ISSN 0042-9007 (Print)
ISSN 1423-0410 (Online)

Volume 118 Number 3 MARCH 2023

Vox Sanguinis

The International Journal of Transfusion Medicine

IN THIS ISSUE

WILEY

ISBT International Society of Blood Transfusion

Vox Sanguinis

International Journal of Blood Transfusion

Official Journal of the International Society of Blood Transfusion

Founded 1956 by J. J. van Loghem, L. P. Holländer, J. Dausset, A. Hässig and J. Julliard (formerly Bulletin of the Central Laboratory of the Blood Transfusion Service of the Dutch Red Cross, founded 1951)

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.

Journal Customer Services

For ordering information, claims and any enquiry concerning your journal subscription please go to https://wolsupport.wiley.com/s/contactsupport?tabset-a7d10=2 or contact your nearest office. **Americas:** Email: cs-journals@wiley.com; Tel: +1 877 762 2974. **Europe, Middle East and Africa:** Email: cs-journals@wiley.com; Tel: +44 (0) 1865 778315; 0800 1800 536 (Germany). **Germany, Austria, Switzerland, Luxembourg, Liechtenstein:** cs-germany@wiley.com; Tel: 0800 1800 536 (Germany). **Asia Pacific:** Email: cs-journals@wiley.com; Tel: +65 3165 0890. **Japan:** For Japanese-speaking support, Email: cs-japan@wiley.com. Visit our Online Customer Help at https://wolsupport.wiley.com/s/contactsupport?tabset-a7d10=2.

Information for Subscribers: *Vox Sanguinis* is published in 12 issues per year. Institutional subscription prices for 2023 are: Print & Online: US$2262 (US), US$2637 (Rest of World), €1579 (Europe), £1225 (UK). Prices are exclusive of tax. Asia-Pacific GST, Canadian GST/HST and European VAT will be applied at the appropriate rates. For more information on current tax rates, please go to www. wileyonlinelibrary.com/ tax-vat. The price includes online access to the current and all online backfiles for previous 5 years, where available. For other pricing options, including access information and terms and conditions, please visit https://onlinelibrary.wiley.com/library-info/products/price-lists. Terms of use can be found here: https://onlinelibrary.wiley.com/terms-and-conditions.

Delivery Terms and Legal Title: Where the subscription price includes print issues and delivery is to the recipient's address, delivery terms are Delivered at Place (DAP); the recipient is responsible for paying any import duty or taxes. Title to all issues transfers Free of Board (FOB) our shipping point, freight prepaid.

Claims for Missing or Damaged Print Issues: Our policy is to replace missing or damaged copies within our reasonable discretion, subject to print issue availability, and subject to the following terms: Title to all issues transfers Freight on Board ("FOB") to the address specified in the order; (1) Freight costs are prepaid by Wiley; and (2) Claims for missing or damaged copies must be submitted by the Customer or Subscription Agent within the claims window, as noted below.

Claims window - General: Claims for missing print issues must be sent to cs-agency@wiley.com (and the Subscription Agent or Customer may be referred to a society) within three months of whichever of these dates is the most recent: date of subscription payment; or date of issue publication.

Claims window - India: Both Subscription Agents and Customers in India have 48 hours after receipt of goods to confirm that all content listed on the packing label has been received. In the event of any discrepancy, SPUR Infosolutions, Wiley's delivery partner in India, needs to be notified within forty-eight (48) hours using this email address: support@spurinfo.com. All claims will be checked against SPUR Infosolutions delivery records before the claim is accepted. The above terms for Wiley's claims policy otherwise apply.

Back Issues: Single issues from current and recent volumes are available at the current single issue price from cs-journals@wiley.com. Earlier issues may be obtained from Periodicals Service Company, 351 Fairview Avenue – Ste 300, Hudson, NY 12534, USA. Tel: +1 518 822-9300, Fax: +1 518 822-9305, Email: psc@periodicals.com

Abstracting and Indexing Services: The Journal is indexed by Abstracts in Anthropology (Sage); Abstracts on Hygiene & Communicable Diseases (CABI); Academic Search (EBSCO Publishing); Academic Search Alumni Edition (EBSCO Publishing); Academic Search Premier (EBSCO Publishing); AGRICOLA Database (National Agricultural Library); BIOBASE: Current Awareness in Biological Sciences (Elsevier); Biological Abstracts (Clarivate Analytics); BIOSIS Previews (Clarivate Analytics); CAB Abstracts® (CABI); CABDirect (CABI); CAS: Chemical Abstracts Service (ACS); CSA Biological Sciences Database (ProQuest); CSA Environmental Sciences & Pollution Management Database (ProQuest); CSA Virology & AIDS Abstracts (ProQuest); Current Contents: Life Sciences (Clarivate Analytics); Embase (Elsevier); Global Health (CABI); HEED: Health Economic Evaluations Database (Wiley-Blackwell); Index Veterinarius (CABI); Journal Citation Reports/Science Edition (Clarivate Analytics); MEDLINE/PubMed (NLM); Nutrition Abstracts & Reviews Series A: Human & Experimental (CABI); Pig News & Information (CABI); ProQuest Central (ProQuest); ProQuest Health & Medical Complete (ProQuest); ProQuest Research Library (ProQuest); Protozoological Abstracts (CABI); PubMed Dietary Supplement Subset (NLM); Review of Medical & Veterinary Entomology (CABI); Review of Medical & Veterinary Mycology (CABI); Rural Development Abstracts (CABI); Science Citation Index (Clarivate Analytics); Science Citation Index Expanded (Clarivate Analytics); Tropical Diseases Bulletin (CABI); Veterinary Bulletin (CABI).

Wiley's Corporate Citizenship initiative seeks to address the environmental, social, economic, and ethical challenges faced in our business and which are important to our diverse stakeholder groups. Since launching the initiative, we have focused on sharing our content with those in need, enhancing community philanthropy, reducing our carbon impact, creating global guidelines and best practices for paper use, establishing a vendor code of ethics, and engaging our colleagues and other stakeholders in our efforts. Follow our progress at www.wiley.com/go/citizenship. Wiley is a founding member of the UN-backed HINARI, AGORA, and OARE initiatives. They are now collectively known as Research4Life, making online scientific content available free or at nominal cost to researchers in developing countries. Please visit Wiley's Content Access – Corporate Citizenship site: http://www.wiley.com/WileyCDA/Section/id-390082.html. The journal to which you are submitting your manuscript employs a plagiarism detection system. By submitting your manuscript to this journal you accept that your manuscript may be screened for plagiarism against previously published works.

VOX SANGUINIS (Online ISSN: 1423-0410 Print ISSN: 0042-9007) is published monthly. Postmaster: Send all address changes to VOX SANGUINIS, Wiley Periodicals LLC, C/O The Sheridan Press, PO Box 465, Hanover, PA 17331, USA. For submission instructions, subscription and all other information visit: www.wileyonlinelibrary.com/journal/vox. Printed in the UK by Hobbs the Printers Ltd.

Vox Sanguinis (2023) **118**, 179–252

118/3/2023

Contents

WILEY

This journal is available online at *Wiley Online Library*. Visit wileyonlinelibrary.com to search the articles and register for table of contents e-mail alerts.

Received: 10 October 2022 | Revised: 7 December 2022 | Accepted: 3 January 2023

DOI: 10.1111/vox.13403

COMMENTARY

Vox Sanguinis ISBT International Society of Blood Transfusion

pH is unsuitable as a quality control marker in platelet concentrates stored in platelet additive solutions

In common with many other countries, the United Kingdom has retained pH as a principal indicator of quality in platelet concentrates (PCs), with a specification for $pH_{22^{\circ}C}$ levels ≥6.4 at end of storage. Murphy et al. reported a loss of platelet function and viability when the PC storage media approached a $pH_{22^{\circ}C}$ of 6.0 [1], with subsequent studies describing a clear correlation between lower pH values and low platelet survival upon re-infusion [2]. Low pH levels have thus been associated with the changes observed with platelet storage, which are collectively referred to as the platelet storage lesion (PSL). However, studies with PCs stored at ambient temperature suspended in 60%–70% platelet additive solution (PAS) show a stabilization and even an increase in pH with increasing storage. It has thus become apparent that the use of pH as a routine quality control (QC) indicator at end of storage is unsuitable for these components and this practice requires modification.

Platelets will generate adenosine triphosphate (ATP) via glycolysis even in the presence of adequate supplies of oxygen, though the rate of lactate production is greatly accelerated in oxygen-depleted conditions. In PCs suspended in 100% plasma, pH is principally buffered by the bicarbonate system. With CO_2 able to leave through the gas-permeable storage pack, the removal of hydrogen ions from solution will be promoted as long as bicarbonate is available [3]:

$$H^+ + HCO_3^- \leftrightharpoons H_2CO_3 \leftrightharpoons CO_2 + H_2O.$$

The addition of bicarbonate as a buffer in additive solutions manufactured with steam sterilization techniques has been problematic, and most commercial additive solutions have avoided its use [4]. Solutions such as PAS-E include phosphate as a buffer and acetate as a source of oxidizable fuel. Acetate is preferred over glucose as an energy source by stored platelets [5]. Its metabolism requires conversion to its conjugate acid, with the proton needed to convert acetate to acetic acid derived from the dissociation of carbonic acid, resulting in the generation of a bicarbonate ion. The formation of bicarbonate through acetate metabolism leads to stable pH levels in PCs using storage media comprised of at least 60% PAS-E. The presence of acetate in PAS also reduces lactate production and increases the oxygen consumption of stored platelets [6]. Phosphate in PAS provides additional buffering capacity; though in seemingly contradictory fashion, it has been associated with stimulating glycolysis in platelets collected with anticoagulants lacking phosphate such as acid–citrate–dextrose in a bid to maintain adequate adenine nucleotide levels [7]. The overall effect of the inclusion of compounds such as acetate and phosphate in PAS is to remove the reliance on the plasma-derived bicarbonate buffering system and decouple the levels of extracellular pH in the unit from the viability and functional capacity of the stored platelets. This poses a problem for blood establishments that have used pH as the principal quality indicator for routine end-of-storage quality monitoring of their PCs.

The decrease in pH in PCs suspended in 100% plasma corresponds to an increase in lactate concentration due to glycolysis. Johnson et al. reported $pH_{22^{\circ}C}$ levels above 7 after 21 days of storage at ambient temperature in PCs suspended in 60% PAS-E, with an increase in pH from day 14 coinciding with the depletion of glucose in the storage media. Despite this, a marked increase in markers of platelet activation, such as annexin V binding to aminophospholipids and soluble P-selectin levels, as well as decreased clotting time and aggregation responses to collagen were noted [8]. Studies with earlier PAS formulations (PAS-B and PAS-C), which extended the storage period beyond the point at which glucose is depleted, have also reported an increase in pH concomitant with a cessation in lactate production [9, 10]. It is possible that the observed rise in pH is due to bicarbonate generated from the metabolism of acetate and warrants further investigation [11, 12].

Over the period of storage, ATP levels in PC are observed to decrease, reducing the platelets' ability to perform energetically demanding processes. The loss of the glycolytic pathway could contribute to this loss of function, but the picture is complicated by the inclusion of acetate, with its role as a preferential fuel for ATP generation and a buffer [12, 13]. Beyond the maintenance of ATP stores, glucose may be involved in other metabolic pathways required for platelet viability such as maintenance of glutathione levels [14]. Glucose levels are also suggested as an alternative QC measure because of the association of glucose depletion with increased expression of markers coupled with a pro-apoptotic phenotype, including increased expression of aminophospholipids such as phosphatidylserine on the platelet surface, increased intracellular calcium levels and microparticle generation [15, 16]. The measurement of glucose is thus a more predictive quality marker than pH in PC suspended in 60%–70% PAS and can be quickly and easily measured on a biochemistry analyser. Lactate can be similarly measured. However, unlike depletion of glucose, which provides a definitive endpoint, it would be more difficult to define a numeric limit for lactate that could be applied as an end-of-storage QC indicator

across the various PC components. Pro-apoptotic markers of the PSL often require specialized equipment and expertise, with assay and donor variability making it difficult to define a numeric end-of-storage specification. They continue to be useful in evaluations of novel components where measurements can be made throughout the storage period.

This discussion has concentrated on PCs stored at ambient temperature but could be extended to the resurgent interest in cold storage of PCs. Cold storage results in reduced glucose consumption and lactate production. Patterns of platelet activation and haemostatic function are quite dissimilar in cold-stored platelets compared to ambient temperature PCs [8, 17]. Further work is required to identify an appropriate routine end-of-storage QC indicator for cold-stored PC, since 'classic' markers like swirling, pH and platelet activation indices may not be appropriate.

In summary, the accelerated expression of several indicators of the PSL is observed to occur after glucose levels are depleted. We therefore recommend that further investigations be undertaken to confirm the suitability of glucose as a routine end-of-storage QC marker for PCs stored in PAS at ambient temperature, and to define a practical specification that can be used by advisory bodies to guide blood establishments with the introduction of this change.

ACKNOWLEDGEMENTS

J.N. and C.V.S. designed the paper and wrote the manuscript; C.G. revised the final version.

FUNDING INFORMATION

The authors received no specific funding for this work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Jamie Nash
Christine V. Saunders
Chloë George

Component Development and Research Laboratory, Welsh Blood Service, Llantrisant, UK

Correspondence
Jamie Nash, Component Development and Research Laboratory, Welsh Blood Service, Llantrisant, UK.
Email: jamie.nash@wales.nhs.uk

ORCID

Jamie Nash https://orcid.org/0000-0002-5984-6268
Christine V. Saunders https://orcid.org/0000-0002-7010-6068

REFERENCES

1. Murphy S, Sayar SN, Gardner FH. Storage of platelet concentrates at 22 C. Blood. 1970;35:549–57.
2. Goodrich RP, Li J, Pieters H, Crookes R, Roodt J, Heyns AP. Correlation of in vitro platelet quality measurements with in vivo platelet viability in human subjects. Vox Sang. 2006;90:279–85.
3. Shimizu T, Murphy S. Roles of acetate and phosphate in the successful storage of platelet concentrates prepared with an acetate-containing additive solution. Transfusion. 1993;33:304–10.
4. Gulliksson H. Platelet storage media. Vox Sang. 2014;107:205–12.
5. Jóhannsson F, Guðmundsson S, Paglia G, Guðmundsson S, Palsson B, Sigurjónsson ÓE, et al. Systems analysis of metabolism in platelet concentrates during storage in platelet additive solution. Biochem J. 2018;475:2225–40.
6. Murphy S, Shimizu T, Miripol J. Platelet storage for transfusion in synthetic media: further optimization of ingredients and definition of their roles. Blood. 1995;86:3951–60.
7. Gulliksson H, Larsson S, Kumlien G, Shanwell A. Storage of platelets in additive solutions: effects of phosphate. Vox Sang. 2000;78:176–84.
8. Johnson L, Vekariya S, Wood B, Tan S, Roan C, Marks DC. Refrigeration of apheresis platelets in platelet additive solution (PAS-E) supports in vitro platelet quality to maximize the shelf-life. Transfusion. 2021;61:S58–67.
9. Sandgren P, Mayaudon V, Payrat J-M, Sjödin A, Gulliksson H. Storage of buffy-coat-derived platelets in additive solutions: in vitro effects on platelets stored in reformulated PAS supplied by a 20% plasma carry-over. Vox Sang. 2010;98:415–22.
10. Van Der Meer PF, Pietersz RNI, Reesink HW. Storage of platelets in additive solution for up to 12 days with maintenance of good in-vitro quality. Transfusion. 2004;44:1204–11.
11. Vinay P, Cardoso M, Tejedor A, Prud'homme M, Levelillee M, Vinet B, et al. Acetate metabolism during hemodialysis: metabolic considerations. Am J Nephrol. 1987;7:337–54.
12. Slichter SJ, Corson J, Jones MK, Christoffel T, Pellham E, Bailey SL, et al. Exploratory studies of extended storage of apheresis platelets in a platelet additive solution (PAS). Blood. 2014;123:271–80.
13. Whisson M, Nakhoul A, Howman P, Niu X, Guppy M. Quantitative study of starving platelets in a minimal medium: maintenance by acetate or plasma but not by glucose. Transfus Med. 1993;3:103–13.
14. Hervig T, Mansoor M, Farstad M. Endogenous glutathione and platelet function in platelet concentrates stored in plasma or platelet additive solution. Transfus Med. 2001;11:97–104.
15. Johnson L, Schubert P, Tan S, Devine DV, Marks DC. Extended storage and glucose exhaustion are associated with apoptotic changes in platelets stored in additive solution. Transfusion. 2016;56:360–8.
16. Saunders C, Rowe G, Wilkins K, Collins P. Impact of glucose and acetate on the characteristics of the platelet storage lesion in platelets suspended in additive solutions with minimal plasma. Vox Sang. 2013;105:1–10.
17. Zhao HW, Serrano K, Stefanoni D, D'Alessandro A, Devine DV. In vitro characterization and metabolomic analysis of cold-stored platelets. J Proteome Res. 2021;20:2251–65.

How to cite this article: Nash J, Saunders CV, George C. pH is unsuitable as a quality control marker in platelet concentrates stored in platelet additive solutions. Vox Sang. 2023;118:183–4.

Received: 16 November 2022 | Revised: 8 December 2022 | Accepted: 8 December 2022

DOI: 10.1111/vox.13395

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Can volume-reduced plasma products prevent transfusion-associated circulatory overload in a two-hit animal model?

Esther B. Bulle[1,2] | Robert B. Klanderman[1,2,3] | Marit B. de Wissel[2] | Joris J. T. H. Roelofs[4,5] | Denise P. Veelo[3] | Charissa E. van den Brom[1,2,6] | Rick Kapur[7] | Alexander P. J. Vlaar[1,2]

[1]Department of Intensive Care, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

[2]Laboratory of Experimental Intensive Care and Anesthesiology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

[3]Department of Anesthesiology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

[4]Department of Pathology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

[5]Amsterdam Cardiovascular Sciences, Microcirculation, University of Amsterdam, Amsterdam, The Netherlands

[6]Department of Anesthesiology, Amsterdam UMC, VU University, Amsterdam, The Netherlands

[7]Sanquin Research, Department of Experimental Immunohematology, Landsteiner Laboratory, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

Correspondence
Esther B. Bulle, Laboratory of Experimental Intensive Care and Anesthesiology, Amsterdam UMC, University of Amsterdam, Meibergdreef 9, 1100DD Amsterdam, The Netherlands.
Email: e.b.bulle@amsterdamumc.nl

Funding information
Landsteiner Foundation for Blood Transfusion Research, Grant/Award Number: 1931F

Abstract

Background and Objectives: Transfusion-associated circulatory overload (TACO) is a pulmonary transfusion complication and a leading cause of transfusion-related morbidity and mortality. Volume overload and rising hydrostatic pressure as a consequence of transfusion are seen as the central pathway leading to TACO. A possible preventative measure for TACO could be the use of low-volume blood products like volume-reduced lyophilized plasma. We hypothesize that volume-reduced lyophilized plasma decreases circulatory overload leading to a reduced pulmonary capillary pressure and can therefore be an effective strategy to prevent TACO.

Materials and Methods: A validated two-hit animal model in rats with heart failure was used. Animals were randomized to receive 4 units of either solvent-detergent pooled plasma (SDP) as control, standard volume lyophilized plasma (LP-S) or hyperoncotic volume-reduced lyophilized plasma (LP-VR). The primary outcome was the difference between pre-transfusion and post-transfusion left ventricular end-diastolic pressure (ΔLVEDP). Secondary outcomes included markers for acute lung injury.

Results: LVEDP increased in all randomization groups following transfusion. The greatest elevation was seen in the group receiving LP-VR (+11.9 mmHg [5.9–15.6]), but there were no significant differences when compared to groups receiving either LP-S (+6.3 mmHg [2.9–13.4], $p = 0.29$) or SDP (+7.7 mmHg [4.5–10.5], $p = 0.55$). There were no significant differences in markers for acute lung injury, such as pulmonary wet/dry weight ratios, lung histopathology scores or PaO_2/FiO_2 ratio between the three groups.

Conclusion: Transfusion with hyperoncotic volume-reduced plasma did not attenuate circulatory overload compared to standard volume plasma and was therefore not an effective preventative strategy for TACO in this rat model.

Keywords
lyophilized plasma, pulmonary oedema, pulmonary transfusion reaction, transfusion-associated circulatory overload, volume-reduced

Highlights

- There was no significant difference in pulmonary capillary pressure between animals transfused with volume-reduced plasma and animals transfused with a regular volume plasma product.
- Volume-reduced plasma was not effective in attenuating transfusion-associated circulatory overload in this animal model.
- In contrast with what was expected, a volume-reduced hyperoncotic plasma product led to a non-significant trend towards an increase in pulmonary capillary pressure.

INTRODUCTION

Transfusion-associated circulatory overload (TACO) is a pulmonary transfusion complication [1]. TACO is still responsible for about one third of transfusion-related deaths in spite of adopted transfusion practices [2–4]. The proposed pathophysiological mechanism of TACO is that a first hit, like heart or renal failure, results in volume incompliance [5]. Subsequently, transfusion leads to a fluid overload in the vascular system, causing the pulmonary capillary pressure to rise and fluid to extravasate from the capillaries into the lungs. This ultimately leads to pulmonary oedema and respiratory distress. Although other factors like inflammatory processes, glycocalyx injury, colloid osmotic pressure or storage lesion also may play a role in the pathophysiology of TACO, volume overload and increased hydrostatic pressure are seen as central features [6, 7].

Current preventative measures suggest reducing volume overload by using restrictive transfusion thresholds, single-unit transfusions, slow infusion rates and diuretics [8]. Considering volume overload in TACO is a fundamental factor, use of volume-reduced transfusion products could aid in preventing TACO.

Plasma transfusion has been identified as a risk factor for TACO in previous studies [9, 10]. Plasma can be freeze-dried resulting in lyophilized plasma. Lyophilized plasma in a standard volume has been used in military and trauma situations, because of the logistical benefits [11, 12]. It can, however, also be reconstituted in a smaller volume prior to patient administration, resulting in a more concentrated, hyperoncotic volume-reduced product. Especially in patients with volume incompliance and vulnerable for developing TACO, reduced volume transfusion might be a strategy to prevent TACO. The aim of this study was therefore to evaluate if concentrated volume-reduced lyophilized plasma could reduce the increase in pulmonary capillary pressure during transfusion compared to a standard volume plasma product.

MATERIALS AND METHODS

General information

Animal experiments were approved by the Dutch national committee for animal experimentation (project licence number: AVD118002017814) and executed according to the Animal Research Reporting of In Vivo Experiments (ARRIVE) guidelines and local guidelines [13]. Animals were housed in an animal housing facility and were kept on a 12-h light–dark cycle. Animals were fed standard rat chow and water ad libitum. The groups described here are part of a multi-armed randomized controlled trial. The control group (solvent-detergent pooled plasma [SDP]) is the same as previously published by our group. Results from this study including a detailed methods section have been previously published [14].

Animal procedures

In brief, experiments were performed using adult male Wistar rats weighing over 300 g. Animals were anaesthetised using 5% isoflurane with 100% oxygen, followed by an intraperitoneal injection with a mix of racemic ketamine (9 mg/100 g body weight), dexmedetomidine (6.25 μg/100 g) and atropine (0.25 μg/100 g). Then, a continuous intravenous mix of racemic ketamine (50 mg/kg/h) and dexmedetomidine (15 μg/kg/h) was administered through a tail vein cannula.

Animals were ventilated following a tracheotomy using a ventilator (Babylog 3000, Dräger, Lübeck, Germany). The right carotid artery was cannulated and a rat pressure–volume catheter (SPR-838, Millar, Houston, TX, USA) was inserted into the left ventricle. The right jugular vein was cannulated for monitoring central venous pressure and administration of fluids and transfusion products. The left carotid artery was cannulated for monitoring mean arterial pressure (MAP) and blood sampling for arterial blood gas analysis (RapidLab 500, Siemens, Erlangen, Germany).

Isovolemic anaemia was established by the exchange of arterial blood for a colloid solution (Tetraspan 6%, B. Braun, Melsungen, Germany) with a target haematocrit of 30% (±2%). Heart failure was induced by ligating the left anterior descending artery (LAD). The myocardial infarct was confirmed using electrocardiogram monitoring and visual inspection of the blanched myocardium. Animals were allowed to stabilize for 30 min, after which a transfusion product was administered. All animals received intravenous norepinephrine (2–8 μg/h) starting before LAD ligation till the end of the experiment. Animals were followed for 1 h and 30 min after start of transfusion, after which they were exsanguinated.

Experimental groups and donor products

In this study, within the multi-armed trial, animals were randomized to receive either (1) lyophilized plasma (LyoPlas N-W [blood type AB], Germany Red Cross Blood Service West, Hagen, Germany) either as standard volume lyophilized plasma (LP-S) or (2) volume-reduced lyophilized plasma (LP-VR) or (3) solvent-detergent pooled plasma (SDP) (Omniplasma [blood type AB], Octapharma, Laachen, Switzerland). SDP transfusion was used to control for a regular plasma transfusion and LP-S transfusion controlled for possible differences within the lyophilized product. The randomization was executed in blocks, containing two interventions for each randomization group. A single unit of lyophilized plasma was reconstituted in 100 ml of sterile water (half of the recommended volume). From this hyperoncotic solution, 50 ml was stored (LP-VR) while the other half was then diluted to the recommended volume (LP-S). All products originated from human donors. Animals were transfused with an equivalent to 4 units (4 ml of LP-S or SDP or 2 ml of LP-VR). Infusion speed was kept equal in all groups at 8 ml per hour, as previous studies have shown that differing transfusion speeds will confound results [15, 16]. The number of units transfused and the infusion speed have been previously tested and validated when this animal model was developed [17]. Treatment allocation was blinded before administration. This was not possible to maintain during transfusion and follow-up because the LP-VR group received less volume in a shorter amount of time.

Haemodynamic measurements

The primary outcome of this study was the difference between left ventricular end-diastolic pressure (LVEDP) measured before and after transfusion (ΔLVEDP). LVEDP is a surrogate marker for pulmonary capillary pressure [18]. In the LP-S and SDP group, ΔLVEDP was calculated by subtracting the pre-transfusion LVEDP value from the post-transfusion measurement 30 min after start of transfusion. For the LP-VR group, the post-transfusion measurement was 15 min after start of transfusion. Haemodynamic data were recorded using LabChart (version 6.1, AD instruments, Oxford, UK). Blood conductivity was measured using the volume-cuvette procedure. Parallel conductance was measured after hypertonic saline bolus injections. Calibration processes were performed according to previously published protocols [19].

Sample processing and pathology

Secondary outcomes were markers of pulmonary injury. After exsanguination, the right lung was harvested and the lobes were separated. The right upper lobe was fixed in 4% formalin, processed into haematoxylin and eosin–stained slides and scored by an experienced pathologist blinded for intervention groups. Scores ranged from 0 to 3 based on the presence and extensiveness of perivascular and intra-alveolar oedema. The right lower lobe was dried in a dehumidifying stove at 37°C for 7 days and a lung wet/dry weight ratio was calculated.

In order to quantify the myocardial infarct size, the heart was excised and perfused with Evans blue dye. Hearts were frozen, cut transversely into 2-mm slices and counterstained with triphenyltetrazolium chloride. Slices were fixed in 4% formalin and scanned. The percentage of infarcted myocardium was calculated using image analysis software (ImageJ 1.50i, National Institutes of Health, USA) [20].

Sample size calculation

The sample size for the complete multi-armed randomized controlled trial was calculated based on the expected effects of SDP and Ringer's lactate [14]. We calculated a sample size of nine animals in each group would have 80% power to detect a difference in means of ΔLVEDP 4.15 mmHg. Assuming that the common standard deviation is 2.8 using a two-group *t*-test with a 0.05 two-sided significance level, one animal was added (±10%) to each group to account for post-randomization mortality.

Statistical analysis

Animals that died or could not maintain a MAP >65 mmHg before the transfusion intervention were excluded prior to randomization and replaced. If animals dropped out after the measurement 30 min after the start of transfusion, all haemodynamic data from these animals were included for analysis. Secondary pulmonary outcomes were analysed from animals that completed the whole experiment. Non-parametric tests were used for all analyses because of non-normally distributed data and small group sizes ($n = 10$). The Kruskal–Wallis test was used when comparing three groups and the Mann–Whitney *U* test was used for two-group comparisons. The Friedman test was used to test differences between timepoints within groups. All results are presented in medians and interquartile ranges. A two-sided p-value <0.05 was considered as statistically significant. Data were analysed using R (Rstudio, 4.0.3) and figures were created using GraphPad Prism (v9.1.0).

RESULTS

Total fluid input was reduced in the LP-VR group

All baseline characteristics prior to randomization were similar between groups (Table 1). Ten animals were randomized and successfully transfused in each group. The LP-S and LP-VR group both contained one animal that died after randomization. Isovolemic dilution was successful in all groups with an overall median haematocrit of 31% (30, 32) directly after dilution. At the end of the experiment, the total fluid input was significantly lower in the LP-VR group (7.8 ml [7.3, 8.5]) compared to the groups receiving LP-S or SDP (9.9 ml [9.4, 10.7] and 9.5 ml [9.2, 9.8], respectively, $p < 0.001$) due to the reduced transfusion volume.

TABLE 1 Pre-randomization descriptive characteristics

Characteristics	LP-S, $n = 10$ (median, IQR)	LP-VR, $n = 10$ (median, IQR)	SDP, $n = 10$ (median, IQR)
Weight (g)	373 (362, 387)	355 (343, 365)	357 (331, 382)
Vent duration pre-transfusion (h)	2 h 05 min (1 h 45 min, 2 h 14 min)	1 h 50 min (1h 45 min, 1 h 59 min)	1 h 50 min (1 h 45 min, 1 h 59 min)
Fluid input pre-transfusion (ml)[a]	3.1 (3.0, 3.9)	3.0 (2.8, 3.7)	3.2 (2.9, 3.5)
Cardiac infarct size (%)	21 (18, 23)	22 (19, 24)	20 (19, 22)
pH	7.35 (7.33, 7.38)	7.37 (7.35, 7.39)	7.39 (7.35, 7.41)
Lactate (mmol/L)	3.2 (2.8, 3.8)	3.0 (2.6, 3.5)	2.7 (2.0, 3.1)
Hct (%)	36 (33, 39)	38 (36, 39)	36 (35, 39)

Abbreviations: Hct, haematocrit; IQR, interquartile range; LP-S, standard volume lyophilized plasma; LP-VR, volume-reduced lyophilized plasma; SDP, solvent-detergent pooled plasma.

[a]Fluid input was defined as all intravenously administrated fluids, but did not include volume used to achieve isovolemic anaemia, which was only used as volume replacement.

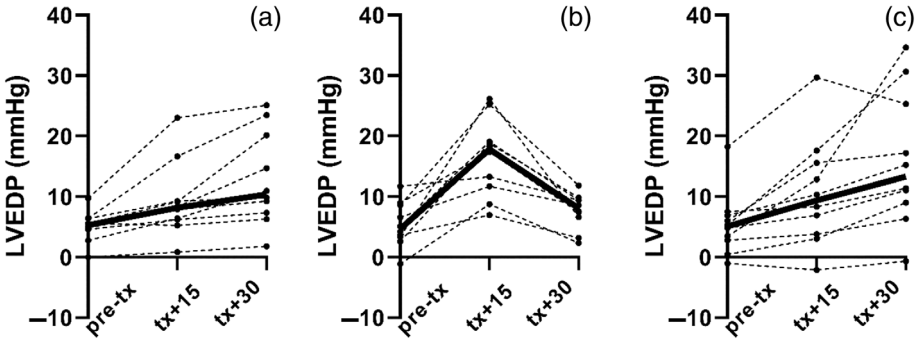

FIGURE 1 Left ventricular end-diastolic pressure (LVEDP) during transfusion. LVEDP values for all individual animals during transfusion (dotted lines) and median values per group (bold lines). (a) Standard volume lyophilized plasma ($n = 10$), (b) volume-reduced lyophilized plasma ($n = 10$) and (c) solvent-detergent pooled plasma ($n = 10$).

Transfusion with LP-VR did not decrease ΔLVEDP

LVEDP did not differ between groups pre-transfusion (median 5.1 mmHg [3.4, 6.6]) and increased significantly in all groups during transfusion with a maximum at the end of the transfusion, $p < 0.001$, $p = 0.002$ and $p < 0.001$ in groups receiving LP-S, LP-VR or SDP, respectively (transfusion of LP-VR ending after 15 min vs. 30 min in the SDP and LP-S groups). Post-transfusion LVEDP did not differ significantly between groups when all groups were compared ($p = 0.47$, Figure 1). ΔLVEDP was similar after 4 units of LP-S or SDP transfusion, 6.3 mmHg (2.9, 13.4) and 7.7 mmHg (4.5, 10.5), respectively ($p = 0.60$). There was a larger increase in ΔLVEDP after two units of LP-VR transfusion, 11.9 mmHg (5.9, 15.6). However, this was not significantly different from either the LP-S or the SDP group ($p = 0.29$ or 0.55, respectively). (Figure 2).

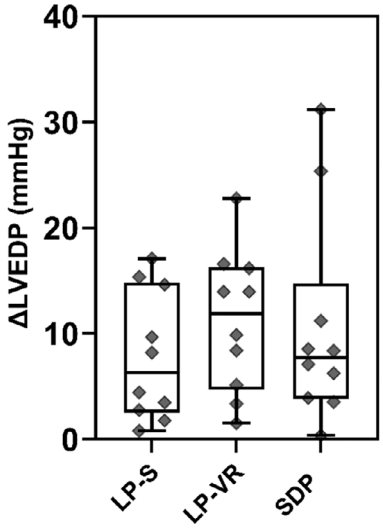

FIGURE 2 Left ventricular end-diastolic pressure (ΔLVEDP). Individual values for ΔLVEDP, boxplots display medians, interquartile ranges and min/max values. Peri-transfusion ΔLVEDP values were calculated as the post-transfusion LVEDP subtracted by the pre-transfusion LVEDP. The post-transfusion measurement for the standard volume lyophilized plasma (LP-S, $n = 10$) and solvent-detergent pooled plasma (SDP, $n = 10$) group was 30 min after start of transfusion, and for the volume-reduced lyophilized plasma (LP-VR, $n = 10$) group, it was 15 min after start of transfusion.

Post-transfusion haemodynamics were not significantly different between groups

Baseline haemodynamic variables did not differ between groups (Table 2). During transfusion, MAP increased significantly within all groups ($p = 0.002$, $p = 0.002$ and $p < 0.001$ in groups receiving LP-S,

TABLE 2 Peri-transfusion haemodynamics

Pre-transfusion	LP-S, $n = 10$ (median, IQR)	LP-VR, $n = 10$ (median, IQR)	SDP, $n = 10$ (median, IQR)
Heart rate (bpm)	305 (280, 330)	280 (265, 317)	297 (277, 334)
MAP (mmHg)	75 (69, 87)	73 (69, 80)	77 (71, 81)
CVP (mmHg)	1.4 (0.8, 2.0)	1.4 (1.0, 2.0)	1.8 (1.2, 3.1)
LVEDP (mmHg)	5.3 (4.7, 6.2)	4.7 (3.5, 8.1)	5.1 (3.0, 6.7)
Cardiac output (ml/min)	17 (14, 24)	19 (15, 22)	20 (16, 30)
15 min after start of transfusion			
Heart rate (bpm)	311 (280, 340)	285 (277, 302)	304 (290, 352)
MAP (mmHg)	105 (81, 118)	109 (95, 127)	121 (102, 133)
CVP (mmHg)	NA	3.0 (2.5–4.0)	NA
LVEDP (mmHg)	8.2 (6.3, 9.3)	17.8 (12.1, 19.0)	9.4 (4.6, 14.9)
Cardiac output (ml/min)	20 (16, 26)	24 (19, 30)	27 (23, 33)
30 min after start of transfusion			
Heart rate (bpm)	333 (284, 339)		346 (311, 375)
MAP (mmHg)	132 (90, 142)		126 (112, 147)
CVP (mmHg)	1.5 (1.0, 2.4)		1.5 (1.1, 2.0)
LVEDP (mmHg)	10.4 (7.8, 18.8)		13.3 (9.5, 23.3)
Cardiac output (ml/min)	19 (16, 24)		25 (16, 31)

Abbreviations: bpm, beats per minute; CVP, central venous pressure; IQR, interquartile range; LP-S, standard volume lyophilized plasma; LP-VR, volume-reduced lyophilized plasma; LVEDP, left ventricular end-diastolic pressure; MAP, mean arterial pressure; NA, not applicable; SDP, solvent-detergent pooled plasma.

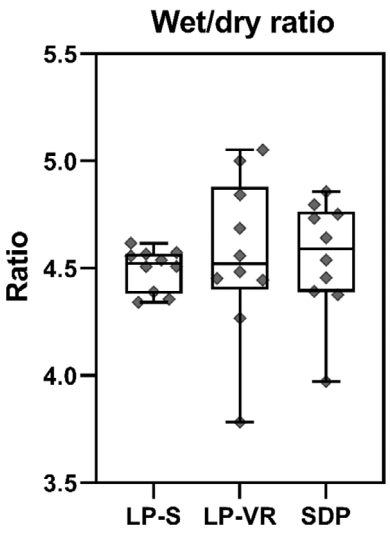

FIGURE 3 Pulmonary wet/dry weight ratio. Individual values for lung wet/dry weight ratio, boxplots display medians, interquartile ranges and min/max values. LP-S, standard volume lyophilized plasma ($n = 9$); LP-VR, volume-reduced lyophilized plasma ($n = 9$); SDP, solvent-detergent pooled plasma ($n = 10$).

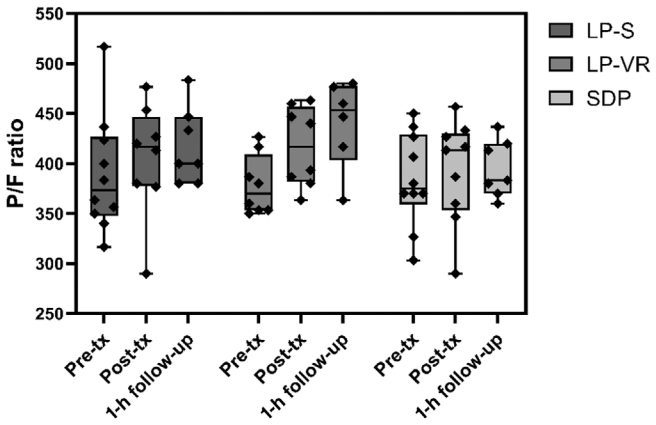

FIGURE 4 PaO_2/FiO_2 ratio (P/F ratio). Individual values for PaO_2/FiO_2 ratio during transfusion and follow-up; black lines display medians and interquartile ranges. LP-S, standard volume lyophilized plasma ($n = 9$); LP-VR, volume-reduced lyophilized plasma ($n = 9$); SDP, solvent-detergent pooled plasma ($n = 10$).

LP-VR or SDP, respectively). Heart rate increased significantly after SDP ($p < 0.001$), but increases in groups receiving LP-S or LP-VR were non-significant ($p = 0.150$ and 0.058, respectively). Hypertension and tachycardia are cardiovascular symptoms in line with TACO's diagnostic criteria. There was no difference in either heart rate or MAP between groups.

Reducing transfusion volume did not reduce pulmonary oedema, histopathological pulmonary injury or PaO_2/FiO_2 ratio

The median pulmonary wet/dry weight ratio was 4.52 (4.42, 4.56) in the LP-S group, 4.52 (4.45, 4.80) in the LP-VR group and 4.59 (4.41, 4.75) in the SDP group ($p = 0.685$) (Figure 3). The median pathology scores for pulmonary injury were 2.0 (2.0, 2.0), 1.0 (1.0, 2.0) and 1.0 (1.0, 1.75) for the group receiving LP-S, LP-VR and SDP, respectively

($p = 0.072$). PaO_2/FiO_2 ratios for the baseline, pre-transfusion, post-transfusion and 1-h follow-up did not differ between groups. They are displayed in Figure 4. At termination, the PaO_2/FiO_2 ratio was 400 mmHg (390, 440) in the LP-S group, 453 mmHg (424, 473) in the LP-VR group and 383 mmHg (375, 417) in the SDP group ($p = 0.155$).

DISCUSSION

Volume overload is seen as a central pathway in the development of TACO; therefore, we hypothesized that concentrated volume-reduced lyophilized plasma would reduce pulmonary capillary pressure and could help preventing TACO. However, our results do not show a reduction in pulmonary capillary pressure after transfusion with LP-VR compared to regular volume plasma products like LP-S or SDP. Also, there was no effect from volume-reduced plasma on pulmonary injury.

Previously, our group developed a two-hit animal model to study the underlying pathophysiology of TACO [17]. In the present experiment, this model was used to study the effect of a volume-reduced plasma product on the development of TACO. Our model was executed successfully as hydrostatic pulmonary capillary pressure (ΔLVEDP) was previously shown to increase significantly in the transfusion groups compared to crystalloid fluids [14]. Also, ΔLVEDP was greater than 4 mmHg in all groups receiving plasma products, which was the predefined threshold for clinical relevance [15]. Most importantly, the volume reduction of the LP-VR product led to a significantly reduced total fluid input in that group. There were no significant differences found between groups receiving LP-S or SDP, indicating that the lyophilized plasma product by itself does not affect TACO development in this animal model.

Interestingly, no differences were found in pulmonary capillary pressure between the three different transfusion products. Although the LP-VR group had a significantly lower volume administered compared to the other groups, this did not lead to a decrease in ΔLVEDP. These results suggest that TACO is more complex than originally thought and that volume overload alone is not the most important factor in rising pulmonary capillary pressure after plasma transfusion in this model. LP-VR is hyperoncotic and therefore able to increase the intravascular colloid osmotic pressure, preventing extravasation of fluids and possibly recruiting volume from the extravascular compartment [21, 22]. While volume recruitment was not an unexpected effect of hyperoncotic LP-VR, the equivalent increase in pulmonary capillary pressure following a low-volume product was. Previous in vitro experiments suggested a minimal volume recruitment, however, previous animal experiments support the observation that colloid osmotic pressure might be involved in rising pulmonary capillary pressure [14, 21]. However, it is unlikely that colloid osmotic pressure alone is responsible for the development of TACO, since we have shown previously a rise in pulmonary capillary pressure after transfusion of red blood cell products and these have a low colloid osmotic pressure [17, 21]. In these cell-containing products, storage lesion could lead to NO scavenging substances, which causes vasoconstriction and rising pulmonary capillary pressure [23]. Other mechanisms that might be involved in TACO development are inflammation and damage of the glycocalyx as a result of the transfusion, however, this needs to be investigated further [6, 7, 24].

Important criteria for TACO are either respiratory compromise or pulmonary oedema [1]. In the present study, we did not find significant differences between groups in any of the markers for acute lung injury, that we measured. An observation that was also seen in previous experiments with this model, where there was no significant increase of pulmonary injury after transfusion [14, 17]. Because there is no additional pulmonary injury, LP-VR cannot reduce it. This absence of transfusion-related pulmonary injury could possibly be explained by the fact that LVEDP does not increase enough to start extravasation of fluids and cause hydrostatic pulmonary oedema. A study in dogs showed that a minimum left atrial pressure of 24 mmHg was required to develop pulmonary oedema [25]. This threshold decreased when the plasma protein concentration was reduced. However, in our experiment we administered plasma proteins, and in the LP-VR group, this was a concentrated hyperoncotic plasma product. This could have possibly raised the pressure threshold for fluid extravasation and prevented pulmonary oedema formation in the LP-VR group, despite a higher LVEDP.

There were some limitations to this study. First, this experiment was part of a multi-armed randomized controlled trial and was not specifically powered to review the effects of low-volume lyophilized plasma. However, since results show a trend towards a harmful effect from the volume-reduced plasma product on pulmonary capillary pressure, it seems highly unlikely that a beneficial effect would have been found in a larger sample size. Second, this experiment was performed with animals that were young and healthy aside from an induced acute myocardial infarction. This situation is not completely translatable from the clinical situation, where TACO patients are more often older with different comorbidities, like renal failure or other chronic cardiovascular problems [3, 5, 9]. Moreover, the fact that these animals did not have an impaired kidney function could contribute to their ability to process fluids and reduce the benefits of a low-volume transfusion product. Also, we transfused human plasma products in rats. This had the advantage that we could study different plasma products that are not available from rat donors like SDP. On the other hand, the use of cross species blood products could lead to a less translatable model. However, human plasma products have been successfully transfused in animal experiments using rats or mice in several other studies [26, 27]. Lastly, as mentioned before, this experimental model lacks pulmonary oedema or respiratory distress, which is the primary clinical symptom of TACO. This was also seen in previous experiments with red blood cells and other plasma products [14, 17]. The model is very suitable for researching changes in pulmonary capillary pressure after transfusion, but the absence of lung injury and oedema limits the possibility to research pathways leading to TACO-like inflammation or storage lesion of blood products [23, 24, 28].

Our study showed no effect on pulmonary capillary pressure from a volume-reduced lyophilized plasma product. We could not research the effect on pulmonary oedema, due to an absence of pulmonary oedema caused by plasma transfusion. Future studies should focus on examining the effect of volume-reduced plasma in a model with pulmonary oedema but should also explore other possible preventative strategies for TACO after plasma transfusion. For example, alternative products for plasma transfusion like prothrombin complex concentrates have been shown to be superior to plasma to restore coagulation [29]. This product and other alternative products could be tested to review their effect on pulmonary capillary pressure and their capability to be an effective preventative strategy for TACO. In conclusion, we wanted to investigate if volume-reduced lyophilized plasma could reduce volume overload and attenuate TACO. In this randomized experimental study in a two-hit rat model, pulmonary capillary pressure increased in all groups during transfusion. A hyperoncotic volume-reduced lyophilized plasma product did not have a beneficial effect but resulted in a trend of larger increase in pulmonary capillary pressure. These data suggest that a volume-reduced plasma product did not attenuate pulmonary capillary pressure required to develop circulatory overload and was therefore not an effective preventative strategy for TACO in this animal model. More research is required to obtain insights into the TACO pathophysiology, in order to identify targetable pathways for preventing TACO.

ACKNOWLEDGEMENTS

This research was funded by a Landsteiner Foundation for Blood Research (LSBR) fellowship grant to A.P.J.V. (number 1931F) from the Landsteiner Foundation for Blood Transfusion Research, Haarlem, The Netherlands.

E.B. designed the study, performed the research, analysed the data and wrote the first draft of the manuscript. R.K. designed the study, supervised the research and reviewed and edited the manuscript. M.W. performed the research and reviewed and edited the manuscript. J.R. performed the research and reviewed and edited the manuscript. D.V. assisted in interpretation of the data and reviewed and edited the manuscript. C.B. assisted in interpretation of the data and reviewed and edited the manuscript. R.K. assisted in interpretation of the data and reviewed and edited the manuscript. A.V. designed the study, supervised the research and reviewed and edited the manuscript.

CONFLICT OF INTEREST

This research was funded by a Landsteiner Foundation for Blood Research grant. The funding body was in no way involved in the study design, collection, analysis and the interpretation of data, nor in writing the manuscript. All other authors declare no conflict of interest.

ORCID

Esther B. Bulle https://orcid.org/0000-0002-1578-2813
Robert B. Klanderman https://orcid.org/0000-0001-5820-4530

REFERENCES

1. Schipperus MR, Wiersum-Osselton JC, Group I-I-ATDR. Updated definitions for respiratory complications of blood transfusion. Transfusion. 2019;59:2482–3.
2. Clifford L, Jia Q, Yadav H, Subramanian A, Wilson GA, Murphy SP, et al. Characterizing the epidemiology of perioperative transfusion-associated circulatory overload. Anesthesiology. 2015;122:21–9.
3. Lieberman L, Maskens C, Cserti-Gazdewich C, Hansen M, Lin Y, Pendergrast J, et al. A retrospective review of patient factors, transfusion practices, and outcomes in patients with transfusion-associated circulatory overload. Transfus Med Rev. 2013;27:206–18.
4. Narayan S, Poles ea D. on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2021 Annual SHOT Report. 2022. [cited 2022 Dec 5]. Available from: https://www.shotuk.org/shot-reports
5. Bosboom JJ, Klanderman RB, Zijp M, Hollmann MW, Veelo DP, Binnekade JM, et al. Incidence, risk factors, and outcome of transfusion-associated circulatory overload in a mixed intensive care unit population: a nested case-control study. Transfusion. 2018;58:498–506.
6. Semple JW, Rebetz J, Kapur R. Transfusion-associated circulatory overload and transfusion-related acute lung injury. Blood. 2019;133:1840–53.
7. Bulle EB, Klanderman RB, Pendergrast J, Cserti-Gazdewich C, Callum J, Vlaar APJ. The recipe for TACO: a narrative review on the pathophysiology and potential mitigation strategies of transfusion-associated circulatory overload. Blood Rev. 2021;52:100891.
8. Frazier SK, Higgins J, Bugajski A, Jones AR, Brown MR. Adverse reactions to transfusion of blood products and best practices for prevention. Crit Care Nurs Clin North Am. 2017;29:271–90.
9. Clifford L, Jia Q, Subramanian A, Yadav H, Schroeder DR, Kor DJ. Risk factors and clinical outcomes associated with perioperative transfusion-associated circulatory overload. Anesthesiology. 2017;126:409–18.
10. Roubinian NH, Hendrickson JE, Triulzi DJ, Gottschall JL, Michalkiewicz M, Chowdhury D, et al. Contemporary risk factors and outcomes of transfusion-associated circulatory overload. Crit Care Med. 2018;46:577–85.
11. Garrigue D, Godier A, Glacet A, Labreuche J, Kipnis E, Paris C, et al. French lyophilized plasma versus fresh frozen plasma for the initial management of trauma-induced coagulopathy: a randomized open-label trial. J Thromb Haemost. 2018;16:481–9.
12. Mok G, Hoang R, Khan MW, Pannell D, Peng H, Tien H, et al. Freeze-dried plasma for major trauma - systematic review and meta-analysis. J Trauma Acute Care Surg. 2021;90:589–602.
13. Percie du Sert N, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, et al. Reporting animal research: explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol. 2020;18:e3000411.
14. Bulle EB, Klanderman RB, de Wissel MB, Roelofs JJTH, Veelo DP, van den Brom CE, et al. The effect of plasma transfusion in an experimental two-hit animal model of transfusion-associated circulatory overload with heart failure. Blood Transfus. 2022.
15. Nand N, Gupta SP, Gupta MS. Hemodynamic evaluation of blood transfusion in chronic severe anemia with special reference to speed of transfusion. Jpn Heart J. 1985;26:759–65.
16. Klanderman RB, Wijnberge M, Bosboom JJ, Roelofs J, de Korte D, van Bruggen R, et al. Differential effects of speed and volume on transfusion-associated circulatory overload: a randomized study in rats. Vox Sang. 2022;117:371–8.
17. Klanderman RB, Bosboom JJ, Maas AAW, Roelofs J, de Korte D, van Bruggen R, et al. Volume incompliance and transfusion are essential for transfusion-associated circulatory overload: a novel animal model. Transfusion. 2019;59:3617–27.
18. Verel D, Stentiford NH. Comparison of left atrial pressure and wedge pulmonary capillary pressure. Pressure gradients between left atrium and left ventricle. Br Heart J. 1970;32:99–102.

19. Pacher P, Nagayama T, Mukhopadhyay P, Batkai S, Kass DA. Measurement of cardiac function using pressure-volume conductance catheter technique in mice and rats. Nat Protoc. 2008;3:1422–34.
20. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012;9:671–5.
21. Klanderman RB, Bosboom JJ, Korsten H, Zeiler T, Musson REA, Veelo DP, et al. Colloid osmotic pressure of contemporary and novel transfusion products. Vox Sang. 2020;115:664–75.
22. Woodcock TE, Woodcock TM. Revised Starling equation and the glycocalyx model of transvascular fluid exchange: an improved paradigm for prescribing intravenous fluid therapy. Br J Anaesth. 2012;108:384–94.
23. Berra L, Pinciroli R, Stowell CP, Wang L, Yu B, Fernandez BO, et al. Autologous transfusion of stored red blood cells increases pulmonary artery pressure. Am J Respir Crit Care Med. 2014;190:800–7.
24. Bosboom JJ, Klanderman RB, Migdady Y, Bolhuis B, Veelo DP, Geerts BF, et al. Transfusion-associated circulatory overload: a clinical perspective. Transfus Med Rev. 2019;33:69–77.
25. Guyton AC, Lindsey AW. Effect of elevated left atrial pressure and decreased plasma protein concentration on the development of pulmonary edema. Circ Res. 1959;7:649–57.
26. Chipman AM, Wu F, Pati S, Burdette AJ, Glaser JJ, Kozar RA. Fresh frozen plasma attenuates lung injury in a novel model of prolonged hypotensive resuscitation. J Trauma Acute Care Surg. 2020;89:118–25.
27. Letourneau PA, McManus M, Sowards K, Wang W, Wang YW, Matijevic N, et al. Aged plasma transfusion increases mortality in a rat model of uncontrolled hemorrhage. J Trauma. 2011;71:1115–24.
28. Blumberg N, Heal JM, Gettings KF, Phipps RP, Masel D, Refaai MA, et al. An association between decreased cardiopulmonary complications (transfusion-related acute lung injury and transfusion-associated circulatory overload) and implementation of universal leukoreduction of blood transfusions. Transfusion. 2010;50:2738–44.
29. Chai-Adisaksopha C, Hillis C, Siegal DM, Movilla R, Heddle N, Iorio A, et al. Prothrombin complex concentrates versus fresh frozen plasma for warfarin reversal. A systematic review and meta-analysis. Thromb Haemost. 2016;116:879–90.

How to cite this article: Bulle EB, Klanderman RB, de Wissel MB, Roelofs JJTH, Veelo DP, van den Brom CE, et al. Can volume-reduced plasma products prevent transfusion-associated circulatory overload in a two-hit animal model? Vox Sang. 2023;118:185–92.

Received: 5 October 2022 | Revised: 7 December 2022 | Accepted: 16 December 2022

DOI: 10.1111/vox.13397

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Non-severe allergic transfusion reaction: A hidden cause of wastage of blood product and laboratory resources

Hasan Hayat | **Qadeer Ahmed** | **Hareem Alam** | **Anila Rashid**

Section of Haematology & Transfusion Medicine, Department of Pathology and Laboratory Medicine, Aga Khan University Hospital, Karachi, Pakistan

Correspondence
Anila Rashid, Department of Pathology and Laboratory Medicine, Aga Khan University Hospital, Stadium Road, Karachi, 74800, Pakistan.
Email: anila.rashid@aku.edu

Funding information
The authors received no specific funding for this work.

Abstract

Background and Objectives: Blood transfusions are often needlessly aborted following a non-severe allergic reaction despite responding well to medication resulting into partial transfusion of the implicated blood product. This results in the wastage of untransfused blood component and resources spent on unnecessary laboratory work-up of these reactions.

Materials and Methods: We aimed to review the amount of blood product and laboratory resource wastage associated with non-severe allergic transfusion reaction (ATR) in a tertiary care hospital.

Results: A total of 174,632 blood products were released and transfused during the study period (2019–2021). There were 336 adverse transfusion reactions with an estimated rate of 1.9 per 1000 blood products administered. Of 336, 145 (43%) were ATR, of which 141 (97%) were non-severe and 4 (3%) were severe. The most commonly associated symptom was found to be urticaria in 31 (22%). All non-severe ATR completely resolved with medication. Seventy-nine percent of the transfusions associated with non-severe ATRs were aborted, of which 37% were followed by additional transfusions. The estimated loss of blood product volume and the cost of non-severe ATR (including transfusion reaction work-up, discarded blood product and additional transfusion) was 11,185 ml (11 L) and Pakistani rupees 1,831,546 ($11,592.06 or €8598.78), respectively.

Conclusion: Non-severe ATR was found to be associated with a significant proportion of laboratory resource wastage and that of blood product in our institution. Revision of institutional guidelines for management and lab work-up of transfusion reactions would be helpful in alleviating this unnecessary loss in a resource-constraint transfusion-setting.

Keywords

allergic, audit, blood wastage, transfusion reaction

Highlights

- Non-severe allergic transfusion reactions (ATRs) are common and often lead to unnecessary complete termination of transfusion despite the patient responding well to medication.
- In a retrospective study, we found that non-severe ATRs led to partial transfusions, with a total loss of 11-L untransfused blood volume and a significant amount of time and cost spent on unnecessary extensive evaluation of these reactions.
- Regular audits, training of staff and revision of policies can alleviate blood product wastage and laboratory resources.

INTRODUCTION

Blood product utilization is expensive for hospitals and for patients in private settings. It not only includes the cost of the blood bag but also incorporates the indirect overhead cost of equipment and direct overhead cost of laboratory testing, including the transfusion scientists and nursing staff required for safe preparation and transfusion of blood product [1]. Therefore, every possible effort should be made to keep blood product wastage to a minimum.

The most common reasons of blood product wastage include improper storage and transport, or products being dispensed but not administered [2, 3]. But the amount of blood components wasted due to transfusion reactions are not well-characterized and not widely studied [4]. Transfusion reactions can vary from being mild to severe life-threatening and in most cases necessitates stopping the transfusion followed by ultimate discard of the implicated blood product [5].

Non-severe allergic transfusion reactions (ATRs), defined as allergic reaction without anaphylaxis or cardiopulmonary compromise are commonly encountered with platelets and plasma transfusion with a reported frequency of 2% and less commonly with red cell with a frequency of 0.1%–0.5% [6–8].

The true prevalence is however not known as these are likely to be under-reported due to their presentation with mild symptoms [9]. ATRs are immunoglobulin-E-mediated hypersensitivity reactions caused by either transfusion recipient's antibody reacting against donor plasma proteins or due to passive transfer of antibody to recipient with subsequent allergen exposure [7].

In most cases, it presents with cutaneous symptoms like rash, urticaria or pruritus and often respond well to anti-allergic medication, which is used as a first-line treatment either with or without steroids [10]. Therefore, resumption of transfusion under direct supervision is a standard practice in non-severe ATR [6]. Resuming and completing transfusion after treatment-responsive non-severe ATR not only prevents unnecessary wastage of the blood product and expense but also prevents additional transfusions, donor exposure and risk of alloimmunization [6]. Despite various published guidelines on the management of ATR, a recent study by Kasim et al. reported high rate of incomplete transfusions and discard rate with non-severe ATR costing the institute an annual loss of $16,500–20,125 for ATR work-up alone [11].

In 2013, a study conducted at our institute on non-infectious complications of transfusion over a period of seven years, reported 458 adverse events with an estimated rate of 1.16 per 1000 blood products, of which non-severe ATR accounted for 57.8% followed by febrile non-haemolytic transfusion reactions (FNHTR) (35.5%) [12].

Since the reported frequency of non-severe ATR was reported to be high in our institution, we aimed to assess how many of such reactions resulted in termination of transfusions leading to wastage of blood product and loss of laboratory resources in the evaluation of these reactions.

MATERIALS AND METHODS

Setting and study design

The study was conducted at the Aga Khan University Hospital, Pakistan, which is a 700-bed academic medical centre with the facilities for trauma, surgery, haematopoietic stem cell transplant and a day-care unit where blood transfusion is given to patients with cancer, haemoglobinopathy and other disorders. The blood units are dispensed only after serological and nucleic acid amplification testing for common transfusion-transmissible infections. The blood products are non-leucoreduced due to cost constraints. However, bedside filters are used for leucoreduction when required.

All blood product requisitions are made through computerized physician order entry. All transfusion reactions are reported to blood bank on a standardized transfusion reaction form. Once reported, the blood bank staff does a complete work-up including clerical check, a visual check for haemolysis, repeat ABO group and compatibility testing on pre- and post-donation samples, a direct antiglobulin test, complete blood count with peripheral blood smear, urine analysis for haemoglobinuria and microbiological culture of the returned bag where indicated. Additional testing depends on the type of reaction suspected. The transfusion reaction is then reviewed and analysed by the haematologist and recommendations for future transfusions are made and documented in the patient's medical record to be reviewed by the primary doctor.

All transfusion reactions are managed under direct supervision of a trained doctor, once the blood recipient complaints of any symptoms, the transfusion is immediately stopped, followed by patient identity, vital signs and clerical check. If the sign and symptoms are suggestive of a non-severe ATR, it is treated first with antihistamines either with or without steroids followed by re-initiation of transfusion at a slow-infusion rate and the patient is monitored more frequently until the transfusion is completed.

Data collection and analysis

The data of transfusion reactions were retrieved from archived records between January 2019 and December 2021. The data extracted from each transfusion reaction included: patients' characteristics, type of blood product transfused, irradiated versus non-irradiated blood products, type of transfusion reaction, the severity of allergic reaction (without anaphylaxis or cardiopulmonary compromise), symptoms, the volume transfused or discarded and whether the transfusion was partial (partially transfused) or complete (entire unit completely transfused), medications used to treat symptoms; and finally whether or not the symptoms resolved with treatment.

All transfusion reactions were classified using Association for the Advancement of Blood & Biotherapies criteria [13]. Additionally, transfusion reaction was termed as 'Not categorized' when the sign or symptoms were relatable to transfusion but cannot be further classified due to either incomplete or missing clinical details or incomplete

radiological and laboratory work-up. The term 'Unrelated to transfusion' was used when the reported sign or symptoms were present prior to the initiation of transfusion at some stage during admission and could be explained by patient's underlying illness rather than strictly related to transfusion.

In this study, to assess the validity of physician's judgement to abort transfusion due to an ATR, we also applied Centers for Disease Control and Prevention grading and attribution criteria for non-severe ATR and divided it further into 'probable' if the patient experienced just one symptom and 'definitive' in case of at least two or more symptoms [14].

Finally, to estimate the loss of laboratory resources associated with non-severe ATR, we calculated the expenditure done on evaluation and analysis of these reactions. In our institution, a complete transfusion work-up, which includes laboratory testing, technologist's time and haematologist's professional fees for interpretation, can cost up to an amount of Pakistani rupees (PKR) 8000–10,000 ($50.63–63.29 or €37.56–46.95). We also estimated the cost of wasted blood product as well as the cost of any additional blood transfusions that occurred within 48 h of the first incomplete transfusion due to non-severe ATR.

The results were expressed as mean ± SD for quantitative variables and qualitative variables are presented as frequency and percentages. Data were analysed using Statistical Package for the Social Sciences, version 21.

Ethical clearance

The study was reviewed and approved by institutional ethical review committee (ERC # 2022-7833-22202).

RESULTS

A total of 174,632 (58,211 annual) blood products were released and transfused over a period of three years, which included $n = 66{,}740$ packed red blood cell (PRBC) units, $n = 63{,}014$ platelets units (whole blood derived and apheresis), $n = 26{,}941$ plasma units and $n = 8707$ cryoprecipitate units.

A total of 336 transfusion reactions were reported with an estimated rate of 1.9 per 1000 blood product. Of 336, 145 (43%) were ATR followed by FNHTR in 124 (38%), transfusion-associated circulatory overload in 6 (2%), hypotensive in 4 (1%), transfusion-associated dyspnoea in 2 (0.6%), transfusion-associated acute lung injury in 1 (0.3%) and haemolytic reaction in 1 (0.3%) transfusion. Thirty-five (10%) transfusion reactions were 'not categorized' while 15 (5%) were found to be 'unrelated to transfusion'.

Of 145 ATRs, 141 (97%) were found to be non-severe in 136 unique patients and 4 (3%) were severe anaphylactic. The symptoms of non-severe ATR included urticaria in 31 (22%), maculopapular rash in 10 (7%), pruritus in 28 (20%), generalized flushing in 6 (4%) and a combination of more than one of these symptoms in 66 (47%) patients hence accounting for a total of 47% of 'definitive' non-severe ATR.

TABLE 1 Patients' characteristics having non-severe allergic reaction

Mean age (years)	**32.6 ± 21.6**
Male/female (*n*)	62/81
Blood group, *n* (%)	
A	38 (27)
B	45 (33)
O	44 (31)
AB	14 (10)
Indications, *n* (%)	Total, $n = 134$
Anaemia	49 (37)
Cancer related	44 (33)
Bleeding	19 (14)
Thrombocytopenia	7 (5)
Trauma	6 (4)
Haemoglobinopathy	6 (4)
Coagulopathy	3 (2)
Implicated blood product, *n* (%)	
PRBC	106 (75)
Irradiated PRBC	14 (10)
Platelets	9 (6)
Plasma	7 (5)
Irradiated platelets	4 (3)
Cryoprecipitate	1 (1)
Treatment, *n* (%)	Total, $n = 126$
Antihistamines	38 (30)
Antihistamines + corticosteroids	34 (27)
Acetaminophen + corticosteroids	33 (26)
Corticosteroids	11 (9)
Acetaminophen + corticosteroids + antihistamines	7 (6)
Acetaminophen	3 (2)

Abbreviations: PRBC, packed red blood cell.

Table 1 shows patients' characteristics and indication of transfusions for patients who experienced non-severe ATR.

Seventy-five percent (106) of non-severe allergic reactions were associated with PRBC followed by irradiated PRBC and platelets (Table 1).

The details on the volume of blood product transfused and discarded were not documented with 17 ATR, therefore, analysis of blood product wastage was done on 126 non-severe ATRs, which showed that 79% ($n = 99$) of transfusions were terminated in 97 unique patients before completion resulting in 'partial transfusions' and wastage of blood products while, the rest of the 21% ($n = 27$) had 'completed transfusion'.

In 99 partial transfusions, the total volume of all products discarded and wasted was 11,185 ml (11 L) (mean 113 ± 62.5 ml), which makes a loss of up to 3.7 L annually, with an estimated cost of PKR

TABLE 2 Wastage of blood volume according to the type of blood product associated with non-severe allergic transfusion reaction

Type of blood product	Total amount wasted, in millilitre, (mean ± SD)
Packed red cells	9135 (119 ± 62)
Platelet	205 (34 ± 8)
Plasma	525 (87.5 ± 41)
Irradiated packed red cells	1320 (132 ± 60.8)

371,656 ($2352.25 or €1744.86), based on institutional negotiated rates of blood products that were used to determine the cost per millilitre of each blood component. The wastage of blood volume according to type of blood product is shown in Table 2.

Information on whether patients received bedside leucodepletion was not provided with any of the reported transfusion reactions.

The details of treatment given were found for 126 non-severe ATRs. Majority of the patients ($n = 38$) received antihistamines as a single agent therapy, followed by patients who received a combination of antihistamines, acetaminophen, and corticosteroids (Table 1). All non-severe ATRs (100%) responded and settled with the medication given.

As per institutional protocol, a complete work-up was performed on all reported transfusion reactions. Therefore, for a total of 141 non-severe ATR work-ups, an approximate amount (adjusted for inflation) of PKR 966,150 ($6114.87 or €4535.92) was spent.

Of 99 partial transfusions, 37 (37%) resulted in additional transfusion of 80 blood products (44 red cell, 21 plasma and 15 random platelet units) within 48 h. The cost calculated for these additional components were PKR 493,740 ($3124.94 or €2318).

Hence, the total computed cost of non-severe ATR including the entire process (of transfusion reaction work-up, discarded blood product and additional transfusion) was PKR 1,831,546 ($11,592.06 or €8598.78).

DISCUSSION

Blood and blood components constitute a vital resource for any healthcare facility. The maintenance of un-interrupted supply and optimal inventory of the blood products always remained challenging for transfusion services. Moreover, coronavirus disease 2019 (COVID-19) pandemic has adversely affected donor recruitment due to pandemic-mediated lockdown and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in otherwise healthy potential donors [15–17]. Therefore, it is important to practice blood transfusion judiciously and avoid unwanted wastage of the blood products.

To date, few studies have highlighted and reported the impact of ATR on the wastage of blood products and lab resources [10, 11]. All transfusion reactions warrant pausing the transfusion, assessment of sign and symptoms and patient's identity check followed by either resumption or discard of remaining blood product in the bag depending on the severity of reaction and response to treatment. Non-severe ATR falls into one such category of transfusion reaction that presents with mild cutaneous symptoms and mostly responds well to medication, where it is reasonable to hold the transfusion briefly until the symptoms subside followed by completion of transfusion [6, 13].

We analysed non-severe ATRs at a large academic tertiary care hospital to gain an insight into its association with wastage of blood products and resources spent on work-up for these reactions. Additionally, we anticipated to identify areas of improvement for effective blood utilization and hence to reduce the wastage of valuable transfusion services related to ATRs.

In this study, we found that non-severe ATR was the most reported transfusion reaction in our institute and hence the most worked-up transfusion reaction. Additionally, 79% of transfusions associated with non-severe ATR were terminated and resulted in partial transfusion despite responding well to the treatment resulting in wastage of significant volume which otherwise would have been transfused to achieve the desired effect. Although the time it took for resolution of symptoms was not documented with any reported transfusion reaction, we assumed that this significant proportion of partial transfusions might have resulted from exceeding the 4-h limit for the completion of a blood product. Also, the high rate (53%) of 'probable' non-severe ATRs indicated that clinical staff are too sensitive and well-cognizant of transfusion reactions, but they might lack the understanding regarding the preferable option of pausing the transfusion, treating the patient and resuming transfusion on resolution of symptoms. This was also reflected by the low rate of complete transfusions that occurred in just 21%. Hence, the clinical staffs' knowledge, understanding and management of transfusion reaction was identified as an important aspect that needs improvement by regular education sessions.

A study conducted by Adkins et al. over a period of one year on symptomatology and wastage related to mild allergic reactions revealed similar findings. Mild cutaneous symptom (urticaria, 54.7%) was the most common, and the rate of partial transfusion (63.9%) and total volume wastage (6892.3 ml at a cost of $12,507.96) was high, which also explains the high frequency (55%) of repeat transfusions in their study [10]. Also in our study, out of 99 partial transfusions, 37% resulted in additional transfusions. However, we could not exclude the other possible clinical indications that could have also led to these repeat transfusions like haemoglobinopathies, persistent bleeding etc.

Another study by Kasim et al. [11] also found majority of the allergic reactions as non-severe (75%) presenting with urticaria as the most common symptom, resulting in termination and partial transfusion in 61% with an annual loss of about 2.9 L annually, which is comparable to what we have observed at our institution.

All non-severe ATRs in our study presented with cutaneous symptoms and responded well to anti-allergic medication, some also required combination treatment with steroids and acetaminophen. This rate of response to medication is in consensus with that published by Adkins et al. and Kasim et al. where they have observed 100% and 96% improvement in symptoms, respectively [10, 11]. This

again consolidates the fact that transfusion does not always need to be completely aborted for such reactions. Additionally, for patients who experience recurrent ATR, pre-medication or plasma reduction of red cells and platelets might be helpful for making the transfusion uninterrupted and uneventful [18].

ATRs are widely reported to be associated with plasma and platelets, but in this study ATRs were higher with red cells. Although the number of plasma and platelets released and transfused were more than that of red cells, it was assumed that the 'number of patients' who received red blood cell transfusions were approximately two times more than patients who received platelets or plasma, as was observed by the number of requests received for different blood products during the study period. Secondly, predisposition to ATR depends on both recipient and donor factors, which might explain that in this study it is the patients/recipients who received red cells were more prone to develop ATR [19, 20]. The history of prior ATR also increases the chance of recurrence irrespective of the type of blood product, since we did not have the patients' historical data on previous transfusion reactions, this could not be further consolidated.

There are various aspects of lab resources that constitutes the cost required to maintain and deliver the safe transfusion, and mostly includes the cost of lab equipment for collection and preparation of blood component, reagents, serological testing and so forth. Recently, the cost of unnecessary transfusion reactions work-up was highlighted by Kasim et al. [11] where the estimated annual cost of $16,500–20,125 was found to be spent on unnecessary lab work-up for non-severe ATR. We also found an additional unexpected and unnecessary cost of $6114.87 (€4535.92) for ATRs work-up. For low- and middle-income countries with limited resources of budget, consumables, donors and donor attendants, this is much more important to consider and evaluate this hidden cost associated with unnecessary lab work-up for transfusions reactions.

Apart from logistics, human resource including qualified and competent blood technical staff is critical for any transfusion services [21, 22]. A review by Chaffe et al. mentioned that about 53% of the blood bank is short of staff and this has been identified as a challenging situation to provide quality services with minimum error rate [23]. Needless to say, the situation at our blood bank is not any different. The unnecessary lab testing puts additional burden on the already understaffed transfusion services. Another pertinent aspect is the cost of staff hiring, which in many instances is the second greatest cost for any transfusion services, following the blood products themselves.

While analysing the results we also noticed that about 5% of the transfusion reactions were classified as 'Unrelated to transfusion' and hence identified as an additional and potential cause for incomplete transfusions and unnecessary lab testing, emphasizing the need for updating the clinical staff's knowledge of identifying and management of transfusion reactions. At present, when a transfusion reaction is reported at our institution, the technical staff performs a complete lab work-up for each reaction, before being reviewed by a haematologist. To minimize wastage of both blood products and laboratory technologists' time, we have planned to revise our workflow and criteria for transfusion reaction work-up. According to the revised protocol, the haematology resident or consultant will be notified first of any transfusion reaction, who will then review and suggest technologist the lab work-up that needs to be performed and excludes the serological tests (like direct antiglobulin test, red cell antibody screening and cross match) when cutaneous symptoms are not accompanied by fever, pain or cardiopulmonary symptoms. Secondly, teaching sessions of nurses/physicians will be arranged on regular intervals to update the knowledge about identification and management of transfusion reactions. It will also be reinforced through institutional blood transfusion policy that in case of mild allergic reactions with urticaria as the only symptom, transfusion with same blood unit can be re-challenged after giving antihistamine and resolution of symptom. Finally, we will present our findings and improvement strategies to hospital's blood utilization committee that will help us to implement the proposed strategy.

This is one of the most comprehensive studies on detailed cost analysis of wastage of transfusion service resources due to non-severe ATRs. However, there were few limitations with respect to the cost of medication for treating ATRs and extended hospital stay or repeated day-care admissions due to additional transfusions which were not included. So, the actual financial loss to both the transfusion service and patients might have been much more than that presented here. Also, the analysis in this study was done on transfusion reactions that were documented and reported and might not represent the actual number of reactions due to under reporting.

In conclusion, this study provides information about significant loss of blood product and cost attached with non-severe ATR work-up from a resource limited country.

We are now in the process of updating our protocol for transfusion reaction work-up and educational sessions on proper management of transfusion reaction to minimize the wastage. It will also be incorporated as a separate quality indicator and will be shared with all stakeholders regularly in the quarterly-held institutional blood utilization committee.

ACKNOWLEDGEMENTS

H.H. designed and supervised the research study, reviewed and edited the manuscript; H.A. and Q.A. acquired and analysed the data and wrote the first draft of the manuscript; A.R. designed the research study, acquired and analysed the data, wrote and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ORCID

Hareem Alam https://orcid.org/0000-0003-2716-0007
Anila Rashid https://orcid.org/0000-0003-3866-3902

REFERENCES

1. Shander A, Hofmann A, Ozawa S, Theusinger OM, Gombotz H, Spahn DR. Activity-based costs of blood transfusions in surgical patients at four hospitals. Transfusion. 2010;50:753–65.

2. Zoric L, Daurat G, Demattei C, Macheboeuf M, Boisson C, Bouix O, et al. Blood wastage reduction: a 10-year observational evaluation in a large teaching institution in France. Eur J Anaesthesiol. 2013;30:250–5.
3. Heitmiller ES, Hill RB, Marshall CE, Parsons BJ, Berkow LC, Barrasso CA, et al. Blood wastage reduction using Lean Sigma methodology. Transfusion. 2010;50:1887–96.
4. Collins RA, Wisniewski MK, Waters JH, Triulzi DJ, Yazer MH. Effectiveness of multiple initiatives to reduce blood component wastage. Am J Clin Pathol. 2015;143:329–35.
5. Delaney M, Wendel S, Bercovitz RS, Cid J, Cohn C, Dunbar NM, et al. Transfusion reactions: prevention, diagnosis, and treatment. Lancet. 2016;388:2825–36.
6. Tinegate H, Birchall J, Gray A, Haggas R, Massey E, Norfolk D, et al. Guideline on the investigation and management of acute transfusion reactions. Prepared by the BCSH Blood Transfusion Task Force. Br J Haematol. 2012;159:143–53.
7. Savage WJ, Tobian AA, Savage JH, Wood RA, Schroeder JT, Ness PM. Scratching the surface of allergic transfusion reactions. Transfusion. 2013;53:1361–71.
8. Goel R, Tobian AAR, Shaz BH. Noninfectious transfusion-associated adverse events and their mitigation strategies. Blood. 2019;133:1831–9.
9. Kracalik I, Mowla S, Basavaraju SV, Sapiano MRP. Transfusion-related adverse reactions: Data from the National Healthcare Safety Network Hemovigilance Module - United States, 2013-2018. Transfusion. 2021;61:1424–34.
10. Adkins BD, Lawicki S, Johnson M, Eichbaum Q. Mild allergic transfusion reactions: impact of associated clinical symptoms? Am J Clin Pathol. 2019;151:344–8.
11. Kasim J, Aldarweesh F, Connor JP. Blood product and laboratory resource wastage in non-severe allergic transfusion reactions: an opportunity for improvement. Transfus Med. 2019;29:338–43.
12. Karim F, Moiz B, Shamsuddin N, Naz S, Khurshid M. Root cause analysis of non-infectious transfusion complications and the lessons learnt. Transfus Apher Sci. 2014;50:111–7.
13. Crowder LA, Steele WR, Stramer SL. Infectious disease screening. In: Cohn CS, Delaney M, Johnson ST, Katz LM, editors. Technical manual. 20th ed. Bethesda: AABB Press; 2020. p. 173.
14. Centers for Disease Control and Prevention. National Healthcare Safety Network Biovigilance Component Hemovigilance Module Surveillance Protocol v2.6. Available from: www.cdc.gov/nhsn/pdfs/biovigilance/bv-hv-protocol-current.pdf. Accessed on 12 Aug 2022.
15. Ngo A, Masel D, Cahill C, Blumberg N, Refaai MA. Blood banking and transfusion medicine challenges during the COVID-19 pandemic. Clin Lab Med. 2020;40:587–601.
16. Rafiee MH, Kafiabad SA, Maghsudlu M. Analysis of blood donors' characteristics and deferrals related to COVID-19 in Iran. Transfus Apher Sci. 2021;60:103049.
17. Elnasser Z, Obeidat H, Amarin Z, Alrabadi N, Jaradat A, Alomarat D, et al. Prevalence of COVID-19 among blood donors: the Jordan University of Science and Technology experience. Medicine (Baltimore). 2021;100:e27537.
18. Tobian AA, Savage WJ, Tisch DJ, Thoman S, King KE, Ness PM. Prevention of allergic transfusion reactions to platelets and red blood cells through plasma reduction. Transfusion. 2011;51:1676–83.
19. Savage WJ, Hamilton RG, Tobian AA, Milne GL, Kaufman RM, Savage JH, et al. Defining risk factors and presentations of allergic reactions to platelet transfusion. J Allergy Clin Immunol. 2014;133:1772–5.
20. Savage WJ, Tobian AA, Fuller AK, Wood RA, King KE, Ness PM. Allergic transfusion reactions to platelets are associated more with recipient and donor factors than with product attributes. Transfusion. 2011;51:1716–22.
21. Bolton-Maggs P, Mistry H, Glencross H, Rook R, UK Transfusion Laboratory Collaborative. Staffing in hospital transfusion laboratories: UKTLC surveys show cause for concern. Transfus Med. 2019;29:95–102.
22. Novis DA, Nelson S, Blond BJ, Guidi AJ, Talbert ML, Mix P, et al. Laboratory staff turnover: a College of American Pathologists Q-probes study of 23 clinical laboratories. Arch Pathol Lab Med. 2020;144:350–5.
23. Chaffe B, Glencross H, Jones J, Staves J, Capps-Jenner A, Mistry H, et al. UK Transfusion Laboratory Collaborative: minimum standards for staff qualifications, training, competency and the use of information technology in hospital transfusion laboratories 2014. Transfus Med. 2014;24:335–40.

How to cite this article: Hayat H, Ahmed Q, Alam H, Rashid A. Non-severe allergic transfusion reaction: A hidden cause of wastage of blood product and laboratory resources. Vox Sang. 2023;118:193–8.

Received: 10 October 2022 | Revised: 9 December 2022 | Accepted: 26 December 2022

DOI: 10.1111/vox.13402

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

The impact of revised definitions for transfusion-associated circulatory overload and transfusion-related acute lung injury on haemovigilance reporting

Yin Yuan [1,2] | Peta M. Dennington [1] | James Daly [1] | Shoma Baidya [1] | John-Paul Tung [1,3,4]

[1]Clinical Services and Research, Australian Red Cross Lifeblood, Kelvin Grove, Australia

[2]WEHI, Melbourne, Australia

[3]Faculty of Health, Queensland University of Technology, Brisbane, Australia

[4]Faculty of Medicine, University of Queensland, Brisbane, Australia

Correspondence
John-Paul Tung, Australian Red Cross Lifeblood, 44 Musk Avenue, Kelvin Grove, Queensland 4059, Australia.
Email: jtung@redcrossblood.org.au

Present address
Yin Yuan, Faculty of Medicine, University of Queensland, Brisbane, Australia.

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

Abstract

Background and Objectives: Transfusion-associated circulatory overload (TACO) and transfusion-related acute lung injury (TRALI) are serious adverse transfusion reactions. Standardized surveillance definitions are important to ensure consistent reporting of cases. Recently, revised definitions have been developed for TACO and TRALI, the latter of which has not yet been widely implemented. This study aimed to assess the impact of the new TACO and TRALI definitions on haemovigilance reporting.

Materials and Methods: The Australian Red Cross Lifeblood Adverse Transfusion Reaction database was accessed to identify all cases of suspected or confirmed TACO and TRALI referred from 1 July 2015 to 30 June 2019. Cases were assessed against both the former and new definitions and the results were compared.

Results: A total of 73 cases were assessed. There were 48 TACO cases identified. Only 26 of 48 cases strictly met the former 2011 International Society of Blood Transfusion (ISBT) definition of TACO; 6 cases did not meet the definition and 16 cases lacked sufficient clinical details. In comparison, 46 cases met the revised 2018 ISBT definition, with only 2 cases having insufficient details. There were 24 cases of TRALI according to the existing 2004 Canadian Consensus Conference (CCC) definition compared with 25 cases according to the proposed 2019 revised definition.

Conclusion: The revised TACO definition captured more cases than the former definition. No significant differences were observed in the number of TRALI cases under the proposed new definition. This is the first study to provide validation data for the revised TRALI definition.

Keywords

haemovigilance, surveillance definition, TACO, TRALI

Highlights

- The 2018 International Society of Blood Transfusion (ISBT)/International Haemovigilance Network (IHN)/Association for the Advancement of Blood & Biotherapies (AABB) definition of transfusion-associated circulatory overload captures more cases than the former 2011 ISBT/IHN definition.

- There appears to be no significant difference in the number of transfusion-related acute lung injury (TRALI) cases under the proposed 2019 redefinition compared with the 2004 Canadian Consensus Conference definition currently in use.
- This is the first study to provide validation data for the proposed revised TRALI definition.

INTRODUCTION

Transfusion-associated circulatory overload (TACO) and transfusion-related acute lung injury (TRALI) are serious adverse transfusion reactions (ATRs) [1]. The most widely accepted definitions have been the 2011 International Society of Blood Transfusion (ISBT) and International Haemovigilance Network (IHN) TACO definition [2] and the 2004 Canadian Consensus Conference (CCC) TRALI definition [3] (Table 1). There is a continuous effort to improve and refine these definitions. Recently, the ISBT, in collaboration with the IHN and the Association for the Advancement of Blood & Biotherapies (AABB; formerly the American Association of Blood Banks), provided an updated definition of TACO [4] (Table 1). Similarly, a revised definition for TRALI was proposed by a consensus panel in 2019 [5] (Table 1).

TACO is the most common respiratory adverse event associated with transfusions and is the leading cause of transfusion-related mortality according to both the UK Serious Hazards of Transfusion (SHOT) [6] and the US Federal Drug Administration (FDA) [7]. The 2011 ISBT/IHN criteria defined TACO as four or more of the following occurring within 6 h of transfusion: (a) acute respiratory distress; (b) tachycardia; (c) elevated blood pressure; (d) acute or worsening pulmonary oedema of frontal chest radiograph and (e) evidence of positive fluid balance [2]. Although the definition was widely used, there were criticisms regarding its restrictive nature [8]. The new 2018 ISBT/IHN/AABB definition addressed these issues and has now been validated and widely adopted [4, 9]. The main changes included increasing onset time from 6 to 12 h, a broadening of supportive cardiovascular signs and formal inclusion of biomarkers.

TRALI incidence is difficult to ascertain due to under-recognition and under-reporting [8], with numbers ranging widely [10]. The reported mortality ranges between 5% and 24% [11]. The 2004 CCC definition defined TRALI as acute onset hypoxaemia and pulmonary oedema occurring within 6 h of transfusion in patients without evidence of left atrial hypertension and no pre-existing or alternative risk factor for acute lung injury (ALI) [3]. Patients with an alternative ALI risk factor were classified with possible TRALI [3]. An expert panel convened in 2019 to propose a revised definition to encompass our improved understanding of TRALI and align with the change in terminology from ALI to acute respiratory distress syndrome (ARDS) [5, 12]. This proposed a new nomenclature of TRALI Type I and TRALI Type II to replace TRALI and possible TRALI, respectively. Furthermore, patients with pre-existing ARDS but stable respiratory status in the 12 h prior to transfusion could be diagnosed with TRALI Type II, whereas they would have been excluded as having possible TRALI. There were also some minor deviations in the list of ARDS risk factors. Unlike the revised TACO definition, this new TRALI definition has not yet been widely implemented. The IHN indicated the need to investigate the suitability of the proposed changes for use in their haemovigilance practice [13].

The pathogenesis of TRALI remains incompletely understood [14]. The majority of TRALI cases (80%) are antibody-mediated (anti-human leucocyte antigen [HLA] class I, anti-HLA class II and anti-human neutrophil antigen [HNA]) [8, 15]. The remaining 20% of cases, in which antibodies are not implicated, are likely caused by 'biological response modifiers' that accumulate in blood components during storage [8, 16]. Blood services have employed various risk mitigation strategies, including leukodepletion, the use of male-only or nulliparous female donors for high plasma volume products and the deferral of donors who have been implicated in TRALI cases [11, 17]. Whilst TRALI remains a clinical diagnosis, the laboratory identification of antibodies assists in donor management decisions [18].

Australian Red Cross Lifeblood ('Lifeblood') is the national blood service provider for Australia. National haemovigilance arrangements require suspected TRALI cases to be reported to Lifeblood due to potential donor implications. Lifeblood is responsible for assessing TRALI cases, performing and interpreting laboratory investigations if required, and ensuring appropriate management of associated donors. The primary aim of this study was to assess the impact of new definitional changes to TACO and TRALI by conducting a retrospective review of cases of TACO and TRALI referred to Lifeblood. The secondary aim was to collate a TRALI case series and describe other aspects of these cases such as associated products, antibody identification and donor outcomes to improve our understanding of TRALI.

MATERIALS AND METHODS

The Australian Red Cross Lifeblood's Human Ethics Committee approved this study. The Lifeblood ATR database was accessed to identify cases of TACO and TRALI reported to Lifeblood from 1 July 2015 to 30 June 2019. The following information was collected: reaction date, patient age and gender, patient's pre-existing medical conditions, type of product implicated, the time of onset and clinical nature of the reaction. Specific clinical features pertaining to TACO and TRALI diagnostic criteria were collated, including absence/presence of pre-existing ARDS or risk factors for ARDS, stability of ARDS if pre-existing, nature and degree of respiratory distress, fluid status, response to diuretics, cardiovascular status, radiological changes, cardiac biomarkers and presence of left heart failure or elevated central venous pressure.

Each case was assessed against both the former and revised TACO or TRALI definitions. Potential outcomes included TACO,

TABLE 1 Comparison of former and revised transfusion-associated circulatory overload (TACO) and transfusion-associated acute lung injury (TRALI) definitions

TACO definitions	
2011 ISBT/IHN definition	**2018 ISBT/IHN/AABB definition**
TACO is characterized by any four of the following: • Acute respiratory distress • Tachycardia • Increased blood pressure • Acute or worsening pulmonary oedema on frontal chest radiograph • Evidence of positive fluid balance occurring within 6 h of completion of transfusion An elevated BNP is supportive of TACO.	Patients classified with TACO (surveillance diagnosis) should have acute or worsening respiratory compromise and/or evidence of pulmonary oedema (A and/or B below) during or up to 12 h after transfusion and presence of a total of 3 or more of the criteria described below: A. Acute or worsening respiratory compromise B. Evidence of acute or worsening pulmonary oedema based on ○ Clinical physical examination, and/or ○ Radiographic chest imaging and/or other non-invasive assessment of cardiac function C. Evidence for cardiovascular system changes not explained by the patient's underlying medical condition, including development of tachycardia, hypertension, widened pulse pressure, jugular venous distension, enlarge cardiac silhouette and/or peripheral oedema D. Evidence of fluid overload including any of the following: a positive fluid balance, response to diuretic therapy and change in the patient's weight in the peri-transfusion period E. Supportive result of a relevant biomarker (e.g., BNP or NT-pro BNP) above the age group-specific reference range and greater than 1.5 times the pre-transfusion value
TRALI definitions	
2004 CCC definition	**2019 revised definition**
TRALI a. ALI i. Acute onset ii. Hypoxaemia PaO_2/FiO_2 ≤ 300 mmHg[a] or SpO_2 < 90% on room air or other clinical evidence of hypoxaemia iii. Bilateral infiltrates on frontal chest radiograph iv. No evidence of LAH (i.e., circulatory overload) b. No pre-existing ALI before transfusion c. During or within 6 h of transfusion d. No temporal relationship to an alternative risk factor for ALI	*TRALI Type I* Patients who have no risk factors for ARDS and meet the following criteria a. ARDS i. Acute onset ii. Hypoxaemia iii. PaO_2/FiO_2 ≤ 300 mmHg[a] iv. or SpO_2 < 90% on room air v. Clear evidence of bilateral pulmonary oedema on imaging (e.g., chest radiograph, chest CT or ultrasound) vi. No evidence of LAH, or if LAH is present, it is judged not to be the main contributor to the hypoxaemia b. Onset during or within 6 h of transfusion c. No temporal relationship to an alternative risk factor for ARDS
Possible TRALI a. ALI b. No pre-existing ALI before transfusion c. During or within 6 h of transfusion d. A clear temporal relationship to an alternative risk factor for ALI	*TRALI Type II* Patients who have risk factors for ARDS or who have existing mild ARDS (PaO_2/FiO_2 of 200–300 mmHg[a]), but whose respiratory status deteriorates and is judged to be due to transfusion based on a. Findings as described in categorizes a and b of TRALI Type I and b. Stable respiratory status in the 12 h before transfusion
	TRALI/TACO Patients in whom TRALI cannot be distinguished from TACO or in whom both conditions occur simultaneously a. Clinical findings compatible with TRALI and with TACO and/or lack of data to establish whether or not significant LAH is present
ALI risk factors Direct • Pneumonia • Aspiration • Toxic inhalation • Lung contusion • Near drowning Indirect • Severe sepsis • Multiple trauma • Acute pancreatitis	*ARDS risk factors* Direct • Pneumonia • Aspiration of gastric contents • Inhalational injury • Pulmonary contusion • Pulmonary vasculitis Indirect • Nonpulmonary sepsis • Major trauma • Pancreatitis

(Continues)

TABLE 1 (Continued)

TRALI definitions	
2004 CCC definition	**2019 revised definition**
• Burn injury • Shock • Drug overdose • Cardiopulmonary bypass	• Severe burns • Noncardiogenic shock • Drug overdose

Abbreviations: AABB, American Association of Blood Banks; ALI, acute lung injury; ARDS, acute respiratory distress syndrome; BNP, brain natriuretic peptide; CCC, Canadian Consensus Conference; CT, computed tomography; IHN, International Haemovigilance Network; ISBT, International Society of Blood Transfusion; LAH, left atrial hypertension; PaO_2/FiO_2, ratio of partial pressure of oxygen to fraction of inspired oxygen; SpO_2, peripheral capillary oxygen saturation.

[a]If altitude is higher than 1000 m, a correction factor should be calculated as follows: $[(PaO_2/FiO_2) \times (\text{barometric pressure}/760)]$.

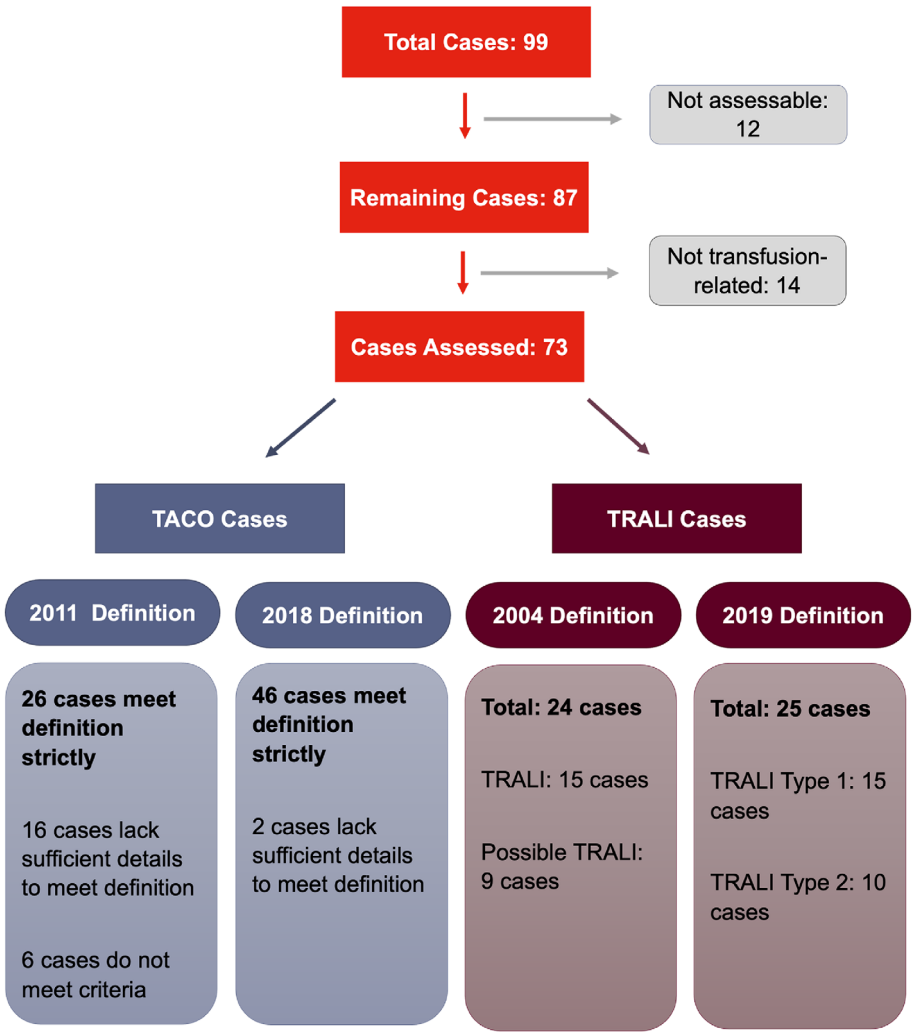

FIGURE 1 Flow chart illustrating assessment process and comparison of transfusion-associated circulatory overload (TACO) and transfusion-related acute lung injury (TRALI) cases based on the definitions used.

TRALI or TRALI Type I, possible TRALI or TRALI Type II, TACO/TRALI, an alternative ATR, or a non-transfusion-related event. Cases with insufficient information available for accurate assessment were noted as such. Cases that were challenging to classify underwent further independent assessment by two transfusion medicine specialists for final assignment.

For confirmed cases of TRALI, further information was collected, including antibody investigation results, age of implicated products,

TABLE 2 Baseline demographics of all patients and patients with transfusion-related acute lung injury (TRALI)

Attribute	TRALI, *n* (% of TRALI)	Total, *n* (% of total)
Gender		
Male	12 (50%)	48 (48.5%)
Female	11 (46%)	50 (50.5%)
Unknown	1 (4%)	1 (1.0%)
Age		
Age range	3 days–90 years	1 day–95 years
Median age	68.5 years	65 years
Paediatric cases (<16 years)		
Number of cases		8 (8%)
Age range		1 day–15 years
Median age		2 years
Patient's underlying condition		
Malignancy	7 (29.2%)	40 (40.4%)
Trauma	0 (0%)	1 (1.0%)
Surgery	7 (29.2%)	18 (18.2%)
Medical	10 (41.7%)	36 (36.4%)
Obstetrics	0 (0%)	2 (2.0%)
Other	0 (0%)	2 (2.0%)
Location		
ICU, intubated	2 (8.3%)	7 (7.1%)
ICU, not intubated	3 (12.5%)	10 (10.1%)
Non-ICU	19 (79.2%)	77 (77.8%)
Unknown	0 (0%)	5 (5.1%)

Abbreviation: ICU, intensive care unit.

associated donor demographics and donor outcomes. Statistical analysis was performed using Fisher's exact test. Significance was determined as $p < 0.05$.

TABLE 3 Product and donor details of transfusion-related acute lung injury (TRALI) cases

Products associated with TRALI cases		
Product	**Number of products**	**Average age of product (days)**
Red cells	25	17.1
Pooled platelets	6	4.2
Apheresis platelets	3	3.5
Fresh frozen plasma	26	59.3
Cryoprecipitate	2	54.0
Donors associated with TRALI cases		
Total number of donors associated		81
Donor gender		
Male		49 (60% of donors)
Female		32 (40% of donors)
Cases with single donor versus multiple donors		
Single		8 (33% of cases)
Multiple		16 (67% of cases)
2 donors		6
3 donors		2
≥ 4 donors		8
Donor outcomes		
Donor deferred		33 (40% of donors)
Gender of deferred donor		
Male		18
Female		15
Antibody detection		
Donors with antibodies detected		28
Donors with no antibodies detected		5
Donor cleared		41 (51% of donors)
Did not present for testing		6 (7% of donors)
Not applicable (cadaveric)		1 (1% of donors)

RESULTS

Comparison of case definitions

Ninety-nine cases were identified in the database (Figure 1). Baseline patient characteristics patients were extracted (Table 2). Twelve cases were excluded from analysis due to insufficient clinical details. A further 14 cases were deemed not to be related to transfusion. The final number of cases assessed was 73.

There were 48 cases classified as TACO within the database. Only 26 cases strictly met the 2011 ISBT/IHN definition. Sixteen cases did not meet the criteria due to insufficient clinical details. Six cases did not technically meet the 2011 definition: five due to the absence of both tachycardia and hypertension and one due to the onset time occurring beyond 6 h (but within 12 h). In contrast, 46 of the 48 cases met the 2018 ISBT/IHN/AABB definition. The remaining two cases did not meet the criteria due to lack of documentation of the time of onset.

There were 24 cases of TRALI under the 2004 CCC definition (15 cases of TRALI and 9 cases of possible TRALI). Following the proposed 2019 redefinition, there would be a total of 25 cases (15 TRALI Type I cases and 10 TRALI Type II cases). There were three cases where the classification was different. One case did not meet the 2004 definition due to pre-existing ARDS from influenza A pneumonia, but could be diagnosed as a TRALI Type II under the revised definition as their respiratory status had been stable for more than 12 h. One case was classified as possible TRALI under the 2004 definition but under the 2019 definition would be classified as TRALI Type I due to cardiopulmonary bypass no longer being listed as an ARDS risk factor. The reverse was true for the last case, which was classified as TRALI under the 2004 definition but as TRALI Type II under the redefinition due to pulmonary vasculitis being added as an ARDS risk factor.

TABLE 4 Antibody investigation results of transfusion-related acute lung injury (TRALI) cases

Antibody detection in TRALI cases	
Frequency of antibody detection	
Cases with no antibodies	8
Cases with antibodies (HNA and/or HLA)	16
Cases with concordant antibodies	8
Cases with only non-concordant antibodies	8
Cases with one class of antibody	3
Cases with more than one class of antibody	13
Frequency of antibody classes	
Cases with HNA antibodies	3
Concordant	2
Non-concordant	1
Cases with HLA class I antibodies	20
Concordant	6
Non-concordant	14
Cases with HLA class II antibodies	19
Concordant	7
Non-concordant	12

Product type and antibody detection			
Product type	**Number of products**	**Number of cases with antibodies**	**Incidence of antibodies**
Low plasma volume			
Red cells	25	8	15/33 (45%)
Pooled platelets	6	6	
Cryoprecipitate	2	1	
High plasma volume			
Apheresis platelets	3	1	10/29 (34%)
Fresh frozen plasma	26	9	

Donor gender and antibody detection			
	Total donors, *n* (% total)	**Male donors, *n* (% male donors)**	**Female donors, *n* (% female donors)**
No. of donors	81	49	32
No. of donors with antibodies	29 (36)	14 (29%)	15 (46%)

Abbreviations: HLA, human leucocyte antigen; HNA, human neutrophil antigen.

TRALI case series

Details of the 24 TRALI cases under the current 2004 CCC definition were extracted from the database (Table A1). In total, 62 blood products from 81 donors were associated with these cases (Table 3). Of these donors, 60% were male and 40% were female. In approximately one-third of cases a single donor was implicated. A total of 33 donors (40%) were deferred, including 18 males and 15 females. Of the remaining donors, 41 (51%) were cleared to donate again, 6 (7%) did not present for testing and 1 (1%) was a cadaveric liver donor. In terms of recipient outcomes, there were 3 deaths, 21 recoveries and 3 unknowns resulting in a case fatality rate of 14%.

There were eight cases where no antibodies were detected (Table 4). Of the 16 cases where antibodies were identified, recipient-specific antibodies were found in 8 cases and only non-concordant antibodies were detected in the remaining 8 cases. In 3 cases, only one class of antibody was identified, whereas in 13 cases multiple antibody classes were detected. The most frequently identified antibodies were non-concordant anti-HLA class I (14 cases) and anti-HLA class II antibodies (12 cases). Recipient-specific anti-HLA class I and anti-HLA class II antibodies were detected in six and seven cases, respectively. Anti-HNA antibodies were detected in only three cases, with two demonstrating recipient specificity (anti-HNA-1a and anti-HNA-2).

There was no difference in the incidence of antibody detection between blood products with low plasma volume (red cells, pooled platelets and cryoprecipitate) compared with products with high plasma volume (apheresis platelets and fresh frozen plasma) (45% vs. 34%, $p = 0.44$). There was a trend towards higher antibody detection rates in female donors (46%) compared with male donors (29%), but this was not statistically significant ($p = 0.10$).

DISCUSSION

The new TACO definition [4] has already been accepted for use internationally [9] and from late 2019, in Australia by Lifeblood. In contrast, the proposed TRALI re-definition [5] has not yet been widely accepted due to the lack of validation studies. This study provided further evidence that the new TACO definition improves upon the 2011 definition [2] in terms of usability and capturing of cases. This is the first study to provide validation data for the TRALI re-definition.

The 2018 TACO definition [4] was found to be broader and more encompassing than the former definition [2]. Sixteen cases did not meet the 2011 definition due to insufficient clinical information, compared with only two cases for the 2018 definition. A further six cases clinically judged to represent TACO did not meet the stringent 2011 definition but met the 2018 definition. This was consistent with the intention of the revised definition, which was developed because many haemovigilance users felt that the 2011 definition was failing to capture cases consistent with TACO clinically [8]. Several SHOT reports highlighted that one of the issues with the 2011 definition was the requirement for at least one of hypertension or tachycardia to be present [19–21]. This was problematic as TACO cases can be accompanied by hypotension rather than hypertension, tachycardia is frequently non-specific, and both can be confounded by other clinical variables [19–21]. For example, one case in this study that did not meet the 2011 definition despite a presentation typical for TACO involved a patient who had concurrent gastrointestinal bleeding. The other restricting criterion of the 2011 definition was the limitation of

reaction onset time to within 6 h of transfusion (one case in this study was excluded on this basis). However, a small but significant proportion of TACO cases (12%–16%) develop beyond this timeframe [19, 20]. To reflect this, the onset time was increased to 12 h in the 2018 definition. Overall, the number of TACO cases reported will likely increase under the broader 2018 definition and be more reflective of the real-world incidence of TACO.

This study did not find any significant variation in the number of TRALI cases between the 2019 [5] and 2004 definitions [3]. Inclusion of patients with mild pre-existing stable ARDS as Type II TRALI patients, after previously being excluded as possible TRALI patients, did not result in a substantial increase in the number of TRALI cases. There are some subtle differences in terms of ALI/ARDS risk factors between the two TRALI definitions, but these had limited impact.

A limitation of our study is the potential for selection bias as data were obtained from a passive haemovigilance system. Clinicians may not have reported cases which did not qualify under the former definitions but would under the new definitions. TACO cases are underrepresented in Lifeblood's data, but our study corroborates previous validation work of the 2018 TACO definition [4]. For TRALI cases, the potential of selection bias is likely considerably lower. As Lifeblood is responsible for the clinical and laboratory assessment of suspected TRALI cases, the number of suspected cases reported is substantially higher than confirmed cases. A previous retrospective review of TRALI cases reported to Lifeblood at a state level over a 20-year period identified only 48 cases of TRALI or possible TRALI of 91 cases referred [22]. Comparison of Lifeblood's ATR database with the Australian Haemovigilance Report, published by the National Blood Authority Australia, during the same period shows that more cases were reported to Lifeblood (24 vs. 18 cases) [23–26]. In the absence of a single national haemovigilance system in Australia, this is likely the most complete dataset for TRALI cases.

Patients with malignancies, particularly haematological malignancies, were overrepresented in our study (40%). This is consistent with previous studies identifying cancer as a risk factor for TRALI [27–30]. Recently, a study has also shown that oncology patients are at higher risk of developing TACO [31]. Interestingly, whilst another series described that 15% of TRALI cases occurred in patients with postpartum haemorrhage [28], we did not observe any cases in obstetric patients. The 14% fatality rate observed was similar to other reports [32].

Amongst the 24 TRALI cases, antibodies were not detected in 8 cases. Of the 16 cases where antibodies were detected, recipient-specific antibodies were only found in 8 cases. This proportion of cases with antibodies was lower than the 80% previously reported [33, 34], and might reflect the impact of risk-reduction strategies, such as male exclusive plasma [18, 22, 35, 36]. The 33% frequency of cognate antibodies was also lower than the data from SHOT, where cognate antibodies were identified in 103 of 187 (55%) of TRALI cases [37]. The most frequently detected antibodies in this case series were anti-HLA class I and II antibodies without cognate recipient antigens. Anti-HNA antibodies were detected in only three cases, with only two of these being against cognate recipient antigens. This is similar to most other case series, where anti-HLA class I and II antibodies were seen more frequently than anti-HNA antibodies (67%–97% vs. 1%–33% of antibody-positive cases) [27, 38, 39].

In conclusion, this study described the impact of the new TACO and TRALI definitions. It provided further evidence that the 2018 definition for TACO is an improvement over the former definition. It also provides the first data to validate the proposed TRALI re-definition. Larger follow-up studies will help finalize this validation.

ACKNOWLEDGEMENTS

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

J.P.T. designed the research study; Y.Y. performed the research and wrote the first draft; P.D, J.D. and S.B. were involved in the analysis of cases; all authors reviewed and edited the manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interests to declare.

ORCID

Yin Yuan https://orcid.org/0000-0002-9353-1702
John-Paul Tung https://orcid.org/0000-0001-7210-0527

REFERENCES

1. Semple JW, Rebetz J, Kapur R. Transfusion-associated circulatory overload and transfusion-related acute lung injury. Blood. 2019;133:1840–53.
2. International Society of Blood Transfusion Working Party on Haemovigilance. Proposed standard definitions for surveillance of non-infectious adverse transfusion reactions [cited 2021 Jul 24]. Available from: https://www.isbtweb.org/resource/proposeddefinitionssurveillancenoninfectiousadversereactionshaemovigilance.html.
3. Kleinman S, Caulfield T, Chan P, Davenport R, McFarland J, McPhedran S, et al. Toward an understanding of transfusion-related acute lung injury: statement of a consensus panel. Transfusion. 2004;44:1774–89.
4. International Society of Blood Transfusion Working Party on Haemovigilance. Transfusion-associated circulatory overload (TACO) definition. 2018 [cited 2021 Jul 24]. Available from: https://www.isbtweb.org/resource/tacodefinition.html.
5. Vlaar APJ, Toy P, Fung M, Looney MR, Juffermans NP, Bux J, et al. A consensus redefinition of transfusion-related acute lung injury. Transfusion. 2019;59:2465–76.
6. Narayan S, editor, on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2019 Annual SHOT Report [cited 2021 Jul 26]. Available from: https://www.shotuk.org/wp-content/uploads/myimages/SHOT-REPORT-2019-Final-Bookmarked-v2.pdf.
7. US Food and Drug Administration. Fatalities Reported to FDA Following Blood Collection and Transfusion Annual Summary for FY2019 [cited 2021 Jul 24]. Available from: https://www.fda.gov/media/147628/download.
8. van den Akker TA, Grimes ZM, Friedman MT. Transfusion-associated circulatory overload and transfusion-related acute lung injury. Am J Clin Pathol. 2021;156:529–39.
9. Wiersum-Osselton JC, Whitaker B, Grey S, Land K, Perez G, Rajbhandary S, et al. Revised international surveillance case definition of transfusion-associated circulatory overload: a classification agreement validation study. Lancet Haematol. 2019;6:e350–8.
10. Toy P, Lowell C. TRALI – definition, mechanisms, incidence and clinical relevance. Best Pract Res Clin Anaesthesiol. 2007;21:183–93.

11. Popovsky MA. Transfusion-related acute lung injury: incidence, pathogenesis and the role of multicomponent apheresis in its prevention. Transfus Med Hemother. 2008;35:76–9.
12. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, et al. Acute respiratory distress syndrome: the Berlin Definition. JAMA. 2012;307:2526–33.
13. International Haemovigilance Network. Revised definitions for respiratory complications of blood transfusion [cited 2021 Jun 01]. Available from: https://www.ihn-org.com/revised-definitions-for-respiratory-complications-of-blood-transfusion/.
14. Tung JP, Chiaretti S, Dean MM, Sultana AJ, Reade MC, Fung YL. Transfusion-related acute lung injury (TRALI): potential pathways of development, strategies for prevention and treatment, and future research directions. Blood Rev. 2022;53:100926.
15. Peters AL, Van Stein D, Vlaar AP. Antibody-mediated transfusion-related acute lung injury; from discovery to prevention. Br J Haematol. 2015;170:597–614.
16. Peters AL, Vlaar AP. Non-antibody-mediated TRALI current understanding. ISBT Sci Ser. 2016;12:260–7.
17. Kuldanek SA, Kelher M, Silliman CC. Risk factors, management and prevention of transfusion-related acute lung injury: a comprehensive update. Expert Rev Hematol. 2019;12:773–85.
18. Vossoughi S, Gorlin J, Kessler DA, Hillyer CD, Van Buren NL, Jimenez A, et al. Ten years of TRALI mitigation: measuring our progress. Transfusion. 2019;59:2567–74.
19. Bolton-Maggs PHB, editor, on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2012 Annual SHOT Report [cited 2021 Jul 26]. Available from: https://www.shotuk.org/wp-content/uploads/myimages/SHOT-Annual-Report-20121.pdf.
20. Bolton-Maggs PHB, editor, PHB on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2013 Annual SHOT Report. Available from: https://www.shotuk.org/wp-content/uploads/myimages/2013.pdf.
21. Bolton-Maggs PHB, editor, PHB on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2014 Annual SHOT Report. Available from: https://www.shotuk.org/wp-content/uploads/myimages/report-2014.pdf.
22. Sivakaanthan A, Swain F, Pahn G, Goodison K, Gutta N, Holdsworth R, et al. Transfusion-related acute lung injury (TRALI): a retrospective review of reported cases in Queensland, Australia over 20 years. Blood Transfus. 2022;20:454–64.
23. National Blood Authority. Australian Haemovigilance Report, Data for 2015-16 [cited 2021 Jul 26]. Available from: https://www.blood.gov.au/system/files/Donor-Haem-Report-15-16-v14.pdf.
24. National Blood Authority. Australian Haemovigilance Report, Data for 2016-17 [cited 2021 Jul 26]. Available from: https://www.blood.gov.au/system/files/Australian%20Haemovigilance%20Report%20-%202016-17_FINAL%20V2.pdf.
25. National Blood Authority. Australian Haemovigilance Report, Data for 2018–2019. Available from: https://www.blood.gov.au/system/files/documents/Australian-Haemovigilance-Report-2018-19-FINAL_v2.pdf.
26. National Blood Authority. Australian Haemovigilance Report, Data for 2017-18 [cited 2021 Jul 26]. Available from: https://www.blood.gov.au/system/files/Australian%20Haemovigilance%20Report%202017-18_FINAL%20v2.pdf.
27. Andreu G, Boudjedir K, Muller JY, Pouchol E, Ozier Y, Fevre G, et al. Analysis of transfusion-related acute lung injury and possible transfusion-related acute lung injury reported to the French Hemovigilance network from 2007 to 2013. Transfus Med Rev. 2018;32:16–27.
28. Ozier Y, Muller JY, Mertes PM, Renaudier P, Aguilon P, Canivet N, et al. Transfusion-related acute lung injury: reports to the French Hemovigilance Network 2007 through 2008. Transfusion. 2011;51:2102–10.
29. Silliman CC, Boshkov LK, Mehdizadehkashi Z, Elzi DJ, Dickey WO, Podlosky L, et al. Transfusion-related acute lung injury: epidemiology and a prospective analysis of etiologic factors. Blood. 2003;101:454–62.
30. Vlaar AP, Juffermans NP. Transfusion-related acute lung injury: a clinical review. Lancet. 2013;382:984–94.
31. Maldonado M, Villamin CE, Murphy LE, Dasgupta A, Bassett RL, Correa Medina M, et al. Oncology patients who develop transfusion-associated circulatory overload: an observational study. Lab Med. 2022;53:344–8.
32. Mazzei C, Popovsky M, Kopko PM. AABB technical manual. 18th ed. Bethesda: AABB Press; 2014.
33. Bux J, Becker F, Seeger W, Kilpatrick D, Chapman J, Waters A. Transfusion-related acute lung injury due to HLA-A2-specific antibodies in recipient and NB1-specific antibodies in donor blood. Br J Haematol. 1996;93:707–13.
34. Middelburg RA, van Stein D, Briët E, van der Bom JG. The role of donor antibodies in the pathogenesis of transfusion-related acute lung injury: a systematic review. Transfusion. 2008;48:2167–76.
35. Reesink HW, Lee J, Keller A, Dennington P, Pink J, Holdsworth R, et al. Measures to prevent transfusion-related acute lung injury (TRALI). Vox Sang. 2012;103:231–59.
36. Otrock ZK, Liu C, Grossman BJ. Transfusion-related acute lung injury risk mitigation: an update. Vox Sang. 2017;112:694–703.
37. Narayan S, editor, on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2018 Annual SHOT Report [cited 2021 Jul 26]. Available from: https://www.shotuk.org/wp-content/uploads/myimages/SHOT-Report-2018_Web_Version-1.pdf.
38. van Stein D, Beckers EA, Sintnicolaas K, Porcelijn L, Danovic F, Wollersheim JA, et al. Transfusion-related acute lung injury reports in The Netherlands: an observational study. Transfusion. 2010;50:213–20.
39. Reil A, Keller-Stanislawski B, Günay S, Bux J. Specificities of leucocyte alloantibodies in transfusion-related acute lung injury and results of leucocyte antibody screening of blood donors. Vox Sang. 2008;95:313–7.

SUPPORTING INFORMATION

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

How to cite this article: Yuan Y, Dennington PM, Daly J, Baidya S, Tung J-P. The impact of revised definitions for transfusion-associated circulatory overload and transfusion-related acute lung injury on haemovigilance reporting. Vox Sang. 2023;118:199–206.

Received: 14 September 2022 | Revised: 21 December 2022 | Accepted: 30 December 2022

DOI: 10.1111/vox.13401

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Validation of a flow-cytometry-based red blood cell antigen phenotyping method

Robert Liwski[1,2] | Gwen Clarke[3,4] | Calvino Cheng[1,2] | Syed Sibte Raza Abidi[5] | Samina Raza Abidi[6] | Jason George Quinn[1,2]

[1]Department of Pathology and Laboratory Medicine, Dalhousie University, Halifax, Nova Scotia, Canada

[2]Nova Scotia Health Authority, Central Zone, Halifax, Nova Scotia, Canada

[3]Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada

[4]Canadian Blood Services, Edmonton, Alberta, Canada

[5]NICHE Research Group, Faculty of Computer Science, Dalhousie University, Halifax, Nova Scotia, Canada

[6]Department of Community Health and Epidemiology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada

Correspondence
Jason George Quinn, 5788 University Avenue, Room 217, Halifax, Nova Scotia B3H 1V8, Canada.
Email: jason.quinn@nshealth.ca

Funding information
The authors received no specific funding for this work.

Abstract

Background and Objectives: Current manual and automated phenotyping methods are based on visual detection of the antigen–antibody interaction. This approach has several limitations including the use of large volumes of patient and reagent red blood cells (RBCs) and antisera to produce a visually detectable reaction. We sought to determine whether the flow cytometry could be developed and validated to perform RBC phenotyping to enable a high-throughput method of phenotyping using comparatively miniscule reagent volumes via fluorescence-based detection of antibody binding.

Materials and Methods: RBC phenotyping by flow cytometry was performed using monoclonal direct typing antisera (human IgM): anti-C, -E, -c, -e, -K, -Jk^a, -Jk^b and indirect typing antisera (human IgG): anti-k, -Fy^a, -Fy^b, -S, -s that are commercially available and currently utilized in our blood transfusion services (BTS) for agglutination-based phenotyping assays.

Results: Seventy samples were tested using both flow-cytometry-based-phenotyping and a manual tube standard agglutination assay. For all the antigens tested, 100% concordance was achieved. The flow-cytometry-based method used minimal reagent volume (0.5–1 µl per antigen) compared with the volumes required for manual tube standard agglutination (50 µl per antigen)

Conclusion: This study demonstrates the successful validation of flow-cytometry-based RBC phenotyping. Flow cytometry offers many benefits compared to common conventional RBC phenotyping methods including high degrees of automation, quantitative assessment with automated interpretation of results and extremely low volumes of reagents. This method could be used for high-throughput, low-cost phenotyping for both blood suppliers and hospital BTS.

Keywords

antigen typing, flow cytometry, phenotyping, RBC phenotyping, transfusion

Highlights

- We have developed and validated a flow-cytometry-based-phenotyping method using existing, commercially available reagents.
- This methodology uses 1%–2% of the antisera that would be used with the conventional agglutination-based phenotyping method and can test multiple samples simultaneously.

- This low-cost, high-throughput method of phenotyping will allow hospitals and blood suppliers to greatly increase the proportion of phenotyped patients and red blood cell units, which presents many potential advantages.

INTRODUCTION

Red blood cell (RBC) phenotyping for non-ABO antigens is commonly performed by both blood suppliers and hospital-based blood transfusion services (BTS) on either donor or recipient RBCs. In Canada, this includes the clinically significant antigens to which alloimmunization is most likely to occur. Rhesus D (D) and Kell are universally evaluated on donor RBCs by Canadian Blood Services (CBS), our national blood supplier. Similarly, hospital BTS routinely test for D and, in selected cases, may phenotype for K (e.g., in patients of childbearing potential). Many other RBC antigens are known to contribute to alloimmunization in some transfused patients and this may complicate future RBC transfusions, result in haemolytic transfusion reactions or haemolytic disease of the foetus and newborn. Common clinically significant antigens associated with alloimmunization include D, C, c, E, e, K, Fy^a, Fy^b, Jk^a, Jk^b, S and s [1].

The extent to which blood suppliers and hospital BTS test for additional antigens is variable. CBS routinely provides phenotype information beyond D and K on donor RBCs in Canada for several additional antigens (Table 1). Hospital BTS phenotyping practice varies. Some hospital BTS will phenotype patient RBCs at the time of antibody identification in those with a current or historical antibody. In certain clinical scenarios, when the provision of phenotype-matched blood is required, phenotyping of patient RBCs would also occur. These circumstances include patients with pan-reactive autoantibodies or those with clinical indications for prophylactic phenotype matching to avoid alloimmunization, such as sickle cell disease [2]. Some centres have adopted a broader approach to patient antigen typing with routine use of phenotype-matched blood in other scenarios or in all chronically transfused patients [3–5].

Presently, RBC phenotyping is performed using techniques that rely on visual detection of RBC agglutination. This assessment is typically performed through the incubation of the test RBCs (donor or patient) with a known antibody corresponding to a single RBC antigen of interest. This can be performed in a variety of detection methods including tubes, gel cards or 'solid phase' plates [6–8]. The latter two detection methods have been automated with instruments including readers that employ photographs and standard interpretation models based on the pattern within the gel card or solid phase plate. All these methods use relatively large volumes of patient RBCs and reagent antisera and the antibodies in order to create visibly detectable agglutination.

Flow cytometry is widely used both as a research and clinical tool. It is commonly used in clinical laboratories to characterize neoplastic cell populations in haematopathology and in the assessment of immune deficiency. Therefore, this technique, including the equipment and expertise to perform it, is already widely available to clinical laboratories. This makes it ideally suited as a potential tool for use in transfusion medicine. Moreover, flow cytometry uses lasers and fluorescent detectors to identify the antigen–antibody interaction, allowing for potential assessment of this endpoint with miniscule amounts of reagent compared to serological methods, while providing a quantitative measurement of fluorescence intensity, which is related to the antigen density and the amount of antibody binding to the antigen. With the advent of 96-well microplates, flow cytometers have the potential for high-throughput, automated testing, which could make the provision of full phenotyping of donor RBCs and a high proportion of phenotyped patient samples more viable from an economic and logistical perspective. This technique could serve both blood suppliers and hospital-based BTS. Despite the widespread availability of flow cytometry in clinical laboratories and potential benefits, use in transfusion medicine has been largely confined to assessment of foetal maternal haemorrhage [9, 10]. The literature does also describe the utility of flow cytometry in resolving ABO discrepancies and in assessment of weak ABO and D antigens [11, 12].

The purpose of this research is to develop a novel and scalable flow-cytometry-based RBC phenotyping method that uses low reagent volumes to achieve a low-cost, high-throughput phenotyping method for common RBC antigens most frequently associated with alloimmunization, on patient RBC samples.

TABLE 1 Percentage of units with phenotypic information provided to hospitals by Canadian Blood Services

Antigen	Percent reported (%)
C	35.19
E	38.48
c	38.9
e	40.56
K	99.96
Jk^a	21.2
Jk^b	20.28
Fy^a	31.34
Fy^b	29.46
S	30.18
s	20.54

STUDY DESIGN AND METHODS

Reagents

RBC phenotyping by flow cytometry was performed using direct typing antisera (IgM): anti-C, -E, -c, -e, -K, -Jk^a, -Jk^b and indirect typing antisera (IgG): anti-k, -Fy^a, -Fy^b, -S, -s (Table 2). All antisera utilized are commercially available and are currently used by the Nova Scotia Health Authority Central Zone Blood Transfusion Service (CZ-BTS)

TABLE 2 List of commercially available blood bank reagents used in flow cytometry validation

Reagents
Anti-human IgG allophycocyanin (Cedar Lane Cat: 109-136-098)
Anti-human IgM phycoerythrin (One Lambda Cat: IGM-PEC1)
Anti-C BioClone® (Ortho. Cat: 713080)
Anti-c BioClone® (Ortho. Cat: 714080)
Anti-E BioClone® (Ortho. Cat: 713180)
Anti-e (Bio-Rad Cat: BT802370)
Anti-K monoclonal IgM BioClone® (Ortho. Cat: 713129)
Anti-k (Ortho. Cat: 721030)
Anti-Jk^a monoclonal BioClone® (Ortho. Cat: 721105)
Anti-Jk^b monoclonal BioClone® (Ortho. CAT: 721480)
Anti-Fy^a monoclonal IgG Gamma-Clone® (Immucor Cat: 4816)
Anti-Fy^b (Ortho. Cat: 725230)
Anti-S (Ortho. Cat: 723030)
Anti-s (Ortho. Cat: 721830)
Dulbecco's phosphate-buffered saline (Life Tech. Cat: 14190250)
Foetal bovine serum, heat inactivated (Life Tech. Cat: A3840301)
Isotonic saline 0.85% w/v, pH >5.5 (Thermo Scientific)
Flow wash buffer

for routine phenotyping of patient and blood donor samples with conventional manual tube standard agglutination techniques. Secondary detection antibodies used for RBC phenotyping by flow cytometry were allophycocyanin (APC) conjugated polyclonal goat anti-human IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA) and phycoerythrin (PE) conjugated polyclonal donkey anti-human IgM (One Lambda, Canoga Park, CA, USA). Flow wash buffer (FWB) composed of Dulbecco's phosphate-buffered saline (DPBS; Life Technologies Inc.) and 2% (v/v) foetal calf serum (Life Technologies Inc.) was used for all washes during the phenotyping procedure. RBC suspensions(3%–5%) and all antisera and secondary antibody cocktails were prepared in DPBS (Life Technologies Inc.). Panel RBCs (3%–5% suspension) were purchased from Ortho Clinical Diagnostics.

Patient RBC preparation

To prepare a 5% RBC suspension from patient samples, 6 ml of EDTA anticoagulated whole blood was centrifuged for 10 min at 1800*g*. Ten microlitre of packed RBCs were collected from the bottom of the centrifuged tube and added to 190 µl of DPBS.

Antiserum cocktail and secondary antibody cocktail preparation

Reagent antisera cocktails consisted of an IgM component and an IgG component as follows: anti-C with -S, -c with -s, -K with -k, -Jka with -Fy^a, -Jk^b with -Fy^b and were diluted in DPBS (Table 3). Anti-E and anti-e antisera (IgM) were diluted in DPBS individually (Table 3). Secondary antibody cocktail consisted of anti-human IgG-APC (1:200) and anti-human IgM-PE (1:100) diluted in DPBS.

TABLE 3 Antisera cocktail suspensions

Cocktail	Anti-IgM antibody		Anti-IgG antibody		DPBS
a	Anti-C	10 µl	Anti-S	20 µl	70 µl
b	Anti-c	10 µl	Anti-s	10 µl	80 µl
c	Anti-E	10 µl	–	–	90 µl
d	Anti-e	10 µl	–	–	90 µl
e	Anti-K	10 µl	Anti-k	20 µl	70 µl
f	Anti-Jk^a	10 µl	Anti-Fy^a	20 µl	70 µl
g	Anti-Jk^b	10 µl	Anti-Fy^b	10 µl	80 µl

Note: A quantity of 5 µl of diluted antisera is required for each test. This recipe provides enough reagent to test approixmately 20 patients.
Abbreviation: DPBS, Dulbecco's phosphate-buffered saline.

As most of the antisera used were combined into a cocktail consisting of an IgM component and an IgG component (anti-E and -e were diluted in DPBS individually), the number of wells in the microplate used for each patient was reduced from 12 to 7 wells. Thus, 12 patient samples could be phenotyped on a single 96-well tray simultaneously.

RBC phenotyping by flow cytometry

RBC phenotyping was performed in 96-well U-bottom Falcon microplates. Briefly, 5 µl of the appropriate antisera cocktail or DPBS (background control) were manually added to the bottom of each well. Next, 2.5 µl of appropriate 5% RBC suspension were manually added to the side of each well. Trays were centrifuged at 1800*g* for 10 s and agitated by vortexing to ensure appropriate mixing of the cells and antisera. Trays were incubated at room temperature (RT) for 5 min and manually washed three times in 200 µl of FWB at 1800*g* for 1 min. A quantity of 20 µl of the secondary antibody cocktail (anti-IgM PE and anti-IgG APC in DPBS) were added to each well, mixed by vortexing and incubated at RT for 5 min. Cells were manually washed twice in 200 µl of FWB at 1800*g* for 1 min and resuspended in 200 µl of FWB before acquisition by flow cytometry. Controls consisted of RBC panel cells with positive and negative antigen expression for antigens being evaluated in each well. Heterozygous controls are used when possible, though for certain antigens homozygous cells may be used.

Cell acquisition and analysis by flow cytometry

RBC events were acquired using the FACSCanto II flow cytometers (BD Biosciences) and analysed with BD FACSDIVA™ software (BD Biosciences) on the 1024 fluorescence channel scale. Data were expressed as the median channel fluorescence shift (MCFS) from the DPBS background control. For all IgM antisera, a cut-off of >100 MCFS from background control was used to classify positive

TABLE 4 Test results for red blood cells negative, heterozygous or homozygous for C, c, E, e, Jk[a], Jk[b] and K analysed using IgM-based antisera

Antigen	Zygosity	*N*	Mean shift[a]	SD	Min shift	Max shift	Median shift	Shift cut-off	% neg.	% pos.
C	Homozygous	17	422	66	294	524	433	100	0	100
C	Heterozygous	19	349	57	241	425	356	100	0	100
C	Negative	34	3	13	−12	59	0	100	100	0
c	Homozygous	34	297	43	205	396	294.5	100	0	100
c	Heterozygous	19	218	47	150	328	215	100	0	100
c	Negative	17	18	21	−6	60	9	100	100	0
E	Homozygous	9	392	22	363	436	393	100	0	100
E	Heterozygous	11	383	44	320	478	374	100	0	100
E	Negative	50	1	6	−20	14	0	100	100	0
e	Homozygous	50	443	62	296	600	447.5	100	0	100
e	Heterozygous	11	400	42	335	483	389	100	0	100
e	Negative	9	−2	7	−13	7	−3	100	100	0
Jk[a]	Homozygous	25	497	28	435	561	501	100	0	100
Jk[a]	Heterozygous	25	445	35	353	484	456	100	0	100
Jk[a]	Negative	20	3	9	−12	22	2.5	100	100	0
Jk[b]	Homozygous	20	353	61	264	470	334	100	0	100
Jk[b]	Heterozygous	25	273	56	200	394	265	100	0	100
Jk[b]	Negative	25	−1	7	−15	12	0	100	100	0
K	Homozygous	2	447	4	444	449	446.5	100	0	100
K	Heterozygous	9	382	24	347	423	382	100	0	100
K	Negative	59	6	12	−10	51	4	100	100	0

Abbreviations: max, maximum; min, minimum; neg, negative; pos, positive; SD, standard deviation.
[a]Median channel fluorescence shift.

TABLE 5 Test results for red blood cells negative, heterozygous or homozygous for Fy[a], Fy[b], k, S and s analysed using IgG-based antisera

Antigen	Zygosity	*N*	Mean shift[a]	SD	Min shift	Max shift	Median shift	Shift cut-off	% neg.	% pos.
Fy[a]	Homozygous	20	258	57	130	362	254.5	50	0	100
Fy[a]	Heterozygous	23	228	45	155	353	225	50	0	100
Fy[a]	Negative	26	4	5	−6	14	2.5	50	100	0
Fy[b]	Homozygous	26	278	57	110	368	280	40	0	100
Fy[b]	Heterozygous	23	237	64	44	312	266	40	0	100
Fy[b]	Negative	20	2	5	−7	15	2	40	100	0
k	Homozygous	59	224	47	103	359	230	50	0	100
k	Heterozygous	9	176	48	129	241	148	50	0	100
k	Negative	2	15	8	9	21	15	50	100	0
S	Homozygous	12	285	37	232	353	281	50	0	100
S	Heterozygous	24	241	51	128	379	241	50	0	100
S	Negative	33	3	9	−18	25	2	50	100	0
s	Homozygous	33	430	54	257	548	435	50	0	100
s	Heterozygous	24	323	56	174	432	330.5	50	0	100
s	Negative	12	2	5	−8	9	1	50	100	0

Abbreviations: max, maximum; min, minimum; neg, negative; pos, positive; SD, standard deviation.
[a]Median channel fluorescence shift.

reactions. The positive cut-off for IgG-based anti-k, -Fy[b], -S and -s IgG antisera was >50 MCFS and anti-Fy[a] antisera was >40 MCFS from background control. Cut-offs were chosen to ensure clear separation between the maximum fluorescence observed for antigen-negative cells and minimum fluorescence observed for antigen-positive cells (see Tables 4 and 5 ranges of channel shifts observed).

The antigen antibody binding on the RBCs was detected by using fluorescently labelled anti-human IgM PE and anti-human IgG APC secondary antibodies. The amount of fluorescence produced as the cells are interrogated by the flow cytometry analyser was measured on the 1024 fluorescence channel scale and expressed as MCFSs from the background control. Presently, results are manually entered into the laboratory information system. Figures 1 and 2 demonstrate examples of the gating strategy employed and representative histograms of the results.

Standard tube agglutination method

The same reagents were used for comparative agglutination-based assays according to the manufacturer's instructions. Briefly, a 5% RBC suspension was prepared from EDTA anticoagulated patient whole blood samples following centrifugation for 10 min at 1800*g*, by mixing RBCs and normal saline. The manufacturer specified volume of sample was mixed with 50 µl of commercial antibody preparation (one tube per antisera). The antisera included human

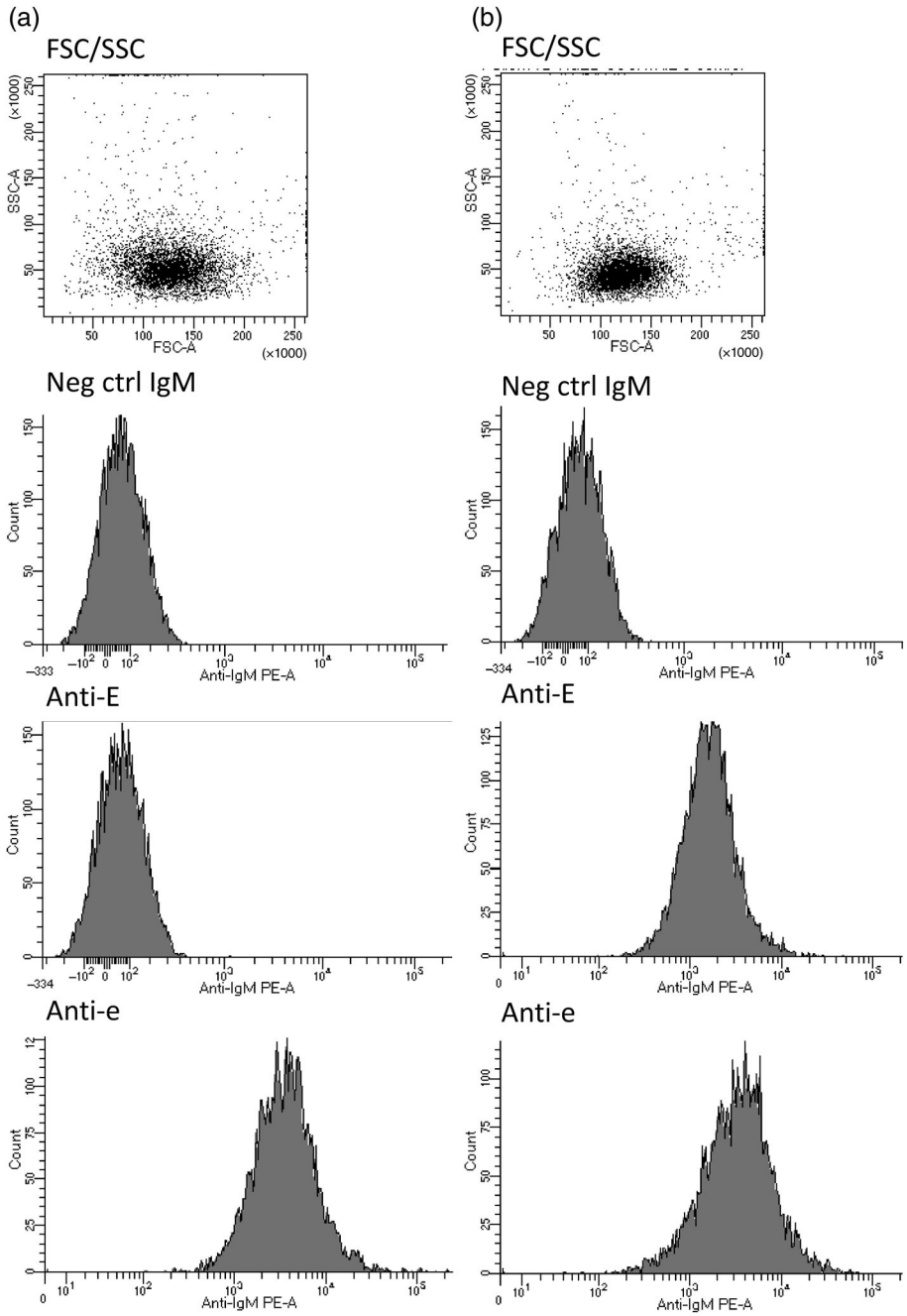

FIGURE 1 The forward and side scatter dot plots showing red blood cell event acquisition, and histograms of channel shift for IgM-based antisera in two patients are shown. A patient with negative expression of E antigen and homozygous positive expression of e antigen (panel a), and a patient with heterozygous expression of E and e antigens (panel b) are shown. PE, phycoerythrin; ctrl, control; neg, negative.

monoclonal IgM blends with the following specificities: anti-C, anti-e, anti-E, anti c, anti-K, anti -Jk^a, anti -Jk^b, anti-S, anti-s and anti -Fy^b. All direct typing reagents were from Ortho Clinical Diagnostics with exception of anti-e (Bio-Rad). Following incubation for 15 min at 37°C, the tubes were washed in a cell washer and the agglutination was read and individually graded. Phenotyping for Fy^a and k were performed using an indirect antiglobulin test (IAT) in micro typing system (MTS) gel cards (Ortho Clinical Diagnostics) according to manufacturer's instructions using anti-Fy^a (Immuncor) and anti-k (Ortho Clinical Diagnostics) indirect antisera.

RBC panel cells with heterozygous and negative antigen expression were used for positive controls and negative controls, respectively (Immuncor).

Statistical analysis

Mean, median and standard deviation of MCFS values from background control as well as Student's *t*-test calculations were performed using Microsoft Excel software.

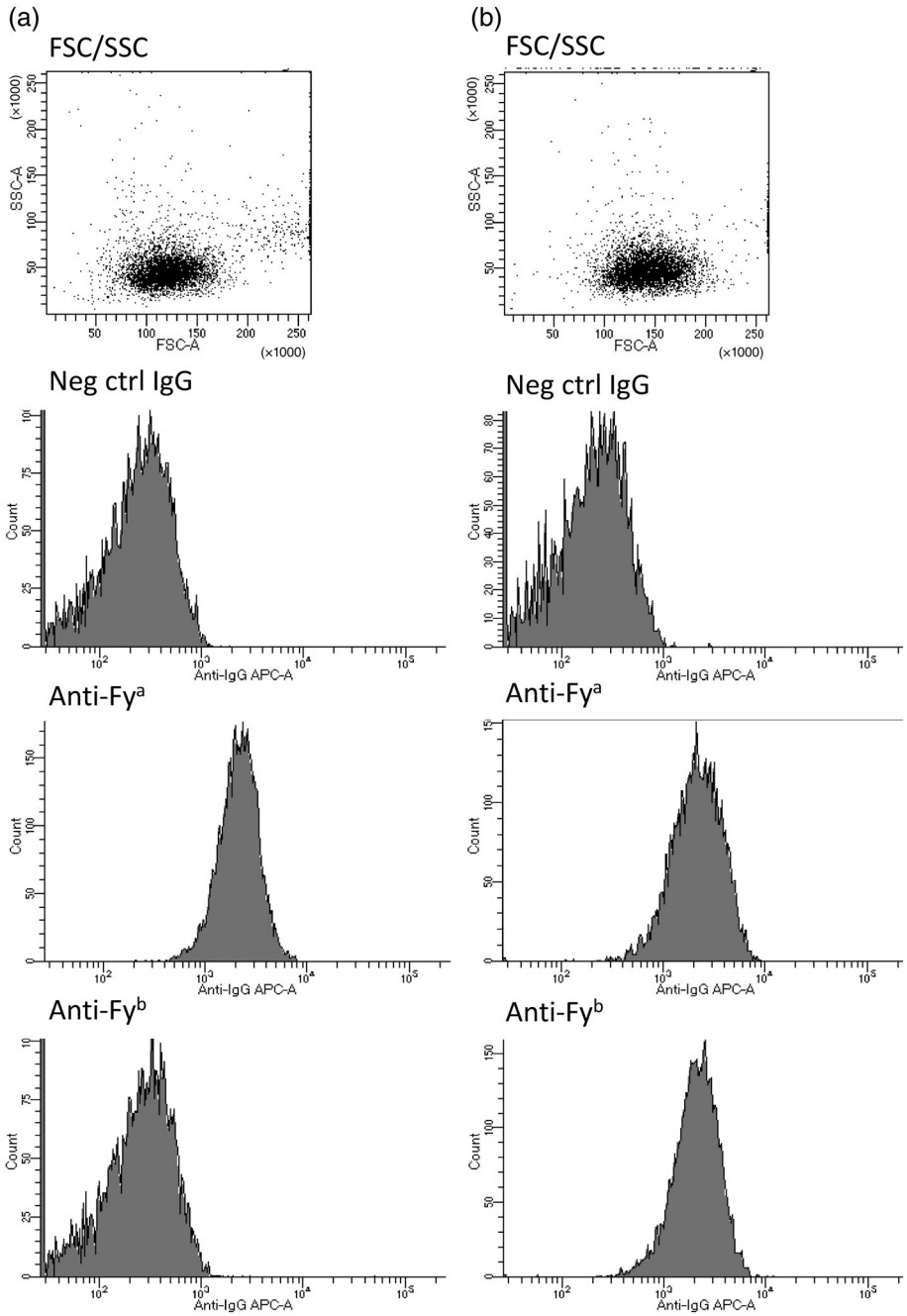

FIGURE 2 The forward and side scatter dot plots showing red blood cell event acquisition, and histograms of channel shift for IgG-based antisera in two patients are shown. A patient with homozygous positive expression of Fy^a antigen and negative expression of Fy^b antigen (panel a), and a patient with heterozygous expression of Fy^a and Fy^b antigens (panel b) are shown. APC, allophycocyanin; ctrl, control; neg, negative.

RESULTS

Red cell phenotyping by flow cytometry involved mixing the RBCs (reagent panel or patient cells) and reagent antisera in 96-well microplates. The volumes used for the purposes of this validation were 20 µl of 5% RBC suspension and 5 µl of the appropriately diluted antisera cocktail or DPBS for the background control. Each 5 µl uses 0.5 or 1 µl (anti-S, anti-Fy^a and anti-k) of antisera per reaction. This compares with 550 µl of 3%–5% RBC suspension and 50 µl of antisera for the comparable tube agglutination technique. This represents 50–100 times reduction in volume of antisera used compared to the manual tube standard agglutination assay. Most of the antisera used for the flow cytometric method were combined into a cocktail consisting of an IgM component and an IgG component (-C with -S, -c with -s, -K with -k, -Jk^a with -Fy^a, -Jk^b with -Fy^b) diluted in DPBS. Anti-E and -e were diluted in DPBS individually. This reduced the number of wells in the microplate used for each patient from 12 to 7 wells. Thus, 12 patient samples could be phenotyped on a single 96-well tray simultaneously.

A total of 40 reagent panel RBCs and 30 patient RBC samples were phenotyped to validate the assay. The results for IgM-based (Table 4 and Figure 3 panel a) and IgG-based (Table 5 and Figure 3 panel b) reagents are shown. There was a clear distinction between positive and negative reactions using both the 100 MCFS and 50 (40 for anti-Fy^a) MCFS for both IgM and IgG reagents, respectively, for both heterozygous and homozygous patient and panel cells tested.

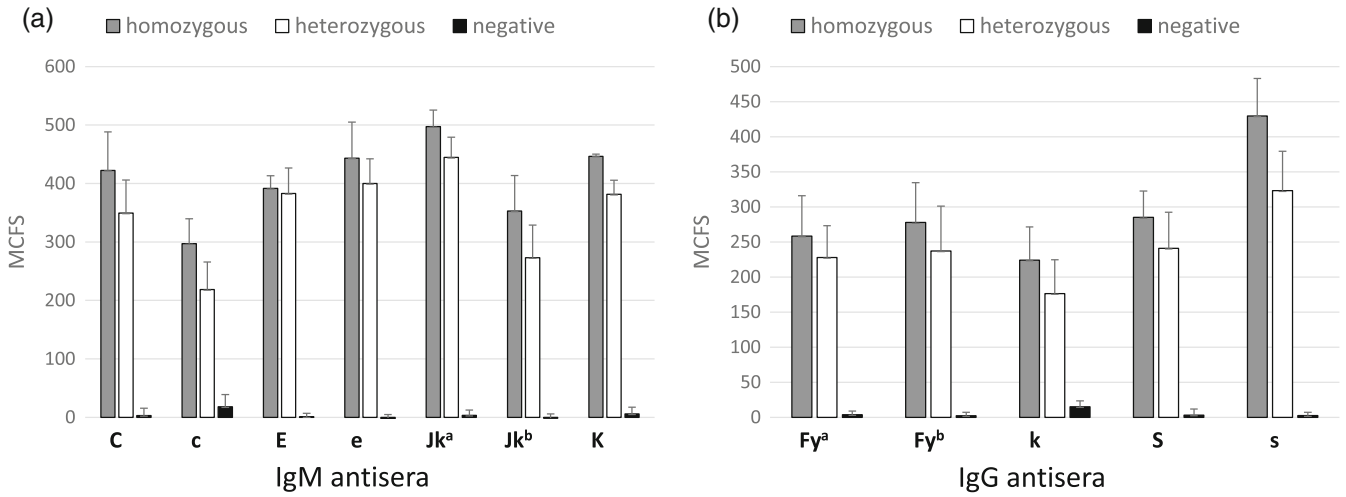

FIGURE 3 Mean median channel fluorescence shift (MCFS) and one standard deviation for homozygous, heterozygous and antigen-negative RBCs tested using IgM-based antisera (panel a) and IgG-based antisera (panel b).

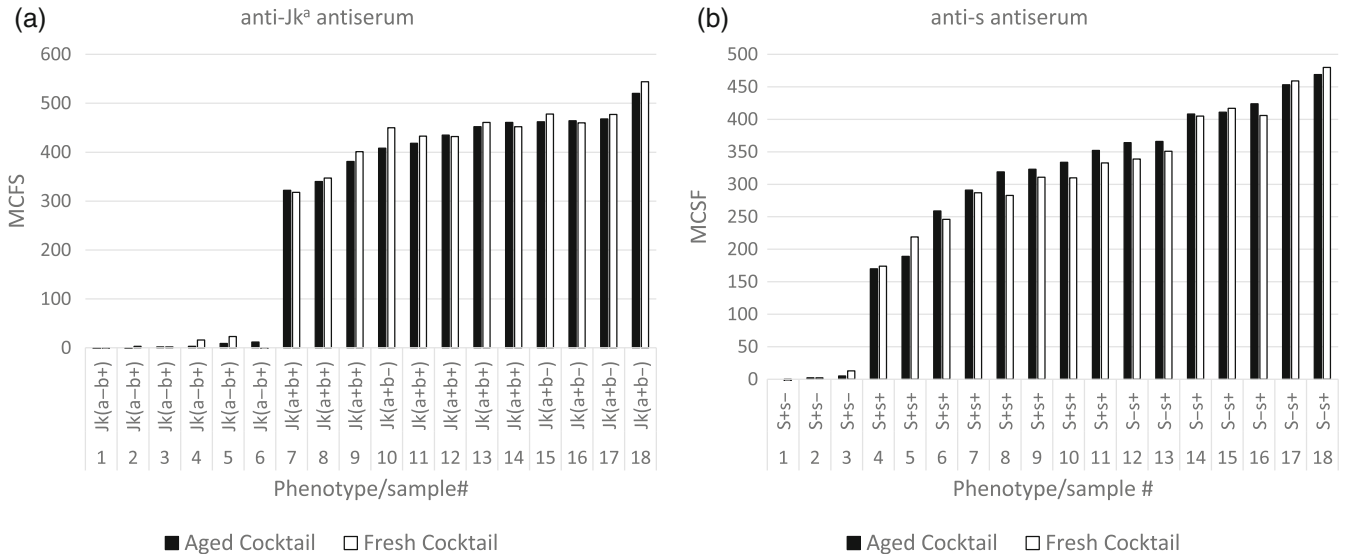

FIGURE 4 Day 1 (fresh cocktail) versus 7-day-old (aged cocktail) antiserum cocktails shown on the *x*-axis and *y*-axis showing median channel shift of specimens negative, heterozygous and homozygous for Jk^a antigen (panel a) and s antigen (panel b) for 18 samples. MCFS, median channel fluorescence shift.

The results of phenotype testing by flow cytometry method indicate a 100% concordance with phenotype results reported by the vendor on panel cell worksheets and with the conventional agglutination-based testing performed on patient samples. Homozygous antigen expression on test RBCs showed significantly higher average MCFS when compared to heterozygous cells for all antigens tested ($p < 0.05$) except for E, but ranges of MCFS overlapped significantly for all antigens, which precludes assessment of zygosity based on MCFS.

The comparison of fresh (Day 1) and aged (stored at 4°C for Day 7) antisera cocktails indicated that there was no appreciable difference between the freshly prepared and stored antisera cocktails. These results are shown in Figure 4.

In addition, this procedure is very fast and requires approximately 30 min to perform the test method outlined in this paper on an available specimen. In addition, multiple patients can be added with minimal marginal increase in test time required.

DISCUSSION

We demonstrated that the monoclonal IgM- and IgG-based antisera used by our blood bank for routine agglutination-based RBC phenotyping using manual tube standard agglutination and IAT methods can be successfully applied to phenotyping by flow cytometry when used in conjunction with fluorescent-labelled secondary anti-human IgM and IgG antibodies as detection reagents. In this paper, we show the successful validation of this phenotyping method which showed 100% concordance with phenotyping results obtained by comparison with both traditional agglutination-based phenotyping method performed in our institution and RBC phenotyping results provided by manufacturers of RBC reagent panel cells.

Due to the small size of each well and because of the sensitive fluorescence-based detection system afforded by flow cytometry, a significantly smaller volume of patient's cells and antisera can be used compared to agglutination-based techniques and multiple patient samples can be phenotyped simultaneously on a single plate. The small volume of sample required may be beneficial in certain situations such as testing on limited patient samples or donor segments from referring institutions.

Conventional techniques based on the visual detection of agglutination require large volumes of donor or recipient RBCs, antisera and other costly reagents, such as gel cards, rely on visual observation with semi-quantitative grading of results, and allow for automation to various extents based on the testing medium employed (i.e., tube vs. gel). Flow-cytometry-based phenotyping used minimal antisera (0.5–1 µl depending on antigen) compared to 50 µl used in our agglutination-based testing, can be easily batched, with reagent cocktails prepared in advance, and allows for automation, with quantitative results reporting contributing to reproducible interpretation. Additionally, the relatively miniscule amount of patient sample (1 µl) required provides potential benefits with respect to reduction of patient sample volumes required for testing when compared with 27.5 µl used in the conventional technique.

Comprehensive cost analysis is beyond the scope of this article and is impacted by a variety of factors which will vary significantly between institutions such as testing volume, technologist salaries, vendor contracts, existing test methods and availability of flow cytometry, currencies, among other things. Given the vast reduction in reagents required using this method compared to conventional agglutination-based assays, it is reasonable to conclude that this method may provide substantial cost benefits to centres with sufficient testing volume. For example, in our centre, the cost of reagents and consumables required to perform our agglutination-based assay on a single patient is $90.60 Canadian (CDN), while testing a single patient using our flow cytometry assay is estimated to cost $17.24 CDN including controls and the tray which was the largest contributor to cost in running a single patient ($6.92 CDN). If batching patients and maximizing the capacity of a tray the cost of reagents and consumables can be reduced to $4.28 CDN per patient.

Problems with agglutination were not encountered during this validation. We speculate that this is likely due to the lower ratio of reagent antisera to RBCs used in this method compared to agglutination assays. Additionally, vortexing was performed as described in the methods which helps to disrupt agglutination.

At CZ-BTS, we have implemented this technology in collaboration with our human leukocyte antigen (HLA) laboratory who provide access and regular care and maintenance for the flow cytometers and trains the BTS technologists in their use. Our current approach is to batch all patients who receive a transfusion and perform phenotyping on the pretransfusion specimen once a transfusion has occurred. This aids in antibody identification following transfusion for those patients who become alloimmunized by transfusion.

A highly phenotyped RBC component inventory provided by blood suppliers in conjunction with a highly phenotyped recipient population has many potential benefits. With regard to blood suppliers, this would aid in identification of units to be used in alloimmunized patients, may lead to identification of rare RBC donors and allows for increased phenotype matching in patients with a recognized indication. From the hospital BTS perspective, having a highly phenotyped recipient pool would allow for faster antibody identification in patients who develop antibodies and prophylactic matching of RBC units for patients with an appropriate indication.

Additionally, the emergence of low-cost, high-throughput phenotyping could be the first step towards a transfusion system where phenotype matching for patients is more broadly utilized. Studies have demonstrated the effectiveness of phenotype-matched blood in reducing immunization following transfusion. In a study of chronically transfused myelodysplastic syndrome patients, matching for C, E and K was associated with lower immunization rates (11% vs. 23%) [3]. It is noted that prevention of alloimmunization in those of childbearing potential by matching for C, E and K may prevent up to 40% of cases of hemolytic disease of the fetus and newborn (HDFN). This routine phenotype matching is performed in the Netherlands [4, 13]. A study of surgical patients, randomized to receive either phenotype-matched or unmatched blood, showed a reduction in alloimmunization of 64% following a single unit transfused in the phenotype-matched group [5].

The two main barriers to universal phenotype matching include the cost of phenotyping for recipient and donor RBCs and the procedural complexity around unit selection and inventory management utilizing this type of approach. The advent of high-throughput, cost-effective phenotyping using flow cytometry techniques widely available to hospital-based BTS largely removes the former of these barriers.

Future directions for this work include the evaluation of flow cytometry to phenotype patients with positive direct antiglobulin testing (DAT), evaluation of additional RBC antigens and a full range of available antisera using this method and flow cytometric RBC antigen phenotyping of RBCs from donor unit segments. All DAT-positive samples showed increased background with anti-IgG negative controls while anti-IgM background was not affected. Therefore, any sample showing increased anti-IgG background would automatically be tested using our routine agglutination-based assay. There is also potential for using flow cytometry as an antibody identification technique utilizing patient plasma and panels of reagent RBCs with a flow cytometric detection system for the antigen–antibody reactions.

Limitations of this technique are that flow cytometers are only likely to be available in a medium or large hospital BTS or blood suppliers. The technique would be impractical in a small hospital as a flow cytometer and/or the attendant expertise and training are unlikely to be available. For maximal cost efficiency, the BTS would have to either access flow cytometry resources elsewhere in the hospital, or purchase and maintain a flow cytometer for dedicated use in BTS, which would introduce considerable cost and complexity only justifiable with high-volume testing.

RBC antigen genotyping for prediction of phenotype using low-cost, high-throughput next-generation sequencing (NGS) and other probe-based genetic assays is a rapidly emerging field [14]. While these techniques may supplant high-volume donor antigen phenotyping in the future, it will only be feasible and cost-effective in a high-volume setting and will likely be best suited for blood suppliers where the volume of testing is high and the turnaround time for testing is less critical than in a hospital transfusion service.

A fully genotyped donor RBC inventory utilizing NGS performed by the blood supplier could be combined with a fully phenotyped pretransfusion patient population using flow cytometric RBC antigen typing at the hospital. This could allow for routine, full phenotype matching of donor and recipient with an expected reduction in alloimmunization and its associated complications.

Finally, the development of directly conjugated antibodies would allow massive volumes of phenotyping for large BTS and blood suppliers that could allow nearly universally phenotyped RBC inventories for common immunogenic antigens, which would represent a major step forward in transfusion medicine.

ACKNOWLEDGEMENTS

The authors thank Kim Johannesson-Veinotte and Craig Rourke for providing technical assistance in the validation of the flow cytometry protocol. The authors thank the HLA and BTS staff and management for support of this validation.

J.G.Q. was involved in design, analysis of data and writing of manuscript; R.L. was involved in design, data acquisition, analysis and manuscript writing; G.C. was involved in data analysis and manuscript preparation; C.C. was involved in design and manuscript preparation; S.S.R.A. was involved in some aspects of conceptual design and manuscript review; S.R.A. was involved in some aspects of conceptual design and manuscript review.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

ORCID

Gwen Clarke https://orcid.org/0000-0001-7738-3378
Jason George Quinn https://orcid.org/0000-0002-6579-4185

REFERENCES

1. Evers D, Middelburg RA, de Haas M, Zalpuri S, de Vooght KM, van de Kerkhof D, et al. Red-blood-cell alloimmunisation in relation to antigens' exposure and their immunogenicity: a cohort study. Lancet Haematol. 2016;3:284–92.
2. Fasano RM, Meyer EK, Branscomb J, White MS, Gibson RW, Eckman JR. Impact of red blood cell antigen matching on alloimmunization and transfusion complications in patients with sickle cell disease: a systematic review. Transfus Med Rev. 2019;33:12–23.
3. Lin Y, Saskin A, Wells RA, Lenis M, Mamedov A, Callum J, et al. Prophylactic RhCE and Kell antigen matching: impact on alloimmunization in transfusion-dependent patients with myelodysplastic syndromes. Vox Sang. 2017;112:79–86.
4. Luken JS, Folman CC, Lukens MV, Meekers JH, Ligthart PC, Schonewille H, et al. Reduction of anti-K-mediated hemolytic disease of newborns after the introduction of a matched transfusion policy: a nation-wide policy change evaluation study in The Netherlands. Transfusion. 2021;61:713–21.
5. Schonewille H, Honohan Á, van der Watering LM, Hudig F, Te Boekhorst PA, Koopman-van Gemert AW, et al. Incidence of alloantibody formation after ABO-D or extended matched red blood cell transfusions: a randomized trial (MATCH study). Transfusion. 2016;56:311–20.
6. Derr DA, Dickerson SJ, Steiner EA. Implementation of gel column technology, including comparative testing of Ortho ID-MTS with standard polyethylene glycol tube tests. Immunohematology. 1998;14:72–4.
7. Eduardo D, Vladislav C. Implementation of gel testing for antibody screening and identification in a community hospital, a 3-year experience. Lab Med. 2005;36:489–92.
8. Weisbach V, Kohnhäuser T, Zimmermann R, Ringwald J, Strasser E, Zingsem J, et al. Comparison of the performance of microtube column systems and solid-phase systems and the tube low-ionic-strength solution additive indirect antiglobulin test in the detection of red cell alloantibodies. Transfus Med. 2006;16:276–84.
9. Farias MG, Bó D, Castro SM, da Silva AR, Bonazzoni J, Scotti L, et al. Flow cytometry in detection of fetal red blood cells and maternal F cells to identify fetomaternal hemorrhage. Fetal Pediatr Pathol. 2016;35:385–91.
10. Davis BH, Davis KT. Laboratory assessment of fetomaternal hemorrhage is improved using flow cytometry; ortho (United States). Lab Med. 2007;38:365–71.
11. Arnoni CP, Muniz JG, Vendrame TAP, Medeiros R, Cortez AJP, Latini FRM, et al. Correlation among automated scores of agglutination, antigen density by flow cytometry and genetics of D variants. Transfus Apher Sci. 2019;58:680–4.
12. Arndt PA, Garratty G. A critical review of published methods for analysis of red cell antigen-antibody reactions by flow cytometry,

and approaches for resolving problems with red cell agglutination. Transfus Med Rev. 2010;24:172–94.
13. Dajak S, Culić S, Stefanović V, Lukačević J. Relationship between previous maternal transfusions and haemolytic disease of the foetus and newborn mediated by non-RhD antibodies. Blood Transfus. 2013;11:528–32.
14. Denomme GA. Prospects for the provision of genotyped blood for transfusion. Br J Haematol. 2013;163:3–9.

How to cite this article: Liwski R, Clarke G, Cheng C, Abidi SSR, Abidi SR, Quinn JG. Validation of a flow-cytometry-based red blood cell antigen phenotyping method. Vox Sang. 2023;118:207–16.

Received: 21 April 2022 | Revised: 8 November 2022 | Accepted: 30 November 2022

DOI: 10.1111/vox.13394

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Evaluation of cell collection efficiency in non-mobilized adult donors for autologous chimeric antigen receptor T-cell manufacturing

Juan A. Piñeyroa[1] | Joan Cid[1,2,3] | Alexandru Vlagea[4] | Gloria Carbassé[1] | Paola Henao[1] | Noemí Bailo[1] | Valentín Ortiz-Maldonado[2,5] | Núria Martínez-Cibrian[5] | Marta Español[4] | Julio Delgado[2,3,5] | Álvaro Urbano-Ispizua[2,3,5] | Miquel Lozano[1,2,3]

[1]Apheresis & Cellular Therapy Unit, Department of Hemotherapy and Hemostasis, ICMHO, Hospital Clínic, Barcelona, Spain

[2]IDIBAPS, Barcelona, Spain

[3]University of Barcelona, Barcelona, Spain

[4]Department of Immunology, CDB, Hospital Clínic, Barcelona, Spain

[5]Department of Hematology, ICMHO, Hospital Clínic, Barcelona, Spain

Correspondence
Joan Cid, Unitat d'Afèresi i Teràpia Cel·lular, Servei d'Hemoteràpia i Hemostàsia, ICMHO, Hospital Clínic, C/.Villarroel, 170, Barcelona, 08036, Catalonia, Spain.
Email: jcid@clinic.cat

Funding information
The authors received no specific funding for this work.

Abstract

Background and Objectives: Data about collection efficiency 1 (CE1), which takes into account blood cell counts before and after collection, thus providing a more accurate estimate, in the collection of autologous T lymphocytes by apheresis for chimeric antigen receptor (CAR) T-cells remain scarce. We evaluated donor- and procedure-related characteristics that might influence the CE1 of lymphocytes.

Materials and Methods: We retrospectively reviewed all mononuclear cell (MNC) collections) performed for CAR T-cell manufacturing in our institution from May 2017 to June 2021 in adult patients. Age, gender, weight, total blood volume (TBV), prior haematopoietic cell transplant, diagnosis, days between last treatment and apheresis, pre-collection cell counts, duration of apheresis, TBV processed, vascular access, inlet flow and device type were analysed as potential factors affecting CE1 of lymphocytes.

Results: A total of 127 autologous MNC collections were performed on 118 patients diagnosed with acute lymphoblastic leukaemia ($n = 53$, 45%), non-Hodgkin lymphoma ($n = 40$, 34%), multiple myeloma ($n = 19$, 16%), and chronic lymphocytic leukaemia ($n = 6$, 5%). The median CE1 of lymphocytes was 47% (interquartile range: 32%–65%). In multiple regression analysis, Amicus device was associated with higher CE1 of lymphocytes ($p = 0.01$) and lower CE1 of platelets ($p < 0.01$) when compared with Optia device.

Conclusion: The knowledge of the MNC and lymphocyte CE1 of each apheresis device used to collect cells for CAR T therapy, together with the goal of the number of cells required, is essential to define the volume to be processed and to ensure the success of the collection.

Keywords

apheresis, CAR T-cell, collection efficiency, mononuclear cell collection

Highlights

- There was a positive correlation between the number of total lymphocytes, CD3+, CD4+ and CD8+ cells in peripheral blood before starting the apheresis procedure and the number of respective collected cells.
- The Amicus device was associated with higher lymphocyte collection efficiency 1 (CE1) and lower platelet CE1 when compared with the Spectra Optia device in non-mobilized donors for autologous chimeric antigen receptor (CAR) T-cell manufacturing.
- When peripheral veins were used, lymphocyte CE1 was equal to that achieved using a central line in non-mobilized donors for autologous CAR T-cell manufacturing.

INTRODUCTION

Mononuclear cell (MNC) collection is an apheresis procedure in which the blood of the donor is passed through a medical device, which separates and collects white blood cells and returns the remainder to the donor [1]. In the complex manufacturing process of producing chimeric antigen receptor (CAR) T-cells, autologous T lymphocytes (CD3+ cells) are required to be collected during the first step of the process [2].

Physicians working in an apheresis unit are familiar with MNC collections performed to collect autologous haematopoietic progenitor cells after mobilization from bone marrow to peripheral blood. However, the collection of autologous CD3+ cells in non-mobilized donors is challenging because it could be technically difficult when performing the apheresis procedure [2]. First, some donors have a complete blood count (CBC) with low number of leukocytes, a frequent situation among refractory non-Hodgkin lymphoma (NHL) and acute lymphoblastic leukaemia (ALL) patients, making it difficult to establish red blood cell (RBC)–white blood cell (WBC) interface. Second, little is known about the collection efficiency 1 (CE1) of CD3+ cells in this setting. Moreover, optimal storage condition, T-cell expansion or final CAR T-cell product could be influenced by the rest of peripheral blood cells in the collected bag [3, 4].

We, therefore, performed this retrospective study at our institution to calculate the CE1. CE1, in contrast to CE2, takes into consideration blood cell counts immediately before and after the procedure, thus providing a more accurate estimate of the efficiency of the procedure. CE1 was calculated in different lymphocyte populations and other peripheral blood cells that are present in the final collected bag, in patients who underwent MNC apheresis for the collection of T-cells required for CAR T-cell therapy. We also evaluated donor- and procedure-related characteristics that might influence this CE1.

MATERIALS AND METHODS

Patients

We reviewed consecutive MNC collections performed from May 2017 to June 2021 in patients who had indication for autologous production of CAR T-cells in the Apheresis and Cellular Therapy Unit at Hospital Clínic, Barcelona. Patients who were less than 18 years old were not included in this analysis. The study was approved by the Ethics Committee of the Hospital Clínic. All donors signed an informed consent to undergo MNC and consented to data release.

Apheresis

Patients were medically assessed before performing apheresis collection. Angiotensin-converting enzyme inhibitors [5], beta blockers and anticoagulant medications [6] were suspended the day before the apheresis procedure. In 94% of cases, a chemotherapy and immunosuppressive treatment wash-out period was performed, defined as at least 2 weeks from the last dose.

Apheresis nurses evaluated peripheral vein access. If they were inadequate to perform the apheresis procedure, a central venous catheter (Softcell catheter 12.5 F, Bard, Access Systems, Inc., Salt Lake City, UT, USA) was placed under ultrasound guidance. MNC collections were performed either continuous MNC (cMNC) with the Spectra Optia v12.0 (Terumo BCT, Lakewood, CO, USA) or with the Amicus v6.0 (Fresenius Kabi, Bad Homburg, Germany) apheresis platforms, following manufacturer's instructions. Amicus was operated through intermittent collection, during a cycle, and MNCs were separated by density centrifugation [7]. Automatically, at a certain volume, the enriched buffy coat was pumped into the chamber to lift and transferred into a collection bag. By contrast, whole blood was separated into RBCs, buffy coat (leukocytes), platelets and plasma by continuous spinning of the centrifuge in cMNC Optia. Manually the place where to suck the buffy coat was established and was continuously collected. Total blood volume (TBV) was calculated according to Nadler et al [8]. We used adenine-citrate-dextrose (ACD-A) as anticoagulant at a rate of up to 1.2 ml/min/L of TBV and at a ratio of 1:12. Routinely, patients received intravenous magnesium sulphate (Mg) and calcium chloride (Ca) solution (1 mol Ca and 0.5 mol Mg per 10 mol citrate), prepared in 100 ml 0.9% saline solution [9]. Adverse events during apheresis, such as hypotension, bleeding, symptomatic hypocalcaemia or allergic reactions, were collected from medical records. TBV to process was calculated, either using the CE1 formula previously reported by our group [10] when a target CD3+ cell quantity was desired or a fixed number of TBVs according to manufacturer's CAR T-cell protocols.

$$\text{Estimate TBV} = \frac{100 \times \text{Desired lymphocytes yield}}{\text{Pre-collection lymphocyte count} \times (\text{CE1} = 39.7)}.$$

Laboratory testing

On the day of the apheresis collection, blood samples were obtained from the patient immediately before starting and immediately after finishing the apheresis procedure. Furthermore, a sample was obtained from the collected bag diluted 1/10 in saline and the collected volume of the product was recorded using device information. From all these samples, a CBC was performed using an autoanalyser (Advia 2120, Siemens AG, Madrid, Spain) as well as lymphocyte populations (CD3+, CD4+ and CD8+ cells) were analysed by flow cytometry (FACSCanto, BD Biosciences, Barcelona, Spain).

Statistical analysis

The CE1 of different cell populations (lymphocytes, CD3+, CD4+, CD8+, MNCs and platelets) was calculated using the following formula:

$$\text{CE1}(\%) = \frac{\text{Product cell count} \times \text{Product volume}}{\left(\frac{\text{Pre-collection cell count} + \text{post-collection cell count}}{2}\right) \times \text{Whole blood processed}} \times 100.$$

Qualitative and quantitative data are reported as number (frequencies) and median (interquartile range [IQR]), respectively. Pearson coefficient and longitudinal linear regression were used to correlate continuous variables.

Potential variables contributing to predict CE1 of different cell populations were identified using multiple regression analysis. Dependent variables taken into consideration were as follows: CE1 of lymphocyte, CD3+, CD4+, CD8+, MNCs and platelets. Potentially, predictors of cell collection efficiencies included (independent variables) donor clinical characteristics (gender, age, weight, TBV, prior haematopoietic cell transplant [HCT], diagnosis, days between last treatment and apheresis), pre-collection cell counts (haemoglobin, WBCs, total lymphocyte count, CD3+, CD4+, CD8+, MNCs—sum of monocytes and lymphocytes—platelet count) and procedure-related characteristics (duration of cell collection, TBVs processed, vascular access, inlet flow and device type).

Data were analysed with software (IBM SPSS Statistics 23, IBM Corporation, Armonk, New York, USA). A p value of less than 0.05 was considered significant.

RESULTS

Our series comprises 118 patients whose main clinical characteristics are shown in Table 1.

Patients underwent 127 apheresis procedures, with no major adverse events occurred during MNC collections. Two and three MNC collections were performed in seven and one patients, respectively. Reasons for second collections were excess of blasts in collected bag, 3 (33%), bacterial contamination, 3 (33%) and insufficient viral transduction, 1 (11%), during academic manufacturing process, change of therapeutic decision towards another CAR T-cells product, 1 (11%) and unknown failure during industrial manufacturing process, 1 (11%).

TABLE 1 Main characteristics of patients

Clinical characteristic	Patients (n = 118)
Gender	
Male	62 (53%)
Female	56 (47%)
Age (years)	51 (30–63)
Weight (kg)	68 (59–80)
Total blood volume (L)	4.4 (3.7–5)
Prior haematopoietic stem cell transplant	
Allogeneic	45 (38%)
Autologous	31 (26%)
None	42 (36%)
Diagnosis	
Acute lymphoblastic leukaemia	53 (45%)
Non-Hodgkin lymphoma	40 (34%)
Multiple myeloma	19 (16%)
Chronic lymphocytic leukaemia	6 (5%)
Days between last treatment and apheresis (days)	50 (25–125)

Note: Values are expressed as number (frequencies) or median (interquartile range).

TABLE 2 Main characteristics of apheresis procedures

Characteristic	All collections (n = 127)
Device	
Amicus	92 (72%)
Optia	35 (28%)
Vascular access	
Peripheral veins	101 (80%)
Central line	26 (20%)
Whole blood processed (L)	4.8 (3.4–7.7)
Number of total blood volume processed	1 (0.8–1.9)
Inlet flow (ml/min)	70 (61–78)
Duration of apheresis procedure (min)	87 (65–146)

Note: Values are expressed as number (frequencies) or median (interquartile range).

Table 2 shows the main parameters of apheresis procedures. Of note, 92 (72%) procedures were performed with Amicus (Fresenius kabi) and peripheral lines were used as vascular access in 101 (80%) cases. According to the need for a second collection (excluding contamination and therapeutic decision) by device, Amicus were involved in three (3%) cases and Optia in two (5%) cases. The volume of ACD-A and Ca-Mg infused was 331 (272–482) ml and 93 (71–158) ml, respectively. Haematocrit calculated in the collection bag was Optia 1 (0–3)% and Amicus 6 (3–12)%. Table 3 shows cell blood counts performed from peripheral blood and calculation of CE1.

TABLE 3 Cell blood counts from peripheral blood and collection efficiency 1 (CE1)

Blood count	*n*	Before collection	After collection	CE1 (%)
Haemoglobin (g/L)	127	112 (96–123)	99 (85–113)	-
Platelets ($\times 10^9$/L)	127	145 (72–203)	111 (54–168)	6 (4–12)
Mononuclear cells ($\times 10^9$/L)[a]	127	1.4 (1–2.3)	1.2 (0.8–1.7)	50 (38–67)
Lymphocytes ($\times 10^9$/L)	127	1.1 (0.6–1.8)	0.8 (0.5–1.5)	47 (32–65)
Monocytes ($\times 10^9$/L)	127	0.3 (0.2–0.5)	0.3 (0.2–0.4)	22 (12–39)
CD3+ cells ($\times 10^9$/L)	120	0.8 (0.4–1.3)	0.6 (0.4–1.1)	38 (20–54)
CD4+ cells ($\times 10^9$/L)	120	0.3 (0.2–0.4)	0.2 (0.1–0.4)	37 (20–56)
CD8+ cells ($\times 10^9$/L)	120	0.5 (0.1–0.8)	0.4 (0.2–0.7)	36 (19–53)

Note: Values are expressed as median (interquartile range).
[a]Mononuclear cells is the sum of lymphocytes and monocytes.

TABLE 4 Multiple regression analyses of patient- and procedure-related variables affecting collection efficiency 1 (CE1) of cells

CE1 (%) of cells	Variable	β standardized	R^2	*p* value
WBCs	Diagnosis (MM vs. other diseases)	−0.26	0.12	0.001
	WBC count before collection	−0.25		
MNCs[a]	MNC count before collection	−0.34	0.15	<0.001
	Diagnosis (MM vs. other diseases)	−0.21		
Lymphocytes	Apheresis device (Amicus vs. Optia)	0.36	0.17	<0.001
	Lymphocytes count before collection	−0.29		
	Diagnosis (NHL vs. other diseases)	0.25		
Platelets	Apheresis device (Amicus vs. Optia)	−0.6	0.39	<0.001
	Weight	−0.23		

[a]Mononuclear cells are the sum of lymphocytes and monocytes.
Abbreviations: MNC, mononuclear cell collection; WBC, white blood cell.

We observed a positive correlation between the number of lymphocytes, CD3+, CD4+ and CD8+ cells in peripheral blood before starting the apheresis procedure and the number of respective collected cells ($r^2 = 0.05$, 0.23, 0.09 and 0.31, respectively; $p < 0.05$) and between the TBV processed and lymphocytes, CD3+, CD4+ and CD8+ yield ($r^2 = 0.04$, 0.13, 0.22 and 0.09, respectively; $p < 0.05$).

Variables contributing to the variance of CE1 of different cell populations were identified using multiple regression analysis. Table 4 shows the variables with predictive value in our multivariate regression model. Interestingly, Amicus device was associated with a higher CE1 of lymphocytes ($\beta = 0.36$; $R^2 = 0.17$; $p < 0.001$) and a lower CE1 of platelets ($\beta = -0.6$; $R^2 = 0.39$; $p < 0.001$) when compared with Optia. However there was no differences between the two separators in the CE1 of MNC, CD3+, CD4+ and CD8+. [Correction added on 07 March 2023 after first online publication: the preceding sentence was added in this version.]

DISCUSSION

We report for the first time a comprehensive study of CE1 of different lymphocyte cell populations in a group of non-mobilized patients who underwent MNC collections by apheresis to produce CAR T-cells.

We observed in the current study, as previously reported [11–14] and because it is somewhat intuitive [15], that higher yield of desired cells is associated with higher pre-collection cells in peripheral blood in the donor and higher TBV processed. However, in order to maximize the collection efficiency of our apheresis procedure and to personalize collection variables for each donor, it is essential to know the CE1 of the apheresis platform.

Collection efficiency refers to the number of cells that are collected from the total number of cells processed by the apheresis device. CE1 is more accurate than CE2, because CE1 is calculated using both pre-collection and post-collection cell counts, not only pre-collection cell count [16]. In our study, median CE1 of lymphocytes was 47% (IQR: 32%–65%) similar to previous data reported in a group of non-mobilized adults diagnosed with ALL, chronic lymphocytic leukaemia (CLL) and NHL (median CE1 of 43%) [12]. When analysing the collection of CD3+ cells, we observed a median CE1 of 38% (IQR: 20%–54%). Other authors reported higher values in children and young adults [11, 14, 17].

Our main finding was that the apheresis device used to collect cells might have an impact in the CE1 of lymphocytes. We report in this study that Amicus device used for collection of MNCs was associated with higher CE1 of lymphocytes and lower CE1 of platelets when compared with Optia. Our group has already published lower CE1 of

platelets with Amicus when compared with Optia in the setting of MNC collections for different cellular therapy protocols [10]. In contrast, other authors did not find a relationship between device type and collection efficiency of MNCs [11, 12], but these results cannot be compared because Amicus was not used in those studies. However, we report here that CE1 of lymphocytes is higher when compared with Spectra Optia in the setting of MNC collections for CAR T-cell manufacturing. This is an important finding because the goal of the apheresis procedure is the collection of enough lymphocytes to produce CAR T-cells. The low content of platelets is also desired to avoid issues with storage conditions, T-cell expansion and characteristics of the final CAR T-cell product, as previously reported [3, 4]. A possible explanation could be a greater collection of MNCs and RBCs with Amicus, as suggested by the higher haematocrit in the collected product. Despite these results, no recommendation can be made on the use of one device over another for two reasons: the strength of correlation between the CE1 of lymphocytes and the device was low and external validation of these results is required.

We also found that patients with multiple myeloma (MM) compared with patients with other diagnoses had lower CE1 of WBCs and MNCs in multivariable regression model, in contrast with a previous report [13]. However, this association was not observed when analysing the CE1 of lymphocytes, CD3+, CD4+ and CD8+ cells, in line with other studies that did not find differences by diagnosis [18]. Moreover, multivariable regression model shows that patients with NHL compared with other diagnoses had higher CE1 of lymphocytes. Similarly to previous research that reported worse lymphocyte CE1 donors diagnosed with ALL versus NHL and CLL [12].

In our study, CE1 of different cell populations was not associated with previous HCT [12, 17] or days between last treatment and apheresis procedure [17]. We must be careful when interpreting timing from last treatment to apheresis procedure result because, in our institution, it was tried to stop chemotherapy administration 2 weeks before starting cell collection. In the present study, only eight patients received chemotherapy within 2 weeks before apheresis.

In a previous study, our group observed that collection of cells using a central line was associated with lower CE1 of MNCs [10]. However, this association has not been observed in the current study. The reasons for the discrepancies between the two articles were unknown. We speculated about the sample difference, $n = 770$ versus 127. Perhaps the strength of the association was smaller and needed a large sample to be observed. Another possible reason was that the characteristics of the samples were not the same. Patients with normal lymphocyte counts were included in the first article and the proportion of apheresis performed with the Optia (63%) and Spectra (21%) devices were higher than the present article. In any case, in light of the absence of statistical differences, as well as to avoid risks associated with central line placement, peripheral vein access should be chosen, if possible. Implementation of ultrasound-guided placement of peripheral venous access and knowledge of experienced nurses are essential to achieve this goal [19].

Our study has some limitations. It was a retrospective study and selection and information biases were possible. Moreover, MNC procedures were performed using different devices and different collection parameters. Of note, in some cases, TBV to be processed was calculated using the CE1 formula when a CD3+ cell target was desired. However, in other cases, TBV was a predefined collection parameter included in the apheresis manual of the CAR T-cell manufacturing process.

In conclusion, Amicus device was associated with higher CE1 of lymphocytes and lower CE1 of platelets when compared with Spectra Optia platforms. The knowledge of the MNC and lymphocyte CE1 of each apheresis device used to collect cells for CAR T therapy, together with the goal of the amount of cells required, is essential to define the volume to be processed and to assure the success of the collection.

Amicus device was associated with higher CE1 of lymphocytes and lower CE1 of platelets when compared with Spectra Optia device. This observation might be useful when collecting MNCs from non-mobilized donors with the aim of preparing CAR T-cells because it will allow us not only to personalize collection parameters for each donor but also to select the optimal apheresis device to collect the desired product with the desired cell content.

ACKNOWLEDGEMENTS

The authors are grateful to the nurses of the apheresis and cellular therapy unit for their excellent work that has made possible the performance of this study.

J.A.P. and J.C. designed the study, collected and analysed the data, and wrote the manuscript. A.V. performed the quantification of lymphocyte populations. G.C., P.H. and N.B. acquired apheresis procedure data and blood samples. V.O.M., N.M.C., M.E., J.D. and A.U.I. contributed to clinical data collection and approved the final version of the manuscript. M.L. supervised the research and reviewed and edited the manuscript.

CONFLICT OF INTEREST

Valentín Ortiz-Maldonado: consultant or advisory role (Kite Gilead, Celgene-BMS, Novartis, Janssen), travel grants (Kite Gilead, Celgene, Novartis, Roche, Takeda, Janssen) and honoraria (Kite Gilead, Celgene-BMS, Janssen). Miquel Lozano on behalf of his institution, Clinic Research Foundation, has received research support from Terumo BCT, Macopharma and Sanofi-Genzyme; speaker honoraria from Grífols and fees as a consultant for Grífols and Fresenius Kabi. The other authors declare that they have no conflicts of interest relevant to the manuscript submitted to *Vox Sanguinis*.

ORCID

Juan A. Piñeyroa https://orcid.org/0000-0003-4576-0540
Joan Cid https://orcid.org/0000-0001-5445-4508
Miquel Lozano https://orcid.org/0000-0003-2593-833X

REFERENCES

1. Padmanabhan A, Connelly-Smith L, Aqui N, Balogun RA, Klingel R, Meyer E, et al. Guidelines on the use of therapeutic apheresis in clinical practice – evidence-based approach from the Writing Committee of the American Society for Apheresis: the eighth special issue. J Clin Apher. 2019;34:171–354.

2. Fesnak A, Lin C, Siegel DL, Maus MV. CAR-T cell therapies from the transfusion medicine perspective. Transfus Med Rev. 2016;30:139–45.
3. Elavia N, Panch SR, McManus A, Bikkani T, Szymanski J, Highfill SL, et al. Effects of starting cellular material composition on chimeric antigen receptor T-cell expansion and characteristics. Transfusion. 2019;59:1755–64.
4. Gniadek TJ, Garritsen HSP, Stroncek D, Szczepiorkowski ZM, DH MK, Collaborative on behalf of the BE for ST (BEST). Optimal Storage Conditions for Apheresis Research (OSCAR): a Biomedical Excellence for Safer Transfusion (BEST) Collaborative study. Transfusion. 2018;58:461–9.
5. Owen HG, Brecher ME. Atypical reactions associated with use of angiotensin-converting enzyme inhibitors and apheresis. Transfusion. 1994;34:891–4.
6. McLeod BC, editor. Apheresis: principles and practice. 3rd ed. Bethesda, MD: AABB Press; 2010. p. 715.
7. Padmanabhan A. Cellular collection by apheresis. Transfusion. 2018;58:598–604.
8. Nadler SB, Hidalgo JH, Bloch T. Prediction of blood volume in normal human adults. Surgery. 1962;51:224–32.
9. Bolan CD, Cecco SA, Wesley RA, Horne M, Yau YY, Remaley AT, et al. Controlled study of citrate effects and response to i.v. calcium administration during allogeneic peripheral blood progenitor cell donation. Transfusion. 2002;42:935–46.
10. Cid J, Carbassé G, Alba C, Perea D, Lozano M. Leukocytapheresis in nonmobilized donors for cellular therapy protocols: evaluation of factors affecting collection efficiency of cells. J Clin Apher. 2019;34:672–9.
11. Jarisch A, Rettinger E, Sörensen J, Klingebiel T, Schäfer R, Seifried E, et al. Unstimulated apheresis for chimeric antigen receptor manufacturing in pediatric/adolescent acute lymphoblastic leukemia patients. J Clin Apher. 2020;35:398–405.
12. Tuazon SA, Li A, Gooley T, Eunson TW, Maloney DG, Turtle CJ, et al. Factors affecting lymphocyte collection efficiency for the manufacture of chimeric antigen receptor T cells in adults with B-cell malignancies. Transfusion. 2019;59:1773–80.
13. Chen J, Goss C, Avecilla ST, Hong H, Walsh E, Wuest D, et al. Evaluation of peripheral blood mononuclear cell collection by leukapheresis. Transfusion. 2019;59:1765–72.
14. Allen ES, Stroncek DF, Ren J, Eder AF, West KA, Fry TJ, et al. Autologous lymphapheresis for the production of chimeric antigen receptor T cells. Transfusion. 2017;57:1133–41.
15. Shi PA. Optimizing leukapheresis product yield and purity for blood cell-based gene and immune effector cell therapy. Curr Opin Hematol. 2020;27:415–22.
16. Neyrinck MM, Vrielink H. Calculations in apheresis. J Clin Apher. 2015;30:38–42.
17. Hutt D, Bielorai B, Baturov B, Z'orbinski I, Ilin N, Adam E, et al. Feasibility of leukapheresis for CAR T-cell production in heavily pre-treated pediatric patients. Transfus Apher Sci. 2020;59:102769.
18. Ceppi F, Rivers J, Annesley C, Pinto N, Park JR, Lindgren C, et al. Lymphocyte apheresis for chimeric antigen receptor T-cell manufacturing in children and young adults with leukemia and neuroblastoma. Transfusion. 2018;58:1414–20.
19. Salazar E, Gowani F, Segura F, Passe H, Seamster L, Chapman B, et al. Ultrasound-based criteria for adequate peripheral venous access in therapeutic apheresis procedures. 2021;36:797–801.

How to cite this article: Piñeyroa JA, Cid J, Vlagea A, Carbassé G, Henao P, Bailo N, et al. Evaluation of cell collection efficiency in non-mobilized adult donors for autologous chimeric antigen receptor T-cell manufacturing. Vox Sang. 2023;118:217–22.

Received: 20 December 2022 | Accepted: 21 December 2022

DOI: 10.1111/vox.13399

INTERNATIONAL FORUM

Vox Sanguinis ISBT International Society of Blood Transfusion

International Forum on Small-Volume Transfusions in Neonates and Paediatric Patients: Summary

Satyam Arora | Ruchika Goel | Arwa Z. Al-Riyami | Abdul Hakim Al-Rawas | Saif Al Hosni | Mauro Montanari | Benedetta Costantini | Christina Lee Lai Ling | Norliza Mustafa | Chan Kok Joo | Hari Krishan Dhawan | Sheetal Malhotra | Ratti Ram Sharma | Helen New | Rachel Moss | Jane Davis | Nancy Robitaille | Valérie Arsenault | Nabiha H. Saifee | Ann-Marie Taroc | Naomi Rahimi-Levene | Victoria Peer | Maha Badawi | Pauline M. Snijder | Elise J. Huisman | Josune Zubicaray Salegui | Julia Ruiz Pato | Julián Sevilla Navarro | José Mauro Kutner | Ana Paula Hitomi Yokoyama | Joyce Ching Mei Lam | Xin Ni Zhong | Mui Ling Heng | Oscar Walter Torres | Aggrey Dhabangi | Anel van Zyl | Nadia Mundey | Vernon Louw | Karin van den Berg | Nancy Dunbar

INTRODUCTION

Blood transfusions are critical therapeutic interventions practiced in hospitalized paediatric and neonatal patients. Red blood cells (RBCs), platelets, plasma and cryoprecipitate are frequently transfused for various indications. Preparation of these blood products for paediatric and neonatal patients includes special considerations surrounding unit selection, preparation of aliquots, neonate-specific unit allocation (e.g., dedicated units), irradiation, use of additive solutions and selection of units based on storage age, especially for foetuses and newborns. Other considerations include the mode of infusion, such as the use of gravity versus syringe/pump, as well as concern for potassium (K^+) levels in RBCs. In this forum, we aim to describe the practice variations in the preparation of small-volume aliquots to paediatric and neonatal patients around the world.

Respondent demography

Responses to the survey were received from 17 institutions, representing a wide geographical distribution over five continents, including the following countries: Argentina, Brazil, Canada, India, Israel, Italy, Malaysia, The Netherlands, Oman, Saudi Arabia, Singapore, South Africa (two responses), Spain, Uganda, United Kingdom (UK) and United States (US). All respondents represent institutes located in urban areas that provide tertiary care services and serve as teaching institutes/hospitals.

The responding institutions include hospitals providing services to both adults and paediatric patients ($n = 10$); maternal and children institutions ($n = 3$; Canada, Argentina and Singapore) and dedicated paediatric and neonatal centres (e.g., children's hospitals, $n = 4$; UK, US, The Netherlands and Spain). The median number of neonatal intensive care unit (NICU) beds in the responding institutes is 30.5 (mean = 31.4) and ranges from 12 beds (South Africa) to 65 beds (Canada). The respondent from Spain reports only paediatric intensive care services without NICU beds.

Aliquoting availability

All of the responding institutions provide small-volume aliquots of blood components to paediatric and neonatal patients. The type of blood components aliquoted varies among the institutions. RBCs are aliquoted at all centres using a sterile connecting device (SCD).

Most of the responding institutions prepare aliquots of most of the blood components with the exception of the respondent from

Uganda where only RBC aliquots are prepared (Table 1). Thirteen institutions prepare plasma aliquots and 11 prepare apheresis platelet aliquots. Platelets from whole blood are aliquoted by only six institutions, probably because the volume of whole-blood-derived platelets is already small. Cryoprecipitate is not aliquoted by any participating centre, possibly due to the availability of single cryoprecipitate units of small volume. The National Health Service Blood and Transplant in the UK specifically reports that they provide components as per adults to paediatric patients above 1 year of age [1].

Selection of blood components

The age of the blood component is one the most important factors considered for selecting a unit for transfusion to neonates. Release of K^+ by aging RBCs is considered clinically significant for neonates receiving large-volume transfusions [2]. The majority of responding institutions select units less than 3–7 days of age for RBC transfusion to neonates. The respondents from Spain and Uganda did not report an age consideration for selecting these units for transfusion.

Anticoagulant with additive solution is used in various parts of the world for extending the shelf life of stored RBCs. Due to mannitol being one of the components of additive solutions, some have expressed reservations regarding its use in neonatal patients [1]. Similar variability in practice is highlighted by responding institutions. Respondents from Malaysia, Saudi Arabia and Israel report the use of anticoagulant alone, whereas the remaining respondents report the use of additive solutions (e.g., Saline, adenine, glucose, mannitol [SAGM]) for neonatal and paediatric transfusions. The respondent from Spain did not report on the use of anticoagulant and additive solutions.

The selection of blood group for blood components used in cases of ABO incompatibility between the mother and the neonate is important to avoid the risk of haemolysis due to passively transferred anti-A/anti-B antibodies. The majority of respondents use group O RBCs (Rh type specific to the neonate) or RBCs that are ABO compatible with both the mother and the newborn. Respondents from the UK, Oman, Singapore, India, Saudi Arabia and Brazil also report the selection of blood group type based on various scenarios depending on the ABO group of the mother and the newborn as well as in case the mother blood group is not known/not available. The respondent from Italy reports the use of group O RBCs for children until 12 years of age.

Leukoreduction (LR) is a blood component modification process that is used to reduce the risk of febrile non-haemolytic transfusion reactions and decrease the risk of cytomegalovirus (CMV) transmission. The majority of responding centres report the universal use of LR for transfusion in paediatric and neonatal patients. The respondent from India reports the use of LR blood products as per availability, while Singapore reports use as per request. The respondent from Uganda reported that LR is not done at their centre. The respondent from Spain did not provide a response to this question.

TABLE 1 Components aliquoted

	Argentina	Brazil	Canada	India	Israel	Italy	Malaysia	The Netherlands	Oman	Saudi Arabia	Singapore	South Africa	South Africa-1	South Africa-2	Spain	Uganda	United Kingdom	United States
Products available as aliquots																		
Red blood cell	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Plasma	×	×	×		×			×	×	×		×	×	×	×		×	×
Platelets, apheresis		×	×	×				×			×	×	×	×	×		×	×
Platelets, whole blood		×	×			×	×	×							×			

Transfusion-transmitted CMV is a concern, especially when transfusing a foetus or a premature neonate as they are at greater risk for severe disease. The majority (9 of 17) of the respondents report that they do not screen for CMV serology with two respondents specifying that they consider leukoreduced RBCs as CMV safe hence do not test further (Table 2). Respondents from Italy, UK, Canada and The Netherlands report selected or specific scenarios for providing CMV seronegative units. Only the respondent from Israel reports universal screening of CMV for all units. The respondent from Spain did not report on this question.

Irradiation of blood components is performed to prevent the transfusion-associated graft-versus-host disease. Policies for the use of irradiated blood products in paediatric and neonatal patients are variable. Timing of irradiation is very important with respect to small-volume aliquots as irradiated units may contain higher level of K^+ and a greater degree of haemolysis in RBCs, increasing over storage [1]. The majority (12 of 17) of respondents report that they irradiate the aliquots just before issue. Respondents from Israel, Spain and Brazil report that they keep pre-irradiated aliquots as part of their available inventory. The respondent from Canada specifies that 'When the patient is at particular risk from hyperkalaemia, it is recommended that red cells be transfused within 24 h of irradiation or that cells have at least undergone centrifugation and supernatant plasma removal' [3]. The respondent from Spain reports that their centre does not have an irradiation facility onsite and thus all units are pre-irradiated. The respondent from Uganda reports that they do not have irradiation available at all.

Pathogen reduction (PR) is a technology developed to improve blood safety. While its use is standard in some countries, others are assessing feasibility for implementation. Many of the responding centres (10 out of 17) report that the PR technology is not yet available or not yet in use at their institutions (Table 2). Respondents from UK and The Netherlands are using PR blood components but not for paediatric or neonatal patients. The Netherlands also provide parvovirus B19 safe units to extremely premature and very low birth weight infants. Respondents from Italy, The Netherlands and UK report the use of solvent detergent plasma for paediatric and neonatal patients. Respondents from US and Saudi Arabia report the use of PR platelets for paediatric and neonatal patients.

Aliquot preparation

Fifteen of 17 respondents report that small-volume aliquots are prepared by the blood centre resulting in standardized and uniform aliquots. Respondents from US and Spain report that aliquots are prepared at the clinical facility (e.g., hospital blood bank). Five of 17 responding centres follow both the strategies of keeping a ready inventory of aliquots as well as preparing them when they receive a specific request. Of the remaining 12 responding centres, 6 of them reported that they keep them ready as part of their inventory and 6 centres prepare them based on the request received (Table 3). Eight of 17 respondents report that they prepare the volume of the aliquot based on the volume requested by the clinician. The remaining nine respondents report that they keep prepared aliquots of 'prefixed'/standard volumes, which may range from 45 to 90 ml. Three respondents report dividing the RBC bag into equal parts (India: 3; Canada: 4; UK: 6).

When preparing a small-volume aliquot, a discard volume may be added to compensate for the loss of the blood component in the tubing or the filter of the transfusion set. Twelve of 17 respondents report that they do not add any standard discard volume when preparing a small-volume aliquot (Table 3). The remaining respondents report adding a prefixed blood volume to the small-volume aliquot (ranging from 10 to 30 ml).

Aliquots for small-volume transfusion may be prepared using a SCD with transfer to a satellite bag or syringe. The majority of respondents (14 of 17) report the use of satellite/transfer bags of varying volumes. The respondent from Uganda reports the use of a component bag (already attached to the mother bag, without the use of SCD) to prepare the aliquot. Respondents from US and Brazil report the use of syringes to prepare the small-volume aliquots (<45 and 50 ml, respectively).

Two respondents report quality control testing for small-volume aliquots in addition to routine quality control measures done as per the regulatory requirements (UK: measures volume, haematocrit and haemoglobin content and checks for haemolysis; Argentina: measures K^+ for aliquots prepared for Intrauterine Transfusion (IUT) and checks for haemolysis). The remaining respondents report the use of available inventory of blood components for aliquot preparation, without additional quality control screening.

Unit allocation and transfusion/infusion practices

Policies and factors which influence RBC units allocated for small-volume transfusion are variable. Some respondents use any RBC unit available in the inventory, whereas 12 of the 17 respondents provide a dedicated unit for multiple future aliquoting for a single patient whenever possible (Table 3).

Small-volume aliquot preparation may result in change in date of expiry for the aliquot and/or the mother bag. There is some variability in this practice due to the method adopted for aliquot preparation. The majority of respondents (15 of 17) report that they do not change the date of expiry. Two respondents report that they do change the date of expiry after aliquot preparation since they use a manual method for providing the aliquots in syringes (Brazil: 4 h since aliquot is prepared manually; South Africa: 21 day from donation).

The majority of respondents (15 of 17) report the use of the remaining volume of blood component in the mother bag for further aliquoting after they prepare the initial aliquots. Respondents from Israel and Uganda report that they discard the remaining volume after preparing the aliquots.

Transfusion of small volumes may be done using gravity or by infusion/syringe pumps. Various type of infusion and syringe pumps are used. For neonates, all respondents report the use of infusion/

TABLE 2 Strategies to reduce transfusion-transmitted infections

	Argentina	Brazil	Canada	India	Israel	Italy	Malaysia	The Netherlands	Oman	Saudi Arabia	Singapore	South Africa-1	South Africa-2	Spain	Uganda	United Kingdom	United States
CMV negative practices																	
Provide CMV safe or CMV negative to all					×												
Provided to select populations			×			×		×								×	
Intrauterine transfusion			✓					✓									
All neonates/infants						✓										✓	
All paediatric haematology-oncology						✓											
Select indications								✓									
Not available, testing not performed	×		×		×		×		×		×	×	×		×		
Leukoreduced RBCs considered CMV safe (testing not done)										×							×
Information not provided														×			
Pathogen reduced practices																	
Not available or not used	×		×	×	×	×				×		×	×	×		×	
Technology available; not offered to neonates and infants								×								×	
Technology available; offered to neonates and infants						×				×							×
Solvent detergent plasma						✓		✓								✓	
Psoralen/UV-treated platelets										✓							✓
Riboflavin/UV-treated platelets										✓							
Information not provided							×							×			

Abbreviations: CMV, cytomegalovirus; RBC, red blood cell; UV, ultraviolet.

TABLE 3 Aliquot preparation practices

	Argentina	Brazil	Canada	India	Israel	Italy	Malaysia	The Netherlands	Oman	Saudi Arabia	Singapore	South Africa-1	South Africa-2	Spain	Uganda	United Kingdom	United States
Aliquot preparation																	
All aliquots pre-prepared in advance					×			×				×	×	×	×		
All aliquots prepared upon request	×		×			×			×	×							×
Both pre-prepared aliquots and upon request				×	×		×				×					×	
Aliquot volume																	
Standard					×		×	×			×	×	×	×	×	×	
Based on request	×		×	×	×		×		×	×							×
Discard volume added?																	
Yes	×		×				×		×								×
No/not applicable, standard volumes				×	×	×		×		×	×	×	×	×	×	×	
Aliquots prepared from a dedicated unit?																	
Yes, when practical or upon request	×		×		×	×		×	×	×		×	×			×	×
No				×			×				×			×	×		

syringe pumps. In contrast, aliquot transfusions in paediatric patients are variable. Nine of 17 respondents report the exclusive use of infusion pumps with five reporting the use of infusion pumps for all blood components except platelets and remaining three respondents report the use of gravity as sole method to transfuse aliquots to paediatric patients. Very small-volume transfusions are only given by syringe pump in Malaysia (<10 ml), UK and US (<50 ml) and Brazil (<50 ml). Details of the use of pumps or gravity were not specified by the reporting centre from Israel.

Significant variability was seen in the rate of transfusion of aliquots within the respondents based on the type of blood component used as well as the clinical condition. Some centres start transfusion for RBCs at 1 ml/kg/h for first 15 min and then gradually increase the rate of transfusion, as compared to 10–20 ml/kg/h for other blood components. Other centres report a standard rate of 5 ml/kg/h with transfusion over 2–4 h. Many respondents also report that they modify the rate of transfusion based on the clinical condition of the patient.

DISCUSSION

Neonatal and paediatric transfusions are provided by hospitals and blood centres around the world. With advances in component therapy for neonates, blood product modifications (irradiation, LR, CMV screening, aliquoting, PR, etc.) along with improved understanding of transfusion outcomes improve the ability to provide safe and effective transfusion support to paediatric and neonatal patients. Blood centre practices for neonatal transfusion are variable [4, 5]. This forum illustrates the current status of these practices around the world.

New et al. published one of the most comprehensive guidelines for supporting the transfusions in foetus, newborns and young children [1]. Respondents report that they refer to these guidelines for their centres. However, this forum highlights that adaption of these guidelines has not been uniform and local/regional practices may be variable. The types of blood component aliquoted vary significantly among our respondents and likely reflect the demand, clinical practice and resource availability.

Most of the responding centres used Citrate-phosphate-dextrose solution with adenine/Citrate-phosphate-dextrose (CPDA/CPD) anticoagulant for collection and SAGM as the additive solution to store the RBCs used for routine neonatal transfusions (e.g., 'top up'). Selection of ABO blood group for neonatal transfusion also shows marked variation. Many respondents maintain a ready inventory of group O RBCs while others provide RBCs based on ABO compatibility of the mother and infant.

Practices also vary for the use of irradiated blood products, LR and CMV screening of donors. Most of the responding centres report that they do not consider CMV serological status for providing blood to neonates since the seropositivity in donors in their region is very high. This is a significant finding since most of the guidelines from western world recommend using 'CMV safe' units for neonatal transfusions [1].

Irradiation practices are also highly variable as some respondents report that they use irradiated cellular blood components for all neonates below the age of 4 or 6 months, whereas other respondents report the use of irradiated components only for specific indications. Similarly, majority of respondents report irradiating aliquots just before the issue of the units while others report that they maintain a pre-irradiated inventory. LR is standard for most respondents but only provided on request or based on availability by some.

PR is not available in most of the responding institutions. In countries where PR technology is available, a few respondents provide PR units to both adults and paediatric and neonatal patients (Italy, US and Saudi Arabia), whereas others provide PR units to adults only (The Netherlands and UK).

These is variability with respect to aliquoting practices. Almost all the respondents report that the aliquots are prepared at the blood centre and few respondents add a discard volume to the aliquots. A change of date of expiry is made only when components are aliquoted in syringes using a manual method. SCD use is uniformly available with all responding institutions using a SCD to prepare an aliquot using transfer/satellite bags. Some responding institutions report the use of syringes for providing small volume of transfusions. None of the responding institutions report screening for supernatant electrolytes (mainly K^+) and majority responding institutions do not perform any additional quality control testing for prepared aliquots.

Allocation of unit is also a variable since very few responding institutions report the use of a dedicated donor unit and instead allocate units based on other factors. Transfusion of aliquots occurs mainly by infusion/syringe pumps at most responding institutions for neonates and by gravity for paediatric patients.

CONCLUSION

This forum summarizes the responses from large academic institutions in 16 countries (17 institutions) across the five continents who support paediatric and neonatal patients. It highlights an immense degree of variation in issues like consideration of unit age for preparation of aliquots, anticoagulant additive solutions used, selection of ABO blood group for aliquot preparation, volume of aliquot prepared, irradiation, unit allocation, mode of transfusion (gravity vs. pumps) and rates of transfusion. In general, uniformity is seen in the site of aliquot preparation, LR, CMV serology screening practices, expiry date of the aliquots, types of aliquot prepared (syringe and bags), quality control screening of aliquots and the fate of the remaining volume in the mother bag. The information from this international forum is probably just the 'tip of an iceberg' regarding the degree of variability in component preparation or manipulation by blood centres for paediatric and neonatal patients. To completely understand this variability, a more detailed survey is recommended. This information may help to identify and guide research as well as inform the development or refinement of existing guidelines, which may be adaptable to all regions of the world.

ORCID

Satyam Arora https://orcid.org/0000-0002-9048-5624
Ruchika Goel https://orcid.org/0000-0001-9653-9905
Arwa Z. Al-Riyami https://orcid.org/0000-0001-8649-0650
Ratti Ram Sharma https://orcid.org/0000-0002-7415-4665
Nabiha H. Saifee https://orcid.org/0000-0003-1646-9201
Naomi Rahimi-Levene https://orcid.org/0000-0003-3411-886X
Maha Badawi https://orcid.org/0000-0003-3272-2393
José Mauro Kutner https://orcid.org/0000-0003-3784-6731
Aggrey Dhabangi https://orcid.org/0000-0002-5615-3925

REFERENCES

1. New HV, Berryman J, Bolton-Maggs PH, Cantwell C, Chalmers EA, Davies T, et al. Guidelines on transfusion for fetuses, neonates and older children. Br J Haematol. 2016;175:784–828.
2. Yamada C, Edelson M, Lee A, Saifee NH, Bahar B, Delaney M. Transfusion-associated hyperkalemia in pediatric population: prevalence, risk factors, survival, infusion rate, and RBC unit features. Transfusion. 2021;61:1093–101.
3. National Advisory Committee on Blood and Blood Products. Recommendations for Use of Irradiated Blood Components in Canada. [cited 2022 Apr 14]. Available from: https://nacblood.ca/en/resource/recommendations-use-irradiated-blood-components-canada.
4. Reeves HM, Goodhue Meyer E, Harm SK, Lieberman L, Pyles R, Rajbhandary S, et al. Neonatal and pediatric blood bank practice in the United States: results from the AABB pediatric transfusion medicine subsection survey. Transfusion. 2021;61:2265–76.
5. Arora S, Dua S, Goel R. Neonatal and pediatric transfusion practices and policies in India: a survey-based cross-sectional assessment of blood centers. Transfusion. 2022;62:1000–9.

Guest Editors
Satyam Arora
Postgraduate Institute of Child Health, Noida, India
Email: satyamarora83@gmail.com

Ruchika Goel
Simmons Cancer Institute at SIU School of Medicine, Springfield, IL, USA
Email: rgoel71@siumed.edu

International Forum Editor
Nancy Dunbar
Dartmouth-Hitchcock Medical Centre, Lebanon, NH, USA
Email: nancy.m.dunbar@hitchcock.org

How to cite this article: Arora S, Goel R, Al-Riyami AZ, Al-Rawas AH, Al Hosni S, Montanari M, et al. International Forum on Small-Volume Transfusions in Neonates and Paediatric Patients: Summary. Vox Sang. 2023;118:223–9.

Received: 20 December 2022 | Accepted: 21 December 2022

DOI: 10.1111/vox.13398

INTERNATIONAL FORUM

Vox Sanguinis ISBT International Society of Blood Transfusion

International Forum on Small-Volume Transfusions in Neonates and Paediatric Patients: Responses

Satyam Arora | Ruchika Goel | Arwa Z. Al-Riyami | Abdul Hakim Al-Rawas | Saif Al Hosni | Mauro Montanari | Benedetta Costantini | Christina Lee Lai Ling | Norliza Mustafa | Chan Kok Joo | Hari Krishan Dhawan | Sheetal Malhotra | Ratti Ram Sharma | Helen New | Rachel Moss | Jane Davis | Nancy Robitaille | Valérie Arsenault | Nabiha H. Saifee | Ann-Marie Taroc | Naomi Rahimi-Levene | Victoria Peer | Maha Badawi | Pauline M. Snijder | Elise J. Huisman | Josune Zubicaray Salegui | Julia Ruiz Pato | Julián Sevilla Navarro | José Mauro Kutner | Ana Paula Hitomi Yokoyama | Joyce Ching Mei Lam | Xin Ni Zhong | Mui Ling Heng | Oscar Walter Torres | Aggrey Dhabangi | Anel van Zyl | Nadia Mundey | Vernon Louw | Karin van den Berg | Nancy Dunbar

SULTANATE OF OMAN

Arwa Z. Al-Riyami, Abdul Hakim Al-Rawas & Saif Al Hosni

Section 1

Our hospital is located in the capital city of the country.

This hospital is a tertiary care referral and teaching hospital that is university affiliated. It has its own hospital-based blood transfusion service/blood centre responsible for recruiting donors; screening and selecting blood donors; whole blood (WB) and apheresis collection; testing, processing and storage of blood components; pre-transfusion testing, and provide clinical transfusion exclusively for use within the hospital. In addition, the hospital gets a fraction of its blood supply from an external blood supplier.

The hospital treats adult, paediatric and neonatal patients.

The hospital has a neonatal intensive care unit (NICU) with 18 beds.

Section 2

a. Our centre supports aliquoting/small-volume transfusions in neonates and paediatrics.

b. We provide small-volume transfusions for red blood cell (RBC) and plasma components by aliquoting the requested volume of RBCs and plasma in addition to a small volume to account for tubings.

For platelet (PLT) and cryoprecipitate components, no aliquoting is performed due to the small volume of the units and the requested transfusion volume. Most PLT units in our inventory are buffy coat-random donor PLTs.

Section 3A

i. For neonatal transfusion: fresh units that are <5 days old are used. However, units within 7 days of the collection can be used if needed.

For paediatric transfusion: fresh units (<10–14 days old) are used for patients with underlying haemoglobinopathies (e.g., thalassemia patients).

ii. All packed red blood cell (PRBC) units stored in the blood bank are collected in citrate-phosphate-dextrose solution with adenine (CPDA) anticoagulant and stored in Saline, Adenine, Glucose,

Mannitol (SAGM) additive solution, whether if used for adults or paediatric/neonatal patients.

iii. All neonates get ABO/D blood group typing and a direct antiglobulin test (DAT) performed to determine the type of units to be selected. Material samples are tested for blood group, antibody screen and antibody identification if needed. Historical records are also checked and compared.

iv. All neonates (<4 months old) receive group O RBC units, regardless of their blood group results. If the neonatal and maternal blood group is O positive, O-positive RBC units are selected. If neonatal blood group is of any other blood group, or if maternal blood group is unknown/sample is unavailable, O-negative RBC units are selected.

If the mother has a previously detected antibody, neonatal DAT test is positive and/or the antibody screen on the mother is positive, additional testing is performed to identify the present antibody. Antigen(s)-negative units for the implicated antibody(ies) are selected, and full indirect antiglobulin test (IAT) cross-match is performed using the maternal or neonatal sample prior to issue. In the absence of a maternal sample, a full IAT cross-match is performed using the neonatal sample and O-negative RBC units. For the rest of paediatric patients, RBC units selected are either ABO-matched or identical and antigen-negative for any historical or detectable antibodies. Type of cross-match performed is as per the procedure done for adult patients (immediate spin in the absence of current/historical antibody, full IAT cross-match in the presence of either).

v. All cellular blood components used for neonates are leukoreduced.

vi. Cytomegalovirus (CMV) testing is not performed on blood donors at our centre or other blood suppliers in the country. However, all cellular blood components used for adults and paediatric/neonatal patients in our hospital are leukoreduced.

vii. All cellular blood components issued for neonates are irradiated.

For other paediatric patients, irradiation is performed upon request from the ordering physician and according to existing hospital guidelines for irradiation of blood and blood components. Unit irradiation is performed prior to issue.

viii. Pathogen reduction in our centre is not used for paediatric/neonatal transfusion. All in-house collected PLT components are tested for bacterial contamination using BacT/Alert microbial identification system.

Section 3B

a. In our centre, unit aliquots are prepared by trained blood bank staff.

b. Unit aliquots are prepared upon receiving the request. These aliquots are made from a pre-set neonatal inventory of reserved RBC components. This inventory includes group O-positive and O-negative RBC units that are pre-storage leukoreduced, fresh <5–7 days old, and negative for sickle cell and G6PD screen tests.

c. The volume to be aliquoted is based on the requested volume with an additional volume to account for the tubings.

d. A pre-fixed additional volume of 25 ml is added to account for the tubings.

e. Irradiation is performed on the unit aliquoted to be issued, while the remainder of the un-irradiated unit is returned to the inventory for future use.

Irradiation is performed before issue of the cellular blood components. This is facilitated by having an irradiator in-house.

f. Unit aliquots are prepared in a separate satellite/transfer bag. A new sterile satellite/transfer bag is connected to the original bag using the sterile docking device. For neonates, aliquots are prepared in syringes.

g. A sterile docking device is used to maintain the sterility of the units during aliquoting in a bag.

h. No additional quality testing is done on the aliquots. All units stored in the blood inventory are tested for all quality requirements as per local standard operating procedures. All units reserved for neonates in the neonatal inventory of RBC units are additionally screened for G6PD and sickle cells.

i. Aliquots are not tested for K^+ levels. Instead, all units used for neonates are selected to be fresh (<5 days), and irradiation is performed only prior to issue.

Section 4

a. Once an aliquot is prepared from a PRBC unit, the unit is reserved for future use for the same neonate, until it passes the 5-day period. Thawed plasma units are kept for 5 days from time of thaw. Plasma units can, however, be used for another neonate if needed.

b. Once aliquoted and irradiated, the expiration date of the aliquot is the same as for any irradiated blood component (28 days for RBCs). However, considering that aliquoting is done when blood is requested for transfusion and the units are irradiated prior to issue, units are transfused on the same day of irradiation.

c. The remaining volume is reserved for use for the same neonate until it passes 5 days from time of collection. After that, it is taken out of the neonatal inventory and added to the general inventory of PRBC components for use for other patients. Thawed plasma units are kept for 5 days from time of thaw, before they are discarded. It, however, can be used for other neonates if needed.

Section 5

a. For neonates: syringes are infused using a syringe pump.

For paediatrics: PLT units are transfused by gravity. PRBCs and thawed fresh frozen plasma (FFP) units are infused using infusion pumps.

b. For neonatal: Alaris™ CC Plus Syringe Pump with Guardrails™ (BD) are used for the syringes.

For paediatrics: Hospira Plum A+ (Abbott) are used for the units.

c. PRBC components: Units start at a slow rate; 1 ml/kg/h, for the first 15 min. Infusion rate is then increased to 4–5 ml/kg/h if well-tolerated. Infusion should be completed within 4 h.

PLTs: 10–20 ml/kg/h over 30 min.
Thawed FFP: 10–20 ml/kg/h over 2–4 h.
Cryoprecipitate: 10–20 ml/kg/h over 30–60 min.

Arwa Z. Al-Riyami
Sultan Qaboos University, Sultan Qaboos University Hospital, Muscat, Sultanate of Oman
Email: arwa@squ.edu.om

Abdul Hakim Al-Rawas
Sultan Qaboos University, Sultan Qaboos University Hospital, Muscat, Sultanate of Oman
Email: alrawas@squ.edu.om

Saif Al Hosni
Sultan Qaboos University, Sultan Qaboos University Hospital, Muscat, Sultanate of Oman
Email: saif883@squ.edu.om

ITALY

Mauro Montanari & Benedetta Costantini

Section 1

The hospital is located in a city, and the catchment area can be considered as urban.
This hospital is a tertiary centre affiliated with the local medical school; hence, it is a teaching hospital.
Our trust is made by three hospitals, one of which is a dedicated maternity/children hospital.
The children hospital has a dedicated NICU with 31 beds.

Section 2

a. Yes, our centre does aliquot of blood components.
b. Yes, we do provide small-volume transfusions for RBC and PLT, not for FFP.

Section 3A

i. The unit chosen is generally freshly collected. If it needs to be irradiated, the limit is 14 days prior the irradiation day (e.g., if it is requested and needs to be irradiated on 14 February), we choose a unit collected from 01 February onwards [1].
ii. SAGM for RBC, 70% platelet additive solution (PAS) for PLT.
iii. For children up to 12 months of age, the blood group assigned is O (Rh positive or Rh negative according to the child's blood group).
iv. All our blood components are leukoreduced pre-storage.
v. For children younger than 12 months and for all the haemato-oncology paediatric patients, we try to give CMV-negative units.
vi. Irradiation is upon request. For any child younger than 6 months, we irradiate RBC and PLT. Blood components are irradiated for all paediatrics haemato-oncology patients.
vii. Ultraviolet light (UV light) and solvent/detergent for FFP (Kedrion).

Section 3B

a. The blood centre prepares the paediatric aliquots.
b. There are no paediatric aliquots kept in the inventory. Each paediatric aliquot is prepared upon request, based on the volume needed by the patient.
c. The volume to be aliquoted is prepared upon request by the clinical team. There are no paediatric aliquots kept in our repository.
d. Generally, an amount of 10 ml is added for the tubing.
e. We do not keep pre-irradiated units in our repository. The units are irradiated just before transfusion.
f. We do prepare paediatric aliquots in a satellite bag, which is attached in a sterile way to the mother bag.
g. The satellite bag is attached by a sterile docking device. We do not manually puncture the bag, hence no need to work under a laminar flow hood.
h. We do not conduct quality control (QC) testing of the aliquot prepared. If there is a blood transfusion reaction (febrile or not), we follow our local protocol.
i. We do not routinely screen the aliquots for supernatant electrolyte levels (the reason is that we always use fresh 'mother units' and we do not pre-emptively irradiate blood components).

Section 4

a. When a newborn needs a blood transfusion (RBC), we routinely allocate a single unit in all cases.
b. The aliquot prepared has the same expiration date of the mother bag. The expiration date of the mother bag never changes, We do not irradiate mother bag older than 14 days.
c. The volume in the mother bag is kept in a separate inventory along with mother bags for other infants. Further aliquots are provided from the same mother bag till there is enough volume and/or for maximum 14 days since the date the bag was collected (date of donation).

Section 5

a. Our NICU uses a pump to transfuse the aliquots.
b. The type of pump is Braun.
c. The infusion rate depends on the clinical conditions of the patients.

REFERENCE

1. Spinella PC, Tucci M, Fergusson DA, Lacroix J, Hébert PC, Leteurtre S, et al. Effect of fresh vs standard-issue red blood cell transfusions on multiple organ dysfunction syndrome in critically Ill paediatric patients: a randomized clinical trial. JAMA. 2019;322:2179–90.

Mauro Montanari
Azienda Ospedaliero Universitaria Ospedali Riuniti Umberto I – G. M. Lancisi – G. Salesi, Ancona, Italy
Email: mauro.montanari@ospedaliriuniti.marche.it

Benedetta Costantini
Azienda Ospedaliero Universitaria Ospedali Riuniti Umberto I – G. M. Lancisi – G. Salesi, Ancona, Italy
Email: benedetta.costantini@ospedaliriuniti.marche.it

MALAYSIA

Christina Lee Lai Ling, Norliza Mustafa & Chan Kok Joo

Section 1

Our hospital is a 1600 bedded University Hospital, located in a city.
It is a tertiary teaching hospital.
Our hospital treats both adults and children.
Our NICU has 27 beds.

Section 2

a. Yes, our centre support aliquoting/small-volume transfusions in neonates and paediatrics.
b. We provide small-volume transfusions for RBCs and random donor PLTs.

Section 3A

i. Our unit age is less than 5 days.
ii. Anticoagulant/additive solution is citrate-phosphate-dextrose (CPD).
iii. For neonates and infants less than 4 months:

Group O RhD-positive aliquoted units are provided for all infants if they require small-volume transfusions. If either the mother or neonate is O RhD negative, we will provide group O RhD-negative units.

For paediatric patients above 4 months:
We will provide group specific units.

iv. For units issued to infants less than 4 months, we provide leukoreduced units.

For patients above 4 months of age, leukoreduced units are supplied based on clinical needs and indications.

v. CMV testing is not performed routinely.
vi. Blood products issued for neonatal exchange transfusion are irradiated on-site. Irradiated blood is also provided for any indications that fall within specified guidelines.
vii. Not applicable.

Section 3B

a. Aliquots are prepared in the blood component preparation facilities, which are attached to the transfusion laboratory.
b. We aliquot a set of RBC pedipack (split 4–6), group O RhD-positive minimum of one set, at any time for inventory usage. Whenever we have special case request (i.e., RhD-negative infant, or other blood group request, we will prepare on demand).
c. We prepare a standard volume of 50–60 ml.
d. No, we do not add a standard discard amount in aliquots to account for the tubing and so forth.
e. We irradiate on demand, as per indication, right before transfusion.
f. We prepare aliquot RBC concentrate (RCC) in satellite/transfer bag. We will use PLT transfer bag if there is a clinical request of smaller volume PLT.
g. We use sterile docking device during aliquoting.
h. We do not conduct any QC testing of the aliquots prepared. QC is based on testing of the parent RBC units.
i. We do not screen the PRBC unit aliquots for potassium level.

Section 4

a. We do allocate a single adult unit for one newborn, when there is special request, especially in a frequently transfused newborn.
b. As we aliquot using close system, the expiration date and time remain the same as the mother bag. However, if we perform irradiation on the blood bag, we will shorten the expiry date as per irradiation protocol.
c. We keep providing further aliquots until expiration date.

Section 5

a. Both methods are employed. For blood volume 10 ml and less, we will use the infusion/syringe pump method. For blood volume exceeding 10 ml, we will use the gravity method.

b. We are currently using Terumo, Nur Medic and Agilia infusion/syringe pumps.
c. We aim to complete the transfusion over 4 h. For example, if 10 ml of blood volume is required, we will administer at a rate of 2.5 ml/h.

Christina Lee Lai Ling
University Malaya Medical Centre, Kuala Lumpur, Malaysia
Email: christina.lee@ummc.edu.my

Norliza Mustafa
University Malaya Medical Centre, Kuala Lumpur, Malaysia
Email: norliza@ummc.edu.my

Chan Kok Joo
University Malaya Medical Centre, Kuala Lumpur, Malaysia
Email: chankj@ummc.edu.my

INDIA

Hari Krishan Dhawan, Sheetal Malhotra & Ratti Ram Sharma

Section 1

Our hospital is a regional tertiary care teaching and training hospital catering to seven neighbouring states in North India.
It is tertiary care hospital with teaching courses for specialization and superspecialization in all medical and surgical branches.
Our hospital treats both adults and children, but we have an advanced paediatric centre for catering exclusively to children. We also have a dedicated neonatal unit including NICU and a thalassemia unit.
Yes, we have NICU with 40 beds.

Section 2

a. Yes, our centre provides PRBC aliquots for small-volume transfusion.
b. We prepare PRBC aliquots, and for PLT transfusion, we provide random donor PLTs derived from WB. In addition, we also prepare aliquots from single-donor apheresis platelet (SDAP) units mainly for haemato-oncology paediatric patients, usually three paediatric units (volume 70–80 ml each) from one SDAP unit (volume 220–240 ml).

Section 3A

i. We use less than 7-day-old units for aliquots [1].
ii. We prefer additive solution bags as they are also leukoreduced.
iii. Neonatal group: for paediatric patients until 4 months of age, we issue blood components that are ABO compatible with both maternal and the neonatal blood group [1]. Pre-transfusion compatibility testing is also done with both maternal and neonatal blood sample. In addition, we perform DAT on the neonatal sample and antibody screen on the maternal sample. In case it is positive, further immunohaematology work-up is performed.
iv. We prefer leukoreduced bags, but as we are not doing universal leukoreduction, we sometimes use non-leukoreduced PRBC.
v. We do not check for CMV seronegative status.
vi. We irradiate for intrauterine transfusion (IUT) but not for top-up transfusions. Irradiation is also done for exchange transfusions in patients for whom IUT was performed [2]. We sometimes issue irradiated units if requested by the clinicians for specific indications.
vii. We do not perform pathogen reduction.

Section 3B

a. The blood centre prepares the aliquots.
b. For Rh-positive groups, we maintain the inventory of aliquots, but for Rh-negative groups, we prepare them on-demand.
c. As we maintain an inventory of Rh-positive groups, we usually divide the adult unit into three equal parts so the volume of our pedibags varies from 70 to 90 ml. For Rh-negative groups, we prepare volume of the pedibag as requested by the clinical team.
d. We have pre-fixed aliquot volumes and the transfusion service uses as much volume as needed. For paediatric patients less than 1 year of age, the volume for pedi units is 70–90 ml. For patients in the age group of 1–3 years, the volume is 100–120 ml, that is two units are prepared from a single adult PRBC unit.
e. We irradiate the aliquots for IUT right before the transfusion.
f. We either use a new sterile transfer bag or two satellite bags separated from a new sterile triple bag having Y port attached to two bags. (This save us on the cost of one sterile connection.)
g. We use a sterile connecting device.
h. We do not perform QC for paediatric aliquots as we are testing 1% of our adult bags for QC.
i. We do not screen the PRBC units for supernatant electrolyte levels (K^+) before transfusion in newborns, unless specified. In our setting, the transfusion practice is to issue fresh (up to 7 days old) PRBC units for neonatal transfusion.

Section 4

a. We allocate a single adult unit for a newborn if multiple transfusions are anticipated as in severe anaemia, top-up transfusions for haemoglobin build-up for a major surgery (cardiac/abdominal surgery), coagulopathies, disseminated intravascular coagulation, patients on extracorporeal membrane oxygenation (ECMO). As we prefer relatively fresh units for neonatal transfusions, it is not feasible to routinely reserve dedicated donor units for all the neonates except for the above indications.

b. As we prepare the RBC aliquots by close system using sterile connecting device, the expiry date of the units is not altered.
c. We use the entire volume of the adult unit for preparing the paediatric units. Some volume (10–15 ml) is left in the tubing of the main bag that is discarded. We store the aliquots until the expiration date.

Section 5

a. We use an infusion/syringe pump for transfusion in neonates.
b. We use a syringe pump at our centre.
c. The infusion rate of RBC aliquot is 1–2 ml/min, over 4 h.

REFERENCES

1. New HV, Berryman J, Bolton-Maggs PHB, Cantwell C, Chalmers EA, Davies T, et al. Guidelines on transfusion for foetuses, neonates and older children. Br J Haematol. 2016;175:784–828.
2. Robinson S, Harris A, Atkinson S, Atterbury C, Bolton-Maggs P, Elliott C, et al. The administration of blood components: a British Society for Haematology Guideline. Transfus Med. 2018;28:3–21.

Hari Krishan Dhawan
Post Graduate Institute of Medical Education and Research Chandigarh, Chandigarh, India
Email: hkdpgimer@gmail.com

Dr Sheetal Malhotra
Post Graduate Institute of Medical Education and Research Chandigarh, Chandigarh, India
Email: sheetalmalhotra50@gmail.com

R. R. Sharma
Post Graduate Institute of Medical Education and Research Chandigarh, Chandigarh, India
Email: rrsdoc@hotmail.com

UNITED KINGDOM

Helen New, Rachel Moss & Jane Davis

Section 1

Our hospital is located in a city. The blood service supplier (NHS Blood and Transplant [NHSBT]) is national, located in several cities across England.
The hospital is a national paediatric tertiary referral centre.
The hospital is a dedicated children's hospital. NHSBT supplies blood products for both adults and children.
The hospital has a NICU with 14 beds, a cardiac intensive care unit with 21 beds that takes newborns for cardiac surgery and ECMO.

Section 2

a. NHSBT provides small-volume components with a 'neonatal/infant' specification for neonates and infants [1]. Children from the age of 1 are provided with the same components as adults.
b. NHSBT provides small-volume RBCs (six splits from a single donation pack), PLTs (four splits from a single apheresis donation), FFP (four splits from a single donor) and cryoprecipitate (single donation).

Section 3A

i. Routinely, NHSBT will choose the freshest units available to split into aliquots to fulfil hospital requests and stock requirements. RBCs can be split at any time up to Day 35 of the primary component shelf life. If irradiation is required, it must not occur past Day 14 following donation. Apheresis PLTs can be split at any time up to Day 7 of the primary component shelf life, in practice the earliest is Day 2 after donation, following receipt of bacterial screening results (irradiation can take place at any time during the split component shelf life). Plasma can be split at any time during the shelf life of the primary component (1095 days) and the split component retains the same shelf life. Plasma is frozen (pending testing results) and defrosted prior to splitting and re-freezing.
ii. RBCs for small-volume aliquots are in SAGM (saline–adenine–glucose–mannitol). PLTs for neonates and infants are in plasma with 20% SSP + PAS.
iii. RBCs for small-volume aliquots are mostly provided to hospitals as group O D-negative. A small proportion are group O D-positive, and even fewer are group A or B, requested by only a few hospitals. Our national transfusion guidelines [1] specify that units must be ABO and D compatible with both maternal and infant groups for infants less than 4 months of age.

PLTs for neonates and children are recommended to match the recipient ABO group wherever possible and to be compatible for D. If it is necessary to use non-identical ABO PLTs, they should have tested negative for high-titre anti-A and anti-B. A hierarchy of alternative choices of ABO group is given, minimizing the use of PLTs with minor ABO incompatibility, in particular avoiding group O for non-O recipients [1].

Plasma components for neonates and children are recommended to be compatible with recipient ABO group. If in emergency alternative groups are given, the principles of the alternative choices are as for PLTs, but group O plasma is only given to group O paediatric recipients.

iv. Blood components in the United Kingdom are universally leukoreduced.
v. RBCs and PLTs for neonates and infants are provided from CMV seronegative donors.
vi. RBCs for small-volume transfusions and PLTs for neonates and infants are irradiated if there has been a previous IUT (for up to 6 months of life) or if there is evidence of a severe congenital T-cell immunodeficiency [2]. Other indications that apply to older recipients also apply to infants where relevant.

vii. NHSBT does not provide pathogen-reduced blood components for neonates and infants, However, solvent/detergent FFP is commercially available and is used by this hospital. Further information on blood components provided for neonates and children in the United Kingdom is in our national guidelines [1, 3].

Section 3B

a. The aliquots are prepared by the blood centre (NHSBT).
b. The aliquots prepared by NHSBT will mostly be held in stock for issue but will be prepared on request if the appropriate component is not available.

The hospital requests and holds in stock six group O D-negative aliquots from the same donation, and issues them as required to patients.

c. For RBCs, a standard volume, mean 45 ml, is prepared by evenly splitting the volume from a single donation into six. Similarly, for PLTs and plasma, the volumes are determined by evenly splitting individual donations. However, the PLTs are split after the addition of PAS to the original apheresis PLT donation, giving a mean final aliquot volume of 63 ml.
d. See above.
e. At this hospital, irradiation is done immediately before the aliquot is issued for transfusion. However, it is important to note that the majority of hospitals in the United Kingdom do not have the facility to irradiate blood components.

NHSBT irradiates either single aliquots or sets of six RBC aliquots from the same donor at the time of the request from the hospital, depending on the number of aliquots requested. The process is the same for small-volume PLTs.

f. All aliquots are prepared by docking a set of connected transfer bags (four for PLTs and plasma or six for RBCs) to the primary component using a sterile connection device.
g. Neonatal/infant small-volume components are all produced by NHSBT in a closed system using integrated blood sets designed for the purpose, attaching them to the primary pack with a sterile connection device.
h. Quality monitoring by NHSBT is undertaken for all blood components according to the 'Guidelines for the Blood Transfusion Services in the UK' [4] at a sampling frequency determined by statistical process control rather than the alternative option of a fixed 1% of the total components produced. Testing for parameters not impacted by splitting is performed on the primary pack, for example, residual white cell counts to confirm conformance to leukodepletion specification.

The individual components have specific additional quality monitoring parameters:

RBCs for neonatal small-volume aliquots: Volume, haematocrit and haemoglobin content. Haemolysis is only measured at expiry with annual process revalidation of 20 units.

PLTs: Volume and PLT yield. pH is only measured at expiry on 1% of units (a minimum of 90% tested should be at least pH 6.4).

FFP: Volume and factor VIII. The primary adult-dose FFP having additionally been tested for total protein, residual PLT count, and residual RBC count.

Cryoprecipitate: Volume, factor VIII and fibrinogen, factor VIII.

i. The hospital does not screen.

Section 4

a. For the majority of general hospitals with neonatal patients requiring transfusions, the transfusion laboratory will usually allocate a number of aliquots from the same donation, based on the baby's gestational age (e.g., born before 30 weeks), as these patients may require repeated RBC top-ups (often due to iatrogenic causes). The hospital used for this survey is a dedicated children's hospital, which manages a high number of neonates, both pre-term and term, requiring treatment for a number of medical and surgical conditions. These patients are complex requiring frequent support of blood (both aliquots and large-volume packs) and blood components. Therefore, this hospital issues aliquots from the same donation for each transfusion episode but does not allocate dedicated units for each patient in advance.
b. The expiration date/time of the aliquots prepared are the same as that of the original donation—while splitting occurs during the shelf life of the primary component, the aliquot shelf life must not extend past the expiry of the original component. For small-volume RBCs, it is 35 days; for PLTs, it is 7 days and for FFP and cryoprecipitate are 36 months while frozen.

If RBCs are irradiated, the shelf life is reduced to 14 days following irradiation (a maximum of 28 days following donation, depending on the date of irradiation). PLT shelf life is unaffected by irradiation.

c. As described above, the mother bag is entirely split between the individual aliquots during preparation.

Section 5

a. Small-volume transfusions are delivered using a small-volume blood giving set (1.5-ml prime) with a 200-μM filter, which is then attached to a syringe and the blood is given using a syringe pump.
b. We use a Becton Dickinson Alaris neXus CC syringe pump.
c. The infusion rate recommended at our centre is:

RBCs 5 ml/kg/h.
PLTs 10–20 ml/kg/h.
Plasma 10–20 ml/kg/h.

REFERENCES

1. New HV, Berryman J, Bolton-Maggs PH, Cantwell C, Chalmers EA, Davies T, et al. Guidelines on transfusion for fetuses, neonates and older children. Br J Haematol. 2016;175:784–828.
2. Foukaneli T, Kerr P, Bolton-Maggs PHB, Cardigan R, Coles A, Gennery A, et al. Guidelines on the use of irradiated blood components. Br J Haematol. 2020;191:704–24.
3. New HV, Stanworth SJ, Gottstein R, Cantwell C, Berryman J, Chalmers EA, et al. British Society for Haematology Guidelines on transfusion for fetuses, neonates and older children (Br J Haematol. 2016;175:784–828). Br J Haematol. 2020;191:725–7.
4. Guidelines for the Blood Transfusion Services UK. [cited 13 Apr 2022]. Available from: https://www.transfusionguidelines.org/red-book.

Helen New
NHS Blood and Transplant, London, UK
Email: helen.new@nhsbt.nhs.uk

Rachel Moss
Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
Email: rachel.moss@gosh.nhs.uk

Jane Davies
NHS Blood and Transplant, Bristol, UK
Email: jane.davies@nhsbt.nhs.uk

CANADA

Nancy Robitaille & Valérie Arsenault

Section 1

The Centre Hospitalier Universitaire (CHU) Sainte-Justine is located in a metropolitan area.

It is a tertiary academic hospital affiliated to the Université de Montréal.

The hospital is a dedicated mother and child facility; the main patient population is paediatric, but pregnant women and women with reproductive issues are also treated.

The hospital has a 65-bed NICU.

Section 2

a. CHU Sainte-Justine's blood bank performs its own aliquoting in neonates and paediatrics. We have two sterile docking devices on site. In the event we could not support aliquoting, our local blood supplier, Héma-Québec, can provide a container with five satellite bags attached to the AS-3 RBC bag or to a unit of apheresis PLTs. The hospital can divide the volume as required using a sterile connection.
b. We provide small-volume transfusion for RBCs and PLTs. Aliquoting is performed for PLTs as they are received as apheresis PLTs or as buffy-coat-derived PLTs pooled from five different donors.

Section 3A

i. There is no restriction regarding the age of the unit for performing aliquoting.
ii. For RBCs units, either AS-3 RBC prepared from WB collected in a CP2D anticoagulant or SAGM RBC collected in CPDA anticoagulant are distributed by our blood centre. Pooled PLTs are prepared from WB collected in a CPDA anticoagulant solution, whereas apheresis PLTs are collected in acid citrate dextrose (ACD)-A [1]. All the anticoagulant/additive solutions are deemed adequate to perform small-volume aliquots.
iii. There is always one group O Rh-positive and one group O Rh-negative RBC unit aliquoted ready for distribution for newborns in the NICU. For neonates, group O RBC units are used for the majority of patients except for patients undergoing cardiovascular surgery where same group RBC transfusion is allowed if there is no anti-A and/or anti-B of maternal origin in the newborn's plasma/serum in accordance with CSA-Z902:20CSA Standards [2]. For recipients other than neonates, aliquoting with respect to blood group is performed on demand provided that the patient's blood group has been determined on two different samples.
iv. Since 1999, all labile blood products, except for granulocytes, are leukoreduced pre-storage by the blood supplier.
v. Since all RBC units and PLTs are leukoreduced, the risk of CMV transmission through transfusion is negligible. The only indication for the use of CMV seronegative products suggested by The National Standard of Canada on Blood and Blood Components, CAN/CSA-Z902:20 is IUT [2].
vi. As our institution has an irradiator on site, irradiation of aliquots is performed prior to distribution to the ward to avoid hyperkalaemia. However, in the event that aliquoting occurs post-irradiation, our local policy is to remove the supernatant if the unit has been irradiated for more than 24 h and the recipient is less than 10 kg. This policy is aligned with the recommendations for use of irradiated blood components in Canada, which states that 'Where the patient is at particular risk from hyperkalaemia, it is recommended that RBCs be transfused within 24 h of irradiation or that cells have at least undergone centrifugation and supernatant plasma removal' [3].
vii. At the time of this survey, pathogen reduced blood components are not available in the province of Quebec.

Section 3B

a. The aliquoting is done in the hospital blood bank by accredited medical laboratory technologists.
b. There is always one group O Rh-positive and one group O Rh-negative RBC unit aliquoted and ready for distribution for newborns in the NICU. Apart from these two units, aliquoting with respect to blood group is performed on demand.
c. For RBCs intended for paediatric patients, the volume to be aliquoted is based on the volume requested by the clinical team. The

usual volume is 15 ml/kg. For neonates, our procedure is to split the unit in four equal parts. An aliquot can be divided again in two other parts if clinically indicated.

For PLTs, the volume in the transfer bag is also based on the volume requested by the clinical team with the caveat that a minimum of 100 ml must be kept in the mother bag, as per the manufacturer's package insert. Therefore, the clinical team knows that the aliquot may contain a greater quantity than what was ordered. Since all transfusions are done by infusion pump, the exact amount to be transfused is programmed by the nursing staff.

d. For RBCs, the volume in each aliquot is pre-fixed in the sense that the volume in the mother bag is split equally between the satellite bags (usually two or four satellite bags). Thus, no discard amount is added, but the technologists verify that the volume of the aliquot is slightly above the volume requested. The nursing staff programs the volume on the infusion pump according to the physician's order. The procedure is similar for PLTs, but we split only into two aliquots.
e. As our institution has an irradiator on site, irradiation of aliquots is performed prior to distribution to the ward to avoid hyperkalaemia. However, in the event that aliquoting occurs post-irradiation, our local policy is to remove the supernatant if the unit has been irradiated for more than 24 h and the recipient is less than 10 kg or for massive transfusions.
f. For RBCs and PLTs, aliquots are prepared by attaching a new sterile transfer bag. Aliquots in a syringe were prepared in the past for NICU patients, but this procedure was abandoned many years ago. The pentabag is available from our blood supplier, but we do not use it routinely.
g. All aliquoting procedures are performed at the blood bank using a sterile docking device to attach the transfer or satellite bag to the mother bag. If the sterile connection is compromised during the procedure, then the bag will be punctured manually under a laminar flow hood and transferred into a new transfer bag.
h. The National Standard of Canada on Blood and Blood Components, CAN/CSA-Z902:20, clause 7.3 provides regulatory guidelines on sterile connecting devices [2]. At our institution, QC for the sterile docking devices is provided by the manufacturer and is done 3 times/year. As per the CSA Standard, each sterile weld is tested for integrity.
i. As per CSA-Z902:20 Standard, clause 7.12, RBCs shall be irradiated only up to 28 days after collection and should be transfused as soon as possible. They shall be transfused no later than 14 days after irradiation and no later than 28 days after collection [2]. RBCs are not screened for supernatant electrolyte levels before transfusion in newborns. However, if the RBC unit has been irradiated for more than 24 h, the supernatant will be removed by centrifugation for children less than 10 kg to avoid hyperkalaemia. Supernatant removal is also done for massive transfusions in children less than 10 kg.

Section 4

a. Routine dedicated donor units for one newborn were used routinely until February 2014. Based on the publication of the ARIPI study [4], we changed our procedure to the following: one group O Rh-positive and one group O Rh-negative RBC unit are aliquoted and ready for distribution for newborns in the NICU. The units chosen are usually less than 7 days of age when aliquoting is performed, but it is allowed up to 15 days. Given the high demand for RBC units, newborns in the NICU are transfused with relatively fresh units. If requested by the clinical team, a dedicated unit for a specific newborn is available. However, no requests have been made since we changed our procedure in 2014.
b. The expiration date/time of the aliquot prepared is the same as for the mother bag if the aliquoting is done using a sterile docking device and if the weld maintains its integrity. If the weld is unable to maintain its integrity, the blood component is considered an open system and the expiration time is reduced to 24 h for RBCs and to 4 h for PLTs.
c. For RBCs and PLTs, we keep providing aliquots until the expiration date. For PLTs, we aim to keep 100 ml or more in the mother bag, as per the manufacturer's package insert. If there is less than a 100 ml in the mother bag, it is discarded.

Section 5

a. As per our hospital policy, all transfusions should be infused through an infusion pump, including aliquots for neonates and paediatric patients.
b. At our institution, the infusion pumps currently in use are the BBraun (Infusomat).
c. The recommended infusion rate is 1 ml/kg/h for the first 15 min, and after that, the infusion rate is calculated by dividing the remaining volume to be transfused by the duration of the transfusion ordered by the physician (usually 3 h for elective top-up transfusion).

REFERENCES

1. Héma-Québec. Circular of information for the use of labile blood products. [cited 2022 Apr 11]. Available from: https://www.hema-quebec.qc.ca/userfiles/file/media/anglais/hospitals/PUB-00038.pdf.
2. CAN/CSA-Z902: 20 Blood and blood components. 4th ed. Toronto, Canada: CSA Group; 2020.
3. National Advisory Committee on Blood and Blood Products. Recommendations for use of irradiated blood components in Canada. [cited 2022 Apr 14]. Available from: https://nacblood.ca/en/resource/recommendations-use-irradiated-blood-components-canada.
4. 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.

Nancy Robitaille
CHU Sainte-Justine, Montreal, Canada
Email: nancy.robitaille@umontreal.ca

Valérie Arsenault
CHU Sainte-Justine, Montreal, Canada
Email: valerie.arsenault.med@ssss.gouv.qc.ca

UNITED STATES

Nabiha H. Saifee & Ann-Marie Taroc

Section 1

Our hospital is located in a city. As the only academic paediatric medical centre for five states (Washington, Wyoming, Alaska, Montana and Idado), our hospital services the largest geographic area of any children's hospital in the United States.

Our hospital is a tertiary care/teaching hospital with over 370 beds and over 1000 total physicians in training. We are a major paediatric extracorporeal life support (ECLS) referral centre with about 50 children per year receiving ECLS support in our intensive care units (ICUs). We perform over 500 heart surgeries in paediatric patients per year. We also have active solid organ transplant services with over 40 liver, kidney and small bowel transplants per year and about 10–20 heart transplants per year. We support ABO-incompatible heart, liver and haematopoietic progenitor cell transplants. We support over 100 haematopoietic progenitor cell transplants per year and a growing number of patients receiving CAR T-cell therapies for both blood and solid organ tumours.

Our hospital is primarily a dedicated children's hospital, but does treat a small number of young adults with cancer for both haematopoietic progenitor cell transplants and CAR T-cell therapies.

Yes, we have a 36-bed level IV NICU that can provide multiple services for complex conditions and complications that present at birth or develop soon after delivery including ECMO, therapeutic hypothermia, continuous renal replacement therapy, peritoneal dialysis, paediatric medical and surgical subspeciality services.

Section 2

a. Yes, we support aliquoting of RBCs and PLTs. Generally, aliquots are provided for volumes less than 150 ml.
b. We provide small-volume transfusion for all blood components, but only aliquot RBCs and PLT on-site. Small-volume RBC and PLT orders less than 45 ml for the NICU are provided in syringes with 10–20 ml extra volume provided for priming of tubing. We receive divided group AB plasma with about 50 ml per paediatric plasma unit from our blood supplier. Our blood supplier also provides single cryoprecipitate units with volumes of about 10–20 ml. Granulocyte transfusion orders are usually ~10 ml/kg and a request for smaller volume collection can be made to blood supplier.

Section 3A

i. Fresh RBC units less than 7 days from collection are generally used for small-volume aliquots unless a patient has a RBC unit already allocated (or dedicated) to them.
ii. We utilize RBC units stored in AS-1 or AS-3 for all transfusion indications whether small-volume or large-volume transfusion expected.
iii. For patients less than 4 months of age, we provide group O Rh-type specific blood components that are negative for detected antibodies (presumed to be passively acquired from mother). For patients ≥4 months of age, ABO-type-specific or compatible RBCs may be provided.
iv. All of our RBC and PLT units are leukoreduced by the blood supplier.
v. We consider leukoreduced RBCs to be CMV safe and do not provide CMV-negative RBCs for any indications at our institution [1].
vi. All of our PLTs are either irradiated or psoralen-treated. All patients at our institution will receive irradiated RBCs until irradiation requirement is removed from patient transfusion profile by a transfusion service physician. Irradiation requirement is kept on patient record for patients less than 4 months of age, with known immunodeficiency, with diagnosis of malignancy, with congenital heart disease under 2 years of age, or with haematopoietic progenitor cell transplant.
vii. We do not have pathogen-reduced RBCs, but we do utilize pathogen-reduced psoralen-treated PLTs in patients. We do not limit pathogen-reduced psoralen-treated PLTs to any particular patient group and use them interchangeably with conventional PLTs. We preferentially utilize PLTs in plasma instead of PLTs in PAS-C for massive transfusion, cardiac surgery, liver surgery and patients otherwise coagulopathic receiving plasma transfusions. If patients are noted to receive poor increment with a psoralen-treated PLT in PAS-C, we may recommend utilizing PLTs in plasma to improve increments.

Section 3B

a. We prepare aliquots on-site in our clinical facility.
b. We prepare the aliquots when we receive the request.
c. The volume to be aliquoted is based on the volume requested by the clinical team for RBCs and PLTs. For small-volume orders of plasma or cryoprecipitate, we provide a paediatric 50-ml plasma unit or a number of single cryoprecipitate units with about 10–20 ml per single cryoprecipitate.
d. For RBC and PLT aliquots, we provide extra 10–20 ml to account for losses in tubing. For plasma orders, we can provide a fixed volume of about 50 ml per paediatric plasma unit. Our blood supplier only divides group AB plasma; thus, a full-size adult ABO-type-specific plasma unit may be provided for patients requiring frequent plasma transfusion.

e. We irradiate RBC units and aliquots right before transfusion and consider RBC units freshly irradiated if irradiated within 8 h from expected transfusion time. Apheresis PLTs that are not psoralen-treated are pre-irradiated.
f. We generally prepare aliquots of RBCs and PLTs by sterile welding a single new sterile transfer bag to the parent bag. After irradiation of RBC aliquot in the transfer bag, the RBC aliquot less than 45 ml may be transferred to a syringe using a syringe set including a 150-μm filter. For small-volume PLT orders less than 45 ml, a syringe set may be directly sterile welded to the parent bag.
g. We always sterile weld the transfer bag or syringe to the parent bag. Each weld is visually inspected for alignment and leakage to ensure that the systems remain closed.
h. We do not routinely conduct any QC testing on the prepared aliquots other than the examination of each sterile weld.
i. We do not screen RBC units or aliquots for supernatant electrolyte levels (K^+) before transfusion.

Section 4

a. Yes, we do allocate single adult RBC units for neonates when requested and for patients receiving antigen-matched RBC transfusions (e.g., patient receiving transfusions for haemolytic disease of the foetus and newborn or sickle cell anaemia). Two patients may be assigned to the same dedicated RBC unit to allow for better inventory utilization. Rarely, we aliquot a PLTs for a patient less than 20 kg receiving HLA- or HPA-matched PLTs.
b. Our RBC units are in either AS-1 or AS-3 additive solutions. We allow aliquoting of dedicated non-irradiated RBC units up to Day 42 and retain 42-day expiration of non-irradiated parent and divided RBC units. RBC aliquots that are irradiated will have expiration shortened to 28 days from date of irradiation if the component has more than a 28-day shelf life at the time of irradiation; otherwise, if RBC aliquot is irradiated after aliquoting from a unit with 28 days or less shelf life, then the original shorter expiration is retained. Two patients may be assigned to a dedicated RBC unit to allow for better inventory utilization.

We currently only have apheresis PLTs in our inventory. Our apheresis PLTs with psoralen-treatment in PAS-C or plasma have a 5-day shelf life, and we retain the 5-day shelf life as long as the parent Intercept® blood system bag has minimum volume of 170 ml. This minimum volume is conservative and based on personal communication from Cerus. We are considering internal validation of a lower minimum volume for PLTs suspended in PAS-C or plasma in the Intercept® bag to maintain 5-day shelf life as the bag surface area to volume ratio can impact PLT quality. PLTs collected in the Fenwal Amicus® separator system and suspended in PAS-C with large volume delayed sampling for mitigation of bacterial contamination have a 5-day outdate, and we utilize a minimum storage fluid volume of 100 ml for these Amicus® PLTs collected in PAS-C to retain 5-day shelf life of the parent bag. For PLTs suspended in plasma stored in the Terumo BCT extended life PLT storage bags, we retain a 7-day shelf life per Terumo BCT bag manufacturer instructions/validation. Since our PLT aliquots are divided into transfer bags that are not gas permeable or divided into syringes, we assign a 4-h outdate to each PLT aliquot. In accordance with the U.S. Food and Drug Administration–recognized Circular of Information for the Use of Human Blood and Blood Components, our transfusion service allows the care team to start the aliquot transfusion before expiration and then allows 4 h after start of transfusion to complete the transfusion.

c. We will continue to aliquot RBCs from the parent bag until it is exhausted or expired. We will continue to aliquot PLTs from the parent bag until the date of expiration as long as the minimum storage volume for our institution is met: 100 ml for PLTs in the Fenwal Amicus® or Terumo BCT PLT storage bags and 170 ml for PLTs in Intercept® blood system PLT bags. If the parent bag volume falls below the minimum storage volume, then the parent bag is given a 4-h outdate and must be issued for transfusion before expiration or discarded.

Section 5

a. Our organization's policy recommends administration of aliquots via an infusion pump. The procedure may vary, depending on the aliquot volume. Small volumes (less than 50 ml) may require the transfusionist to draw the products through a neonatal/paediatric syringe set to filter into a syringe. RBCs prepared in a pre-filtered syringe, and aliquots drawn into a syringe infuse via the pump through macrobore tubing. Larger volume aliquots are delivered via an infusion pump with blood tubing set.
b. Our organization currently uses BD Alaris™ PC unit with both the large volume and syringe modules.
c. At our centre, the rate of transfusion starts at 2 ml/kg/h (limited to 120 ml/h) for at least the first 15 min. If transfusion is tolerated without signs of transfusion reaction after 15 min, the transfusion rate can be increased to a maximum of 8 ml/kg/h for PLTs, cryoprecipitate or plasma. For RBCs, the maximum transfusion rate is 5 ml/kg/h up to 300 ml/h.

REFERENCE

1. Delaney M, Mayock D, Knezevic A, Norby-Slycord C, Kleine E, Patel R, et al. Postnatal cytomegalovirus infection: a pilot comparative effectiveness study of transfusion safety using leukoreduced-only transfusion strategy. Transfusion. 2016;56:1945–50.

Nabiha H. Saifee
Seattle Children's Hospital, Seattle, WA, USA
Email: nabiha.saifee@seattlechildrens.org

Ann-Marie Taroc
Seattle Children's Hospital, Seattle, WA, USA
Email: annmarie.taroc@seattlechildrens.org

ISRAEL

Naomi Rahimi-Levene & Victoria Peer

Section 1

The hospital is located in a city.
It is a teaching hospital.
The hospital treats both adults and children.
Yes, we have a NICU, with 19 beds.

Section 2

a. Yes. The centre supports small-volume transfusions.
b. We provide small-volume transfusions for plasma and for packed cells.

Section 3A

i. Unit age: Up to 1 week from donation.
ii. Anticoagulant/additive solution: CPDA.
iii. Packed cells: Only O positive/negative. PLTs: random blood group. Plasma: AB.
iv. Leukoreduced packed cells and PLTs.
v. CMV seronegative status: CMV negative or CMV-safe blood products.
vi. We use irradiated packed cells and PLTs.
vii. Pathogen reduction status: Not in use.

Section 3B

a. The aliquots are prepared by the National Blood Services, Magen David Adom.
b. The aliquots are the part of the inventory.
c. The standard volume aliquots are prepared in the National Blood Services, Magen David Adom.
d. We have fixed aliquot volumes and the medical personnel uses as much volume as needed.
e. All units are pre-irradiated by Magen David Adom, Israeli Blood Services Organization.
f. Aliquots are prepared by using new sterile satellite bags. They are not attached with the mother bag but connected by sterile connector device.
g. The aliquots are prepared by the National Blood Services, Magen David Adom. They ensure sterility by using a sterile connector device.
h. Magen David Adom performs QC testing of the mother bags.

There are regulatory guidelines for QC parameters.

The parameters for packed cells are: volume (1% of all packed cells being tested), haematocrit (at least four bags a month), haemoglobin (at least four bags a month) and haemolysis at the end of storage (at least four bags a month).

i. No, we do not screen K^+ levels before transfusion.

Section 4

a. Yes, we make efforts to dedicate one adult packed cell unit divided into four satellite bags for one newborn.
b. We do not determine the expiration date and do not change the expiration date of mother bag.

We try to use an aliquots for a week after the donation of mother bag.

c. We discard the main bag with remaining volume.

Section 5

a. The hospital unit uses the regular infusion method for transfusion.
b. Not applicable.
c. Transfusion rate is 10–15 ml/kg for 2–3 h and the transfusion should be completed within 3 h.

Naomi Rahimi-Levene
Blood Bank, Shamir (Assaf Harofeh) Medical Center, Zerifin, Israel
Email: nrlevene@shamir.gov.il

Victoria Peer
Blood Bank, Shamir (Assaf Harofeh) Medical Center, Zerifin, Israel
Email: victoriap@shamir.gov.il

SAUDI ARABIA

Maha Badawi

Section 1

Our hospital is located in the city of Jeddah, Saudi Arabia.
Our hospital is a tertiary care academic centre.
Our hospital treats adults and children.
Yes. The NICU has 25 beds.

Section 2

a. Yes. Our centre supports aliquoting for neonates and paediatric patients using sterile techniques.

b. We provide aliquoting mostly for RBCs. Whole-blood derived PLTs are not typically aliquoted because of their small volume, and plasma is rarely aliquoted as this is not cost effective in our centre with a shelf life of 24 h after thawing plasma from frozen state.

Section 3A

i. For neonatal transfusions (<4 months of age), we aim to provide units that are less than 7 days from collection. For older children, we do not have a similar restriction apart from the expiry date of the unit.
ii. For neonatal transfusions, our policy states use of CPD collected blood (without adenine or SAGM). We do not specify similar requirements for older children.
iii. When the maternal and neonatal samples are both available, our policy states providing RBCs that are compatible with both blood groups. If the maternal sample is not available, O RBCs are chosen. For PLT transfusions, the donor plasma in the PLTs unit should be compatible with ABO of the neonate.
iv. All of our blood components are leukoreduced. Leukoreduction is required according to our policy for transfusion of neonates.
v. We consider our blood components of low risk for CMV given they are leukoreduced.
vi. We require irradiation of cellular blood components to all neonates who received IUTs, in addition to those with T-cell deficiency. We follow the British Society of Haematology Guidelines regarding indications for irradiated blood components [1].

We do not irradiate PLTs that undergo pathogen reduction.

vii. Most of the PLT units in our inventory undergo pathogen reduction either with riboflavin/UV light or amotosalen/UV-light-based technologies. We do not routinely perform pathogen reduction of plasma.

Section 3B

a. Our hospital blood bank/transfusion service performs all the steps starting from blood collection to issuing.
b. Aliquots are only prepared on demand.
c. The volume of aliquots is based on the volume requested by the clinical team.
d. We do not routinely add a standard discard amount.
e. Irradiation is performed on-demand within 24 h before the transfusion. No irradiator is available on-site, and units must be transported to a neighbouring hospital for irradiation based on need.
f. We routinely use new sterile satellite/transfer bags for aliquoting.
g. Satellite/transfer bags are attached using a sterile docking device.
h. No specific QC testing is performed on aliquots. All other components undergo QC testing according to the most recent edition of the Standards for Blood Banks and Transfusion Services published by Association for the Advancement of Blood & Biotherapies.
i. We do not screen RBC units for supernatant potassium levels.

Section 4

a. Specific adult units are set for certain newborns if they are expected to use many units (e.g., cardiac surgery patients and patients on ECMO).
b. Since the aliquot is prepared in a closed system, the aliquot is assigned the expiration date/time of the mother bag.
c. The main mother bag undergoes additional aliquoting according to patient need until expiration.

Section 5

a. Infusion pumps are used around the whole hospital for administration of all units including to paediatric patients.
b. Most of the pumps used in the hospital are volumetric infusion pumps.
c. We recommend the following rates for non-emergency transfusion in paediatrics:

RBCs: 1–5 ml/kg/h (maximum 150 ml/h).
PLTs, plasma, cryoprecipitate: 10–20 ml/kg/h.

REFERENCE

1. Foukaneli T, Kerr P, Bolton-Maggs P, Cardigan R, Coles A, Gennery A, et al. Guidelines on the use of irradiated blood components. Br J Haematol. 2020;191:704–24.

Maha Badawi
King Abdulaziz University, Jeddah, Saudi Arabia
Email: mbadawi2@kau.edu.sa

THE NETHERLANDS

Pauline M. Snijder & Elise J. Huisman

Section 1

Our hospital is located in a city.
Our hospital is a University Medical Centre, this means it is a tertiary and teaching hospital.
Our hospital is a dedicated children's hospital. Our children's hospital is part of the University Medical Centre where adults are also treated.
Yes, there is a NICU. We have 30 beds available.

Section 2

a. Our centre supports aliquoted/small-volume transfusions in neonates and paediatrics. There are units available with a smaller volume.
b. Our hospital provides small-volume transfusions for RBCs (4 × 1/4 U), plasma (FFP 150 ml for neonates instead of normal 300 or 200-ml solvent/detergent plasma) and PLTs (2 × 1/2).

Section 3A

i. The preparation of all small-volume units for neonatal or paediatric patients is done by the National Blood Supply. The transfusion laboratory from our hospital then orders these units at the National Blood Supply. In specific groups, the age of the unit is of importance, mainly because of potassium levels. RBC units are <14 days when used for premature/very low birthweight infants; <5 days for infants on ECMO or if an exchange transfusion is needed. PLTs are aged <7 days, for quarantine plasma this is not applicable (max storage is 2 years).
ii. RBC units in SAGM [1].

PLTs in Pas-E [1].
Plasma, not applicable.

iii. For RBC units, small aliquots are prepared from units with blood groups O and A, both positive and negative.

For PLTs, all blood groups are selected.
For FFP, all blood groups are selected.
The blood group of the products should be compatible with the blood group of the baby. IAS screening is performed in mother and baby until the age 3 months [1].
Donors have anti-A and anti-B titres <1:128 for PLT donations.

iv. All transfusion products prepared in our country are leukoreduced, so automatically the smaller units are leukoreduced [1].
v. Since all products are leukoreduced, products are not prepared according to their CMV sero status. In our hospital, the only two indications for CMV seronegative products are IUTs or infants with severe severe combined immunodeficiency before a haematopoietic bone marrow transplantation [1].
vi. The irradiation status does not determine the unit selection from which small-volume aliquots are to be prepared. There are separate (clinical) criteria for irradiation, for example, IUTs. Small volumes though can be irradiated on indication [1].
vii. No pathogen reduction strategy is used in our country other than in SD plasma, which is not primarily given to neonates due to larger unit volume. Parvo B19 safe blood is given to extreme premature and very low birthweight infants [1].

Section 3B

a. The aliquots are prepared by the National Blood Supply. In our hospital, the transfusion laboratory only orders these units. The transfusion volume is then prescribed by a physician.
b. Aliquoted RBC units and FFP in smaller volumes are on stock, neonatal apheresis PLTs should be ordered at the National Blood Supply.
c. Standard (pre-determined) volumes are prepared by the National Blood Supply. From these standard volumes, the physician decides the volume that is transfused to the patient.
d. No, not applicable.
e. We can irradiate in our own hospital because it is a large University Hospital. Therefore, it is irradiated right before use. This is not the case in other Dutch hospitals, they order irradiated products at the National Blood Supply.
f. The smaller volumes are prepared at the National Blood Supply in new, sterile satellite bags (splitted in new units). On the NICU, the nurses draw the tight volume from the standard bags in syringes.
g. A sterile docking device is used, no laminar flow hood is used. This preparation can only be done by trained National Blood Supply employees.
h. QC checks are performed only on the mother bag.
i. No, we do not, but we do not allow neonatal units to be >14 days old to avoid extremely elevated potassium levels.

Section 4

a. Allocating a single adult unit for one newborn is not done routinely. We consider this if we have to give many transfusions for a longer period of time.
b. Not applicable for the mother bag. The new, satellite units have an expiration date and are scanned before given out to the clinic.
c. Not applicable, because we do not make aliquots from the mother bag.

Section 5

a. We use syringe pumps for transfusing the aliquots to newborn patients on the NICU. The units are delivered in bags to the department by the transfusion laboratory. The exact amount that needs to be transfused to the patient is determined by the physician. That amount is transferred to a syringe by the nurse.

On the contrary, babies on the general wards receive their transfusions from the satellite bag through gravity.

b. We use the Braun Space perfusor pump for the neonates on the NICU, and for term babies on the wards, the Braun gravity system + safeline (large filter) is used.
c. For children, the advised infusion rate is 5 ml/kg/h during 3 h [1].

REFERENCE

1. National Blood transfusion Guideline. Guideline from the database of the Federation of Medical Specialists, the Netherlands. [cited 2022 April 15]. Available from: https://richtlijnendatabase.nl/richtlijn/bloedtransfusiebeleid.

Pauline M. Snijder
Erasmus Medical Centre – Sophia Children's Hospital, Rotterdam, The Netherlands
Email: p.snijder@erasmusmc.nl

Elise J. Huisman
Sanquin, Rotterdam, The Netherlands
Email: e.huisman@sanquin.nl

SPAIN

Josune Zubicaray Salegui, Julia Ruiz Pato & Julián Sevilla Navarro

Section 1

Our hospital corresponds to a health service located in a city.
It is a teaching hospital.
Our hospital treats only paediatric patients.
No, we just have a paediatric intensive care unit.

Section 2

a. Yes, we use small or adjusted volume transfusions, so that we support aliquoting.
b. We use small-volume transfusions for all the blood components [1].

Section 3A

i. No response given.
ii. No response given.
iii. We only aliquot O blood group units.
iv. No response given.
v. No response given.
vi. No response given.
vii. No response given.

Section 3B

a. Aliquot preparation is done onsite in the clinical facility, blood transfusion service of the hospital, there is a general blood centre for the region.
b. We prepare aliquot on demand.
c. We have pre-fixed aliquot volumes.
d. We have pre-fixed aliquot volumes.
e. We usually have pre-irradiated units in your inventory.
f. We usually attach a new sterile satellite bag to divide different aliquots for a unit.
g. We attach the satellite bag using a sterile docking device.
h. Quality transfusion standards (CAT - Certificación de Calidad en Transfusión) [2].
i. Not applicable.

Section 4

a. No, we do not allocate a single adult unit for one newborn.
b. We use the same expiration date (mother bag) for the aliquot.
c. We keep the mother bag as the B0 aliquot with the original and the same expiration date.

Section 5

a. We usually use a gravity method for PLTs and infusion pump for RBC [3].
b. PLUM 360™ ICU MEDICAL.
c. The infusion rate will depend on the patient.

REFERENCES

1. New HV, Berryman J, Bolton-Maggs PHB, Cantwell C, Chalmers EA, Davies T, et al. Guidelines on transfusion for fetuses, neonates and older children. Br J Haematol. 2016;175:784–828.
2. Robinson S, Harris A, Atkinson S, Atterbury C, Bolton-Maggs P, Elliott C, et al. The administration of blood components: a British Society for Haematology Guideline. Transfus Med. 2018;28:3–21.
3. Guía sobre la transfusion de componentes sanguíneos y derivados plasmáticos. 5ª edición. Sociedad Española de Transfusión Sanguínea y Terapia Celular. 2015. ISBN: 978-84-606-8950-8.

Josune Zubicaray Salegui
Hospital Infantil Universitario Niño Jesús, Madrid, Spain
Email: josune.zubicaray@salud.madrid.org

Julia Ruiz Pato
Hospital Infantil Universitario Niño Jesús, Madrid, Spain
Email: julia.ruiz@salud.madrid.org

Julián Sevilla Navarro
Hospital Infantil Universitario Niño Jesús, Madrid, Spain
Email: julian.sevilla@salud.madrid.org

BRAZIL

José Mauro Kutner & Ana Paula Hitomi Yokoyama

Section 1

Our hospital is located in the city of Sao Paulo, Brazil.

Our hospital is a tertiary/teaching hospital.
We treat both adults and children at our hospital.
We have a 22-bed NICU.

Section 2

a. Yes, we do provide aliquots/small-volume transfusion in neonates and paediatrics units.
b. We provide small-volume transfusions for all the blood components.

Section 3A

i. For neonates and infants undergoing cardiac surgery, large-volume transfusions (>80 ml/kg over 24 h), IUTs and neonatal exchange transfusions, RBC units up to the end of Day 5 are selected [1].
ii. Our standard additive solution is SAGM. In case of large-volume transfusions, IUTs and neonatal exchange transfusions, RBC units are washed to reduce mannitol toxicity.
iii. Regarding ABO selection, for neonates and infants less than 4 months of age, RBC units are compatible with maternal and neonatal ABO and clinically significant maternal antibodies. For infants older than 4 months of age, selected RBC units must be compatible with recipient's ABO and any RBC alloantibodies.
iv. All units are pre-storage leukoreduced.
v. We do not provide CMV seronegative units. Since in Brazil more than 90% of the population is CMV seropositive, Brazilian National Health Agency recommends pre-storage leukoreduced units as a surrogate for CMV seronegative units [2]. In our hospital, we provide universal pre-storage leukoreduction.
vi. All units are irradiated. In case of neonatal exchange transfusion and IUTs, a 24-h shelf life after irradiation is the standard, in order to reduce hyperkalaemia.
vii. We do not provide any pathogen-reduced units.

Section 3B

a. The blood centre prepares the aliquots.
b. We prepare the aliquots on demand.
c. The volume of the aliquot is based on the request provided by the clinicians.
d. We do add a standard discard amount in our aliquots for the tubing, as follows:

Set for infusion pump for syringe = 2 ml.
Set for infusion pump with transfer bag = 20 ml.
Regular tubing set = 10 ml.

e. Units for IUT, neonatal exchange or large-volume transfusions are irradiated right before issue because of the 24-h expiry period after irradiation. Units for top-up transfusions are pre-irradiated. This pre-irradiation is performed daily on Day 13 or 14 of collection date.
f. For prescriptions of up to 50 ml of RBCs, we prepare the aliquots in syringes.

For prescriptions above 50 ml, we prepare the aliquots in transfer bags using sterile connection devices.

g. To ensure sterility while preparing aliquots in syringes, we have a two-step procedure: (1) volume is transferred to a transfer bag using sterile connection device, so that the system in mother bag remains closed and (2) the transfer bag is manually punctured under laminar flow to transfer the volume for the syringe.

To ensure sterility when preparing aliquots in transfer bags, they are prepared using sterile connection devices.

h. No, we do not conduct any QC testing of the aliquots.
i. No, we do not screen PRBC for supernatant electrolyte levels for transfusions in newborns.

Section 4

a. Yes, we routinely allocate a single unit for top-up transfusions for newborns in order to avoid multiple donor exposure. However, we contact the clinicians by the time the unit reaches 7 days of collection to check if they prefer a fresher unit or if we can proceed with the previously allocated unit.
b. Syringes expire within 4 h of preparation, mother bags maintain the same expiry date because the system remains closed. We transfer the volume of the aliquot to a transfer bag unit using sterile connection device and the transfer bag is manually punctured to transfer the volume to the syringe.

Aliquots in transfer bags prepared with sterile connection devices maintain the original expiry date.

c. For top-up transfusions, we keep providing further aliquots until the bag reaches 7 days. Then, we contact clinicians in order to determine if they prefer a fresh RBC unit or if we can proceed with the primary unit so that multiple donor exposure is avoided.

Section 5

a. For prescriptions of up to 50 ml of RBCs, we prepare the aliquots in syringes and then an infusion pump for syringe is used for transfusion.

For prescriptions above 50 ml, we prepare aliquots in transfer bags and then the gravity method is used. Infusion pumps can also be used with aliquots in transfer bags, at the clinician's discretion.

b. We use the B.Braun® syringe pump.
c. For infusion in syringe pumps, the recommended rate is 25 ml/h.

REFERENCES

1. New HV, Berryman J, Bolton-Maggs PH, Cantwell C, Chalmers EA, Davies T, et al. British Committee for Standards in Haematology. Guidelines on transfusion for fetuses, neonates and older children. Br J Haematol. 2016;175:784–828. Epub 2016 Nov 11. PMID: 27861734.
2. Portaria de Consolidação n° 5, de 28 de setembro de 2017. Ministério da Saúde, Brasil.

José Mauro Kutner
Hospital Israelita Albert Einstein, São Paulo, Brazil
Email: kutner@einstein.br

Ana Paula Hitomi Yokoyama
Hospital Israelita Albert Einstein, São Paulo, Brazil
Email: ana.yokoyama@einstein.br

SINGAPORE

Joyce Ching Mei Lam, Xin Ni Zhong & Mui Ling Heng

Section 1

My hospital is located in a city.
It is an academic medical centre offering a full suite of tertiary services.
My hospital is an acute hospital that treats both adults (obstetrics and gynaecology) and children.
Yes. 42 beds.

Section 2

a. Yes, our centre supports aliquoting/small-volume transfusions in neonates and paediatrics.
b. We provide small-volume transfusions for specific components, for example, leukoreduced RBCs, apheresed paediatric PLTs and single cryoprecipitate units.

Section 3A

i. Units that are within 7 days from donation will be selected.
ii. The anticoagulant used is SAGM.
iii. If the maternal and neonatal blood groups are the same, group-specific units will be selected. If the maternal and neonatal blood groups are different, group O units will be selected.
iv. Leukoreduced units are selected upon request.
v. The blood centre does not test for CMV status from donors.
vi. Routine irradiation is not performed.
vii. Pathogen reduction is not performed.

Section 3B

a. The blood centre prepares the aliquots. For Singapore, this will be the Blood Services Group at Health Sciences Authority.
b. Aliquots are prepared at the blood centre upon request, but there is also an inventory of leukoreduced small-volume RBC units on site at the clinical facility.
c. Each aliquot has a standard (pre-determined) volume.
d. The aliquot volumes are pre-fixed and the transfusing service use as much volume as needed.
e. The blood centre irradiates the unit/aliquot only upon request, and the irradiated unit is then transported to the clinical facility for transfusion.
f. Aliquots are prepared in a satellite/transfer bag. A new sterile satellite/transfer bag is attached for preparation of aliquots.
g. Satellite bags are attached using a sterile docking device.
h. No, we do not conduct any QC testing of the aliquots prepared.
i. No, we do not screen the PRBC unit aliquots for supernatant electrolyte levels (K^+) before transfusion in newborns.

Section 4

a. No, we do not allocate a single adult unit for one newborn.
b. The expiration date/time of the aliquot follows the mother bag. There is no change to the expiration of the mother bag.
c. Further aliquots are provided until the expiration date.

Section 5

a. All three methods are used.
   i. Gravity method: Regulate the drop rate according to the drop factor on the packaging of the blood administration set with filter. The usual drop rate is 20 drops per minute.
   ii. Infusion pump: A compatible blood administration set with filter is used to transfuse the product via an infusion pump.
   iii. Syringe pump: The blood is withdrawn to the syringe via a blood administration set with filter, then transfused via a syringe pump. This is commonly used in the neonatal setting.
b. Infusion pump: B. Braun Infusomat Space pump.
   i. Syringe pump: B. Braun Perfusor Space pump.
c. For all blood and blood products, the infusion rate should not exceed 5 ml/kg/h.
   i. RBCs: Transfuse over 2–4 h.
   ii. PLTs and plasma: Transfuse over 30–60 min, not exceeding 4 h.
   iii. Cryoprecipitate: Transfuse over 30–60 min.

Joyce Ching Mei Lam
KK Women's and Children's Hospital, Singapore
Email: joyce.lam.c.m@singhealth.com.sg

Xin Ni Zhong
KK Women's and Children's Hospital, Singapore
Email: zhong.xinni@kkh.com.sg

Mui Ling Heng
KK Women's and Children's Hospital, Singapore
Email: heng.mui.ling@kkh.com.sg

ARGENTINA

Oscar Walter Torres

Section 1

Our institution is located in Buenos Aires City.
It is a hospital dedicated for maternal and neonatal health services only.
It is the unique maternal hospital in Buenos Aires, which means we treat only pregnant women and newborns patients. In some cases, also gynaecologist patients.
The hospital has a NICU with 35 beds.

Section 2

a. The Transfusion Medicine Service is able to provide small-volume/aliquoting transfusions if it is required.
b. We provide small-volume transfusions for all the blood components, specially RBCs or FFP. In case of PLTs or cryoprecipitated antihemophilic factor (CRYO), we deliver the unit without aliquoting, because the volume to be administered is regulated by the paediatrician.

Section 3A

i. Although the unit selection according to the age is controversial [1], we consider the clinic situation in order to choose which is unit age to be administered (e.g., for IUT: no more than 48 h, up to 5 days for exchange transfusion) and sometimes up to 10 days for small volume in other cases.
ii. We use both, CPDA and additive solution indistinctly.
iii. In case of maternal transfusion, we provide RBCs ABO and Rh/K-compatible phenotype. For PLT transfusion, if the patient is RhD negative, not immunized to the D antigen and receives RhD positive PLTs, Rh immune globulin is indicated.

For neonatal transfusions, all the newborns receive RCCs group O, RhD positive or negative depending on each case. PLT and CRYO must be ABO compatible.

iv. All of our patients (women and newborns) receive pre-storage leukoreduced units by filtration.
v. We do not take in account the CMV serological status.
vi. According to the national or international recommendations, we irradiate cellular components for IUT, exchange transfusion, newborns who have received IUT, pre-term neonates, neonates weight <1200 g and any other patient at risk for transfusion-associated graft versus host disease [2].
vii. Technique not available in our country.

Section 3B

a. Blood components aliquoting are prepared by the Transfusion Medicine Service. The phlebotomy is performed in quintuple blood bags.
b. We prepare the aliquots only when the transfusion is required.
c. The aliquots are prepared in base of the volume required by the clinical team, but after consulting with the Transfusion Medicine Service.
d. The aliquots are prepared taking in account the 30 ml that corresponds to the tubing.
e. As we do not have the irradiator in our institution, we prepare the aliquot just before being transfused.
f. As mentioned above, we use quintuple blood bags, so aliquots are taken from the mother bag. Once the aliquot was done, the unit is reserved for the same patient for 10 more days, for probable future transfusions (for RCC only).
g. In order to ensure the closed circuit, we attach the satellite. If we need more aliquots than the blood bag has, we use a transfer bag with a sterile connection device [3].
h. We have a QC for RBCs only. We measure haematocrit, (K^+ in IUT only), and sometimes haemolysis when unit age is near 10 days from the phlebotomy.
i. As the K^+ level is not an important parameter to consider in RBC aliquot, we do not screen it routinely [4].

Section 4

a. The purpose of creating small-volume aliquots is to limit donor exposures, avoiding circulatory overload, and potentially decreasing donor-related risks. So, we have this transfusion practice routinely.
b. Once the aliquot is done, it is labelled with the same expiration date and unit number of the original blood bag [5].
c. We maintain the main mother bag up to 10 days from the phlebotomy. After that, depending on the remaining volume, it could be discarded or not. In some cases, the remaining volume, if it is important, it could be destinated to an adult patient. Note: These criteria are applicable for RCC only.

Section 5

a. The transfusion practice is through a gravity method.
b. Not applicable.
c. The rate of infusion depends on the clinical condition, but each unit must be administered in no more than 2–4 h.

REFERENCES

1. Fergusson DA, Hebert P, Hogan DL, et al. Effect of fresh RBC transfusions on clinical outcomes in premature, very-low birth-weight infants: The ARIPI randomized trial. JAMA. 2012;308:1443–51.
2. Guidelines on the use of irradiated blood components. BJHaem. 2020;191:704.
3. Roseff SD. Pediatric blood collection and transfusion technology. In: Herman JK, Manno CS, editors. Pediatric transfusion therapy. Bethesda, MD: AABB Press; 2002. p. 217–47.
4. Strauss RG. Data-driven blood banking practices for neonatal CGR transfusions. Transfusion. 2000;40:1528–40.
5. Maier RF, Sonntag J, Walka MW, et al. Changing practices of RBC transfusions in infants with birth weights less than 1000 g. J Pediatr. 2000;136:220–4.

Oscar Walter Torres
Hospital Materno-Infantil 'Ramón Sardá', Buenos Aires, Argentina
Email: owtorres@gmail.com

UGANDA

Aggrey Dhabangi

Section 1

Location: Located in the City, Kampala, Uganda.
Type of hospital: National Referral and Teaching hospital.
Treats both adults and children.
NICU has 46 beds.

Section 2

a. Yes.
b. Whole components from the National Blood Transfusion Service (NBTS) are WB derived.
   i. For RBCs, we receive paediatric blood bags, each 80–100 ml.
   ii. For PLTs, we receive single donor PLT bags, each 50–70 ml.

Section 3A

i. Not used.
ii. Both the paediatric RBCs and PLTs bags are made by the NBTS. The WB from which these components are made is collected in CPD/SAGM quadruple bags.
iii. Not used.
iv. N/A: All the blood components in the country are not leukocyte reduced.
v. N/A: CMV testing is not available locally.
vi. N/A: Irradiation of blood is not yet available in the country.
vii. N/A: Pathogen inactivation/pathogen reduction is not yet available in the country. There is only one ongoing clinical trial using PRT of WB.

Section 3B

a. The Blood Centre/National Transfusion Services (NBTS).
b. All received from the NBTS and kept as part of the inventory.
c. Standard volumes (ranges) as provided by the NBTS.
d. Pre-fixed volumes, and so, the hospital uses as much volume as needed.
e. N/A: Irradiation not available in the country.
f. From one WB unit collected into a quadruple bag, three satellite paediatric RBC bags are made by the NBTS and one PLT bag. The hospital does not have transfer bags.
g. The NBTS uses sterile docking device and sealant, during preparations. Also, the quadruple bags have in-tube seals between the bags; these seals once broken allow blood to flow from the parent bag, into the satellite bags, then the sealant is used to close the tubes again.
h. The blood Centre (NBTS) performs QC, following African Society for Blood Transfusion, 'AfSBT' guidelines, namely:
   i. For RBC bags (1%); haemoglobin (Hb).
   ii. PLT bags (1%); PLT count and bacterial culture (aerobic only).

i. No.

Section 4

a. No. The three RBC bags from a single donor may be used to transfuse up to three babies.
b. Expiration dates issued by NBTS remain unchanged.
c. Often, the remaining volume of the RBC bags—each 80–100 ml—is discarded.

Section 5

a. On the paediatric wards—we use gravity method. In the NICU, currently we use infusion pumps (previously we used syringes).
b. Two types:
   i. The 'KellyMed' type.
   ii. The SK-600 II—'Mindray'.
c. 5 ml/kg/h.

Aggrey Dhabangi
Makerere University College of Health Sciences, Kampala, Uganda
Email: adhabangi@gmail.com

SOUTH AFRICA—CAPE TOWN

Anel van Zyl, Nadia Mundey & Vernon Louw

Section 1

Tygerberg Hospital is located in the northern suburb of the Cape Metropole, South Africa.
Tygerberg Hospital is a tertiary/teaching hospital.
The hospital treats both adult and children patients.
There are various neonatal wards at Tygerberg Hospital including an ICU. The NICU has eight beds and it is linked to a four-bed high-care area.

Section 2

a. Yes. Tygerberg Hospital Blood Bank supply small volumes of RBCs, plasma and PLTs to neonatal and paediatric patients.
b. RBC products are produced from an adult WB O-positive or O-negative unit. The adult unit is made into plasma and RBC components. The RBCs are leukodepleted and divided equally into four smaller bags. The bags are labelled as infant RBC unit 1–4 with an individual volume of ±55 ml. The RBCs are also available in a slightly larger volume of ±130 ml, labelled as paediatric RBC units 1 and 2.
c. FFP are made from a male donor that is group AB. The AB plasma is divided into smaller volumes of ±130 ml each and labelled as paediatric FFP 1 and 2.

The PLTs are made from an apheresis unit (group O positive) and divided into four smaller bags with an individual volume of 40–60 ml.

Section 3A

i. The RBC unit is processed into smaller aliquots within 72 h of collection from the donor.

The FFP are processed within 18 h of collection from the donor.
The PLTs are divided into smaller aliquots within hours of collection from the donor.

ii. The RBC units have CPD and SAGM as the additive solution.

Plasma products have CPD. The PLTs contain CPDA.

iii. The infant and paediatric units are either group O positive or negative.

Plasma products are group AB.
PLTs are group O positive, low titre.

iv. The RBCs and PLTs are leukoreduced.
v. Products are not tested for CMV. However, as they are leukoreduced, the risk is reduced.
vi. Infant and paediatric RBC and PLT units are not irradiated. Any irradiated units must either be a leukoreduced WB or adult unit of RBCs or PLTs.

Plasma units are not irradiated.

vii. We do not currently do pathogen reduction or inactivation.

Section 3B

a. The aliquots are prepared by the Blood Service. The units are sent to the relevant blood bank for distribution to patients as required.
b. Tygerberg Blood Bank have small-volume units available as part of our inventory.
c. It is a standard volume for each product.
d. In general, the clinicians do not take the tubing waste into consideration for paediatric and neonatal patients as the volume in the bags supplied by the Blood Service is sufficient for the patients transfusion needs.
e. Units are irradiated just before transfusion. If that is not possible, the unit may be irradiated up to 12 h before issue.
f. The Blood Service prepares the RBC aliquots using a transfer bag set that is attached to the leukoreduced unit via sterile welding. The bags are weighed as the blood is separated into each bag.

The paediatric plasma is prepared by dividing the plasma between the satellite bags that are already present as part of the quad pack that is used. No additional transfer bags are attached.

In paediatric haematology/oncology, we transfuse from the bag and rarely waste any blood. When we order, we round off to the closest unit. We also rarely have small babies who need small amounts of blood. However, if we had to do that, we would probably use a buretrol to measure the volume when transfusing from the bag.

In NICU, the blood is transfused directly from the bag. We do not draw any blood products into a syringe.

g. Sterile docking or welding is used to maintain sterility.
h. No additional QC is performed on the aliquots as the smaller volumes are prepared from an adult unit. No changes are made to the content of the aliquots.
i. Not applicable.

Section 4

a. The Blood Service has a limited donor exposure program also known as LDEP. An adult RBC unit is divided into four smaller bags. The units can be reserved two or four units at a time for a single newborn. The units are usually reserved for those neonates that will require multiple transfusions in the first few weeks after birth. The requesting clinician will indicate how many units to reserve for the neonate. The units can either be kept for the neonate until expiry or it will be kept for the neonate for 20 days, after which the units will be cancelled and used for another neonate if required.
b. The RBC product has an expiry date of 42 days from the time it was bled as it was prepared under sterile conditions.

The plasma is stored at −18°C for a year. Once thawed, it has an expiry time of 6 h from the time that the thawing process started.

Infant PLTs have an expiry of 5 days as the PLTs were bled into a closed sterile system.

c. Not applicable.

Section 5

a. Paediatrics—an infusion pump is used.

NICU—The blood will run through an infusion pump where the amount we want to give is entered into the pump.

b. Aguilla Infusamed infusion pumps.
c. Paediatrics—We usually transfuse over 4 h for a child who is not in heart failure and over 6 h for a child in heart failure due to the risk of volume overload and worsening of heart failure.

In an emergency situation (e.g., Hb of 1 or 2 and collapse imminent), we would initially transfuse faster and then slow the rate to the above.

NICU—Most packed cell transfusions run over 4 h. The volume is usually between 10 and 20 ml/kg in total, run over the 4 h.

Anel van Zyl
Stellenbosch University and Tygerberg Hospital, Cape Town, South Africa
Email: vanzyla@sun.ac.za

Nadia Mundey
Western Cape Blood Service, Cape Town, South Africa
Email: nmundey@wcbs.org.za

Vernon Louw
University of Cape Town and Groote Schuur Hospital, Cape Town, South Africa
Email: vernon.louw@uct.ac.za

SOUTH AFRICA—ROODEPOORT

Karin van den Berg

Section 1

We provide blood transfusion services to hospitals in large metropolitan cities, regional centres as well as small district hospitals in remote areas.

We provide blood transfusion services to academic, tertiary, regional and district level hospitals.

Most of the hospitals to whom we provide blood transfusion services treat both adults and children, but we have a few dedicated children's hospitals as well.

Some of our hospitals do have NICUs, the size of which varies from a few beds (2–3) to very large units with 20 or more beds.

Section 2

a. Yes, our centre supports aliquoting/small-volume transfusions in neonates and paediatrics.
b. We provide small-volume, routinely leukoreduced RBC products.

The small-volume PLT products are produced from aliquots of SDAPs that are leukoreduced at time of collection.

We also produce small-volume FFP products aliquoted from plasma recovered from a WB donation.

Section 3A

i. Paediatric small-volume RBC and plasma products are produced from WB collections processed within 24 h. Small-volume RBC products less than 5 days old are available on request, but generally we do not have separate limitations on unit age for small-volume RBC products.
ii. Small-volume RBC products are produced from WB collections, which are collected in CPDA. Single-donor apheresis products are collected using ACD and stored in PAS.
iii. We mostly produce small-volume RBC products from group O and group A donations, although of these the majority are group O products.
iv. All small-volume RBC products are produced from leukoreduced WB donations.
v. We do not test for CMV and do not carry a CMV negative inventory.
vi. Irradiated small-volume products are produced from irradiated WB donations, but only upon request.
vii. We do not perform pathogen reduction in South Africa.

Section 3B

a. We (the blood centre) produce the aliquots.
b. We (the blood centre) maintain an inventory of small-volume RBC, plasma and PLT products that are issued upon request.
c. Small-volume RBC products are produced by aliquoting a leukoreduced RBC collection into three aliquots by equally distributing the volume between three paediatric bags. The final volume of these small-volume products are standardized to 120 ± 30 ml.
d. We have a pre-fixed aliquot volumes with the treating clinician transfusing as much as needed.
e. We do not keep an inventory of irradiated small-volume products. Both small-volume RBC and PLT products are irradiated upon request, with the 'mother' unit being irradiated and then aliquoted into the smaller units.
f. After filtration of a fresh adult RBC product, a set of four paediatric bags are sterile docked on to the 'mother' unit and the volume equally distributed among three of the four bags. The four bag may be used in cases where we have a directed donation.
g. Sterility is maintained by using a sterile docking device in combination with a strict infection prevention control policy and procedure and routine environmental monitoring, including of the sterile docking devices.
h. Yes, we test 1% of all products produced. We follow the 'Standards of Practice for Blood Transfusion in South Africa' regulations. We measure volume, white cell count and sterility.
i. No, we do not screen for supernatant electrolyte levels.

Section 4

a. If the treating clinician either request that we reserve all three aliquots for a specific patient or if (s)he indicates that the patient will require repeated transfusion, we will reserve all three aliquots for one specific patient.
b. As we have standardized processes for producing small-volume products, they have standard expiration dates, based on the date of donation. For small-volume RBC products, the expiration date is 21 days from date of donation. Small-volume PLTs and plasma products have the same expiry as the routine products.
c. For small-volume RBC products, we do not have a remaining volume. Small-volume PLT products are produced from the remainder of a SDAP collection that is too little to produce an adult unit.

Section 5

a. Practices in the different hospitals differ, but where available, the preference is the use of an infusion or syringe pump.
b. Different units use different types of pumps.
c. The rate of transfusion is determined by the treating clinician, as the blood service we recommend that the transfusion of a particular unit (or part thereof) should be completed within 4 h of commencing the transfusion [1].

REFERENCE

1. Medical Directors of the South African Blood Transfusion Services, Clinical Guidelines for the use of blood products in South Africa. Fifth ed. 2014.

Karin van den Berg
South African National Blood Service, Roodepoort, South Africa
Email: karin.vandenberg@sanbs.org.za

ORCID

Satyam Arora https://orcid.org/0000-0002-9048-5624
Ruchika Goel https://orcid.org/0000-0001-9653-9905
Arwa Z. Al-Riyami https://orcid.org/0000-0001-8649-0650
Ratti Ram Sharma https://orcid.org/0000-0002-7415-4665
Nabiha H. Saifee https://orcid.org/0000-0003-1646-9201
Naomi Rahimi-Levene https://orcid.org/0000-0003-3411-886X
Maha Badawi https://orcid.org/0000-0003-3272-2393
José Mauro Kutner https://orcid.org/0000-0003-3784-6731
Aggrey Dhabangi https://orcid.org/0000-0002-5615-3925

How to cite this article: Arora S, Goel R, Al-Riyami AZ, Al-Rawas AH, Al Hosni S, Montanari M, et al. International Forum on Small-Volume Transfusions in Neonates and Paediatric Patients: Responses. Vox Sang. 2023;118:230–51.

DOI: 10.1111/vox.13307

Vox Sanguinis ISBT International Society of Blood Transfusion

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

