 mothers and all of them showed the presence of anti-D in their breast milk with a titre ranging from 2 to 8. This phenomenon has not been described previously. Similarly, we detected anti-D in the breast milk of Rh-D negative alloimmunized mothers. The presence of anti-D and it causing prolonged haemolysis in breast-fed neonates have been described in the literature. IgG anti-H with a titre of 4 was demonstrated in the breast milk of the Bombay group mother.

Boorman et al. have observed some correlation between titres of agglutinins in colostrum and milk and between those of maternal serum and colostrum [7]. Our study, however, does not show an association between plasma titres and breast milk titres, thus making it impossible to predict which mother may transfer a larger amount of these haemagglutinins. The process of transfer of IgG from plasma to milk in rodent models has shown that a decrease in plasma IgG occurs concomitantly with the accumulation in milk [8]. Studies in rodents have also suggested that, as in the placenta, neonatal Fc receptor (FcRn) can be involved in IgG transfer across the mammary gland epithelium. Absorption of these secreted antibodies in breast milk during the neonatal period is also mediated by the FcRn on the intestinal brush border that binds ingested IgG antibodies from maternal milk and transports them through the enterocytes to the systemic circulation [9]. FcRn not only mediates the transport of IgG into the lumen but also prevents degradation in a pH-dependent cellular recycling mechanism [10]. It therefore remains a possibility that these natural haemagglutinins can get absorbed and cause ongoing haemolysis in the neonate.

Infancy and early childhood are vulnerable age groups for anaemia throughout the world and is a potentially preventable condition. Preterm infants can experience a decline in haemoglobin more abruptly and more profoundly than in term infants owing to decreased RBC lifespan and a sub-optimal erythropoietin response [11]. Similarly, it is estimated that 19.44% of the Indian neonates are anaemic [12]. While the cause for infancy and neonatal anaemia can be multifactorial, transfer of natural isohaemagglutinins can significantly exacerbate an existing physiological anaemia or be the primary cause for ongoing haemolysis. In a previous study from our institute (unpublished data), among 575 "O" group mothers, prevalence of ABO-incompatible pregnancies was found to be 35.3% ($n = 203$). In the present study, among 147 "O" group mothers with a negative antibody screen, 38.1% ($n = 56$) had ABO-mismatched babies. Although Boorman et al. found that group A babies of group O mothers in some of whom the colostrum titres were 512–4000 showed no sign of jaundice or anaemia, there remains a theoretical possibility for continued haemolysis in these babies with breastfeeding.

The novel experiment designed to determine the subtype of immunoglobulins of anti-A and anti-B present showed a fascinating range of values. Analysis of the breast milk eluate in 11 patients showed predominantly IgG in 63.6% ($n = 7$) and predominantly IgA in 36.4% ($n = 4$). Except for two samples (samples 4 and 11), the immunoglobulin subtypes between plasma and breast milk were comparable. A study by Hauschner et al. in immune thrombocytopenia (ITP) patients diagnosed during pregnancy showed the transfer of clinically significant amounts of anti-platelet IgA antibodies in human breast milk [13]. A murine model by Santhanakrishnan et al. has also shown anti-RBC IgA antibody in breast milk [1]. However, this study showed IgG as the predominant antibody secreted in all the mothers, with only a few mothers showing IgA predominance. IgM levels remained undetectable for most of samples tested, possibly due to the 37°C incubation which would be unfavourable for the adsorption of IgM on red cell surface.

Our study was limited by lack of follow-up of the neonates to evaluate for worsening of anaemia/ongoing haemolysis/prolonged anaemia after the initiation of exclusive breastfeeding. Neonates were not checked for the presence of isohaemagglutinins after a period of 1 month, which could have detected the transfer through breast milk. Serial monitoring of titres in the breast milk along with titres in the neonates' plasma was not performed, which would have given corroborative evidence to the transfer of the isohaemagglutinins through milk. All these were not possible because of the ongoing pandemic.

In conclusion, antibodies of the ABO blood group system appear to be consistently present in breast milk. Our study demonstrates the presence of a wide range of titres for IgG antibodies of the ABO blood group system in breast milk. The clinical impact of this finding needs to be studied further, as it assumes great relevance in developing countries where anaemia continues to challenge young infants.

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D.D., L.J., A.R., M.M.B., J.M., M.K., S.S. and A.A. designed the research study; D.D., S.K., J.E.R. and V.T.D. acquired and analysed the

data; D.D. and A.P. provided laboratory support; H.Y., A.R., M.M.B., J.M., M.K., S.S. and A.A. provided clinical support.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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

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REFERENCES

1. Santhanakrishnan M, Tormey CA, Natarajan P, Liu J, Hendrickson JE. Clinically significant anti-KEL RBC alloantibodies are transferred by breast milk in a murine model. Vox Sang. 2016;111:79–87.
2. Li M, Blaustein JC. Persistent hemolytic disease of the fetus and newborn (HDFN) associated with passive acquisition of anti-D in maternal breast milk. Transfusion. 2017;57:2121–4.
3. Leonard A, Hittson Boal L, Pary P, Mo YD, Jacquot C, Luban NL, et al. Identification of red blood cell antibodies in maternal breast milk implicated in prolonged hemolytic disease of the fetus and newborn. Transfusion. 2019;59:1183–9.
4. DeMoss P, Asfour M, Hersey K. Anti-K1 (Kell) antibody expressed in maternal breastmilk: a case report of a neonate with multiple intrauterine transfusions and postnatal exposure to Kell antibody in maternal breastmilk. Case Rep Pediatr. 2017;2017:6927813–4.
5. Kaya Z, Yasar Y, Goral S, Tokgöz A, Muluk C, Sever T. Hemolysis in two newborns due to isohemagglutinins passively transferred from maternal breast Milk. Indian J Hematol Blood Transfus. 2020;36:580–1.
6. Rasalam JE, Kumar S, Amalraj P, Bal HS, Mathai J, Kumar M, et al. Do red cell alloantibodies continue to challenge breast fed babies? Transfus Med. 2020;30:281–6.
7. Boorman KE, Dodd BE, Gunther M. A consideration of colostrum and milk as sources of antibodies which may be transferred to the newborn baby. Arch Dis Child. 1958;33:24–9.
8. Israel EJ, Taylor S, Wu Z, Mizoguchi E, Blumberg RS, Bhan A, et al. Expression of the neonatal fc receptor, FcRn, on human intestinal epithelial cells. Immunology. 1997;92:69–74.
9. Pyzik M, Sand KMK, Hubbard JJ, Andersen JT, Sandlie I, Blumberg RS. The neonatal fc receptor (FcRn): a misnomer? Front Immunol. 2019;10:1540.
10. Patel DD, Bussel JB. Neonatal fc receptor in human immunity: function and role in therapeutic intervention. J Allergy Clin Immunol. 2020;146:467–78.
11. Kett JC. Anemia in infancy. Pediatr Rev. 2012;33:186–7.
12. Sareen A, Singh S, Mahajan K. Maternal anemia and its effect on cord hemoglobin. Ind Med Gaz. 2013;161–3.
13. Hauschner H, Rosenberg N, Seligsohn U, Mendelsohn R, Simmonds A, Shiff Y, et al. Persistent neonatal thrombocytopenia can be caused by IgA antiplatelet antibodies in breast milk of immune thrombocytopenic mothers. Blood. 2015;126:661–4.

SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Efficacy and safety of a reduced dose of plerixafor in combination with granulocyte colony-stimulating factor in healthy haploidentical stem cell donors

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Abstract

Background and Objectives: Implementation of the technique of immunomagnetic selection requires the procurement of a large number of CD34+ cells from haploidentical donors within a single apheresis procedure. The release of stem cells with granulocyte colony-stimulating factor (G-CSF) alone is unsatisfactory in a number of donors, and plerixafor, a CXCR4 chemokine receptor antagonist, could be used as an additional mobilization agent. The aim of our study was to examine whether a lower dose of plerixafor (0.12 mg/kg) can provide sufficient increase in CD34+ cells in the peripheral blood of allogeneic healthy donors in comparison with a historical control group. In addition, we assessed the risk of inability to provide the recipient with a transplant containing the optimal dose of 8–10 $\times$ 10^6 CD34+ cells/kg body weight of the recipient.

Materials and Methods: In a prospective, single-arm study, we examined the results of 105 mobilizations in healthy adult haploidentical donors with G-CSF and plerixafor at a dose of 0.12 mg/kg. The historical control group consisted of 106 mobilizations with G-CSF and plerixafor at 0.24 mg/kg.

Results: The median increase in the number of CD34+ cells from day 4 to day 5 of mobilization was 69 cells/μl (range, 28–240) versus 77 cells/μl (24–217) in the groups of 0.12 and 0.24 mg/kg of plerixafor, respectively (*p*-value 0.255). The apheresis products contained a median of 14.4 $\times$ 10^6/kg recipient body weight CD34+ cells versus 12.9 $\times$ 10^6/kg in the groups that received 0.12 and 0.24 mg/kg of plerixafor, respectively (*p*-value 0.118). The obtained differences were not significant, which means the application of a decreased dose of plerixafor did not affect the results of mobilization.

Conclusion: The obtained differences in collection were not significant, and thus the application of a decreased dose of plerixafor did not affect the results of mobilization.

KEYWORDS
allogeneic healthy donor, apheresis, plerixafor, stem cell mobilization

Highlights

- Haploidentical stem cell transplantation (SCT) with TCRαβ immunomagnetic depletion requires the collection of a large number of CD34+ cells.
- Mobilization with granulocyte colony-stimulating factor (G-CSF) does not always allow the collection of the defined numbers of CD34+ cells.
- Plerixafor in reduced dose in combination with G-CSF shows equal efficacy in healthy adult haploidentical donors.

INTRODUCTION

Allogeneic transplantation of haematopoietic stem cells (HSCs) from haploidentical donors is essential for the treatment of many malignant and non-malignant diseases as well as rare congenital syndromes, and has shown its effectiveness and safety [1–4]. Granulocyte colony-stimulating factor (G-CSF) is widely used to mobilize CD34+ cells in healthy voluntary donors; however, the release of cells into the peripheral blood (PB) is unsatisfactory in 5%–30% of them [5]. Moreover, it has been shown previously that more than 10^7/kg weight of CD34+ cells are needed for successful engraftment and rapid immune reconstitution after haploidentical stem cell transplantation (SCT) with T-lymphocyte depletion [4, 6].

Plerixafor, a CXCR4 chemokine receptor antagonist, is actively used to mobilize autologous HSCs [7–9]. The results on the safety and efficacy of HSC mobilization using plerixafor have been published in healthy volunteers [10, 11], but its use in healthy stem cells donors is still rarely available. Published studies mainly describe data obtained from small groups of healthy donors and relate to both the results of the use of plerixafor as a 'rescue therapy' in the case of a failed first apheresis [12, 13] and its effect on the results of mobilization and apheresis [14, 15]. Also, there is very little data on the use of plerixafor as a single stem cell mobilizing agent [14, 16].

Most of the published data concerning the effect of plerixafor in healthy donors relate to monotherapy mobilization (subcutaneous or intravenous administration); data on the combined use of G-CSF and plerixafor are so far insufficient. Lemery et al. investigated the effect of increasing plerixafor doses (two doses to each subject in increments of 0.08 mg/kg) but did not find statistically significant differences in the medians of peak CD34+ values in PB. Moreover, the fact that in 5 out of 18 donors the maximum CD34+ value was recorded after a lower dose of plerixafor suggests that for smaller dose increments, intra-subject variability in CD34+ counts following plerixafor administration may make interpretation of the dose–response difficult [15]. Pantin et al. demonstrated that the peak values of circulating CD34+ cells were significantly higher after administration of 0.48 mg/kg of plerixafor in 16 out of 20 healthy donors [14]. According to a number of studies, mobilization with G-CSF (10 μg/kg) is more effective than with plerixafor (0.24 mg/kg) as a single agent [15, 17].

In our centre, since 2012, the main platform for the preparation of the haploidentical graft has been the immunomagnetic depletion of TCRαβ/CD19+. This process not only results in partial loss of cells during processing but also limits the cellular composition of the apheresis product [18]. The standard TCRαβ/CD19+ immunomagnetic depletion protocol has been designed to process no more than 24×10^9 TCRαβ+ cells among no more than 60×10^9 total nuclear cells (TNCs), so that the concentration of CD34+ cells in the apheresis product should be as high as possible. Low concentration of CD34+ cells among high numbers of TNCs, collected over several apheresis sessions, will lead to an increase in residual TCRαβ cells in the final depletion product with the risk of fatal acute graft versus host disease (GVHD) and unjustified increase in the cost of the procedure. The difficulty in obtaining an adequate number of cells may be associated not only with poor donor mobilization but also with the weight disparity between the donor and recipient [19, 20]. Unlike the transplantation of untreated HSCs, where the required dose of CD34+ cells can be obtained in several sessions of leukapheresis, the use of T-cell depletion techniques implies obtaining a quality product by a single apheresis, which is due to technical limitation of the immunomagnetic purging technology. Therefore, the use of plerixafor is justified not only in 'poor mobilizing' donors but also in cases of weight disproportion between the donor and recipient.

There are several studies evaluating the effectiveness of low doses of plerixafor [7, 21, 22]. Hubel et al. demonstrated a sufficient increase in the level of CD34+ cells after a single subcutaneous injection of plerixafor (80 mg/kg) in healthy volunteers [23]. Boulad et al. in their dose-escalating study were able to show the efficacy of low-dose plerixafor for CD34+ cell mobilization in patients with inherited bone marrow diseases [24].

Unlike patients with inherited diseases and lymphoproliferative cancers, allogeneic donors are not pre-treated. We hypothesized that 50% of the standard dose of plerixafor recommended for HSC mobilization in combination with G-CSF would be sufficient to achieve the target level of cells.

In the current study, we aimed to provide evidence that reducing the dose of plerixafor by half (up to 0.12 mg/kg) in healthy related haploidentical donors, in whom adequate mobilization of CD34+ cells with G-CSF alone could not be effected, will not lead to a significant decline in the release of CD34+ cells in comparison with a similar group of healthy donors who had received the standard dose of 0.24 mg/kg (historical control). In addition, we assessed the risk of inability to provide the recipient with a transplant containing the optimal dose of $8–10 \times 10^6$ CD34+ cells/kg body weight of the recipient.

MATERIALS AND METHODS

A comparative analysis of the results of mobilization and peripheral blood stem cell (PBSC) apheresis with the use of plerixafor and G-CSF in healthy adult donors was carried out for the period from July 2012 to March 2021. In total, 211 PBSC mobilizations from healthy, adult, haploidentical, related donors were performed with the use of a combination of plerixafor and G-CSF. All collected products were used for haploidentical transplantation for paediatric patients (age, 0.5–17 years) with different malignant and non-malignant diseases according to treatment protocols.

From March 2018, after the interim analysis of a pilot group results (first 10 donors with reduced dose of plerixafor), all haploidentical adult donors (parents), with the indication for additional stimulation, received plerixafor at a dose of 0.12 mg/kg of body weight. The group that received the standard dose of plerixafor (0.24 mg/kg) was used as a historical comparison group. The use of the combined PBSC mobilization protocol with reduced dose of plerixafor in healthy haploidentical donors was approved by the institutional expert board and local ethics committee. All donors of stem cells signed informed consent to participate in the study.

The main characteristics of donors who were mobilized with the combination of G-CSF and plerixafor are presented in Table 1.

Stem cell mobilization

Standard mobilization was performed with G-CSF (Leucostim, ZAO Biocad, Russia; Zarsio, Ai Di Ti Biologika GMBH, Germany) at a dose of 10 μg/kg/day for five consistent days, administered subcutaneously in the morning. The last dose of G-CSF was administered 2–4 h before the start of apheresis. The control of CD34+ cells in PB was carried out twice—on the 4th day from the start of G-CSF stimulation and immediately before the onset of apheresis (on the 5th day from the start of G-CSF stimulation). Indications for additional stimulation with plerixafor were determined based on CD34+ cells enumeration on day 4 of the mobilization and considering the recipient's body weight. The main indication for the addition of plerixafor was less than 20 CD34+ cells/μl in the donor's PB on the 4th day (72–76 h) of mobilization with G-CSF. Also, plerixafor was added when the predicted collection of CD34+ cells in the apheresis product might have been less than 8×10^6/kg body weight of the recipient (due to the large body weight of the recipient). For a more adequate comparison, donors were divided into subgroups, considering the differences in the number of CD34+ cells in the PB before the administration of plerixafor, as well as the body weight of recipients (Table 2).

Plerixafor (Mozobil, SanofiGenzyme, UK) was administered subcutaneously, 9–10 h before the start of apheresis. Initially, plerixafor was used at a standard dose of 0.24 mg/kg of the donor's body weight. From March 2018, the dose was reduced to 0.12 mg/kg of body weight.

All adverse reactions, attributed to mobilization, were recorded daily, graded and summarized according to National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.

TABLE 1 Main donor characteristics

Total number	$n = 211$
Gender (male/female)	58/153
Age, years (median; range)	36 (18–55)
Donor body weight, kg (median; range)	66 (40–124)
TBV, ml (median; range)	4075 (2911–7121)
Recipient body weight, kg (median; range)	40.5 (6.3–128)

Abbreviation: TBV, total blood volume.

Stem cell apheresis

Leukapheresis was performed using a mononuclear collection programme (MNC collection protocol) with a Spectra Optia cell separator (Terumo BCT Inc, Lakewood, CO; software versions 6, 9 and 11). Acid citrate dextrose formula A (ACD-A, Haemonetics, Haemonetics Corp., Boston, MA) was used as an anticoagulant. Apheresis was planned on the 5th day from the beginning of mobilization of the CD34+ cells in the PB.

To assess the efficiency of CD34+ cell collection, the CE2 index was used, as calculated by the previously described formula:

CE2 (%) = (number of CD34+ cells [$\times 10^6$] in the apheresis product/(number of CD34+ cells before apheresis $\times$ processed blood volume) $\times$ 100 [11].

We tried to collect no less than 8×10^6 CD34+ cells/kg of the recipient body weight among ≤60×10^9 total nucleated cells.

CD34+ cell enumeration

To determine the number of CD34+ cells in the PB and in the apheresis product, we adopted the ISHAGE (International Society of Hematotherapy and Graft Engineering) research protocol [20]. Briefly, the absolute number of CD34+ cells was calculated using a two-platform method: multiplying the percentage of stem cells and the absolute number of leukocytes obtained by flow cytometry (BD FACS Canto TM II, Becton-Dickinson, Franklin Lakes, NJ) and a haematology cell counter (Sysmex Corporation XS800i, SYSMEX, Japan), respectively. The number of HSCs was determined by the expression of membrane markers (CD34 and CD45) in a direct immunofluorescence reaction with monoclonal antibodies labelled with phycoerythrin and fluorescein isothiocyanate (Becton-Dickinson, Franklin Lakes, NJ). Staining with primary labelled monoclonal antibody was performed according to the manufacturer's instructions. After incubation, the erythrocyte lysing solution (FACS PharmLyse, BD, USA) was added to the suspension and then washed with phosphate-buffered saline (Cell Wash, BD, USA).

Statistical analysis

The primary endpoint was to assess the delta (difference) in the number of CD34+ cells/μl in the PB on days 4 and 5 of mobilization with

plerixafor at a dose of 0.12 mg/kg. Secondary endpoints were as follows:

1. To assess the difference in the number of CD34+ cells on days 4 and 5 of mobilization with plerixafor at a dose of 0.12 mg/kg as compared to the historical control group (0.24 mg/kg);
2. To evaluate the risk of collecting less than 8×10^6 CD34+ cells/kg of recipient body weight in a single apheresis;
3. To assess the frequency and severity of side effects after the administration of plerixafor in two different dosages.

To describe the results, medians and first and third quartiles were chosen. The comparison of the obtained results of the CD34+ cells mobilization obtained in two main groups of donors, treated with different doses of plerixafor, was carried out using the Mann–Whitney *U*-test through the construction of boxplots to display the difference between cell mobilization and collection in the comparative groups. The Kruskal–Wallis test was used to compare the effects of plerixafor in four subgroups depending on the intermediate level of mobilization (day 4). The Fisher exact test in the contingency tables was used to assess the effect of plerixafor dosage on the incidence of adverse reactions. All statistical analyses were conducted with the XLStat 2021.1.1 software (Addinsift, Paris, France), and a *p*-value of <0.05 was considered statistically significant.

TABLE 2 Donor subgroups

Subgroup	CD34+ cells on day 4 (cells/μl)	Recipient body weight, kg
A	0–10	–
B	11–20	–
C	21–30	≥30
D	31–45	≥50

RESULTS

During the study period, plerixafor was administered at a dose of 0.24 mg/kg to 106 donors and at 0.12 mg/kg to 105 donors. Of the total number, 157 donors (74%) can be attributed to the poor mobilizers (subgroups A and B, Table 2). In the other cases, plerixafor was added in order to obtain an optimal quality product for recipients heavier than 30 kg (subgroup C) and 60 kg (subgroup D). All mobilizations with plerixafor in reduced dose led to an increase in the number and concentration of CD34+ cells in the PB, which made it possible to perform a successful apheresis with a mean duration of 219 min (180–282 min). In the study group of donors who received 0.12 mg/kg of plerixafor, the median number of CD34+ cells in the PB by day 5 was 86 cells/μl.

When comparing the two different doses of plerixafor used in the mobilization protocol, no significant differences were found in the main parameters of mobilization and apheresis results (Table 3). The median increase in CD34+ cells after administration of 0.12 mg kg of plerixafor was 69 cells/μl (range, 28–240) versus 77 cells/μl (range, 24–217) in the historical control group (*p*-value 0.255).

When comparing the results in subgroups A, B, C and D, statistically significant differences were revealed. The absolute number of CD34+ cells in PB by day 5, CD34+ cell increase, and the total number of collected CD34+ were expectedly lower in the subgroups of 'poor mobilizers' (subgroups A and B) (all $p < 0.001$, Table 4). However, no statistically significant differences were found within each of the subgroups between the two-dosage regimens of plerixafor (Figure 1a–c).

TABLE 3 Mobilization and apheresis results with plerixafor

	Plerixafor, mg/kg	Median	Q1	Q3	Standard deviation	*p*-value
CD34+ day 4 (cells/μl)	0.12	14.6	9.0	19.7	8.74	0.409
	0.24	12.7	8.2	20,8	8.52	
CD34+ day 5 (cells/μl)	0.12	86	61	107	42.08	0.320
	0.24	92	68	121	37.29	
CD34+ increment (cells/μl)	0.12	69	51	91	36.26	0.255
	0.24	77	56	99	33.11	
CD34+ (%) cells in the apheresis product	0.12	0.75	0.56	0,98	0.31	0.582
	0.24	0.77	0.58	1,0	0.29	
Collected CD34+ cells $\times 10^6$	0.12	525	342	654	251	0.329
	0.24	541	369	698	233	
Collected TNC $\times 10^9$	0.12	65.2	55.3	82,8	23.8	0.365
	0.24	67.1	57.6	84,0	18.9	
Collected CD34+/kg r.b.w. $\times 10^6$	0.12	14.4	10.8	19,5	8.3	0.118
	0.24	12.9	9.8	18	8.8	

Abbreviations: r.b.w., recipient body weight; TNC, total nuclear cells.

TABLE 4 Main results of mobilization and apheresis in subgroups depending on the initial response to G-CSF stimulation (4th day)

	Subgroup	A		B		C		D	
	Plerixafor	0.24 mg/kg	0.12 mg/kg	0.24 mg/kg	0.12 mg/kg	0.24 mg/kg	0.12 mg/kg	0.24 mg/kg	0.12 mg/kg
Donor	$n = 211$	41	33	38	48	21	18	6	6
	Gender F/M	9/32	4/29	12/26	17/31	5/16	6/12	2/4	2/4
	Age, years	34 (18–51)	34 (23–41)	37 (18–55)	36 (26–49)	39 (21–51)	39 (22–50)	39 (22–45)	40 (21–51)
	Weight, kg	64 (46–99)	57 (40–90)	66.5 (44–120)	66.5 (46–114)	79 (60–104)	70.8 (44–124)	72.5 (53–99)	88 (60–124)
CD34+ in PB	%CD34+ day 4	0.03 (0.01–0.07)	0.03 (0.013–0.06)	0.04 (0.03–0.08)	0.05 (0.02–0.09)	0.07 (0.05–0.1)	0.07 (0.04–0.11)	0.1 (0.037–0.12)	0.09 (0.03–0.13)
	%CD34+ day 5	0.16 (0.07–0.3)	0.14 (0.07–0.28)	0.18 (0.08–0.6)	0.19 (0.1–0.46)	0.23 (0.12–0.33)	0.22 (0.13–0.35)	0.26 (0.17–0.46)	0.29 (0.2–0.38)
	CD34+ day 4 (cells/μl)	7 (2–10)	7 (3–10)	14 (11–20)	15 (11–20)	23 (21–30)	25 (21–29)	39 (32–44)	41 (31–45)
	CD34+ day 5 (cells/μl)	75 (29–133)	61 (33–123)	93 (42–227)	91 (42–232)	125 (72–172)	112 (86–163)	142 (121–175)	161 (112–217)
	CD34+ increment (cells/μl)	66 (24–124)	52 (28–114)	76 (32–217)	75 (28–212)	104 (50–150)	86 (62–139)	103 (80–131)	123 (101–169)
Apheresis	Processed TBV	2.1 (1–4.2)	2.1 (1.1–4.2)	2.3 (1.2–3.7)	2.1 (0.8–4.7)	2 (1.4–3.0)	2.1 (1.2–2.9)	2.2 (1.8–2.9)	1.8 (1.4–2.7)
	Apheresis time (min)	240 (116–485)	209 (104–357)	301 (185–486)	236 (100–408)	226 (177–371)	232 (161–420)	297 (216–334)	210 (176–252)
	CE2 (%)	68 (44–120)	66 (36–136)	64 (30–89)	67 (44–131)	67 (40–78)	63 (39–108)	56 (49–79)	58 (53–82)
Product collection	TNC ($\times 10^9$)	62.4 (29–112)	58.1 (26–115.8)	69.5 (42–120)	67 (44.4–140)	79.9 (47.1–106)	83.7 (46–125)	72.2 (62.4–91.4)	77.7 (60.3–98)
	CD34+ ($\times 10^6$)	376 (149–867)	309 (105–777)	563 (219–1308)	538 (228–992)	688 (411–1249)	676 (445–1400)	770 (541–1157)	905 (633–1145)
	CD34+ ($\times 10^6$/kg r.b.w.)	14.5 (5–54)	16.4 (5.6–40.7)	12.6 (6.6–24)	12.6 (5.9–55.3)	12.4 (7.8–26.5)	15.3 (8.6–21.9)	11.9 (6.3–15.2)	15 (11.2–18.8)

Note: Subgroup A: 0–10 cells/μl; B: 11–20 cells/μl; C: 21–30 cells/μl; D: 31–45 cells/μl.
Abbreviations: F/M, female/male; PB, peripheral blood; TBV, total blood volume.

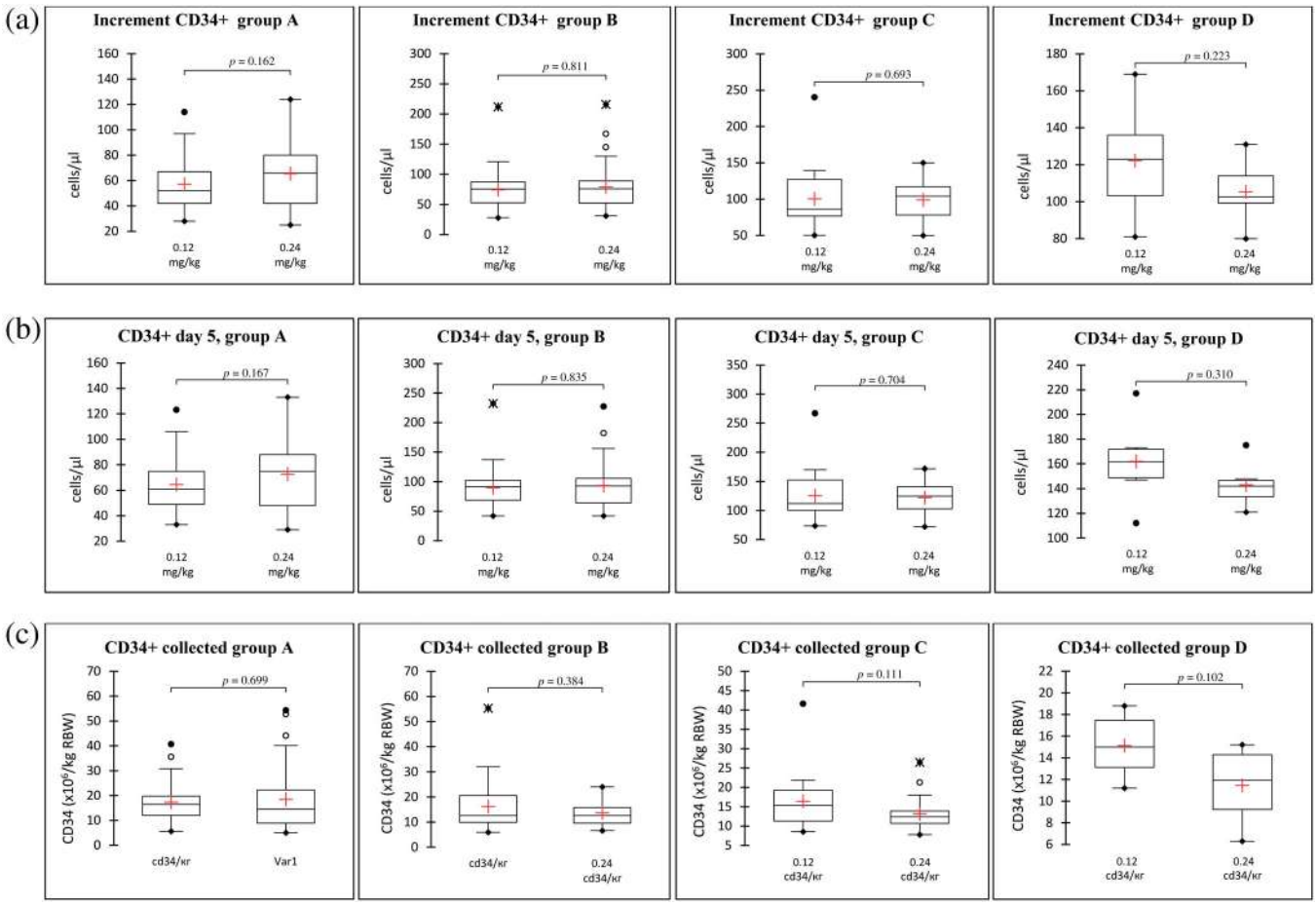

FIGURE 1 Effect of plerixafor depending on the response to granulocyte colony-stimulating factor (G-CSF) by day 4 of stimulation: (A) 0–10 cells/μl; (B) 11–20 cells/μl; (C) 21–30 cells/μl; (D) 31–45 cells/μl

Sub-optimal results of mobilization and apheresis (collection ≤8 CD34+ $\times 10^6$ cells/kg recipient body weight [r.b.w.]) were noted in 16 donors (7.6% of cases), with no significant difference between two plerixafor dosing regimens (0.24 mg/kg, 10 cases; 0.12 mg/kg, 6 cases; *p*-value 0.436). Fifteen donors could be attributed as 'poor mobilizers' (subgroups A and B), with the median dose of collected CD34+ cells was 6.8 $\times$ 10^6 (5.0–7.8 $\times$ 10^6/kg r.b.w.) (Table 5).

Mobilization side effects

G-CSF toxicity was mild and occurred in 125/211 donors (59%). In most cases, it manifested with malaise, muscle, bone and joint pain. Headaches were noted in 50 donors and dyspeptic disorders in 5 cases (nausea, epigastric pain and diarrhoea).

Side effects developed after the addition of plerixafor in 92 donors (43%); 26 of them had no previous toxic effects from G-CSF administration. The most common adverse effects were mild to moderate nausea, headache, bone, joint, muscle pain and diarrhoea (Table 6). Other side effects were much less common, and their severity was mild. The frequency of side effects among donors who received 0.24 mg/kg of plerixafor was significantly higher: 58 cases out of 106 (55%) versus 34 out of 105 (32%) who had received a lower dose of plerixafor (*p*-value 0.001). There was significant decrease in the frequency of nausea (*p*-value 0.041), headache (*p*-value 0.029), paresthesia (*p*-value 0.033) and subcutaneous tissue swelling (*p*-value 0.035) in the group of donors with reduced plerixafor dose. The severity of symptoms was mild to moderate in all cases, and there was no serious complications after plerixafor injection in both dosages. Treatment of all side effects was symptomatic, if necessary. The development of side effects did not affect the possibility of an apheresis procedure.

DISCUSSION

The focus on plerixafor in the context of allogeneic HSC transplantation with graft modification has grown significantly in recent years due to the possibility of obtaining a transplant with different immunological characteristics, a shorter period of donor mobilization, and the need to optimize the dose of transfused CD34+ cells.

Since the protocols for immunomagnetic depletion have their own peculiarities (limit on the number of nuclear cells, TCRαβ+ cells, CD19+ cells; higher doses of CD34+ cells in the primary product),

TABLE 5 Sub-optimal mobilization and collection results

No.	Plerixafor, mg/kg	CD34+ day 4 (cells/μl)	CD34+ day 5 (cells/μl)	Apheresis, min	Processed TBV	CE2	Collected CD34+ $\times 10^6$	Collected CD34+ $\times 10^6$/kg r.b.w.
1	0.24	13	96	407	3.1	36.8	461	7.8
2	0.24	7	41	211	3	73.3	393	6
3	0.24	10	84	316	2.2	57.9	469	7.7
4	0.24	42[a]	141	216	1.8	51	759	6.3
5	0.24	25	121	228	1.9	46.6	547	7.8
6	0.24	11	45	304	2.7	52.9	296	6.6
7	0.24	4[a]	36	329	3.4	62	306	5
8	0.24	16	104	332	2.1	89	1308	7.2
9	0.24	8	44	327	2.8	64.3	324	7.4
10	0.24	2	32	287	3.4	82	267	6.5
11	0.12	19	59	316	2.6	72.5	540	7.1
12	0.12	11	55	272	3.2	55.3	355	5.9
13	0.12	5	68	296	3.6	57.3	538	7.6
14	0.12	14	43	334	2.9	56.3	341	7.4
15	0.12	5	33	211	2.1	49.5	125	5.6
16	0.12	3	39	258	3.1	43.6	185	5.8

[a]Recipient body weight (r.b.w.) ≥119 kg.

TABLE 6 Frequency of side effects after administration of plerixafor

$n = 92$ donors[a]	Plerixafor 0.12 mg/kg ($n = 34$)	Plerixafor 0.24 mg/kg ($n = 58$)	*p*-value
Nausea	6	16	0.041
Headache	3	11	0.029
Paresthesia	2	10	0.033
Tissue swelling	1	8	0.035
Bone and joint pain	6	6	1.0
Diarrhoea	15	25	0.113
Malaise	5	6	1.0
Vomiting	0	4	0.121
Dizziness	0	2	0.498
Hypersalivation	0	3	0.246
Stomach ache	1	5	0.212
Hypertension	1	1	1.0
Flatulence	1	1	1.0

[a]50% of donors had a combination of different side effects.

most centres that actively use this method of graft processing have developed their own protocols of SCT mobilization. For example, Rutella et al. classified donors as 'poor mobilizers' with indications for plerixafor if the number of CD34+ cells in the PB by the 4th day of mobilization did not reach 40 cells/μl and/or the predicted dose of CD34+ cells per recipient weight in the apheresis product was less than 12×10^6 [19]. Similar data were published by Jaiswal et al., according to whose protocol donors with less than 50 cells/μl by the 4th day of mobilization with G-CSF were stratified as 'poor mobilizers' with indications for plerixafor [20]. The possibility of plerixafor dose reduction in the context of the mobilization of autologous HSCs was of interest to some authors. Sanikommu et al. used reduced dose of plerixafor in combination with G-CSF for mobilization of HSC in adult patients with plasma cells disorders [25]. Since the median body weight of these patients was 88 kg (62.4–149.5 kg), it can be easily calculated that the dose of plerixafor varied between 0.19 and 0.08 mg/kg, and the number of collected CD34+ cell did not differ between the groups of patients. Gutiérrez-Aguirre et al. conducted a 'proof-of-concept' study to evaluate the efficacy of reduced dose of plerixafor (0.12 mg/kg) in the mobilization protocol for adult patients with lymphomas and multiple myeloma [22]. The authors showed that a reduced dose of plerixafor was sufficient for effective mobilization in 85% of patients. If we consider that there are no significant differences in the peak CD34+ values when using plerixafor doses below 0.24 mg/kg in heavily pretreated patients with, it is possible to conclude that a lower dose of plerixafor in combination with G-CSF would be sufficient for mobilization for healthy donors as compared with 0.24 mg/kg.

Since March 2018, we began to use a reduced dose of 0.12 mg/kg s.c. in the case of a poor or sub-optimal mobilization in healthy allogeneic haploidentical donors, detected on the 4th day of G-CSF administration, instead of the standard conventional dose of plerixafor (0.24 mg/kg). When comparing the results of mobilization and apheresis obtained after the administration of 0.12 mg/kg of plerixafor with a similar historical control group, treated with plerixafor at a dose of 0.24 mg/kg, no statistically significant differences were detected. Thus, the median multiplicity of CD34+ cells in the PB between days 4 and 5 was 6.1 and 6.6 times in the study and

historical control groups, respectively, which is slightly lower than the 8-fold increase described by other authors [19, 20]. In the apheresis product, with comparable medians of CD34+ cells concentration (0.75% and 0.77%, respectively) and collection efficiency (65% and 66%, respectively), the amounts of collected CD34+ cells in the study group were slightly higher than in historical control, but the difference did not reach statistical significance, which could be explained by insignificant differences in the medians of body weight of the recipients.

When comparing the effect of plerixafor in those subgroups of donors that differed in the level of response to G-CSF stimulation, the 'poor mobilizers' were expectedly characterized by a lower level of CD34+ cells in the PB and CD34+ cell concentration in the apheresis product after the addition of plerixafor. At the same time, there was no dose-dependent differences within each subgroup (all *p*-values > 0.05), which confirms the validity of our hypothesis. The cumulative risk of sub-optimal collection ($<8 \times 10^6$/kg r.b.w.) was relatively low and did not depend on the plerixafor dosage.

The use of plerixafor was quite safe. Side effects that could be attributed to plerixafor were noted in 92 donors (43%). The manifestation of symptoms was mild to moderate in most cases, which is consistent with published data [14–17]. At the same time, the frequency of side effects among donors who received 0.24 mg/kg of plerixafor was significantly higher: 58 cases out of 106 (55%) versus 34 out of 105 (32%) who received the lower dosage of plerixafor (*p*-value 0.001). Statistically significant differences in the frequency of occurrence of side effect have been proven for nausea (*p*-value 0.041), headache (*p*-value 0.029) and paresthesia (*p*-value 0.033). Since about 60% of stem cell donors experience G-CSF toxicity, reducing the likelihood of new additional unpleasant effects being added may be very important.

In conclusion, based on the present study for the combined use of plerixafor in conjunction with G-CSF in a cohort of healthy haploidentical allogeneic donors, the use of lower doses can be recommended. Our data were obtained with paediatric cohort of recipients with the average weight of the donor being higher than that of the recipient. This may be a limitation for the approach to be used in adult transplantation and needs further investigation. The risk of ineffective mobilization and apheresis did not show a significant dependence on the dose of plerixafor, and, despite the absence of differences in the severity of side effects, the toxicity profile of the lower dose of plerixafor was favourable. This approach requires controlled randomized trials to confirm both efficacy and safety.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Laberko A, Sultanova E, Gutovskaya E, Shipitsina I, Shelikhova L, Kurnikova E, et al. Mismatched related vs matched unrelated donors in TCRαβ/CD19-depleted HSCT for primary immunodeficiencies. Blood. 2019;134:1755–63.
2. Locatelli F, Vinti L, Palumbo G, Rossi F, Bertaina A, Mastronuzzi A, et al. Strategies to optimize the outcome of children given T-cell depleted HLA-haploidentical hematopoietic stem cell transplantation. Best Pract Res Clin Haematol. 2011;24:339–49.
3. Maschan M, Shelikhova L, Ilushina M, Shekhovtsova Z, Khismatullina R, Kurnikova E, et al. Outcome of αβ T cell-depleted transplantation in children with high-risk acute myeloid leukemia, grafted in remission. Bone Marrow Transplant. 2020;55:256–9.
4. Martelli M, Reisner Y. Haploidentical 'megadose' $CD34^+$ cell transplants for patients with acute leukemia. Leukemia. 2002;16:404–5.
5. To L, Levesque J, Herbert K. How I treat patients who mobilize hematopoietic stem cells poorly. Blood. 2011;118:4530–40.
6. Martelli M, Aversa F, Bachar-Lustig E, Velardi A, Reich-Zelicher S, Tabilio A, et al. Transplants across human leukocyte antigen barriers. Semin Hematol. 2002;39:48–56.
7. Flomenberg N, Devine S, Dipersio J, Liesveld J, McCarty J, Rowley S, et al. The use of AMD3100 plus G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone. Blood. 2005;106:1867–74.
8. Calandra G, McCarty J, McGuirk J, Tricot G, Crocker S-A, Badel K, et al. AMD3100 plus G-CSF can successfully mobilize CD34+ cells from non-Hodgkin's lymphoma, Hodgkin's disease and multiple myeloma patients previously failing mobilization with chemotherapy and/or cytokine treatment: compassionate use data. Bone Marrow Transplant. 2008;41:331–8.
9. DiPersio J, Stadtmauer E, Nademanee A, Micallef I, Stiff P, Kaufman J, et al. Plerixafor and G-CSF versus placebo and G-CSF to mobilize hematopoietic stem cells for autologous stem cells transplantation in patients with multiple myeloma. Blood. 2009;113:5720–6.
10. Hendrix C, Flexner C, MacFarland R, Giandomenico C, Fuchs E, Redpath E, et al. Pharmacokinetics and safety of AMD3100, a novel antagonist of the CXCR-4 chemokine receptor, receptor, in human volunteers. Antimicrob Agents Chemother. 2000;44:1667–73.
11. Liles W, Broxmeyer H, Rodger E, Wood B, Hubel K, Cooper S, et al. Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist. Blood. 2003;102:2728–30.
12. Cid J, Monsalvo S, Castillo C, Pascual C, Moreno-Jimenez G, Lopez-Parra M, et al. Addition of plerixafor to G-CSF in poor mobilizing healthy related donors overcame mobilization failure: an observational case series on behalf of the Grupo Espanol de Trasplante Hematopoyetico (GETH). Transfus Apher Sci. 2021;60:103052. https://doi.org/10.1016/j.transci.2021.103052
13. Gattillo S, Marktel S, Rizzo L, Malato S, Malabarba L, Coppola M, et al. Plerixafor on demand in ten healthy family donors as a rescue

strategy to achieve an adequate graft for stem cell transplantation. Transfusion. 2015;55:1993–2000.
14. Pantin J, Purev E, Tian X, Cook L, Donohue-Jerussi T, Cho E, et al. Effect of high-dose plerixafor on CD34$^+$ cell mobilization in healthy stem cell donors: results of a randomized crossover trial. Haematologica. 2017;102:600–9.
15. Lemery S, Hsieh M, Smith A, Rao S, Khuu H, Donohue T, et al. A pilot study evaluating the safety and CD34+ cell mobilizing activity of escalating doses of plerixafor in healthy volunteers. Br J Haematol. 2011;153:66–75.
16. Schroeder M, Rettig M, Lopez S, Christ S, Fiala M, Eades W, et al. Mobilization of allogeneic peripheral blood stem cell donors with intravenous plerixafor mobilizes a unique graft. Blood. 2017;129:2680–92.
17. Devine S, Vij R, Rettig M, Todt L, McGlauchlen K, Fisher N, et al. Rapid mobilization of functional donor hematopoietic cells without G-CSF using AMD3100, an antagonist of the CXCR4/SDF-1 interaction. Blood. 2008;112:990–8.
18. Schumm M, Lang P, Bethge W, Faul C, Feuchtinger T, Pfeiffer M, et al. Depletion of T-cell receptor alpha/beta and CD19 positive cells from apheresis product with the CliniMACS device. Cytotherapy. 2013;15:1253–8.
19. Rutella S, Filippini P, Bertaina V, Li Pira G, Altomare L, Ceccarelli S, et al. Mobilization of healthy donors with plerixafor affects the cellular composition of T-cell receptor (TCR)-αβ/CD19-depleted haploidentical stem cell grafts. J Transl Med. 2014;12:240–54.
20. Jaiswal S, Bhakuni P, Joy A, Murli N, Bharadwaj P, Zaman S, et al. Impact of single-dose plerixafor as an adjunct to granulocyte colony-stimulating factor–based peripheral blood stem cell mobilization on the graft composition and outcome for T cell–replete haploidentical peripheral blood stem cell transplantation with post-transplantation cyclophosphamide: a comparative study. Biol Blood Marrow Transplant. 2018;24:542–8.
21. Cashen A, Rettig M, Gao F, Smith A, Abbound C, Stockerl-Goldstein K, et al. Phase I/II study of intravenous plerixafor added to a mobilization regimen of granulocyte colony–stimulating factor in lymphoma patients undergoing autologous stem cell collection. Biol Blood Marrow Transplant. 2017;23:1282–9.
22. Gutiérrez-Aguirre C, Alvarado-Navarro D, Palomares-Leal A, Mejía-Jaramillo G, Salazar-Riojas R, Gómez-DeLeón A, et al. Reduced-dose plerixafor as a mobilization strategy in autologous hematopoietic cell transplantation: a proof-of-concept study. Transfusion. 2019;59:3721–6.
23. Hubel K, Liles W, Broxmeyer H, Rodger E, Wood B, Cooper S, et al. Leukocytosis and mobilization of CD34$^+$ hematopoietic cells by AMD3100, a CXCR4 antagonist. Support Cancer Ther. 2004;1:165–72.
24. Boulad F, Shore T, van Besien K, Minniti C, Barbu-Stevanovic M, Wiener Fedus S, et al. Safety and efficacy of plerixafor dose escalation for the mobilization of CD34$^+$ hematopoietic progenitor cells in patients with sickle cell disease: interim results. Haematologica. 2018;103:770–7.
25. Sanikommu S, Reese E, He J, Le C, Ai J, Butler C, et al. Cost saving, patient centered algorithm for progenitor cell mobilization for autologous hematopoietic cell transplantation. J Clin Apher. 2021;36:553–62.

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SHORT REPORT

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Transfusion double whammy? Adrenaline-takotsubo-anaphylaxis-Kounis complex post transfusion?

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Abstract

Background and Objectives: The adrenaline-takotsubo-anaphylaxis-Kounis, or the ATAK complex, where there are clinical and pathophysiological overlaps between takotsubo and Kounis syndromes, in which histaminergic, adrenergic and other mediators may play roles, was recently described. The objective of this report was to describe three cases where the ATAK complex was suspected to have occurred after transfusion.

Materials and Methods: Three cases were recently reported to the New Zealand Blood Service haemovigilance programme that appeared to have features in common suggestive of the ATAK complex.

Results: All three patients had had a blood component transfused, an initial severe allergic reaction, treatment with adrenaline or a congener, subsequent acute left ventricular failure or transfusion-associated circulatory overload, and features suggestive of takotsubo cardiomyopathy.

Conclusions: Although rarely described, transfusion-associated ATAK complex may be occurring more often than believed. Circumstances during a transfusion may predispose to it. It should be suspected if the sequence of events described above occur. Its characteristics need to be better understood. Risk factors for it may be modifiable.

KEYWORDS
ATAK complex, Kounis syndrome, takotsubo syndrome, transfusions

Highlights

- Transfusion-related severe allergic reactions may quickly develop into transfusion-associated circulatory overload in some patients.
- The features are suggestive of the recently described adrenaline-takotsubo-anaphylaxis-Kounis or ATAK complex.

INTRODUCTION

Three patients with common features showed a rare sequence of transfusion-associated adverse reactions (henceforth, reactions or reaction). Initially, all had respiratory and other features suggesting severe allergic reactions. They were treated as such. Shortly afterwards, the nature of the reactions changed to resemble transfusion-associated circulatory overload (TACO). None fitted a recognized reaction category.[1, 2]

CASE 1

A 71-year-old female with platelet count less than 10×10^9/L (probable immune thrombocytopenic purpura) received an ABO-identical, non-pathogen-reduced, 7-day-old unit of pooled platelets in platelet additive solution. Pre-transfusion temperature (T) was 36.3°C, heart rate (HR) 96 beats/min, blood pressure (BP) 138/82 mmHg, respiratory rate (RR) 17/min and peripheral oxygen saturation (SpO_2) 97%

on room air (RA). After 230 ml of transfusion, she developed breathlessness, restlessness, extensive skin rash, chills, rigours, hypotension (BP = 78/62 mmHg), tachycardia (HR = 103 beats/min), tachypnoea (RR = 29/min) and hypoxia (SpO_2 = 95% on O_2 at 6 L/min). She was given metaraminol for the hypotension. Shortly afterwards, her observations were T = 36°C, HR = 145 beats/min, BP = 201/148 mmHg, RR = 31/min and SpO_2 = 90% on RA. The electrocardiogram (ECG) showed segment elevation in the anterior leads, and troponin T and B-natriuretic peptide (BNP) were elevated (510 ng/L and 953 pg/ml, respectively). Chest X-ray (CXR) taken 30 min post the event showed no pulmonary oedema. A chest CT scan taken 6 h post the event showed bilateral apical and lower lobe ground-glass attenuation, suggesting haemorrhage or oedema. Trans-thoracic echocardiography 9 h after the event was suggestive of a new takotsubo cardiomyopathy with an estimated left ventricular (LV) ejection fraction (EF) of 40%–45% (Figure 1). CT coronary angiography 10 days post the event showed normal coronary arteries.

CASE 2

An 89-year-old female presented with breathlessness of short duration. Initial observations were T = 36.2°C, HR = 89 beats/min, BP = 180/65 mmHg, RR = 23/min and SpO_2 = 100% on RA. CXR taken shortly after admission showed clear lungs, normal cardiac size and no pleural effusions. Baseline blood results were consistent with iron-deficiency anaemia (haemoglobin 76 g/L, mean corpuscular volume 80 fl, mean corpuscular haemoglobin 24 pg, serum ferritin 14 μg/L and transferrin saturation 4%). She was admitted for an elective red blood cell (RBC) transfusion. She also had type 2 diabetes mellitus (T2DM), hypertension, hyperlipidaemia, osteoarthritis, gout, cataracts and glaucoma.

RBC transfusion (ABO-identical, 20-day-old) started 8 h post presentation. Pre-transfusion observations were T = 36.7°C, HR = 88 beats/min, BP = 210/75 mmHg, RR = 28/min and SpO_2 = 93% on RA. After 40 min and 57 ml of the transfusion, she became breathless, tachypnoeic, wheezy, desaturated, hypotensive (systolic BP = 60–70 mmHg) and febrile (T = 38.5°C), but there was no skin rash.

Adrenaline (1 mg im × 2), hydrocortisone 100 mg and fluids intravenously (iv) were given for anaphylaxis. CXR taken 30 min post the event showed small volume lungs, bilateral, confluent airspace opacities consistent with acute LV failure but no cardiomegaly. During pre-intubation laryngoscopy, foamy material, but no oedema, was seen in the oropharynx. CXR taken in the intensive care unit (ICU) 90 min later showed bilateral, extensive and confluent opacities. She was given esmolol 20 mg iv (because HR = 130 beats/min and BP = 210/130 mmHg), became hypotensive, and was given further adrenaline. In the ICU, she needed circulatory support with noradrenaline and vasopressin, and piperacillin + tazobactam for persistent fever. Clinical and radiological improvement started within 24 h. The next day, she was extubated, but she still needed intermittent respiratory support following discharge to the ward.

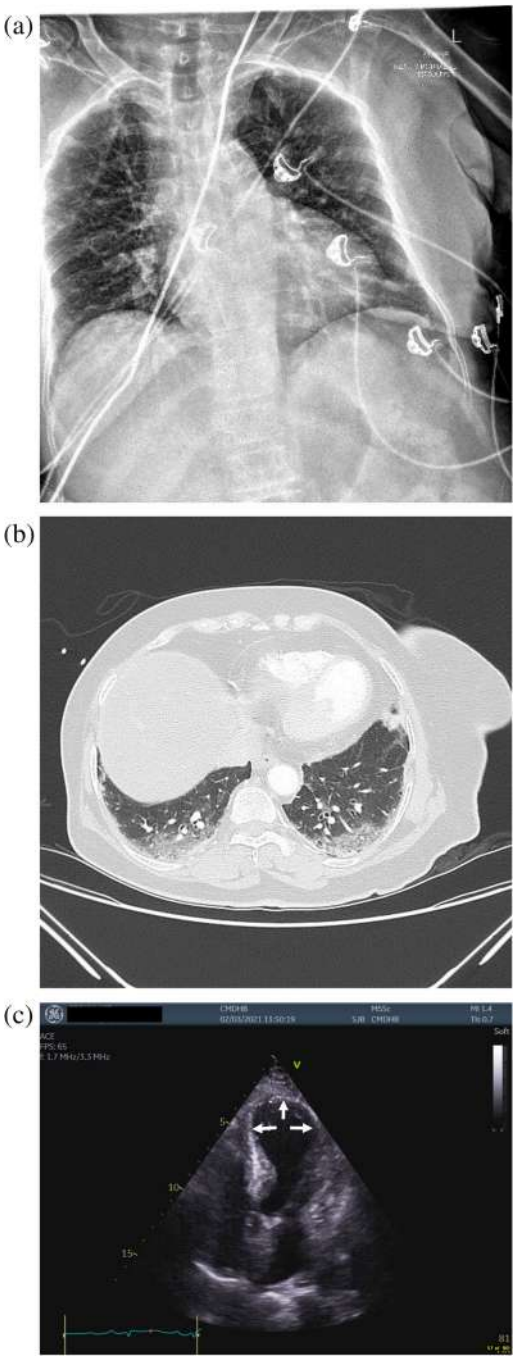

FIGURE 1 Case 1 (a) Chest X-ray taken 30 min after the initial allergic reaction. The lung fields are clear, and there is nothing to suggest pulmonary oedema. (b) Contrast-enhanced chest CT taken 6 h after the initial reaction, showing ground-glass attenuation in both lower lobes with nodular foci. The overall appearance, taken in context, is suggestive of pulmonary oedema or haemorrhage. (c) Transthoracic echocardiogram, apical four-chamber view, 9 h after the event, suggestive of regional wall motion abnormalities (left ventricular apical hypo-/dys-kinesia and basal hyper-kinesia), leading to the 'takotsubo' (octopus pot) appearance (arrows)

Serologic anomalies suggestive of a haemolytic transfusion reaction and evidence of bacterial contamination of the transfusion were absent. Patient blood cultures were negative. There was evidence of stage 2 acute kidney injury, likely secondary to hypoperfusion (serum creatinine 90 μmol/L at admission rising to 160 μmol/L 2 days later). Troponin T rose from 28 ng/L on admission to 243 and 1139 ng/L at 3 and 11 h post the event, respectively, with no cardiac ischaemia symptoms or new ECG changes (there was evidence of a chronic left bundle branch block). This was considered to be consistent with type 2 NSTEMI. Trans-thoracic echocardiography 18 h post the event showed moderate left atrial dilatation and global LV impairment with estimated LVEF of 35%–40%, significant mitral regurgitation, and mild aortic stenosis and tricuspid regurgitation. N-terminal pro-BNP at admission was 507 pmol/L (suggesting heart failure). No post-event result was available.

CASE 3

A 42-year-old male with spondylodiscitis, myositis, gluteus maximus abscesses, end-stage renal failure (ESRF), anaemia secondary to ESRF, T2DM, peripheral neuropathy, chronic diarrhoea, gout, hyper- and dys-lipidaemia, cardiomyopathy and raised BMI with a baseline haemoglobin of 69 g/L was receiving an ABO-identical, 20-day-old RBC transfusion. Pre-transfusion observations were T = 36.2°C, HR = 81/min, BP = 108 beats/42 mmHg, RR = 18/min and SpO_2 = 99% on RA. After about 1 h and 246 ml of the transfusion, he developed a choking sensation in the throat (but not shortness of breath initially) and abdominal and left-sided chest pain, and he became anxious, restless and nauseous. Later, he developed extensive flushing, dyspnoea, increased work of breathing, bilateral wheezes, chills and rigours. He had no skin rash or oro-pharyngeal or peripheral oedema. At this point his observations were T = 37.9°C, RR = 25/min, SpO_2 = 79% (RA), HR = 108 beats/min and BP = 158/60 mmHg. He was given 0.5 mg adrenaline im, 5 mg salbutamol nebulized, 10 mg loratadine orally and 100 mg hydrocortisone iv for anaphylaxis. ECG was normal. CXR taken 50 min post the event showed a new atelectatic band in the left mid-zone and prominent pulmonary arteries with upper lobe redistribution, but no air-space shadowing, pleural effusions or cardiomegaly. The next day he was found to have bilateral basal chest crepitations. Echocardiography was not done.

DISCUSSION

Our cases suggest that initial severe allergic reactions can sometimes quickly change to TACO and may be examples of the recently described adrenaline-takotsubo-anaphylaxis-Kounis (ATAK) complex.

Takotsubo syndrome (TS) is a well-recognized cause of acute reversible heart failure often precipitated by 'stressors' such as sudden BP drops, asthma attacks, medical procedures, pain, surprises and so on. It is especially common in post-menopausal women. The defining feature is regional ventricular dysfunction, causing characteristic echocardiographic abnormalities. Atherosclerotic coronary artery disease is absent, but ECG and biochemical features often suggest cardiac ischaemia. Symptoms include chest pain, breathlessness, dizziness and other features of heart failure.[3]

Catecholamines (e.g., noradrenaline) and stress-related neuropeptide Y are believed to play central roles in TS causing not only cardio-inhibitory effects but also acute microvascular dysfunction.[3] Interestingly, asthma exacerbation, especially after treatment (short-acting ß2 adrenergic receptor agonist, adrenaline and intubation), is well described as a TS trigger.[4, 5]

Kounis syndrome (KS) is an acute coronary syndrome precipitated by severe allergic events. In KS, it is postulated that mast cell mediators such as histamine, platelet-activating factor, cytokines, and so on cause coronary artery spasm, atheromatous plaque rupture or coronary artery stent thrombosis. Many drugs, foods, and so on—although not yet blood or its derivatives—have been implicated. Clinical, biochemical, ECG and angiographic features overlap allergic reactions and cardiac ischaemia.[6]

Lately, the ATAK complex, which overlaps TS and KS, has been described. Exogenous adrenaline may also play a role.[7] Recent reports include a female aged 60 years receiving gelofusine who became breathless and sweaty and received adrenaline; a male aged 48 years in Addisonian crisis treated with steroids and noradrenaline; and a 44-year-old anaesthetised female who received adrenaline for peri-anaesthesia anaphylaxis.[8–10]

Our patients showed initial features of significant allergic reactions. One had extensive rashes; another, significant hypotension; the third, bilateral wheezes and a choking sensation. Two patients received RBC and one patient received platelets. Two received adrenaline, and the third metaraminol (a direct α-1 and an indirect β-1 adrenoreceptor agonist). CXR in all was consistent with TACO. In one, there was also echo evidence of TS. In another, echocardiography was done 18 h post the event and global LV impairment was seen. In the third, echocardiography was not done. Early echocardiography is recommended because changes can be transient. Mitral regurgitation (as seen in one) is known in TS, and CXR may show features of pulmonary oedema, cardiomegaly and upper lobe diversion (as seen in all our patients).[11, 12]

We came across one other similar transfusion-associated case. A 48-year-old female developed acute reversible heart failure with TS features and urticaria while receiving platelets. Adrenaline was not given. The authors suggested combined histaminergic and (endogenous) adrenergic elements, causing the cardiac effects.[13]

In conclusion, some transfused patients, some or all of the following may occur together (the 'perfect storm'):

1. Borderline cardiac function
2. Catecholamine release (secondary to stress of the underlying disorder, the transfusion or the reaction to it)
3. Histamine release (in allergic reactions)
4. Treatment with adrenaline
5. Volume overload.

This may cause the ATAK complex and TACO. An epidemiological study on KS-associated TS suggests that older people, females, non-

Whites, hypertensives and the dys-lipidaemics may be at risk.[14] Although rarely described in transfused patients, they may be at particular risk. Transfusion-associated ATAK complex (TAAC) should be considered in patients with the following sequence: severe allergic reactions, treatment with adrenaline or congener and TACO. TAAC may be occurring more often than is known. Its incidence, pathophysiology, transfusion correlates and clinical course need to be better understood.

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Krishna G. Badami conceived, wrote, reviewed, made changes to, and approved the final version of the paper.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. International Society of Blood Transfusion. Working party on Haemovigilance. Proposed standard definitions for surveillance of non-infectious adverse transfusion reactions.
2. Kopko PM, editor. Transfusion reactions. 5th ed. Bethesda: AABB Press; 2021.
3. de Chazal HM, Del Buono MG, Keyser-Marcus L, Ma L, Moeller FG, Berrocal D, et al. Stress cardiomyopathy diagnosis and treatment: JACC state-of-the-art review. J Am Coll Cardiol. 2018;72:1955–71.
4. Saito N, Suzuki M, Ishii S, Morino E, Takasaki J, Naka G, et al. Asthmatic attack complicated with Takotsubo cardiomyopathy after frequent inhalation of inhaled corticosteroids/long-acting beta2-adrenoceptor agonists. Intern Med. 2016;55:1615–20.
5. Pontillo D, Patruno N, Stefanoni R. The tako-tsubo syndrome and bronchial asthma: the chicken or the egg dilemma. J Cardiovasc Med. 2011;12:149.
6. Kounis NG. Kounis syndrome: an update on epidemiology, pathogenesis, diagnosis and therapeutic management. Clin Chem Lab Med. 2016;54:1545–59.
7. Kounis NG. Attack the ATAK: "ος ὁ θεὸς συνέζευξε, ἄνθρωπος μὴ χωριζέτω" (ous o theos synezeuxe anthropos me horizeto) "what therefore god hath joined together, let not man put asunder". Int J Cardiol. 2016;203:960–1.
8. Marganato D, Abete R, Di Giovine G, Delfino P, Massimiliano G, Mazzetti S, et al. Takotsubo cardiomyopathy associated with Kounis syndrome: a clinical case of the "ATAK complex". J Cardiol Cases. 2019;20:52–6.
9. Singh G, Manickam A, Sethuraman M, Rathod RC. Takotsubo cardiomyopathy in a patient with pituitary adenoma and secondary adrenal insufficiency. Indian J Crit Care Med. 2015;19:731–4.
10. Alshaeri T, Gupta DE, Nagabhushana AN. Peri-anesthesia anaphylaxis (PAA): we still have not started post-PAA testing for inciting anesthesia-related allergens. Middle East J Anaesthesiol. 2016;23:465–9.
11. Izumo M, Akashi YJ. Role of echocardiography for Takotsubo cardiomyopathy: clinical and prognostic implications. Cardiovasc Diagn Ther. 2018;8:90–100.
12. Rahman A, Liu D. Broken heart syndrome a case study https://www.racgp.org.au/download/documents/AFP/2012/JanFeb/201201rahman.pdf. Accessed 03 Dec 2021.
13. Zhou JQ, Choe E, Ang L, Schnittger I, Rockson SG, Tremmel JA, et al. Stress-induced cardiomyopathy associated with a transfusion reaction: a case of potential crosstalk between the histaminic and adrenergic systems. Exp Clin Cardiol. 2011;16:30–2.
14. Desai R, Singh S, Kounis NG, Sachdeva R, Kumar G. Epidemiology of Takotsubo syndrome associated with Kounis syndrome in the United States. Int J Cardiol. 2020;310:32.

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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

Breast milk contains red cell isohaemagglutinins: Doubts and dilemmas

I am writing this letter in response to the article published by Kumar et al. [1]. Although the study consists of nice data, there are few questions that remain unaddressed. Herein I would like to highlight those areas that could pave the way for future research on this interesting topic.

1. The antibody titres were estimated in breast milk by column agglutination technology (CAT), but breast milk is not included in the standard sample acceptance criteria for CAT. The micro-columns using glass beads as the column ingredient or presence of polyethylene glycol in the cassettes could interact with the paraprotein present in breast milk and give false-positive results, which might lead to overestimation of the ABO antibody levels. Therefore, it would be better to use conventional tube technique rather than CAT until satisfactory validation data are available for antibody titration in breast milk.
2. Although the method for testing the levels of ABO immunoglobulin isotypes in elutes was intriguing, it would have been further appreciated if the IgG subtypes were classified. It is reported that foetal anaemia correlates well with the maternal IgG1 and IgG3 alloantibodies [2] but due to some unknown reasons, IgG2 antibodies, which poorly activate complement, are commonly formed against carbohydrate A and B antigens [3]. Therefore, estimation of IgG subtypes in breast milk might help in predicting the clinical significance of ABO antibodies.
3. The FcRn plays a central role in establishing humoral immunity in mammalian offspring, but there remain the species-specific differences. In humans, most of the maternal IgGs are transmitted by placental transfer, whereas in rats and mice uptake of IgG predominantly occurs across the intestine via FcRn-mediated pathway [4]. Therefore, one must remain mindful before translating the murine or rodent models to humans due to the differences in FcRn receptors, which transfer IgG significantly more efficiently from intestine to circulation than human receptors.
4. In contrast to plasma, human breast milk contains a significant amount of IgA, which is transcytosed across the gut wall via the polymeric immunoglobulin receptors without any involvement of FcRn [5]. As FcRn prevents pH-dependent degradation of the human IgG, it remains in the circulation for 19–21 days, whereas IgA has a half-life of 5–7 days only. Therefore, the doubt remains regarding the clinical impact of this short-lived IgA type of ABO antibodies in neonates.
5. Finally, the maternal antibodies are transported through the enterocytes to the portal circulation, not to the systemic circulation, as mentioned by the authors. Moreover, the liver eliminates complex macromolecules from the circulation; therefore, absorbed immunoglobulin might be catabolized in the liver before entering into the systemic circulation, which could further reduce the clinical significance of red cell isohaemagglutinins.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Kumar S, Rasalam JE, David VT, Yenuberi H, Amalraj P, Jeyaseelan L, et al. Breast milk contains red cell isohaemagglutinins: an observational study of 176 mothers. Vox Sang. 2022;117:847–52.
2. Lambin P, Debbia M, Puillandre P, Brossard Y. IgG1 and IgG3 anti-D in maternal serum and on the RBCs of infants suffering from HDN: relationship with the severity of the disease. Transfusion. 2002;42:1537–46.
3. Lee KH, Won DI, Yook JM, Kim KY, Park SM, Park JH, et al. Distribution of anti-ABO immunoglobulin G subclass and C1q antibody in ABO-incompatible kidney transplantation. Transplant Proc. 2018;50:1063–7.
4. Paveglio S, Puddington L, Rafti E, Matson AP. FcRn-mediated intestinal absorption of IgG anti-IgE/IgE immune complexes in mice. Clin Exp Allergy. 2012;42:1791–800.
5. He W, Ladinsky MS, Huey-Tubman KE, Jensen GJ, McIntosh JR, Bjorkman PJ. FcRn-mediated antibody transport across epithelial cells revealed by electron tomography. Nature. 2008;455:542–6.

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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

Reply to Datta: Breast milk contains red cell isohaemagglutinins: Doubts and dilemmas

We appreciate the interest of Dr. Datta on our recently published article 'Breast milk contains red cell isohaemagglutinins: An observational study of 176 mothers' and for highlighting the many areas of research that could be pursued on this front.

We wish, however, to clarify a few of the issues raised.

With regard to the suggestion of using the tube technique as opposed to the column agglutination technology platform for testing breast milk, while we agree with the author that validation data of the same are not available for the latter, neither is there data of testing breast milk using the tube technique. Breast milk is an unusual sample used in the setting of detecting isohaemagglutinins. This has been recognized by Leonard et al., who compared the two platforms in his publication and found similar results [1]. Our study describes the use of negative controls with every breast milk sample to ensure that no non-specific positivity interfered with our interpretation, a concern raised by the author. However, we do agree that a formal validation of the same would offer greater confidence when using breast milk for testing on either of these platforms.

The title of this study is self-explanatory, in that it demonstrated breast milk 'contains' red cell isohaemagglutinins [2]. The testing for immunoglobulin types was directed towards demonstrating IgG in breast milk, and it was a deliberate decision to not define subtypes at this stage, particularly as clinical follow-up was hampered by the pandemic. We are in full agreement with the author that demonstrating subclasses coupled with neonatal assessments will definitely enhance our understanding of the clinical significance of the IgG antibodies identified.

The author provides a strong theoretical explanation regarding differences between rodents and humans in terms of antibody transfer, and also highlights the complexities of absorption and processing in humans through the portal circulation, which could render many of these antibodies clinically insignificant [3]. As mentioned in our paper, a major limitation was the inability to follow-up neonates due to the pandemic. However, earlier literature from our institution, as well as the paper by Leonard et al., have clearly demonstrated the transfer of alloantibodies to neonates via breast milk, the presence of these antibodies in neonate serum, and in most instances causing significant haemolysis well past 1 month of life [1, 4]. We thank Dr. Datta for his insightful comments.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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REFERENCES

1. Leonard A, Boal LH, Pary P, Mo YD, Jacquot C, Luban NL, et al. Identification of red blood cell antibodies in maternal breast milk implicated in prolonged hemolytic disease of the fetus and newborn. Transfusion. 2019;59:1183–9.
2. Kumar S, Rasalam JE, David VT, Yenuberi H, Amalraj P, Jeyaseelan L, et al. Breast milk contains red cell isohaemagglutinins: an observational study of 176 mothers. Vox Sang. 2022;117:847–52.
3. Santhanakrishnan M, Tormey CA, Natarajan P, Liu J, Hendrickson JE. Clinically significant anti-KEL RBC alloantibodies are transferred by breast milk in a murine model. Vox Sang. 2016; 111:79–87.
4. Rasalam JE, Kumar S, Amalraj P, Bal HS, Mathai J, Kumar M, et al. Do red cell alloantibodies continue to challenge breast fed babies? Transfus Med. 2020;30:281–6.

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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

Massive transfusion protocols during the COVID-19 pandemic

Recently, Yang and colleagues [1] described the impact of the COVID-19 pandemic on massive transfusion protocol (MTP) activations at a trauma centre. The authors experienced no change in the number of MTPs activated during the pandemic. Likewise, our previous study [2] found that 60% of surveyed institutions ($n = 50$) across the United States had no change in activations, while 24% experienced an increase in monthly MTP activations compared to the same period before the pandemic. Persistent activation of the MTP at pre-pandemic levels, and an increase at some hospitals, may not only reflect differences in region- and nation-specific virus control measures but also demonstrates the impact of societal pressures on hospital workflow and the character of traumas.

The authors discovered there was an increased amount of time from MTP initiation until the first unit was transfused. Their hypothesis was that the use of enhanced personal protective equipment required more time. Despite this increased time, no significant change in the amount of blood product wastage was observed. In contrast, our study found that 27% of institutions experienced increased wastage, with multiple institutions attributing this to staffing shortages leading to poor product handling [2]. To that end, if indeed there were challenges in maintaining adequate staff in Taiwan, these issues may have contributed to the increased time from MTP activation to transfusion as well; this underscores the importance of an adequately trained trauma care staff.

Additionally, the authors report that most traumas that required an MTP activation were due to motor vehicle trauma [1]. We analysed the number of road traffic accidents, injuries and fatalities in Taiwan [3] based on the authors' study time-period. Despite an average of almost 25% fewer traffic accidents (31,341 vs. 23,560), approximately 25% fewer traffic injuries (41,972 vs. 30,990), and 8% fewer fatalities (159 vs. 146) during the pandemic compared to earlier times, the reported number of MTP activations did not change. This may be related to increased high-speed open road accidents, as seen among some international drivers during the pandemic [4], as opposed to more minor 'fender-bender' type incidents, given the reduced road traffic during quarantines. Moreover, recent literature has also described an increase in drug and alcohol use as well as a reduction in law enforcement staff internationally, contributing to an increase in both severe accidents [5] and violent crime, as seen in the United States. While specific details of traffic accidents are not captured in the Taiwanese database, the impact of the pandemic on daily life cannot be ignored. Understanding developing blood supply and staffing issues, as well as the nature of trauma during a pandemic, should inform services about the importance of adequate preparedness for continued MTP activations.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Yang CX, Lin PC, Chang CC, Tsai HW, Transfusion Committee, Sun JT. The impact of the COVID-19 outbreak on activation of the massive transfusion protocol in the emergency department. Vox Sang . Published online January 4 2022. https://doi.org/10.1111/vox.13238
2. Jacobs JW, Booth GS. Blood shortages and changes to massive transfusion protocols: survey of hospital practices during the COVID-19 pandemic. Transfus Apher Sci. 2021;61:103297. https://doi.org/10.1016/j.transci.2021.103297
3. Ministry of Transportation and Communications. Monthly Transportation Report - Ministry of Transportation and Communications, R.O.C. [cited 2022 Jan 25]. Available from: https://www.motc.gov.tw/en/home.jsp?id=609&parentpath=0%2C154&mcustomize=statistics301.jsp
4. Yasin YJ, Grivna M, Abu-Zidan FM. Global impact of COVID-19 pandemic on road traffic collisions. World J Emerg Surg. 2021;16:51.
5. FBI. Uniform crime reporting (UCR) program. FBI [cited 2022 Jan 26]. Available from: https://www.fbi.gov/services/cjis/ucr#Latest-Releases

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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

Reply to Jacobs et al.: Massive transfusion protocols during the COVID-19 pandemic

We thank Jacobs and colleagues for their comments on our article 'The impact of the COVID-19 outbreak on activation of the massive transfusion protocol in the emergency department' [1]. Jacobs and colleagues found that half of the 50 surveyed institutions had no change of monthly massive transfusion protocol (MTP) activation in the previous study [2], which was similar to our results. Jacobs and colleagues also analysed the number of traffic accident cases according to the nationwide database released by the Ministry of Transportation and Communications, Taiwan, as our study found that the most common reason for triggering MTP for traumatic cases was motor vehicle accidents. They found that traffic accidents were seemingly fewer in our study period of COVID-19 outbreak. However, our study was performed in single medical centre, which had an MTP in northern Taiwan. Hence, it may be somewhat difficult to explain totally why there was no remarkable change in the monthly MTP activation before and during COVID-19 outbreak in our study. Furthermore, we have read the article by Jacobs and colleagues with high interest and found that 12 of the 50 surveyed institutions had experienced an increase of monthly MTP activation since the COVID-19 pandemic began in the United States (March 2020) compared to the pre-pandemic period [2]. Though the aetiology of MTP activation was not disclosed in this survey, the increase of monthly MTP activation may be due to the increasing cases of gun-violence-related deaths and injuries in the recent 2 years (gun-violence-related deaths: 43,652 in 2020 and 44,912 in 2021; gun-violence-related injuries: 39,519 in 2020 and 40,540 in 2021) as disclosed in the Gun Violence Archive [3]. In contrast, most Taiwanese citizens are not allowed to have guns because of the 'Controlling Guns, Ammunition and Knives Act'. These could explain why the most common cause of MTP activation for traumatic cases in our study was due to motor vehicle accidents rather than trauma due to violence. As a result, the strategies for MTP and patient management could vary from country to country.

Noteworthy, Jacobs and colleagues wondered whether the increased interval from MTP activation to transfusion was associated with challenges in maintaining adequate staff in Taiwan. The COVID-19 outbreak in mid-May 2021 mainly happened in northern Taiwan and a majority of COVID-19 patients with severe illness were admitted to our hospital [4, 5], which was divided into the so-called 'dirty zone' and 'clean zone' for optimized patient management. Of these, the critical care area was set as part of the dirty zone. Medical staff intending to enter into the critical care area should be equipped with adequate personal protective equipment (PPE) first, including eye protection, N95 or P100 respirator and gown. As was the case with the time-consuming process of wearing PPE, it inevitably took time to deliver blood products into the critical care area in MTP. Importantly, however, it ensured the staff safety in case of exposure to severe acute respiratory distress syndrome coronavirus 2 (SARS-CoV-2) in the dirty zone.

Finally, we appreciate this comment by Jacobs and colleagues on our findings and hope that our work could contribute to the growing body of knowledge concerning the MTP practice during the COVID-19 pandemic.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Yang CX, Lin PC, Chang CC, Tsai HW, Transfusion Committee, Sun JT. The impact of the COVID-19 outbreak on activation of the massive transfusion protocol in the emergency department. Vox Sang. 2022 Jan 4. https://doi.org/10.1111/vox.13238

2. Jacobs JW, Booth GS. Blood shortages and changes to massive transfusion protocols: survey of hospital practices during the COVID-19 pandemic. Transfus Apher Sci. 2021;61:103297.
3. Gun Violence Archive. Past Summary Ledgers. U.S.A [cited 2022 Feb 22]. Available from: https://www.gunviolencearchive.org/past-tolls
4. Huang CY, Tsai HW, Liu CY, Liu TH, Huang HL, Chang CC, et al. The predictive and prognostic role of hematologic and biochemical parameters in the emergency department among coronavirus disease 2019 patients. Chin J Physiol. 2021;64:306–11.
5. Tsai HW, Hung FM, Liao CH, Lin HF. Hotel-based quarantine center as a rapid response to COVID-19 outbreak, New Taipei, Taiwan, May to July 2021. J Formos Med Assoc. 2022 Jan 10. S0929-6646(22)00001-8. https://doi.org/10.1016/j.jfma.2022.01.001

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CORRIGENDUM

Vox Sanguinis ISBT International Society of Blood Transfusion

In the article by Putter and Seghatchian [1], published in the July 2021 issue of *Vox Sanguinis*, reference 42 was incorrect. The correct reference is as follows:

42. Harrison CN, Koschmieder S, Foltz L, Guglielmelli P, Flindt T, Koehler M, et al. The impact of myeloproliferative neoplasms (MPNs) on patient quality of life and productivity: results from the international MPN Landmark survey. Ann Hematol. 2017; 96:1653–65.

The online version has been corrected.

The authors apologize for this error.

REFERENCE

1. Putter JS, Seghatchian J. Polycythaemia vera: molecular genetics, diagnostics and therapeutics. Vox Sang. 2021;116:617–27.

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DIARY OF EVENTS

See also http://www.isbtweb.org/congresses/

10.2.2022	The European Hematology Association (EHA) and the European Society for Blood and Marrow Transplantation (EBMT) - 4th edition of the jointly organized European CAR T-cell Meeting.
15-16.3.2022	The IPFA/EBA Symposium on Plasma Collection and Supply will take place fully physical in Amsterdam, the Netherlands on March 15 - 16, 2022.
23.3.2022	Eye Drops from Human Origin - First EDHO Workshop on Current Standards and Future Developments organized by the ISBT Working Party Cellular Therapies.

