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Aims and Scope

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

1. Blood Component Collection and Production: Blood collection methods and devices (including apheresis); blood component preparation; inventory management; collection and storage of tissues; quality management and good manufacturing practice; automation and information technology; plasma fractionation techniques and plasma derivatives;
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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;

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REVIEW

Vox Sanguinis ISBT International Society of Blood Transfusion

Should adding pain, oxygen saturation and physical assessment to vital signs become the new standard of care for detecting blood transfusion reactions?

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Abstract

Background and Objectives: Clinicians sought to ascertain what frequency of vital signs best detects blood transfusion reactions. This review discusses early and delayed blood product transfusion reaction detection through the lens of scientific literature.

Methods: A comprehensive appraisal of published literature was conducted using Integrative Research Review methodology through June 2022 not limited to English or research in Cumulative Index to Nursing and Allied Health Literature, Cochrane Library of Systematic Reviews, Medline and PubMed.

Results: Full-text articles in the final sample included four articles discussing vital signs detecting blood transfusion reactions and four articles reporting the importance of adding physical assessments for early reaction detection. None of the studies provided evidence regarding how often vital signs should be monitored to detect transfusion reactions. No studies included identical screening components for detecting blood product transfusion reactions. Main themes emerged including variations in what was included in vital signs, importance of respiratory assessment, inclusion of physical assessment, nurse documentation and reporting compliance, and patient and family inclusion in transfusion reaction recognition.

Conclusion: Vital sign components varied across reviewed studies. Respiratory rate and pain were not always included in 'vital signs' to identify transfusion reactions. Only low-level data and no clinical trials loosely informing frequency of vital sign monitoring to transfusion reaction detection were found. Respiratory (to include oxygen saturation, lung sounds and respiratory rate) and pain assessment emerged as crucial to acute and delayed transfusion reaction recognition. The disconnect between 'vital signs' and the varied vital sign components reported to detect transfusion reactions in scientific literature requires further exploration.

Keywords

adverse event, blood safety, Integrative Research Review, nurse assessment, transfusion reactions, vital signs

Highlights

- The findings of this review suggest that respiratory rate, pulse oximetry and pain are crucial to detecting clinical deterioration and should be officially incorporated into vital sign assessment to detect both early and late transfusion reactions.
- No standard contextual vital sign derangement guidelines were identified to inform clinicians as to what should be viewed as 'abnormal' or to direct them towards transfusion reaction consideration.
- This review communicates the need for further research to define: the frequency of the vital sign measurement needed to detect blood component transfusion events; what assessments should be included when using the term 'vital signs' and what deviation from baseline represents an 'abnormal' finding.

INTRODUCTION

Much like other medical emergencies, blood transfusion reaction detection may improve patient outcomes at the earliest possible time. The U.S. transfusion adverse event rate associated with 20,933,000 blood products transfused during 2011 was 0.24%; thus, demonstrating a time burden on nurses administering blood products, monitoring for transfusion reactions and managing reaction events while already overstretched in complex health systems [1, 2]. Being able to recognize and intervene rapidly in medical emergencies remains imperative for frontline nurses despite many tasks needing to be completed. Competing interests for a nurse's time make early, accurate and evidence-based blood transfusion reaction detection methods necessary.

With reported blood transfusion reaction rates ranging from 0.14% to 2.1% in reviewed literature [1–4], nurses require clear guidance on vital sign measurement frequency, vital sign composition and abnormal findings to recognize a patient reacting to a transfusion promptly [5, 6]. Incomplete inclusion of vital signs (especially respiratory rate, pain and pulse oximetry), lack of guidelines to interpret the data [5, 7], absence of physical assessment (especially lung sounds) and not including family members and patients as partners in detection contribute to adverse events. This review examined best practices for reaction detection published in peer-reviewed literature.

Background

Screening criteria for transfusion reactions must be developed based on sound evidence to improve the quality of patient care and decrease costs to treat. Using translational research methodologies, healthcare agencies are committed to implementing evidence-based process solutions for accurate and timely recognition and management of adverse transfusion reaction events. Our hospital in the Southwestern United States recorded 51/12,077 (0.422%) transfusion-related adverse reactions in 2017, highlighting the importance of discovering evidence to support ways to detect transfusion reactions. Blood transfusion reaction detection exhibits clinical implications for practitioners and patients. Our institutional transfusion reaction rate was in the lower quartile of rates found in reviewed literature; however, even one transfusion reaction event can represent a life-changing incident for a patient.

Significance

Early reaction detection during blood product administration may reduce or even mitigate adverse transfusion-related events. Although the U.S. Federal Drug Administration reported one fatality per 697,767 (0.00013%) blood component transfusions [8], one study reported a 3% mortality rate [3]. Despite mortality rate variance in reviewed literature, the fact remains blood transfusions cause severe complications and fatalities [3, 9]. Adverse reactions may occur immediately or within several hours post-transfusion, depending on the type of reaction [3, 4, 9–11]. Consequently, clinical observations, patient verbalized complaints and visitor recognized changes are pivotal to patient survival.

Aims

This integrative research literature review aimed to examine the frequency of vital sign measurement needed to identify blood transfusion reactions. The review and evaluation of literature were conducted to examine how often vital sign assessment and what physiologic and physical parameters ought to be included to identify blood transfusion reactions in patients. The secondary purpose of this project was to ensure best transfusion reaction detection practices are employed by nurses administering blood products by partnering frontline acute care nurses with doctorally prepared nurses to conduct the structured literature review.

METHODS

Design

A comprehensive review of peer-reviewed published literature was used as the study design to guide this project. This literature review

was conducted using Integrative Research Review (IRR) methodology adapted from Whittemore and Knafle [12] and Brown [13]. This methodology permitted inclusion of studies reporting both qualitative and quantitative findings and a narrative approach for synthesizing results from a wide range of study designs. Additionally, sources using theoretical approaches allowed for narrative synthesis through data reduction, display and comparison to draw conclusions in alignment with IRR methodology [12].

The International Prospective Register of Systematic Reviews (PROSPERO) was searched to identify if any similar systematic review is currently being conducted. The literature search was guided by a clearly formulated clinical question, 'In individuals receiving a blood product transfusion, what frequency of vital sign measurement should be used to monitor for a reaction?' to find all relevant articles addressing the clinical issue of interest by reviewers. Specifically, the structured and organized method of identifying and evaluating the body of peer-reviewed literature was framed using a population, intervention, comparison, outcome (PICO) statement.

Search methods

Six frontline nurses, four academic partners (nurse scientists, research librarian and Doctor of Nursing Practice) and two institutional Doctor of Nursing Practice leaders evaluated and synthesized evidence to identify transfusion reaction screening best practices using keywords determined by the research team. To increase consistency among reviewers, all reviewers vetted publications, independently weighed levels of evidence and rated the quality of the same sample of articles before coming together to discuss results as a group. Articles and data included in the final sample were agreed upon through consensus after dialogue.

A research evidence table was compiled to amalgamate significant discoveries and aid in synthesizing published findings. The Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) 2020 checklist guided researchers through the literature appraisal process [14]. The PRISMA Statement assisted investigators in evaluating effectiveness and unintended consequences of the healthcare intervention of blood product transfusion reaction screening to assure transparency of literature review reporting.

Search strategy

The Boolean search strategy included using the key search terms of 'blood transfusion' AND 'vital signs' OR 'nurse assessment' AND 'reaction' as MeSH (Medical Subject Headings) major word terms. The databases and clinical sources searched included Cumulative Index to Nursing and Allied Health Literature, Cochrane Library of Systematic Reviews, National Institutes of Health U.S. National Library of Medicine (Medline) and The National Library of Medicine database (PubMed).

A research librarian conducted the preliminary and final searches; cross-verified the search strategy and confirmed that all applicable existing publications were included. All existing peer-reviewed articles, not limited to English or research, through June 2022 were included. Applying manual searches of reference lists for included studies allowed for expansion of the search coverage. After removing duplicates, a nurse scientist and research librarian screened titles and abstracts for potential relevancy. Eight additional reviewers met as a group to further examine the remaining full-text manuscripts for inclusion or exclusion.

Inclusion criteria applied for literature selection of studies, guidelines and reports used for this review were qualitative, quantitative, mixed methods and clinical practice guidelines (CPGs). Non-empirical studies such as reviews, letters, commentaries and governmental documents were not included. Because no studies were found to match the clinical question, reviewers agreed the findings from continuing nursing education, reports with a focus on detection of blood transfusion reactions and manuscripts discussing strategies and methods for nurses to identify blood transfusion reactions in patients were helpful in informing the consensus opinion of the group. Publications without relevance to blood product transfusion reaction detection or associated adverse events were excluded.

Search outcome

Published articles identified through database searching were initially evaluated and selected by screening titles and abstracts. Inclusion criteria required selecting publications involving individuals being screened for blood product transfusion reactions using vital signs. Additional application of inclusion criteria involved selecting articles relevant to answering the clinical question or validating blood transfusion reaction detection criteria. Manuscripts were reviewed separately before conducting discussions to make the final selection decisions. Discrepancies in reviewer perspectives led to discussing the disputed manuscripts for selection determination by the team.

Quality appraisal and risk of bias assessment

The 11 reviewers independently read, evaluated and appraised each article. Investigators grouped publications by key contexts reported and summarized settings, populations and study designs on a standardized research table. Research tables including author(s), date, purpose(s), reported outcome(s), sample size, study design and level of evidence were completed independently by each reviewer to ensure inter-rater reliability of levels of evidence, avoid selection bias and avoid influencing conclusions. Reviewers explored characteristics and methodological quality of each evaluated manuscript's content using the Enhancing the Quality and Transparency of Health Research (EQUATOR)'s Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement guidelines for reporting observational studies according to the studies' designs [15].

Levels of evidence were independently determined by reviewers using the 'Quick Guide to Designs in an Evidence Hierarchy' [16]. As

a group, a comparison of assigned levels of evidence and quality appraisal tool scores were completed to reach consensus for each article included in the final sample. Collective opinions regarding significance for inclusion to inform the study aims guided final decisions. Due to the low quality of evidence discovered, assessments of risk of bias in studies were addressed by examining pre-intervention, at-intervention and post-intervention elements of each study as a group.

Data extraction and knowledge synthesis

Researchers critically evaluated all physiological parameters and physical assessment findings reported in the literature to validate significance for inclusion in blood product transfusion reaction screening criteria. Gathered data from literature reviewed were independently evaluated, organized and recorded on a standardized evidence table used by all reviewers. Major themes were extracted and documented as they emerged from the final sample of manuscripts and key discoveries were assigned to appropriate major themes to guide synthesis of publicized findings (see Table 1). The authors discussed and reached agreement through consensus of emerging themes and the allocation of manuscripts into the appropriate theme groupings.

Data synthesis

Reviewers met as a group to synthesize data from the included studies. Findings were organized by theme in a Microsoft Word document

TABLE 1 Themes emerging from literature reviewed

Theme	Description of findings
Respiratory assessment importance	• 67% ($n = 4$) of participants had hypoxaemia and 50% ($n = 3$) had dyspnea [3] • 5% of participants had shortness of breath, 3% chest pain and 2% facial oedema ($n = 128$) [2] • 20.5% ($n = 117$) participants had transfusion-associated circulatory overload [11] • Six participants had tachypnea and/or dyspnea [4] • Pulse oximeter readings during first 15 min of trauma predicted early massive transfusion and mortality better than currently used vital signs [17]

Vital sign measurement frequency	• Before, 15 min after start and at the end [9]
	• Before, 15 min after start and at the end. Vital signs were added up to 1 h post [3]
	• Forty percent had vital sign changes after patients reported symptoms with variance noted in vital sign guidance [2]
	  ○ Cleveland Clinic (2014) before, after 15 min and at completion
	  ○ Joint Commission (2011) before, after 15 min and within 1 h of completion
	  ○ New York State Department of Health (2012) before, after 15 min and at completion
	  ○ United Kingdom Guidelines for Blood Transfusion (2012) before, after 15 min and at completion
	• Before and after every transfusion [1]

Inclusion of physical assessment	• Chills: bone, muscle, chest or abdominal pain; nausea, reports of feeling unwell included [9] • Hives/itching, chills, dyspnea, flushing, nausea and back pain recommended added [3] • Suggested less frequent vital sign monitoring when combined with physiologic assessment; only 9% of transfusion reaction detection using routine vital sign monitoring alone [2] • Reactions only detected with an assessment or oxygen saturation [11] • 8% ($n = 12$) experienced severe chest, back or proximal extremity pain [4]

Nurse documentation and reporting compliance	• Vital signs were documented correctly 88% of the time at all three required intervals [2]
	• 77% of reactions were not reported to blood bank [1]
	  ○ Lack of compliance with policy regarding temperature increase of more than 1°C noted
	  ○ Temperature recorded for 95.5% (2241/2346) of patients, pulse rate recorded for 96.6% (2241/2346) of patients, SBP recorded for 95.5% (2242/2346) of patients, DBP recorded for 95.2% (2234/2346) during red blood cell transfusion
	  ○ Temperature recorded for 94% (618/657) of patients, pulse rate pre and post recorded for 95.7% (629/657) of patients, SBP recorded for 94.7% (622/657) of patients, DBP recorded for 93.6% (615/657) during plasma transfusion
	  ○ Temperature recorded for 95.6% (455/476) of patients, pulse rate pre and post recorded for 96.2% (458/476) of patients, SBP recorded for 94.5% (450/476) of patients, DBP recorded for 94.5% (450/476) during platelets transfusion
	• 7272 nurse notes revealed 65% complete documentation with most frequent non-conforming items reported were:
	  ○ Vital signs after blood transfusion (83.3%)
	  ○ Time of completion of blood transfusion and initial vital signs (57.3%) [18]

Patient and family inclusion in transfusion reaction recognition	• Patient/family members provided written and verbal directions to guide reporting of defined symptoms [9] • Engaged patients and families for identification of signs and symptoms of transfusion reaction [2]

Abbreviations: DBP, diastolic blood pressure; SBP, systolic blood pressure.

during team meetings. Disagreements were resolved through group discussion. Reviewers noted differences in what vital sign and physical assessment criteria were used to screen for transfusion reactions in reviewed manuscripts. Both reported vital sign components and physical assessment criteria used to detect blood transfusion reactions were consolidated into a table (see Table 2). Main themes emerged including variations in what was included in vital signs, importance of respiratory assessment, vital sign measurement frequency, inclusion of physical assessment, nurse documentation and reporting compliance and patient and family inclusion in transfusion reaction recognition leading to further exploration of subthemes.

RESULTS

Peer-reviewed published articles identified through initial search strategies ($N = 41$) contained duplicate articles ($n = 12$) which were removed. The remaining 29 articles were then further screened for inclusion by eight research team members using abstracts and titles. Twelve additional articles were excluded through screening of abstracts. After full-text review, nine additional manuscripts were excluded due to lack of relevance to the clinical question or not addressing search terms (Figure 1).

High levels or quantities of evidence were not found to inform investigators' question of best practices in detecting blood product transfusion reactions. Since only four studies were identified to loosely inform vital sign measurement frequency recommendations during blood transfusion, other sources of knowledge were included to further explore the scope of available empirical evidence and guidance. No study was excluded based on quality or methodology. Evidence ratings included 12.5% ($n = 1$) Level V, 62.5% ($n = 5$), Level VI and 25% ($n = 2$) Level VII in the final sample.

Selection of studies

Full-text articles evaluated were compared against clinical question components to determine study inclusion using relevancy to the patient population, intervention of interest, comparison of interest, outcome of interest and timeframe (see Table 3). Two excluded publications focused on predictive computer modelling to detect transfusion reactions [19, 20]. Another excluded article looked at blood component transfusion rate only and included the same sample represented in an included publication [21]. Finally, one excluded study investigated the predictive value of vital signs in geriatric trauma patients for massive blood product transfusion [22]. The final sample used for synthesis included the eight remaining manuscripts.

Of the eight articles included in the final sample, none included evidence to answer the clinical question asked by researchers. Six were research reports of descriptive and case study designs. Other included publications were a literature review and a continuing nursing education learning activity (see Table 4). Four articles examined vital signs detecting blood transfusion reactions, and four articles alluded to the importance of adding a physical assessment for early reaction detection. The final sample inclusion criteria included:

1. using all-aged patients being monitored for blood product transfusion reactions ($n = 4$) [1–3, 9];
2. validating or examining physical assessment component inclusion for reaction detection (especially lung auscultation) ($n = 1$) [11];
3. supporting addition of physical assessment (especially pain) to augment vital sign use ($n = 1$) [4];
4. informing use of oxygen saturation in addition to vital signs for transfusion reaction detection ($n = 1$) [17]; and
5. documentation compliance and completeness by nurses of post-transfusion vital signs ($n = 1$) [18].

TABLE 2 Comparison of screening criteria for blood transfusion reactions

Article	Blood pressure	Heart rate	Respiratory rate	Temperature	Pulse oximetry	Physical assessment
Battard Menendez (2016)	Not specified	Not specified	Not specified	Not specified	Not specified	Chills, pain, nausea and reports of feeling unwell
Cortez-Gann (2017)	Systolic blood pressure only	Included	Not included	Included	Not included	Hives, itching, chills, flushing, nausea, pain and cyanosis
DeYoung Sullivan et al. (2015)	Not specified ($n = 6$)	Not specified ($n = 6$)	Not specified ($n = 6$)	Not specified ($n = 6$)	Not specified ($n = 6$)	Not specified ($n = 10$)
Literature review	Included ($n = 4$)	Included ($n = 4$)	Included ($n = 1$) Only if change noted ($n = 1$)	Included ($n = 4$)	Not included ($n = 4$)	
Mackenzie et al. (2014)	Included	Included	Not included	Not included	Included	Not included

Note: Components represent blood component transfusion reaction screening criteria in the four included articles relevant to answering the clinical question.

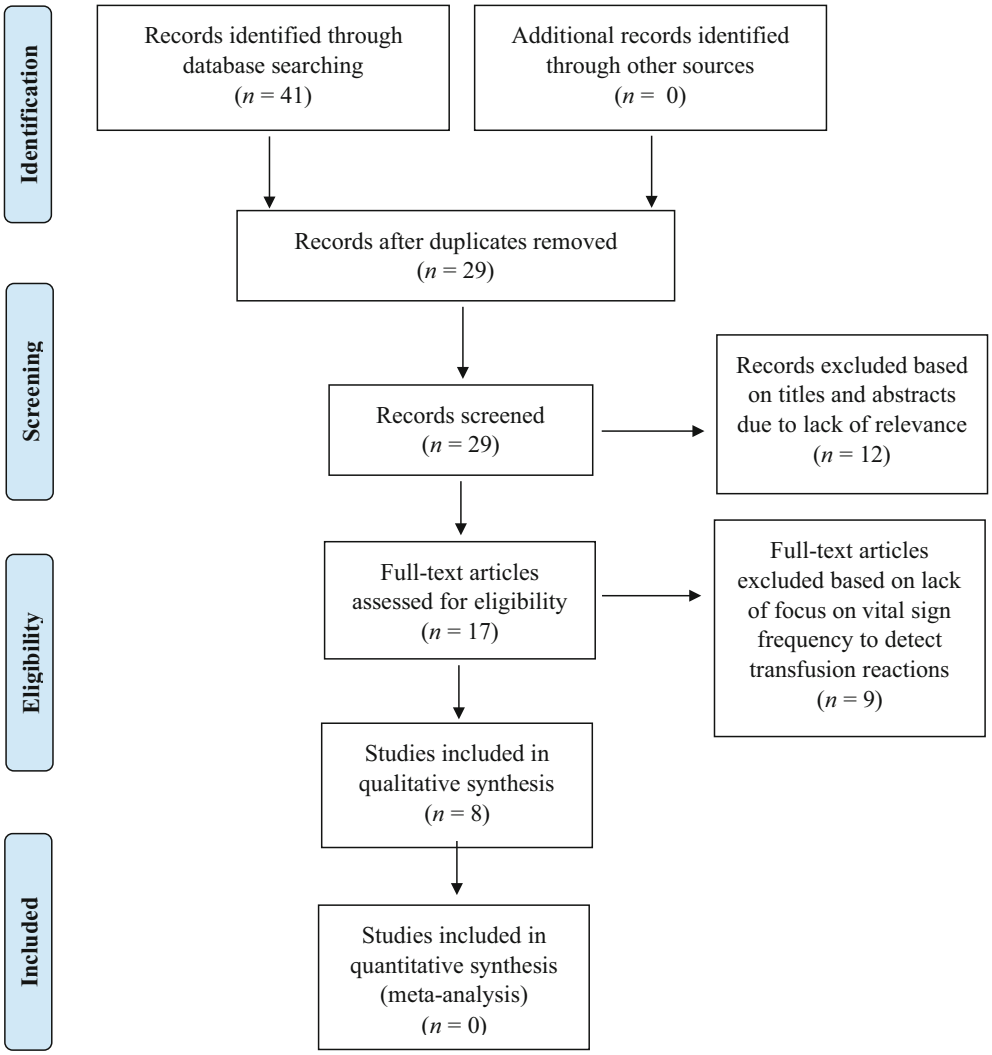

FIGURE 1 Preferred Reporting Items for Systematic Review and Meta-Analyses flow diagram. This figure demonstrates the search methodology used to guide this literature synthesis on detecting blood transfusion reactions.

Characteristics of selected sources of evidence.

None of the four included studies provided evidence regarding how often vital signs should be monitored to detect transfusion reactions or were deemed free of potential bias. No studies included identical screening components for detecting blood product transfusion reactions. 'Abnormal' was found frequently as a vital sign finding to indicate a transfusion reaction. Still, parameters to denote what vital sign variation should be considered 'abnormal' were not found in the literature except for an increased body temperature of 1°C without supporting evidence [1, 6].

Practical considerations of vital sign assessment

Reviewed literature recommended vital signs be obtained before, after 15 min and at transfusion completion [2, 3, 9]. Variations in physiological screening criteria incorporated into 'vital signs' across reviewed publications made metanalysis of quantitative data, such as blood transfusion reaction rates, impossible and presented challenges to meta-synthesis. Investigators identified evidence indicating more than the frequency of vital signs at prescribed intervals was needed to detect blood transfusion reactions.

Only four articles were included for synthesis due to relevance to the asked clinical question. Four additional articles introduced evidence related to themes emerging during the literature review. Instead of discovering what vital sign assessment frequency best detects blood product transfusion reactions, reviewers identified gaps in evidence and practice. Emerging themes guided informative and actionable synthesis of discoveries to guide clinical nursing practice.

Vital signs defined

Vital sign components used to detect transfusion reactions differed across reviewed literature where specific components were reported.

TABLE 3 Comparison of findings to PICO question

Clinical question component	Alignment with literature reviewed
P (population of interest) = patients receiving a blood product transfusion	• Population not specified by authors ($n = 4$) [3, 4, 9, 11] • Population veterans and 99% of men in both articles (exact same population) ($n = 2$) [1, 5] • Population haematology-oncology ($n = 2$) [2, 4] • Population adult trauma ($n = 4$) [15–17, 19]
I (intervention of interest) = receiving a blood component transfusion	• Intervention met by study ($n = 7$) [1–5, 9, 11] • Vital sign prediction of blood transfusion, 3+ day ICU stay or death ($n = 1$) [15] • Predictive value of vital signs in geriatric trauma patients for massive transfusion ($n = 1$) [19] • Pulse oximetry signals prediction of blood transfusion and death ($n = 1$) [17] • Nursing documentation of vital signs during transfusion ($n = 1$) [18] • Automated analysis of pulse oximetry signals with laboratory values and vital signs obtained at triage ($n = 1$) [16]
C (comparison of interest)	• Not receiving a blood component transfusion
O (outcome of interest) = frequency of vital signs	• One article met the outcome of interest of frequency of vital sign measurement during blood transfusion [2]

Note: Literature compared to PICO to guide exclusion of articles for relevancy.
Abbreviation: ICU, intensive care unit.

The only vital sign elements consistently included to detect transfusion reactions were systolic blood pressure, heart rate and body temperature (Figure 2). Respiratory rate was often excluded from 'vital signs' employed to identify reactions to blood product transfusions.

Pain inclusion in vital signs

Pain was repeatedly absent from vital sign assessment across manuscripts despite being frequently mentioned as clinically significant to transfusion reaction detection [2–4, 6, 9]. Only one reviewed manuscript supported including pain in vital sign monitoring to detect a blood product transfusion reaction [4]. In two studies, vital signs included SaO_2 to screen for transfusion-related adverse events [1, 4]. Investigators found lack of a standard definition of what constitutes 'vital signs' led to inconsistency in physiological components included across studies used for blood transfusion reaction detection.

Physical assessment inclusion

One discovery emerging from reviewed literature was the importance of nurses completing physical assessments (especially lung auscultation) to detect transfusion reactions. Results from two publications implied the importance of including oxygen saturation (SaO_2) when monitoring for blood transfusion reaction in addition to periodic vital sign assessment [11, 17]. Another manuscript supported adding physical assessment, pain and respiratory rate to blood reaction monitoring [4]. Literature findings underscored physical assessment inclusion detected indications of transfusion reaction missed by vital signs alone [6]. Clinical assessments incorporating vital sign and physical exam components were found crucial for detecting acute or delayed transfusion reactions.

Documentation of vital signs

Three studies highlighted the importance of nurse documentation compliance and completeness. Reviewed evidence revealed documentation deficiencies of vital sign results during blood product transfusions ranging from 57.3% to 88% of the time [1, 2, 18]. Post-transfusion vital sign documentation was the least documented transfusion-associated assessment by nurses (83.8%) [18]. Nurse incomplete documentation of vital sign assessments alludes to difficulties complying with even the bare minimum standards for transfusion reaction monitoring.

DISCUSSION

Vital signs defined

Across reviewed studies, including more components for blood transfusion reaction detection led to a higher rate of reported transfusion reaction events. These findings suggest comprehensive vital signs with the addition of physical assessment components detect blood transfusion reactions better than incomplete vital sign monitoring with limited vital sign components lacking physical assessment inclusion. Lack of derangement definitions to distinguish 'abnormal' vital sign components or to indicate transfusion reactions also prevent triggering blood reaction recognition and interventions by nurses [6].

The Joint Commission standards direct nurses to record respiratory rate during transfusions only if a significant change from baseline occurs, while notably excluding respiratory rate in vital signs to be monitored [2, 6]. How can clinicians detect 'significant' changes in the respiratory rate during a transfusion without checking a respiratory rate or knowledge of what shift from baseline represents a 'significant' deviation?

Reviewers found a paucity of evidence to guide what components should be included in 'vital signs' to identify transfusion reactions. While some clinicians consider SaO_2 and pain vital signs, other practitioners may consider these components of a physical

TABLE 4 Literature informing vital sign use to detect blood transfusion reaction events

Authors (year)	Purpose(s)	Reported outcome(s)	Sample size	Study design	Level of evidence
Battard Menendez (2016)	Continuing nursing education on best practices for monitoring for acute haemolytic transfusion reactions	None	NA	NA	VII
Cortez-Gann (2017)	Identify relationship of vital sign changes to signs and symptoms of blood product transfusion reaction	Transfusion reaction rate of 0.15% ($n = 116$)	Over 77,800 units of blood product transfusions	Retrospective descriptive design	VI
DeYoung Sullivan et al. (2015)	Examine the evidence regarding the optimum frequency of vital sign monitoring for haematology patients receiving blood products and to ensure current institutional practice was aligned with the evidence	Vital sign monitoring during blood transfusions	Ten studies evaluated and reported by authors at levels of evidence IV, V and VII	Literature review	V
Gehrie et al. (2015)	Evaluate the variation in vital signs observed after blood product transfusion	Pre and post vital signs (temperature, pulse rate and blood pressure) and reported reaction	3496 blood product transfusions including red blood cells ($n = 2359$), platelets ($n = 476$), plasma ($n = 659$)	Retrospective descriptive design	VI
Goodall (2014)	Inform on TACO	TACO	One	Single descriptive case study	VII
Hardwick et al. (2013)	Report blood product reaction in a 34-year-old woman with haemophagocytic lymphohistocytosis	Acute pain transfusion reaction	One	Single descriptive case study	VII
Mackenzie et al. (2014)	Analyse whether pulse oximeter signals predict blood transfusion and mortality in trauma patients	Continuous vital sign data (heart rate, systolic blood pressure and pulse oximetry), blood product transfusion and mortality	556 patients	Descriptive design	VI
Paiva Dos Santos et al. (2013)	Evaluate nursing documentation of blood transfusion monitoring in inpatient units of a hospital for compliance	Vital signs post transfusion, observation 10 min after transfusion started, transfusion end time and nurses' signature.	7272 nurses' documentation notes	Retrospective descriptive design	VI

Note: Level I evidence indicates systematic review or meta-analysis of all relevant randomized controlled trials (RCTs); Level II evidence indicates evidence-based clinical practice guidelines based on systematic reviews of RCTs; Level III evidence indicates evidence obtained from at least one well-designed RCT; Level IV evidence indicates evidence obtained from well-designed controlled trials without randomization and from well-designed case–control and cohort studies; Level V evidence indicates evidence from systemic reviews of descriptive and qualitative studies; Level VI evidence indicates evidence from a single descriptive or qualitative study, and Level VII evidence indicates evidence from the opinion of authorities and/or reports of expert committees.
Abbreviations: NA, not applicable; TACO, transfusion-associated circulatory overload.

FIGURE 2 Vital sign assessment for transfusion reaction. The only vital sign elements consistently included to detect transfusion reactions were systolic blood pressure, heart rate and body temperature.

assessment [6]. Adding pain to vital sign monitoring for transfusion reaction detection aligns with the recommendation by the U.S. Department of Veterans Affairs to add pain as the fifth vital sign in 1999 and The Joint Commission's condition for accreditation to include pain with vital signs in the year 2000 [6, 23, 24]. Standardizing the inclusion of SaO_2 and pain into vital sign assessments would ensure consistency in monitoring for blood transfusion reactions using the same screening criteria globally.

Frequency of vital sign measurement

In correlation with literature findings, the National Institute for Health and Care Excellence (NICE) Blood Transfusion CPGs only state 'vital signs' should be completed before, during and after blood transfusions without specifying what elements constitute vital signs or what results should raise clinician concerns [25]. Notably, the NICE Blood Transfusion CPG recommendations for vital sign monitoring were based only on the opinion of guideline developers without supporting evidence evaluating the clinical effectiveness or hazards [25]. In one study, 40% of the sample had vital sign changes after participants reported symptoms [2]. Consequently, vital sign monitoring at the start, after 15 min and at the conclusion of a transfusion may not detect transfusion-related events and do not detect delayed transfusion reactions.

One 921 bed U.S. hospital added vital signs 1-h post-transfusion to identify delayed reactions due to the study's outcomes [3]. The average blood product transfusion reaction time was over 1.5 h, with a wide variation in times from transfusion to reaction onset in the sample. Adverse transfusion events would go undetected with only 1-h post vital sign completion. These inconsistencies highlight the importance of standardizing vital signs across healthcare settings.

Partnering with patients and family

Only 9% of transfusion reactions in one study were discovered by routine vital sign monitoring alone [2]. Engaging patients and families as partners in detecting transfusion reactions led to earlier identification and management of transfusion-related events [2, 9]. Educating patients and families on what condition changes warrant immediate reporting to caregivers creates partnerships in care to improve recognition, timely management and clinical outcomes. The NICE Blood Transfusion CPG only informs nurses to provide verbal and written information to patients and family members on a list of transfusion-specific information that does not include guidance on detecting or monitoring for acute or delayed transfusion reactions or what warrants contacting clinicians immediately [25].

Respiratory assessment

Including respiratory rate monitoring due to deterioration predictive ability represents a key finding important to incorporate into nursing practice [6]. Oxygen saturation, respiratory rate and lung sound monitoring augment transfusion-related reaction detection. Pulmonary changes may lead to decreased SaO_2, increased respiratory rate and alterations in lung sounds stemming from transfusion-associated circulatory overload (TACO), transfusion-related acute lung injury (TRALI) and transfusion-associated dyspnea (TAD) [2, 3, 6, 11, 17, 26]. The leading cause of transfusion-associated deaths prior to 2016 in the United States was TRALI [8]. Fiscal years 2016 through 2018 saw TACO grow to become the leading blood transfusion-associated cause of death in the United States [8].

National Healthcare Safety Network Hemovigilance Module data from 18,308 reported adverse reactions included 15 (65%) fatalities involving pulmonary complications of TACO, TAD and TRALI [26]. Sixty-one of the reactions involving TRALI included 44 (72.1%) deemed serious and 6 (10%) deaths [26]. Transfusion-reaction assessments including SaO_2, respiratory rate and lung sounds throughout the monitoring period may detect these top transfusion-related conditions leading to death better than mere vital signs.

One U.S. Veterans Affairs hospital study reported that blood transfusion was not associated with significant changes in recipient vital signs [1]. Notably, neither respiratory rate nor SaO_2 was included

in the study's definition of 'vital signs' or applied by practitioners to detect a reaction in 3496 blood component infusions used to determine study results. Had researchers included respiratory rate and oxygen saturation in patient assessments, vital sign changes may have been found significant to detecting transfusion reactions in participants.

Inclusion of physical assessment findings

Focusing only on vital signs may overcloud physical symptoms of a transfusion reaction (Figure 3). Transfusion reaction signs and symptoms include physical findings only detected with a clinical assessment. Acute pain transfusion reaction supports inclusion of pain assessment throughout blood transfusion [4, 9]. Recommendations to add assessments for hives/itching, chills, dyspnea, flushing, nausea and back pain were made to detect transfusion reaction sooner [3]. For example, immediate febrile transfusion reactions not only result in a rise in body temperature but also include the symptoms of chills and malaise [9]. Haemolytic transfusion reactions require immediately stopping a blood transfusion. The haemolytic transfusion reaction may be detected through assessment of chills, pain, nausea, vomiting, shock and dark urine in addition to a rise in body temperature [2, 11]. Allergic reactions may only be detected through detection of urticaria, pruritis or hives. Vital sign monitoring frequency may be less critical than a thoughtful physiologic assessment to transfusion reaction detection at the earliest point of clinical deterioration.

Limitations

Selection and sample biases were controlled through methodological use of independent review and evaluation by research team members before reaching a consensus on inclusion or exclusion of an article or evidence for synthesis of findings. As previously noted, limited evidence and a lack of high-level evidence were found to answer the clinical question being asked. Few relevant studies were identified using the selected search strategy to inform synthesis of discoveries

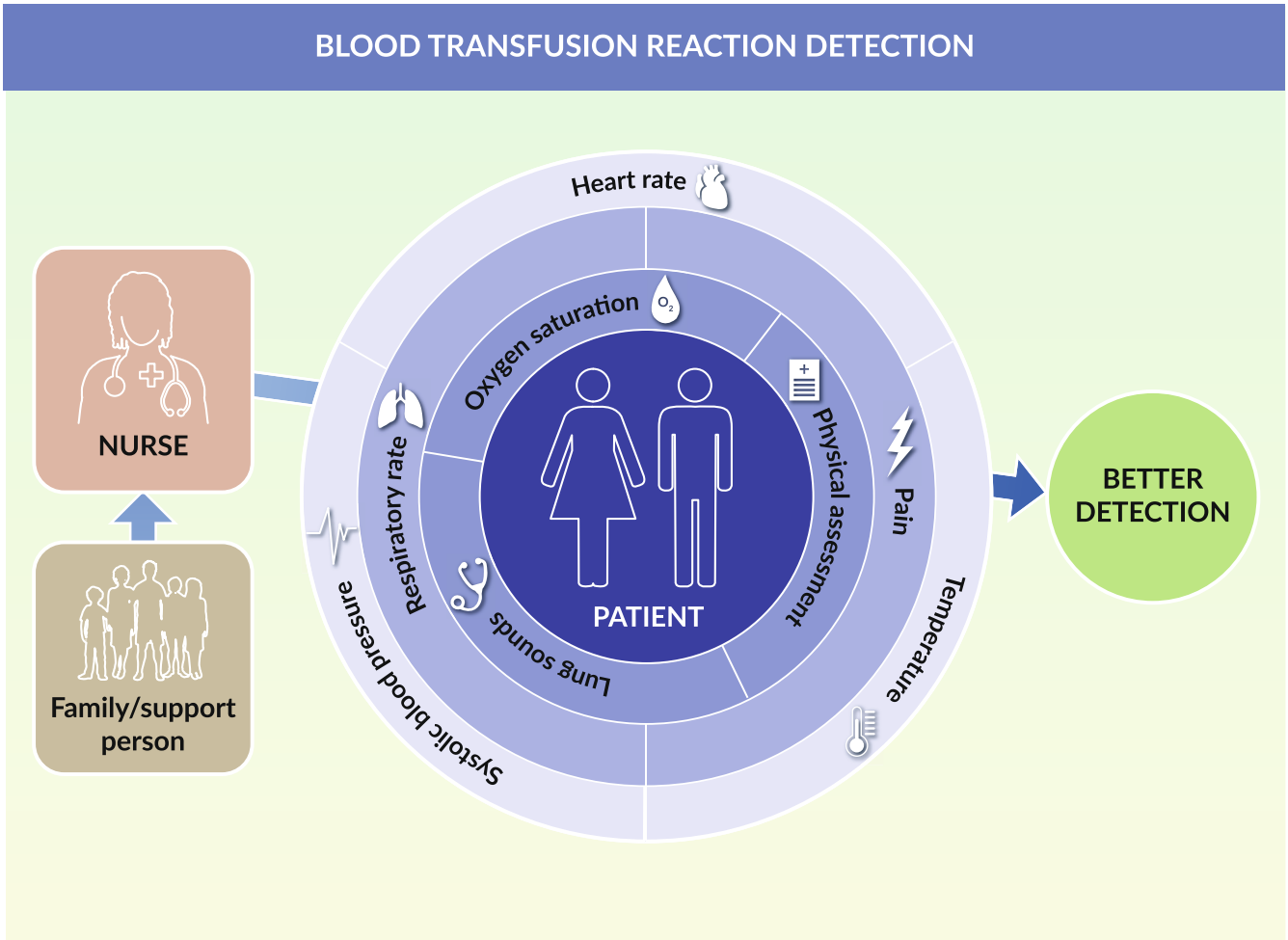

FIGURE 3 Targeted strategies for identifying blood transfusion reactions. Vital sign monitoring frequency may be less critical than which vital sign components are assessed, a thoughtful physiological assessment and partnering with family and significant others to transfusion reaction detection at the earliest point of clinical deterioration.

from published literature. Applying search terms as free text words in addition to MeSH terms may have provided additional evidence to address the clinical question. The quality of research identified relevant to this literature review established new gaps in scientific literature needing further investigation. As a final point, varied study designs, definitions and methods used for calculating transfusion reaction rates in reported publications prevented meta-analysis of quantitative results.

CONCLUSION

While blood transfusion reaction detection remains fundamental for optimal patient outcomes through intervention at the earliest point in care delivery, no high-level evidence was found. Few studies have reported on patient screening criteria to detect transfusion reactions. Due to the lack of high-level evidence and guidelines in peer-reviewed literature to inform nurses of how frequently vital signs should be monitored and what components should be included in 'vital signs' to identify transfusion reactions, an evidence-based answer to the clinical question was not found by researchers. The absence of evidence to support conventional recommendations on the frequency of vital signs during a blood product transfusion led reviewers to more questions than answers.

Relevance for clinical practice

Reviewers explored what should be assessed by nurses to best detect transfusion reactions according to published evidence. Limited evidence and absence of guidance regarding measurable changes in assessment findings indicative of possible transfusion reactions led our team to more quandary than answers. Guidelines defining what deviations from vital sign baselines should raise alarms during transfusions are needed. Without evidence-based advice of what vital sign findings represent 'abnormal', vital signs may have limited usefulness in triggering transfusion reaction detection by nurses. More research needs to be completed to answer how often vital signs should be performed to detect blood transfusion reactions and to develop validated vital sign standards for reaction detection.

Adopting strategies to improve reaction detection such as employing patients and families to help recognize transfusion reaction signs and symptoms during transfusion and post-discharge improve detection of acute and delayed reaction events. Healthcare systems should compare evidence summaries and CPGs to current policies to ensure evidence-based practices are employed by nurses monitoring for blood transfusion reactions and other procedures. When quandaries arise during the investigation of evidence, these difficulties provide unique perspectives for consideration by clinicians and scholars alike. Evidence-based nursing practices may only be introduced to nurses at the point of care delivery with thorough investigation of published literature, synthesis of findings and global application of evidence-based findings.

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CONFLICT OF INTEREST

All authors have no conflicts of interest to disclose.

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

A Danish national, multicentre evaluation of the new donor vigilance system among different staff groups

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Abstract

Background and Objectives: Two years after implementing a new national donor vigilance system, the Danish Haemovigilance Committee conducted a nationwide survey to evaluate the implementation among different staff groups. We present the results here.

Materials and Methods: The study was designed as an anonymous online survey to evaluate the satisfaction with the new registration, understanding of the parameters used and the user-friendliness. The REDCap platform was used. The questionnaire consisted of 22 questions. Ordinal variables were answered using five-point Likert scale (1 = strongly disagree to 5 = strongly agree). The data were analysed using descriptive statistics. Successful implementation was defined as mean overall satisfaction ≥4 and mean understanding of the individual components (adverse reaction category, severity and imputability) in the registration ≥4.

Results: In all, 104 staff members (77.9% donation staff) participated. The mean (SD) overall satisfaction among all participants was 3.96 (0.94), highest among medical doctors (4.43 (0.78)) and lowest for administrative or other personnel (2.78 (1.09)). The mean scores for understanding the adverse reaction categories, severity and imputability were 3.92 (0.94), 3.92 (0.94) and 3.88 (1.00), respectively. Experience with a previous donor vigilance system was associated with lower scores. The most successful implementation programme included a medical doctor for introduction and a contact person.

Conclusion: The goal for successful implementation was not met. However, the overall attitude towards the new registration was positive and indicates that the system is suitable for different staff groups. Our results suggest that implementation could benefit from special attention to administrative staff and those accustomed to another donor vigilance system.

Keywords

donors, donor health, haemovigilance

Highlights

- International standards for the categorization of adverse reactions and for their severity and imputability were found to be easy for donation staff to apply.
- Implementation of a new donor vigilance system is more challenging when a donor vigilance system is already in place.
- Implementation can be improved by an in-person introduction to the system and by having a defined contact person.

INTRODUCTION

Denmark is divided into five healthcare regions. In each, a regional blood establishment (BE) manages blood donation and registration of donation-related adverse reactions in blood donors.

In January 2020, the Danish Haemovigilance Committee implemented a new national donor vigilance system. The system was based on three parameters: (1) adverse reaction categories as defined by the ISBT; (2) severity as defined by the AABB; and (3) a modified version of imputability levels adapted to donor vigilance by the ISBT [1].

In the new donor vigilance system, registration of donation-related adverse reactions is performed on-site at the donation facilities in case of immediate reactions or later in case of delayed reactions.

The registration is done directly in the blood bank IT system and is a standardized code that includes a category number and a severity and imputability score. It is registered in the donor's donation chart, as a separate entry under the corresponding donation. Staff members can access previous registrations using donor id.

The staff member initially notified about the adverse reaction is responsible for ensuring immediate and correct registration. Therefore, donation staff and secretaries perform the majority of registrations. More complicated or severe reactions can be passed on to the attending physician for clinical assessment and subsequent registration of the adverse reaction. The Haemovigilance Committee provided national guidelines that included definitions of the three parameters used and examples of how to rate severity and imputability. This was included in regional standing operating procedures, which staff members had to sign off when read.

The regional BE annually provides the Haemovigilance Committee with data extraction using a predefined template. The committee then prepares the national report.

Currently, two IT systems are used in the Danish BE. One region uses Blodflödet and the remaining four, ProSang. In Blodflödet, three separate entries are made during the registration, one for each parameter. In ProSang, all three parameters are embedded in a single three-digit code.

The ISBT definitions and AABB severity tool have already been validated across different staff groups [2, 3], and users especially found imputability hard to assess. However, participants were predominantly senior staff members.

Following implementation of this new registration in Denmark, the Haemovigilance Committee repeatedly received questions concerning imputability ratings, in particular from staff at donation sites. Therefore, to evaluate the success of the implementation and to identify areas for improvement, a national survey was planned in order to study both the user attitude towards the system and the feasibility including user-friendliness of the system. The survey included all staff members working with blood donors in any of the Danish blood banks and blood collection sites, which enables us to investigate potential differences among staff groups, different age groups and to evaluate the potential differences between educational groups, age groups among staff and to evaluate the different methods of implementation on a national level.

MATERIALS AND METHODS

Survey design

The survey principles by Dillmann et al. [4] and Schleyer and Forrest [5] were followed, while the results were reported in accordance with Eysenbach [6]. The survey design was a structured format comprising a maximum of 22 questions, with adaptive questioning to reduce complexity and volume for the participants. For staff members in the Capital region, two follow-up questions were included due to a different IT system than in the other four regions. Single and multiple-choice questions were included with answer types assigned to nominal, ordinal and ratio scales. Ordinal variables were answered using five-point Likert scale (1 = strongly disagree to 5 = strongly agree). The fully translated questionnaire is included in the Supplementary Material. In the following, we present a brief overview.

The survey-landing page described the survey topics and length, goals and provided information about data handling according to the European General Data Protection Regulation (GDPR). The survey was voluntary, non-incentivized and fully anonymous. Survey information was not suitable to draw any conclusions to the participant, nor were technical identifiers (IP address, or other) stored. To start the survey, participants were required to give consent to their participation. The first survey section included demographical questions related to the respondent's age, gender, professional position, educational background, time of employment in a BE and healthcare region. In the second section, participants were asked a series of questions concerning the previous donor vigilance system and the implementation procedure of the new system. The third section addressed participant satisfaction with different aspects of the system including IT solutions and the three parameters. The fourth section addressed the

system's user-friendliness and understanding both overall and for each of the three parameters. The fifth and final section asked participants whether they were aware of different ways to add comments or do follow up registration and whether they knew that data were routinely published.

Successful implementation was defined from the point of view of feasibility and user satisfaction as an overall satisfaction score of ≥4 and a score of ≥4 in understanding each of the three parameters.

Survey draft and validation

The Haemovigilance Committee drafted the questionnaire. Then, —one or two staff members from each region were invited to participate in a focus group meeting to inquire about: (1) the overall perception and understanding of the donor vigilance system; (2) the course of implementation; (3) expectations to results from the system; and (4) others. The participants in the focus group meeting unanimously commented that the survey should address the following: (1) Are users familiar with the option to provide additional comments if deemed relevant? (2) Do users omit registration due to lack of routine or time? (3) Do users know that results are used to improve donor safety and whether they know where to find more information about the results? (4) Do the users understand how to rate severity and imputability and are they satisfied with the guidelines? Item one through three were addressed in the survey's fifth section and item four in the fourth section as previously described in Section 2.1.

These four points were included in a revised version of the questionnaire. We then asked the focus group to fill out the questionnaires and provide their comments.

We received five responses. They commented on the use of abbreviations and highlighted that questions addressing technical issues in the IT system Blodflödet should be limited to participants from the relevant region. Based on these comments, we revised the questionnaire and asked a new group of staff members to test it and provide their comments. We received nine responses in total from three different regions, five include comments/suggestions and four respondents had no comments.

The respondents reported that the questionnaire took between 5 and 10 min to complete, which we considered acceptable. Furthermore, they asked that the Danish translation of imputability be used. Also, to add the possibility to reply if they themselves did not do the actual registration. Finally, for a couple of multiple-choice questions, they missed the opportunity to reply, "Do not know".

Recruitment

The target group included BE staff members working with the new donor vigilance system and included IT staff, secretaries, medical doctors, nurses, phlebotomists and all other staff members in contact with blood donors. The survey was administered between 01.04.2022 and

TABLE 1 Presentation of the study cohort

Study cohort	Number (percentage)
Sex	
Female	99 (95.2)
Male	5 (4.8)
Age	
20–29	8 (7.7)
30–39	18 (17.3)
40–49	31 (29.8)
50–59	23 (22.1)
>60	24 (23.1)
Education	
Medical laboratory technologist	42 (41.3)
Nurse	42 (40.4)
Medical doctor	7 (6.7)
Other[a]	12 (11.5)
Position in the blood establishment	
Donation staff	81 (77.9)
Medical doctor	7 (6.7)
Other	16 (15.4)
Time employed in the blood establishment	
<1 year	13 (12.5)
1–3 years	13 (12.5)
3–5 years	9 (8.7)
>5 years	15 (14.4)
>10 years	54 (51.9)
Region	
1	43 (41.3)
2	23 (22.1)
3	17 (16.3)
4	17 (16.3)
5	<5 (<4)
Response rate by region	
1	61%
2	33%
3	57%
4	22%
5	29%
National response rate	40%
IT system	
Blodflödet	43 (41.3)
ProSang	61 (58.7)
Previous donor vigilance system	
Yes	66 (63.5)
No	8 (7.7)
Do not know	30 (28.8)

[a]Includes administration, secretary, other health and non-health background.

30.04.2022 (01.05.2022–31.05.2022 in the Zeeland region) via REDCap [7, 8]. REDCap is a freeware system approved for research projects in the Capital Region by the Danish Data Protection Agency (I-suite Nb. 05196).

Information about and a link to the survey was administered from the Danish Society of Clinical Immunology to the Organization of Transfusion Centers in Denmark (OTCD), who distributed the email to their staff members. Reminder emails were sent 2 weeks before the survey ended.

Data exclusion

The estimated target study population was 260 staff members. Only completed questionnaires were included. Of 140 participants, 36 were excluded due to missing data, defined as a questionnaire that had not been fully completed. In total, the completion rate was 74%, and 104 participants were included in the analysis.

Statistical analysis

The main outcome variables of the survey were overall user satisfaction, user-friendliness and understanding of the parameters. All outcome variables mentioned can be assigned to ordinal scales. Descriptive variables include nominal scales (gender, position, professional background, region, implementation and follow-up), ordinal scales (five-point Likert scale for user satisfaction, friendliness, understanding and attitude) and ratio scales (age and time of employment in BE). The percentage of respondents who chose each item was calculated. The descriptive data analysis was carried out using R studio. Results are presented as mean and standard deviation (SD). Groups with less than five individuals are either combined with other groups or presented as <5. Data were analysed in R studio 2022.02.03 Build 492.

RESULTS

Details of the study cohort are given in Table 1. The regional distribution of participants largely follows the size and activity of the BEs, with the highest number of participants coming from the two largest regions. Regions with a larger geographical coverage and fewer centralized donation sites have lower participation. The sex distribution is extremely skewed but is thought to reflect the gender composition of BE staff.

From Table 2 it can be seen that the overall satisfaction on a scale from 1 (very dissatisfied) to 5 (very satisfied) did not reach the goal of a score of 4 or higher. However, all were still above the neutral value of 3, thus in the positive part of the scale. Slightly surprising was the fact that 12%–24% of the included 104 participants were not able to

TABLE 2 Results from the survey presented as mean (SD)

	Number (percentage) of responses	Mean (SD)	Percentage of non-responders
Satisfaction with the new registration			
Overall satisfaction	83 (79.8)	3.96 (0.94)	20.2
Satisfaction with the IT solution	79 (76.0)	4.05 (0.90)	24
Satisfaction with the construction of the registration codes	91 (87.5)	3.76 (0.90)	12.5
Satisfaction with the three categories used (type of complication, severity and imputability)	90 (86.5)	3.79 (0.83)	13.5
User-friendliness and understanding of the new registration			
The new registration is easy to use	88 (84.6)	3.93 (1.05)	15.4
The new registration is not time-consuming	89 (85.6)	3.82 (1.09)	14.4
The adverse reaction categories are easy to use and understand	88 (84.6)	3.92 (0.94)	15.4
The severity categories are easy to use and understand	91 (87.5)	3.99 (0.91)	12.5
The imputability categories are easy to use and understand	91 (87.5)	3.88 (1.00)	12.5
Did you know an annual donor vigilance report is published			
Yes	54 (51.9)	–	
No	50 (48.1)	–	
Do you think the new registration will improve donation safety?			
Yes	17 (16.3)	–	
No	42 (40.4)	–	
Do not know	45 (43.3)	–	

TABLE 3 Responses stratified according to demographics

	Overall satisfaction ($n = 83$)	Overall user-friendliness and understanding ($n = 88$)
Sex		
Female	3.95 (0.95)	3.89 (1.05)
Male	4.20 (0.84)	4.75 (0.50)
Age group		
20–29	4.00 (0.86)	4.13 (0.99)
30–39	4.33 (0.99)	3.88 (1.02)
40–49	3.81 (0.69)	3.85 (1.08)
50–59	3.74 (1.24)	4.05 (1.10)
>60	4.17 (0.86)	3.89 (1.08)
Time employed in BE		
<1 year	4.00 (0.71)	4.09 (0.83)
1–3 years	4.17 (0.94)	4.25 (0.75)
3–5 years	4.33 (0.82)	4.33 (1.21)
>5 years	3.69 (1.18)	3.86 (1.03)
>10 years	3.93 (0.94)	3.78 (1.15)
Position		
Donation staff	4.07 (0.82)	4.04 (0.96)
Medical doctor	4.43 (0.78)	4.50 (0.55)
Other	2.78 (1.09)	2.80 (1.23)
IT system		
Blodflödet	3.75 (0.98)	3.53 (1.08)
ProSang	4.10 (0.90)	4.19 (0.95)
Introduction of the new system		
New standard of operations		
Yes	3.96 (0.83)	3.93 (1.03)
No	3.97 (1.13)	3.94 (1.09)
Introduction by daily leader		
Yes	3.64 (1.15)	4.13 (0.99)
No	4.03 (0.89)	3.89 (1.06)
Introduction by medical doctor		
Yes	4.00 (0.78)	4.15 (0.80)
No	3.96 (0.98)	3.89 (1.09)
Introduction by a colleague		
Yes	4.08 (0.78)	3.82 (1.09)
No	3.92 (1.00)	3.98 (1.03)
No introduction		
Yes	2.50 (0.71)	2.33 (0.58)
No	4.00 (0.92)	3.99 (1.02)
Designated contact person		
Daily leader	4.00 (1.10)	4.06 (0.66)
Appointed medical doctor	4.20 (0.78)	4.43 (0.65)
Appointed colleague	3.90 (0.72)	3.68 (1.04)
Other	4.00 (1.26)	3.71 (1.25)
No	3.67 (1.05)	4.00 (1.32)
Do not know	4.09 (1.04)	3.67 (1.30)

Note: In total, 87 females and 5 males are included in the satisfaction results and 84 females and 4 males in the user-friendliness and understanding results. Results are presented as mean (SD).

give a score and instead replied "Do not know" (here named non-responders). When looking deeper into the groups of non-responders, we found that they had a higher percentage of participants replying that they did not do the registration themselves compared with the responders. For the satisfaction questions listed in Table 2, 14.3% of non-responders versus 6.0% of responders had replied that they themselves did not perform the registration. For user-friendliness, the numbers were 25.0% versus 3.4%, respectively. No difference in demographics between responders and non-responders was seen.

In the three specific questions addressing user-friendliness and understanding of the adverse reaction categories, severity and imputability, 10%, 5.5% and 11%, respectively, answered that they did not find the parameters easy to use or understand. Of these, 70%–75% further replied that this was because they did not understand the grading.

Despite the overall positive responses, only 16.3% believed that the registration would improve donation safety and only half of the participants knew that the Haemovigilance Committee publishes an annual report.

When looking closer into the responses for overall satisfaction and user-friendliness, we observed some interesting patterns (Table 3). First, mean scores were higher among men and medical doctors. For age and employment time in BB, the youngest and newest colleagues were in general more positive towards the new system. Most interesting was that non-donation/medical staff, that is, administrative personnel rated much lower than the other groups and below neutral. The new registration had the largest impact on daily routine for Blodflödet users and they had lower overall satisfaction as well as lower satisfaction with the technical aspect of the registration compared with ProSang users (3.83 (1.05) vs. 4.18 (0.78)).

The method of introduction to the system seemed to have some effect. While new standard of operations, newsletters or information on the intranet did not seem to have an effect, introduction by a daily leader, medical doctor or colleague did. Participants who responded that they had an appointed medical doctor or leader as their primary contact person also had improved satisfaction.

We proceeded to investigate regional variation and the effect of a previous donor vigilance system (Table 4). The higher the percentage of staff members who were acquainted with a previous registration in their region, the lower the scores for the two main parameters. This was not dependent on region, size, time of employment in the BE, IT system or participant age. However, as previously observed, administrative personnel had lower ratings (data not shown). This could indicate that even though familiar with a previous system, performing "bed side" registration in close collaboration with the donor improves the experience compared with those who have simply changed from one system to another without the clinical context.

To access the specific challenges in Blodflödet, where three separate entries must be made per registration, a follow-up question was included for Blodflödet users only. When asked to estimate how often they registered all three parameters, 51% replied "always" and 14% replied "always, if I find all three relevant", 7% registered more than 75% of the time, another 7% registered it half of the time, 12% did not register themselves and the remaining 9% replied "do not know".

TABLE 4 Responses stratified by healthcare region and previous donor vigilance system.

	Region 1	Region 2	Region 3	Region 4	Region 5
Previous registration					
Yes	78.1	40	80	53.8	66.7
No	0	20	6.7	23.1	0
Do not know	21.9	40	13.3	23.1	33.3
Survey responses					
Overall satisfaction	3.75 (0.98)	4.30 (1.03)	3.87 (0.74)	4.15 (0.69)	3.67 (1.53)
Overall user-friendliness	3.53 (1.08)	4.50 (0.802)	3.93 (1.10)	4.15 (0.801)	3.50 (1.29)

Note: Previous registration is presented as percentage and responses by mean (SD).

DISCUSSION

The overall attitude towards the new donor vigilance system in Denmark is positive. Based on this survey, we did not meet our criteria for a successful implementation, although we were extremely close. However, if looking only at donation staff, our primary group of interest, we did succeed.

The donor vigilance system in Denmark builds on validated, international standards. However, the ISBT and AABB validations were limited to senior and academic staff. As the primary users in Denmark are donation staff, the Danish Haemovigilance Committee wished to evaluate their perception of the new system, both to identify areas with a need for revision or better guidelines, and also in the interest of colleagues elsewhere, who are in the process of implementing new guidelines for donor vigilance.

Our results show that even though the system was implemented during the COVID-19 pandemic, most staff members are positive towards the system and its individual components. We initially anticipated that imputability would receive low ratings for user-friendliness and understanding as this was the parameter that sparked the most debate and questions. Nevertheless, even though the ratings were marginally lower, they were still overall positive.

One of the main challenges of the implementation phase was to design a system that could work in two very different IT systems. In particular, the registration in Blodflödet was a concern, as the three parameters (category, severity and imputability, respectively) had to be registered with three separate entries, whereas the staff had previously been accustomed to a three-digit single entry. Given this, it is not surprising that Blodflödet users have the lowest satisfaction and find the new registration harder to use than ProSang users, who were subject to very few changes in their daily registration routine. As Denmark is preparing a national implementation of a shared ProSang-based IT system, no further actions will be taken by the Haemovigilance Committee on this matter.

The new system was differently implemented in all five regions. Based on our results, an in-person presentation improved the user's experience and understanding, whereas a written guideline did not seem to make a difference in how the users perceived and understood the registration.

One concern is the low number of staff members who know that donor vigilance data are compiled and published in an annual report. It could be suspected that this is also one of the reasons why more than 40% did not believe that the new system will improve donation safety. The Haemovigilance Committee is currently working on a strategy to improve the information provided to our staff as well as the possibilities to use the data in, for example, national campaigns to reduce donation-related adverse reactions.

The main strength of our survey is the large number of responses from donation staff. To our knowledge, this is the first time these international standards have been evaluated by this staff group. However, the study also has some important limitations. First, for comparison, a higher number of medical doctors and administrative staff should have been preferred. Second, in the design of the survey, the option to answer, "Do not know" instead of a score does mean that for some of the key questions, we have a high number of non-responders. However, given that not all staff groups use the new registration in the same way, that is, some do not register themselves, this was considered the best option. In addition, it also revealed that some staff members do not consider themselves informed well enough to answer, which also gives cause for reflection.

In total, 36 incomplete responses had to be excluded. As most staff members do not have designated office times, they had to fill out the questionnaire while working at the donation site with the risk of being interrupted. We therefore expect that the incomplete response reflects this, as the design of the survey did not give the possibility to save and resume at another time point.

In conclusion, even though the implementation of the Danish donor vigilance system did not meet our criteria for successful implementation, staff members were predominantly positive towards the new registration in terms of satisfaction, user-friendliness and understanding. Our results show that international standards for adverse reaction categories, severity and imputability are suitable for most staff groups.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

PlasmaCap EBA: An innovative method of isolating plasma proteins from human plasma

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Abstract

Background and Objectives: The growing demand for immunoglobulin (IG) requires development of improved plasma fractionation methods to provide higher yields in a cost effective, scalable manner without compromising product purity and efficacy. A novel protein extraction method, utilizing expanded bed adsorption (EBA) chromatography, has been developed. PlasmaCap IG (10% liquid formulation intravenous IG [IVIG]) is the first plasma-derived product manufactured using PlasmaCap EBA technology.

Materials and Methods: The PlasmaCap EBA platform consists of a series of consecutive columns which bind a target protein, or group of proteins, in their native state directly from cryo-poor plasma. EBA chromatography includes five key steps: (1) expand, (2) sanitize and equilibrate, (3) load, (4) wash and (5) elute. These steps are made possible using high-density tungsten-carbide agarose beads, suspended by upward flow. The PlasmaCap EBA process was evaluated during Evolve's clinical campaign for scalability, product quality and yield.

Results: PlasmaCap EBA technology can be predictably scaled by maintaining the minimum residence time and residence time distribution for EBA columns of different diameters. Scalability of the manufacturing process was demonstrated by the 50-fold volumetric increase from laboratory-scale lots to clinical-scale lots. The process is also associated with enhanced product purity, such as lower aggregates. The PlasmaCap EBA process is expected to have the same or better yield and purity at commercial scale production compared to the clinical campaign.

Conclusion: The PlasmaCap EBA platform was used to successfully develop PlasmaCap IG (10% liquid formulation IVIG) with proven scalability, product quality and yield.

Keywords

chromatography, immunoglobulin, IVIG, manufacturing, plasma fractionation, quality control

Highlights

- Shortages of intravenous immunoglobulin (IVIG) continue to be reported globally and demand has grown over time, creating a need for improved plasma fractionation methods to provide higher yields without compromising purity and efficacy.

- A novel protein extraction method, utilizing expanded bed adsorption (EBA) chromatography, has been developed (PlasmaCap EBA) that utilizes high-density tungsten-carbide agarose beads, suspended by upward flow, to isolate proteins.
- The PlasmaCap EBA platform was used to successfully develop PlasmaCap IG (10% liquid formulation IVIG) for clinical investigation with proven product efficacy, safety, purity and scalability, at a high yield.

INTRODUCTION

Intravenous immunoglobulin (IVIG) and subcutaneous IG (SCIG) preparations are comprised pooled immunoglobulin G (IgG) antibodies from the plasma of thousands of donors and were initially used as an IgG replacement therapy in immunocompromised patients [1]. Increasingly, immunoglobulin (IG) at high doses (1000–2400 mg/kg) is also used as a treatment for a variety of autoimmune and inflammatory conditions. Although some autoinflammatory disorders respond to a single course of IG therapy, the majority of patients require long-term, regular infusions [1].

Shortages of IVIG and SCIG continue to be reported globally [2–4], and demand for IVIG and SCIG has grown continuously since the 1980s [5]. The drivers for increased demand are multifactorial, but are largely due to expanded indications requiring high-dose IG, such as chronic inflammatory demyelinating polyneuropathy, which absorbs approximately a third of all IG produced, according to several surveys [6–8]. Although the volume of source plasma has continued to increase [5], the plasma supply has been negatively impacted by a number of factors, including the global coronavirus disease 19 pandemic [9]. Regulatory agencies are developing mandates and alternative procedures to improve availability and reduce shortages of source plasma [8–10], but these measures may not be sufficient to ensure adequate global supply. The current and ongoing shortages of IVIG and SCIG have exposed the need for increased efficiency in manufacturing to meet global demand. Therefore, the introduction of new products and manufacturing technologies are urgently needed to meet expanding needs worldwide.

The manufacturing of IG is conducted by a fractionation methodology developed in the United States (US) in the 1940s known as the Cohn process with derivations thereof generally referred to as cold ethanol fractionation [11]. Cold ethanol fractionation separates plasma proteins through a succession of precipitation steps [1, 9]. The process usually begins using pools of plasma (2000–4000 L) with an initial cryoprecipitation step (thawing of plasma at 2–3°C to generate the cryoprecipitate) followed by a series of cold-temperature precipitation steps in the presence of ethanol associated with shifts in pH, temperature and osmolality [12]. The resulting selective precipitation of plasma proteins are then separated by centrifugation or filtration [12]. Additional products, including coagulation factors and protease inhibitors, are also manufactured from additional fractions, either precipitates or supernatants, of this core fractionation process [12].

A novel protein extraction method, utilizing chromatography as the primary separation and purification process, has been developed. This technology uses expanded bed adsorption (EBA) chromatography to selectively capture proteins in their native state. Expanded bed chromatography enables the capture of a target protein in a complex unclarified feedstock by introducing the starting material at the base of the column and allowing it to pass through chromatographic media which is suspended by the upward vertical flow. The increase in void volume resulting from expansion permits feedstocks with a high level of solids and lipids to flow unimpeded [13]. PlasmaCap IG (10% liquid formulation IVIG) is the first plasma derived product manufactured using the proprietary process known as PlasmaCap EBA technology.

While there are some advantages to cold ethanol fractionation, including its widespread adoption in the production of the bulk proteins (albumin and IGs) and the bacteriostatic effect of ethanol (and low pH) that limits the risk of bacterial growth and endotoxins, precipitation methods have some disadvantages with respect to sensitivity to process changes and raw material variability. More critically, cold ethanol fractionation has as a maximum potential yield, since selective precipitation of a complex fluid depends on a muti-factorial phenomena where not all the factors can be defined or controlled [14–17]. In addition, precipitation is a multi-stage phenomenon whereby the precipitate initiates around a nucleation site and grows or breaks apart as a function of solubility, shear disruption and time. The mechanical properties of precipitate, including size and density affect the recovery and re-solubilization [15, 18]. Re-solubilization with a high shear mixer can result in the permanent denaturation of plasma proteins, and clarification is typically required to remove non-recoverable protein. In contrast, the binding of IG during a selective chromatographic step depends on known factors which can be tightly controlled, and the multivariate operating space can be characterized, which results in process robustness. In addition, alcohol fractionation cannot be used for the purification of many trace plasma proteins due to limitations of specificity/selectivity [13, 19].

Chromatography is widely recognized as a purification technique capable of obtaining high purity products with high yields. It is easily automated and more versatile than precipitation [13]. Chromatography has yet to displace traditional fractionation for the production of 'non-hyperimmune' IG due to the technical limitations of conventional chromatography media or reduced concentration of some subclasses of IG [20–22]. Evolve's proprietary PlasmaCap EBA purification platform overcomes these technical limitations by creating a stable fluidized bed with a high-density chromatographic media with a ligand that enables high recovery and selectivity. This innovation

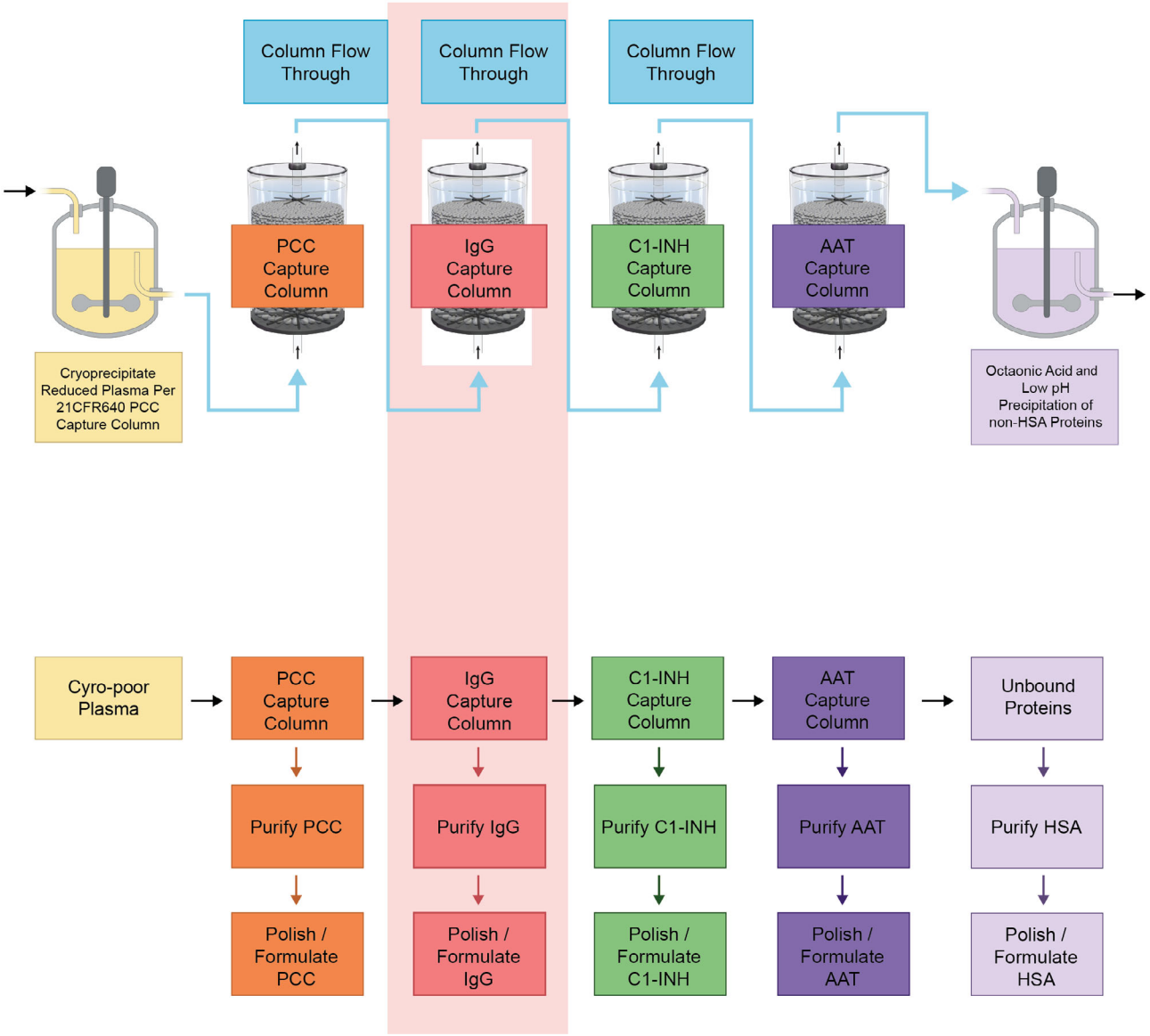

FIGURE 1 PlasmaCap EBA backbone/capture steps and process flow diagram for PlasmaCap IG drug product development. 21CFR640, 21 Code of Federal Regulations Part 640; AAT, alpha-1 antitrypsin; C1-INH, C1 esterase inhibitor; HSA, human serum albumin; IgG, immunoglobulin G; PCC, prothrombin complex concentrate

enables robust and selective capture of native IgG from plasma and can replace cold ethanol precipitation.

A key advantage of EBA manufacturing over cold ethanol fractionation is its scalability. Small-scale manufacturing of PlasmaCap IG for laboratory and toxicological evaluations, was rapidly and successfully expanded to produce clinical-scale (CS) product batches. Further, EBA manufacturing can process large volumes of unclarified raw materials with specificity and high recoveries at the linear flow rate and residence time employed during laboratory and clinical manufacturing [19].

The following report describes the production process of PlasmaCap IG resulting in a high-purity, high-yield product that was rapidly scaled to support evaluation in a pivotal clinical study with positive results.

MATERIALS AND METHODS

Evolve's PlasmaCap EBA platform consists of a series of consecutive columns which bind a target protein, or a group of proteins, in their native state directly from cryo-poor plasma (Figure 1). The unbound fraction from each EBA step is applied to the subsequent column. Prothrombin complex concentrate (PCC) is captured in the first EBA column and IgG is captured in the second EBA capture step. The subsequent EBA steps are necessary to recover other plasma proteins but are not necessary to recover IgG, and were not included during the clinical manufacturing of IgG.

The target protein(s) captured during each step of the PlasmaCap EBA backbone can be eluted at neutral pH in a medium ionic strength aqueous buffer (see Figure 1). The eluate can be concentrated, sterile

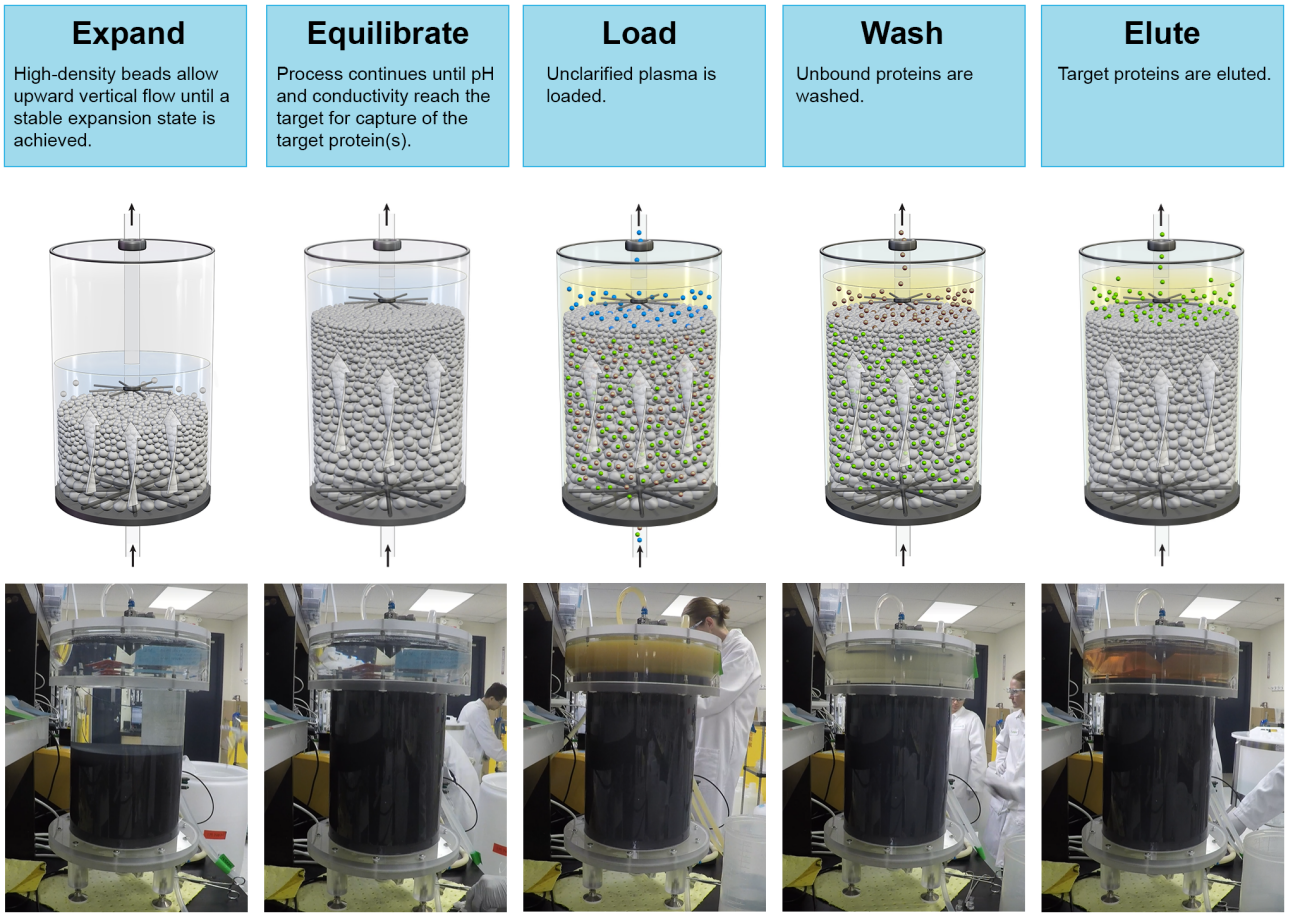

FIGURE 2 The five (5) key steps of PlasmaCap EBA chromatography

filtered, and frozen for further purification, or purified immediately without an intermediate hold step.

The five key steps of the PlasmaCap EBA process are made possible by functionalized high-density tungsten-carbide agarose beads which are suspended by upward flow (Figure 2). The upward flow results in a dynamic expanded bed which permits unclarified plasma to flow through the column with no risk of plugging, and without the need for aggressive cleaning or repacking to achieve a high cycle life.

An overview of the manufacturing process steps for the production of IG are provided in the content below. In terms of scalability of the manufacturing process, it is important to note the rapid expansion of production capabilities from the laboratory-scale (LS) lots to the CS lots. More detailed information regarding each step is provided in the following sections.

Starting material

The purification processes may start from either Source or recovered frozen human plasma. Source plasma from first-time donors was used for development of toxicology product lots, whereas Source plasma from repeat donors was used for all other LS and CS lots. Source plasma can range in volume depending on the donor, but averages approximately 750 ml, collected in 1 L bottles. Prior to use, all plasma was stored at ≤ −30°C.

Cryoprecipitation and recovery of cryo-poor plasma

Cryoprecipitation reduces the concentration of clotting factors, such as Factor VIII and Factor XIII, von Willebrand factor and fibrinogen in the process stream. The precipitation occurs naturally when frozen plasma is thawed at a controlled temperature.

For LS lots, 7 L of thawed plasma was centrifuged using a fixed angle centrifuge and the cryo-poor plasma was decanted. For CS lots, 350 L of pooled thawed plasma was fed through a Sharples AS-26 continuous bowl centrifuge, at 700 ml/min and 16,902 g which separated cryoprecipitate solids and cryo-poor plasma.

EBA PCC capture

The PCC capture chromatography process features a diethylaminoethyl (DEAE) weak anion exchange ligand linked to high density tungsten carbide-agarose beads. The column primarily binds PCC from cryo-poor plasma, allowing the remaining proteins, including the desired IgG molecules, to flow through the column.

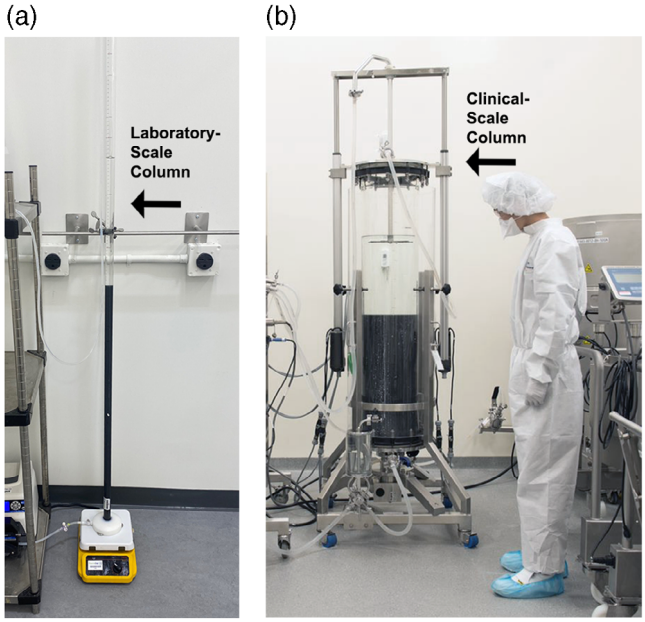

FIGURE 3 (a) The column utilized for the laboratory-scale (LS) immunoglobulin (IG) lots was 2 cm in diameter. (b) The column utilized for the clinical-scale (CS) IG lots was 30 cm diameter

The columns used for LS lots were 2 cm in diameter and packed with 235 ml of Fastline DEAE EBA adsorbent (Upfront Chromatography A/S). For CS lots, a 10 cm diameter column was packed with Upfront Fastline DEAE EBA adsorbent resulting in a settled bed volume (SBV) of 5.9 ± 0.2 L. LS and CS columns were operated at an upward linear flow of 300–600 cm/h and an expansion factor in the range 1.70–2.40. The expansion factor is the ratio of the settled bed height and the expanded bed height. The flow rate and expansion factor vary during each step in the chromatographic sequence.

Sixty (60) cycles of the EBA PCC capture step were performed for the CS lots with cleaning and sanitization with 1 M of sodium hydroxide (NaOH). No change was measured during the clinical campaign for column efficiency, selectivity, expansion factor and pre-use and post-use cleaning verification test results.

Depth filtration

The depth filtration operation was introduced into the process to improve performance in the subsequent tangential flow filtration (TFF) step. Pall Seitz P-series filters were used. For LS lots, an 800 cm^2 of filter area was used. For CS lots, a 2.0 m^2 filter was used. The filtration area was scaled linearly.

pH and conductivity adjustment

The pH and conductivity are adjusted prior to loading onto the EBA IG capture column. The target conductivity is achieved by diafiltration using TFF via 30 kDa Sartorius Sartocube Hydrosart regenerated cellulose cassettes. For LS lots, a 0.2 m^2 was used. For CS lots, a 7 m^2 was used. The operating parameters were scaled linearly.

EBA IG capture

The process of EBA IG capture features a mixed mode ligand, 4-amino benzoic acid, linked to high density tungsten carbide-agarose beads. The resin binds IG with high selectivity between pH 6.0 and 7.0. The IG capture step is the branching point of the IG purification process. The eluted protein is recovered and further processed. The flow through from the IG capture step is depleted of IG and can be used to isolate other plasma proteins.

The columns used for the LS lots were 2 cm in diameter and packed with 170 ± 6 ml MabDirect MM EBA absorbent (Figure 3a). For CS lots, the column used was 30 cm diameter and packed with EBA adsorbent resulting in a SBV of 38.8 ± 1.4 L (Figure 3b). The columns were operated at an upward linear flow of 300–600 cm/h and an expansion factor in the range 1.70–2.40.

In total, 30 cycles of the IG EBA capture step were performed at for CS lots with cleaning and sanitation with 1 M NaOH. No change was measured for column efficiency, selectivity, expansion factor, and pre-use and post-use cleaning verification test results.

Low pH viral inactivation with sodium caprylate

A low pH sodium caprylate incubation step is performed for viral inactivation. Sodium caprylate is added to Column C eluate to a target concentration of 32 ± 2 mM and the pH is adjusted to 4.50 ± 0.10 with 3 M acetic acid. Adjusted Column C eluate is incubated for a minimum of 60 min.

Depth filtration and sterile filtration is performed after incubation, resulting in a stable, partially purified, sterile and viral-treated bulk intermediate.

pH and conductivity adjustment

The pH and conductivity is adjusted prior to loading onto the anion polishing column which is the final purification step in the IG process. The target conductivity is achieved by diafiltration with sodium acetate buffer using TFF with 30 kDa Sartorius Sartocube Hydrosart regenerated cellulose cassettes. The operating parameters were scaled linearly. For LS lots, a 0.2 m^2 filter was used. For CS lots, a 7 m^2 filter was used.

Anion exchange chromatography

The last purification step in the IG process is anion exchange chromatography. Traditional anion exchange packed bed chromatography is performed as a negative capture step, binding residual impurities including IgM, IgA, apolipoprotein H (ApoH) and aggregated IG. The IG does not bind to the resin at the operating pH and conductivity.

The LS and CS columns were packed to a bed height of 20 ± 1 cm. A 5-cm diameter column was used at LS and a 30-cm diameter column was used at CS. Removal of impurities was performed using a linear flow rate of 150 cm/h.

Nanofiltration

Nanofiltration functions as a viral reduction step via size exclusion. A pre-filter is used prior to nanofiltration to improve the performance of nano-filters.

Formulation and sterile filtration

Diafiltration by TFF is the last operation in the IG purification process. The drug product must have total protein and IG concentrations of 100 ± 10 g/L and a pH of 4.70 ± 0.20 with 265 mM glycine.

EBA manufacturing scalability

The PlasmaCap manufacturing process, which relies on EBA capture chromatography for isolation and reproducible capture of target proteins, can be predicably scaled by maintaining the minimum residence time and residence time distribution for columns of different diameters. Scalability of the manufacturing process was demonstrated by the 50-fold volumetric increase in scale from LS to the CS lots.

All critical process parameters related to scale can be increased by the square of the ratio of column diameter. The loading parameters (pH, conductivity, loading factor) have the same relationship with product quality attributes for a 2-cm diameter column and a 30-cm diameter column provided the bed height and linear flow rate are conserved. A 15× increase in column diameter equated to a 225× increase in volumetric flow rate. Column efficiency and residence time distribution can be measured with a pulsed injection of a tracer molecule.

Rigorous drug substance and drug product evaluations were undertaken to ensure similarity of attributes between the LS and CS lots.

RESULTS

PlasmaCap EBA technology provides several advantages over the Cohn/precipitation process. A key advantage of EBA manufacturing over cold ethanol fractionation (Cohn/precipitation process) is its scalability with equivalent or superior product quality as demonstrated in the results detailed below. In addition, EBA manufacturing can process large volumes of unclarified raw materials with specificity and high recoveries. Key differences between the Cohn/precipitation process and PlasmaCap EBA technology are demonstrated in Figure 4, notably elimination of the cold ethanol fractionation/precipitation step.

Product quality

Biochemical investigations and results

PlasmaCap samples were tested at Evolve Biologics, Haematologic Technologies Inc., Haemtech Biopharma Services and/or Haemostasis Reference Laboratory to evaluate critical quality attributes including

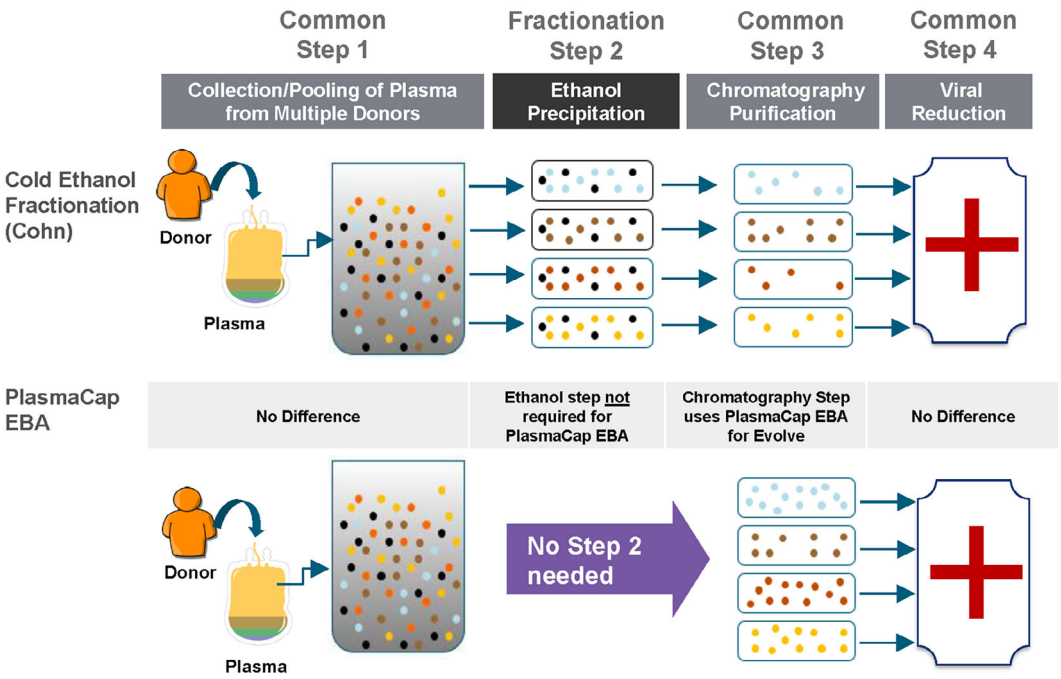

FIGURE 4 Comparison between PlasmaCap EBA and cold ethanol fractionation manufacturing

IgG-related parameters, accompanying plasma proteins, functional parameters, physiochemical parameters, antibody titres, residual coagulation factors and thrombin generation. Specific product attributes of PlasmaCap IG produced at LS and CS are listed in Table 1. Extensive supplementary characterization of three clinical batches was performed (Table 2).

Total protein concentration was determined by bicinchoninic acid assay, while IgG concentration was determined using a quantification assay with protein G biosensors. Concentrations of IgG subtypes were evaluated using a SAX biosensor with the Octet Red96 system, while concentrations of IgA, IgM, ApoH, plasminogen and fibrinogen were evaluated by enzyme-linked immunosorbent assay. The compendial haemagglutination method was used for determination of Fc function. A Calibrated Automated Thrombogram® method was used to measure thrombosis risk. The compendial method for anti-complementary activity was performed. Size distribution was determined by size exclusion chromatography-high performance liquid chromatography (SEC-HPLC).

PlasmaCap IG retained potency as demonstrated by the required potency assays. For example, the anti-measles potency averaged 0.79× the reference lot, which is twice the new threshold (0.36×) allowed for products which may not meet the previous threshold of 0.48× due to declining titres in plasma.

Key aspects of PlasmaCap product quality such as lower aggregates and low levels of impurities such as Factor XIa, may increase quality and decrease the potential for adverse reactions such as thromboembolic events [23]. Notably, an assay with a lower limit of quantitation than the previous industry standard was developed specifically to detect the amount of Factor XIa in samples derived from PlasmaCap EBA technology [23]. The Factor XIa threshold concentration to induce thrombi in the Wessler test lies between 2 and 7 mU/ml; the highest measured value in 13 PlasmaCap batches was 0.21 mU/ml. PlasmaCap IG also compares very favourably to commercially approved IVIG products for residual impurities such as IgA and IgM [24].

Pathogen safety

The PlasmaCap IG process includes three viral inactivation steps; a 60-min incubation with 32 ± 2 mM sodium caprylate at pH 4.5 after

TABLE 1 Comparison of critical quality characteristics for clinical scale and laboratory scale batches

Parameter	Limit	Clinical scale batches ($N = 13$)	Laboratory scale batches ($N = 3$)
Total protein	100 ± 10 g/L	100	101
Total immunoglobulin G (IgG)	100 ± 10 g/L	103	101
IgG purity	≥96%	96.9%	99.6
Molecular size	Mono- and dimeric ≥90%	99.1%	99.9
	Polymeric <2%	0.1%	<0.1
	Fragment <3%	0.7%	<0.1
IgG subclasses	IgG1 ≥55%	68%	63
	IgG2 ≥20%	28%	32
	IgG3 ≥1.5%	2.0%	3.3
	IgG4 ≥1.5%	2.2%	3.5
Immunoglobulin A	≤100 μg/ml	17	15
Immunoglobulin M	≤20 μg/ml	1	<0.2
Apolipoprotein H	≤1.00 mg/ml	0.4	0.1
Fibrinogen	≤500 ng/ml	0.8	0.6
Thrombin generation assay (Factor XIa)	≤1.0 mU/ml	0.1	Not applicable (n/a)
Protein kinase A	NMT 35 IU/ml 3% IgG	0.2	n/a
Anticomplement activity	≤1 CH50 U/mg IgG	0.7	n/a
pH	4.7 ± 0.2	4.8	4.7
Osmolality	280 ± 15 mOs/kg	280	274
Anti-measles antibodies	NLT 0.6× the antibody level of NIH reference measles immune globulin	0.8	n/a
Anti-diphtheria antibodies	NLT 2U of diphtheria antitoxin/ml	10	n/a
Anti-poliomyelitis antibodies	Type 2: NLT 0.25× reference lot Type 1: NLT 0.6 reference lot	1	n/a
Constant fragment function	≥60% of reference material (15 and 30 mg)	102/110	n/a
Endotoxin	≤1.0 EU/ml	≤0.5	n/a

TABLE 2 Key characteristics of three representative clinical batches

Characteristic	Measurement in clinical batches ($N = 3$)
Proteolytic activity	<0.2 U/ml
Alpha-1 antitrypsin	<10 μg/ml
α-2 macroglobulin	<0.1 μg/ml
Albumin	<2 μg/ml
Apolipoprotein A1	<10 Ug/ml
C1-esterase inhibitor	<12 Ug/ml
Ceruloplasmin	<0.03 Ug/ml
Complement C3	<20 Ug/ml
Complement C4	<6 μg/ml
Elastase	22 ± 5 ng/nl
Factor VII antigen	0.1 U/ml
Factor VII activity	<0.05 U/ml
Factor VIII antigen	0.09 U/ml
Factor VIII activity	<0.15 U/ml
Factor IX antigen	<0.009 U/ml
Factor IX activity	<0.12 U/ml
Factor X antigen	<0.008 U/ml
Factor X activity	<0.10 U/ml
Factor XIa	<0.20 U/ml
Haptoglobin	<30 μg/ml
High-density lipoprotein/low-density lipoprotein	<1 μg/ml
Immunoglobulin E	<4 μg/ml
Transferrin	<2.3 μg/ml

the IG capture step, nanofiltration after the final polishing chromatography step and incubation in the final vials for 28 days at 26°C at pH 4.7.

Product yield

As per a report published by Market Research Bureau in June 2021, the yield for Cohn-based fractionation ranges from 42% to 55% [5]. This aligns with statements made by Bertolini et al. [25] that, even with all the enhancements made to the traditional fractionation process in recent years, the maximum yield of purified IG is between 50% and 55% regardless of whether the starting material comes from recovered or Source plasma. It is estimated that 40%–50% of IG is lost in the non-IG supernatants or are coprecipitated with impurities. Data collected during clinical manufacturing of PlasmaCap IG (using Source plasma) has shown an average yield of 67.0% ± 5.1%. While there is potential for differences in concentrations based on modest differences in sampling strategies at clinical and commercial scales, the PlasmaCap EBA process is expected to have the same or better yield and purity of IVIG at commercial scale production with automation and improved process flows compared to the numbers reported above during CS development.

Clinical outcomes

PlasmaCap IG was evaluated in a Phase 3 prospective, open-label, multicentre, study for efficacy, safety and tolerability at 12 sites in the US and Canada [26]. Adult and paediatric subjects with primary

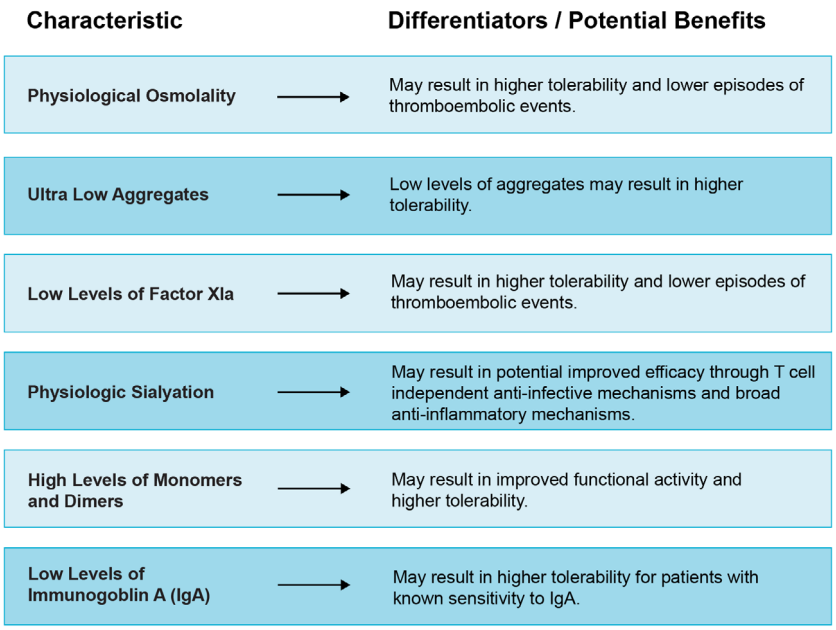

FIGURE 5 PlasmaCap IG characteristics and differentiators/potential benefits

immunodeficiency disorders (PIDD) received 300–900 mg/kg of PlasmaCap IG administered either every 21 or 28 days for a period of 1 year. Patients with PIDD require life-long IG replacement therapy to prevent recurrent infections, notably severe bacterial infections (SBIs) of the respiratory tract [1]. Thus, the primary endpoint of the study was the mean annual acute SBI rate, with secondary endpoints related to safety and quality of life. The primary endpoint of the study was met as there were no SBIs in any age group. Measures affecting quality of life such as days absent from work or school due to an infection (mean 6.5 days/patient/year), hospitalization due to infections (mean 0.2 days/patient/year) or with a fever >38.5°C (mean 0.9 days/patient/year) were all low, indicating that treatment with PlasmaCap IG had a positive effect on secondary measures of efficacy. There were no treatment-related serious adverse events (SAEs) or cases of haemolysis, thromboembolism or renal failure. A total of 127 (17.7%) adverse events were considered related to treatment; 97% were mild or moderate. This study demonstrated that PlasmaCap IG is effective, safe and well-tolerated in the treatment of adult and paediatric patients with PIDD with no reported SBIs or related SAEs.

DISCUSSION

The technology discussed in this report uses EBA chromatography to selectively capture proteins in their native state. In contrast, conventional cold ethanol fractionation (Cohn process) separates plasma proteins through a succession of precipitation steps, and purifies IG from two fractions: Fraction II and Fraction III. Some of the key characteristics and potential benefits offered by PlasmaCap IG are summarized in Figure 5.

Prior to the introduction of PlasmaCap IG, all commercial IVIG and SCIG products were produced from large pools of human plasma by first concentrating the IG by cold ethanol fractionation [27]. As mentioned previously, this technology was first introduced in the early 1940s [11, 28], and has remained relatively unchanged and unchallenged for over 75 years. The results of this study demonstrate that direct capture of IG during the PlasmaCap EBA chromatographic capture step is a promising alternative to legacy technology.

PlasmaCap IG (using Source plasma) has shown an average yield of 67.0% ± 5.1% and 99.1% ± 0.2% monomeric IG. The PlasmaCap EBA process is expected to have the same or better yield and purity at commercial scale production due to automation and improved process flows compared to the drug substance produced for clinical investigation.

The use of chromatography confers other advantages. By controlling average residence time and the distribution of beads, the capture step can be replicated in columns with different dimensions. In addition to process robustness, there is process flexibility, and the option of automation and high throughput strategies can be employed. In contrast, the unintended consequences of minor process changes in a selective precipitation step to improve yield or resulting from raw material changes may have quality and yield impacts which cannot be predicted. Because the PlasmaCap IG manufacturing process relies solely on chromatography, it has a smaller footprint and is more reliable, cost effective and scalable, which will significantly improve the reliability of the supply chain to patients.

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D.M., G.V., O.V. and M.K. conceived and designed the study. D.M. and G.V. collected the data with input and supervision from O.V. and M.K. D.M. drafted the initial manuscript and D.M., G.V., O.V. and M.K. reviewed and revised the manuscript. All authors approved the final version of the manuscript.

CONFLICT OF INTEREST

All authors are employees of Evolve Biologics, Inc.

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Information technology improves the quality of transfusion practice in China

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Abstract

Background and Objectives: Transfusion services in China must establish a quality management system, and regular inspection of quality indicators is an important component of quality management. Although the positive role of information technology in reducing human errors has been widely reported, its role in improving transfusion quality indicators still requires further study. This study explores the role of information technology in improving the quality of transfusion practice.

Materials and Methods: We developed an optimized blood transfusion management information system and then analysed the changes in four quality indicators before and after using the system to clarify the role of information technology in improving the quality of transfusion practice.

Results: After using the optimized system, the completeness rate for transfusion request forms increased from 81.5% to 99.3%; an unqualified doctor's signature was the most common incomplete content (0.45%). The appropriate transfusion rate increased from 87% to 99.4%, and red blood cell and frozen plasma utilization in most surgical departments decreased. Although the reporting rate for adverse transfusion reactions increased from 0.22% to 0.49%, these increases might be partly due to changes in transfusion regulations. The adequacy rate of transfusion medical records increased from 74.8% to 90.4%. Overall, the inadequacy of informed consent for transfusion, pre-transfusion laboratory tests and documentation of the transfusion process were reduced from 6.4%, 6.2% and 12.6% to 1.7%, 2.0% and 5.9%, respectively.

Conclusion: Information technology can play an important role in improving the quality of transfusion practice, as part of a programme of medical education, regular audit and other measures.

Keywords

information technology, quality indicator, quality management, transfusion

Highlights

- A blood transfusion management information system (BTMIS) increased the completeness of transfusion request forms and the quality of transfusion medical records.

Gui-Ping Xu and Li-Fang Wu contributed equally to this work.

- The BTMIS increased the appropriate transfusion rate and decreased red blood cell and plasma utilization in surgical departments.
- The BTMIS seemed to improve reporting practices for adverse transfusion events.

INTRODUCTION

Blood transfusion is an effective treatment but may cause harm to patients. As in other countries around the world, China has promulgated relevant laws and regulations, such as the Blood Donation Act and the Technical Criterion of Clinical Blood Transfusion, to regulate transfusion practice. According to these laws and regulations, every transfusion service must establish a quality management system. The Chinese transfusion quality management system usually adopts the ISO9001 quality management system，which has at its core a quality improvement cycle known as PDCA (plan-do-check-act) [1]. This system provides a scientific management model for continuous quality improvement. Auditing documents related to blood transfusion are an important means for evaluating the quality of transfusion practice. In previous audits, we found that our information system was too simple and only satisfied routine work, such as blood group identification, antibody screening and cross-matching. However, blood transfusion is a complex process, and the use of a simple software design led to insufficient control of some quality management links, so a better information system was required.

At present, many countries use information systems in hospitals. In the United Kingdom, 63% of laboratories had installed information systems prior to 2000, and more than 50% of these information systems could link to other hospital information systems (HISs) [2]. In 2007, the British Committee for Standards in Haematology (BCSH) published the Guidelines for the Use of Information Technology, which drew our attention to the specifications of information systems [3]. In Australia, a system developed to monitor transfusion practice was confirmed as reliable and was largely automated [4]. In 2010, the International Society for Blood Transfusion published the ISBT Guidelines for Validation of Automated Systems in Blood Establishments, which detailed the equipment, facilities, utilities, methods and processes for information systems [5]. Information systems were also reported to improve the compliance rate of transfusion request form [6], reduce the number of errors (wrong component transfusions and wrong blood in tubes) and improve the reporting rate of transfusion reactions [7–9].

The aim of the present study was to explore the role of information technology in improving the quality of transfusion practice. We developed an optimized blood transfusion management information system (BTMIS) and then statistically analysed the changes before and after implementing BTMIS in four quality indicators, including the completeness rate of transfusion request forms, appropriate transfusion rate, reporting rate of adverse transfusion reactions (ATRs) and adequacy rate of transfusion medical records.

MATERIALS AND METHODS

Data sources

All data were collected from the Second Affiliated Hospital of Chongqing Medical University in southwest China from 2015 to 2020. Only inpatients were included in this study. Our institution was a 1600-bed grade A tertiary hospital that had approximately 50,000 discharges per year. The hospital was a single central general medical facility with one transfusion laboratory, which only accepted adult patients. Our institution had about 500 cases of traumas, 100 cases of cardiac operations and several liver and kidney transplants every year. The hospital issued about 8000 units of red blood cells (RBCs), 9000 units of frozen plasma (FP), 1000 therapeutic doses of platelets (PLT) and 500 units of cryoprecipitation every year, of which 35% of the RBCs and 20% of the FP were issued to the surgical departments.

Development of a BTMIS

The BTMIS was developed by Chongqing Meiweier Technology Co. Ltd. (China) and the transfusion department of the Second Affiliated Hospital of Chongqing Medical University. The system incorporated blood requisitions, specimen receipts, compatibility tests and blood receipts and issuances, which are common in traditional information systems. Additionally, our system included pre-transfusion indication evaluations, blood transfusion nursing, reports of ATRs and post-transfusion efficacy evaluation modules (Figure 1). Many procedure restrictions were also added, for example, a transfusion request form could automatically result in the retrieval of a patient's information and latest laboratory results from the HIS and laboratory information system (LIS) (Figure 2). If the laboratory tests were unfinished, special instructions would be marked on the transfusion request form. For example, a specimen had been submitted but testing was incomplete, or the patients refused the tests. The doctors could not print the blood-taking form until they finished the pre-transfusion indication evaluation. If the BTMIS determined that the blood transfusion was inappropriate, the evaluation form would be reviewed by the transfusion department physician. Only after completion of the last post-transfusion efficacy evaluation could the doctors complete the next transfusion request form on the BTMIS.

Classification criteria for transfusion quality indicators

The completeness rate of transfusion request forms, appropriate transfusion rate, reporting rate of ATRs and adequacy rate of

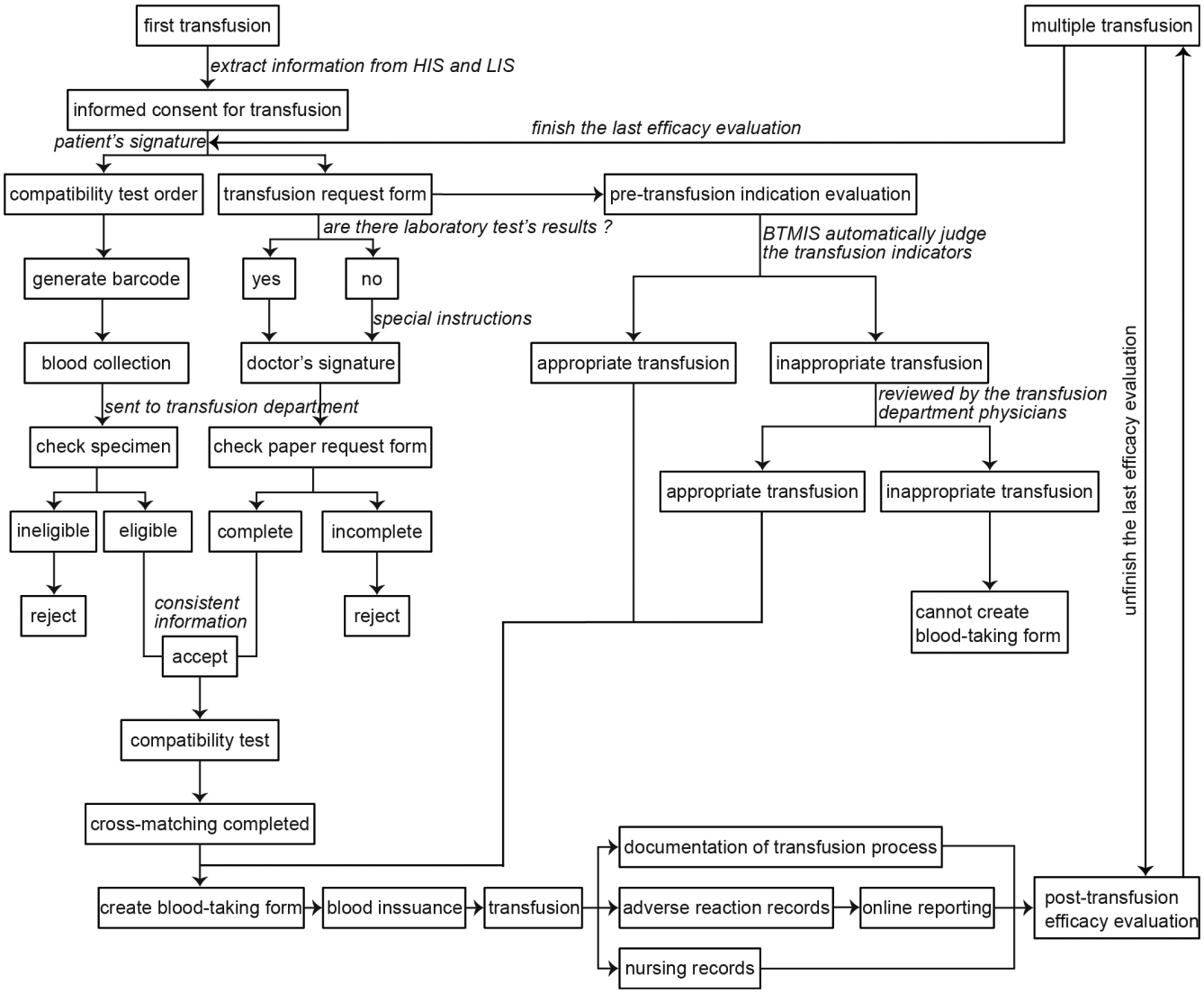

FIGURE 1 The framework and workflow of blood transfusion management information system (BTMIS). When ordering blood components, the BTMIS generated transfusion request form and compatibility test orders at the same time (if it was the first blood transfusion, BTMIS first generated the informed consent form for transfusion), the request form and the compatibility test specimen were sent to the transfusion department together for next test. After completing cross-matching, the doctors could not print the blood-taking form until they finished the pre-transfusion indication evaluation. After the completion of blood transfusion, doctors and nurses wrote the documentation of transfusion process and nursing records and reported adverse transfusion reactions. Next, the post-transfusion efficacy evaluation was carried out, and the patient could not request next blood transfusion until completing the efficacy evaluation.

transfusion medical records were the four main indicators that were given attention by Chinese health authorities. The classification criteria of these indicators are described below.

In China, the transfusion request form should include the gender, age, department, ward, bed number, patient number, transfusion targets, volume, blood component, date for transfusion, blood group, pre-transfusion laboratory tests, transfusion history, obstetrical history, doctor's signature, signing status of informed consent and degree of urgency. If any of these items were missing or incorrect, the transfusion request form was deemed incomplete.

ATRs were classified according to the criteria of the Chinese Hemovigilance Network [10]. During the study, seven ATRs were defined: febrile non-haemolytic transfusion reaction (FNHTR), allergic reaction, transfusion-associated circulatory overload, transfusion-associated dyspnoea, hypotensive transfusion reaction, intoxication of citrate and other/unknown. Once ATRs occurred, doctors must report them online in the BTMIS after the event; these data were reported online to the medical affairs office in the institution after being reviewed online by the transfusion department physicians each day. The reporting rate of ATRs was calculated as the percentage of the number of adverse reactions in total transfusion episodes.

For transfusion medical records, we mainly checked for informed consent for transfusion, pre-transfusion laboratory tests and documentation of the transfusion process. If any of these three items was

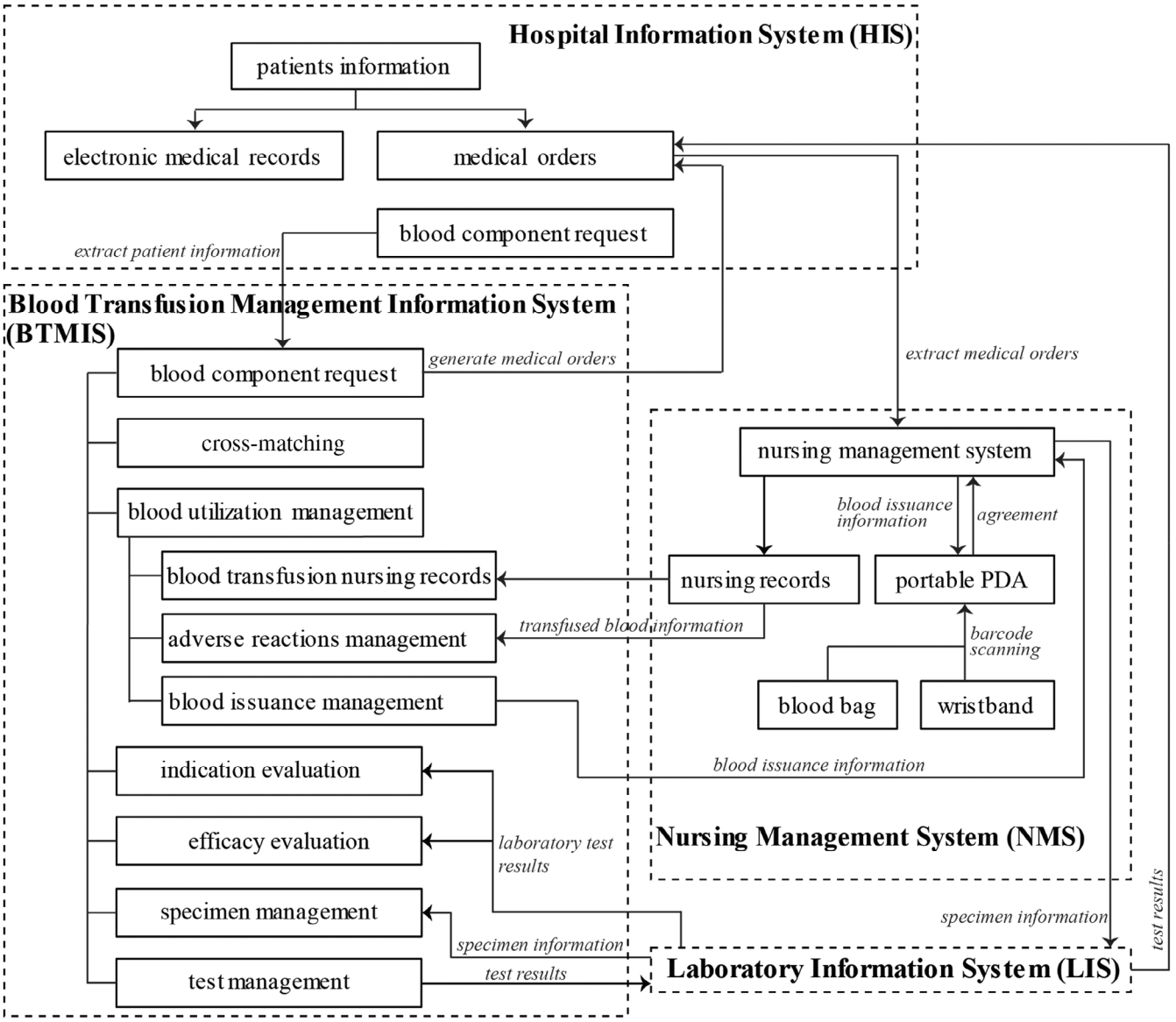

FIGURE 2 The linked modules and elements between blood transfusion management information system (BTMIS) and other systems. When doctors requested blood components in hospital information system (HIS), request module would be linked to the BTMIS and generated compatibility test orders in HIS. The nursing management system (NMS) extracted medical orders from HIS and nurses collected blood. BTMIS extracted specimen information from NMS via laboratory information system (LIS). After the detection was completed, BTMIS sent the results back to LIS, LIS then sent them to HIS and other mobile terminals. After the completion of cross-matching, the doctor conducted the pre-transfusion indication evaluation by extracting the laboratory test results from LIS. After the evaluation, the blood transfusion department issued blood products, and the blood issuance information was sent to NMS. The nurses scanned the barcode of blood bag and wristband with a portable personal digital assistant (PDA), and PDA sent the comparison result to NMS. When the information was consistent, the patients started blood transfusion. After the completion of blood transfusion, the nurses wrote the blood transfusion nursing record and the doctors reported the adverse transfusion reaction.

missing or incorrect, the transfusion medical record was considered inadequate. If medical records did not include informed consent for transfusion or if the contents of the informed consent were incomplete or wrong, informed consent for transfusion was deemed inadequate. Missing or incomplete pre-transfusion laboratory tests and missing laboratory reports were classed as inadequate pre-transfusion laboratory tests. The documentation of the transfusion process was judged inadequate in cases of (1) a lack of documentation of the transfusion process, (2) incomplete or incorrect documentation of the transfusion process, (3) failure to report an ATR to the transfusion department, (4) non-conforming or missing blood loss and transfusion volumes between the operation and anaesthesia records, (5) non-conforming temporary transfusion orders and documentation of the transfusion processes, (6) a lack of a pre-transfusion indication evaluation, (7) a lack of a post-transfusion efficacy evaluation or (8) a lack of nursing records.

The following criteria for appropriate transfusions were stated in China. For RBC transfusion, a haemoglobin (Hb) level below 6 g/dl

TABLE 1 The changes in quality indicators and other factors

	No BTMIS, *n* (%)	BTMIS, *n* (%)	*p* value[a]
Types of incomplete forms			
Missed gender or age	67 (0.17)	0 (0.00)	<0.0001
Missed department or ward or bed number	54 (0.14)	0 (0.00)	<0.0001
Wrong purpose of transfusion	596 (1.5)	10 (0.02)	<0.0001
Wrong number of component requirement	380 (0.95)	23 (0.06)	<0.0001
Wrong type of component requirement	277 (0.69)	12 (0.03)	<0.0001
Wrong time of the transfusion	262 (0.66)	1 (0.00)	<0.0001
Incorrect or missed blood group	996 (2.5)	0 (0.00)	<0.0001
Lack of pre-transfusion laboratory test results	1849 (4.6)	31 (0.08)	<0.0001
Lack of transfusion or pregnancy history	628 (1.6)	1 (0.00)	<0.0001
Unqualified doctors' signature	1675 (4.2)	181 (0.45)	<0.0001
Signing status of informed consent for transfusion	325 (0.81)	22 (0.05)	<0.0001
Improper degree of urgency	185 (0.46)	2 (0.00)	<0.0001
Incorrect blood special requirement	84 (0.21)	13 (0.03)	<0.0001
Total	7378 (18.5)	296 (0.73)	<0.0001
The changes in appropriate blood utilization			
Inappropriate transfusion	3797 (13.0)	213 (0.57)	<0.0001
Appropriate transfusion	25,445 (87.0)	37,416 (99.4)	<0.0001
Total	29,242	37,629	
Adverse events of blood transfusion			
Wrong blood in tube	0	2	
Adverse transfusion reactions	64 (0.22)	184 (0.49)	<0.0001
Febrile non-haemolytic transfusion reaction	16 (0.05)	38 (0.10)	0.0367
Allergic reaction	28 (0.10)	94 (0.25)	<0.0001
Transfusion-associated circulatory overload	2 (0.01)	3 (0.01)	0.8665
Transfusion-associated dyspnoea	1 (0.00)	2 (0.01)	0.7166
Hypotensive transfusion reaction	1 (0.00)	3 (0.01)	0.4502
Intoxication of citrate	4 (0.01)	9 (0.02)	0.3462
Other/unknown	12 (0.04)	35 (0.09)	0.0119
Type of inadequate medical records			
Informed consent for transfusion	161 (6.4)	37 (1.7)	<0.0001
Pre-transfusion laboratory tests	156 (6.2)	44 (2.0)	<0.0001
Documentation of transfusion process	316 (12.6)	131 (5.9)	<0.0001
Total	633 (25.2)	212 (9.6)	<0.0001
Other factors			
Training for blood transfusion (no. per year)	1	1	
Educational level for staff			
Postgraduate or above	1396 (44.4)	1620 (46.6)	0.0759
Undergraduate or below	1747 (55.6)	1857 (53.4)	0.0759
Changes in transfusion regulations			
National regulations	I, II, III, IV	I, II, III, IV	
Provincial regulations	V, VI	V[b], VI	
Hospital regulations	VII	VII, VIII[c]	

Note: I. Blood Donation Act of the People's Republic of China. II. Technical Criterion for Clinical Blood Transfusion. III. Administrative Measures for Clinical Blood Usage in Medical Institutions. IV. National Guidelines for Technical Verification of Blood Safety. V. Blood Donation Act of the Chongqing. VI. Guidelines for Technical Verification of Blood Safety in Chongqing. VII. The Inspection Standard for the Quality of Medical Record. VIII. The Incentives for Reporting Adverse Medical Events.

Abbreviation: BTMIS, blood transfusion management information system.

[a]Chi-square test was performed using GraphPad Prism. A value of $p < 0.05$ was considered statistically significant.

[b]There was a new version of the Blood Donation Act of the Chongqing, but the revised items had little relevance to our research.

[c]For each reported adverse transfusion reaction, the institution awarded doctors ¥10.

was required for internal medicine patients or Hb ≥ 6 g/dl for patients with severe infection or hypoxic symptoms, Hb < 7 g/dl for surgical patients, blood loss exceeding 20% of the self-blood volume, or Hb ≥ 7 g/dl or blood volume loss less than 20% self-blood volume in patients who had severe infection or hypoxic symptoms. For PLT, the criteria were PLT counts below 50×10^9/L, or PLT < 100×10^9/L in patients undergoing brain or eye operations, or massive blood transfusion, or active bleeding during operation. For FP, the criteria were prolongation of prothrombin time (PT) or active partial thromboplastin time (APTT) to 1.5 times the reference value, or an international normalised ratio (INR) ≥1.5, or massive blood transfusion, or an urgent antagonizing anticoagulant effect of warfarin, or therapeutic plasma exchange. For cryoprecipitate, the criteria were fibrinogen below 0.8 g/L, treatment of haemophilia A, supplementation of von Willebrand factor or massive blood transfusion.

Statistical methods

The data collected during 2015–2017, when BTMIS was not implemented, were classified as the 'no BTMIS' group. The data collected during 2018–2020, when BTMIS was implemented, were classified as the 'BTMIS' group. The completeness rate of transfusion request forms, appropriate transfusion rate, reporting rate of ATRs and adequacy rate of transfusion medical records were analysed using the chi-square test, and other measurement data were analysed with the unpaired *t* test. All analyses were conducted using GraphPad Prism. A value of $p < 0.05$ was considered statistically significant.

RESULTS

BTMIS increased the completeness rate of transfusion request forms

Before implementing BTMIS, we used handwritten request forms; there were many mistakes in these forms. Of the 39,890 transfusion requests, 32,512 forms were complete, with a completeness rate of 81.5%. After implementing BTMIS, doctors ordered blood components online and the electronic request forms needed to be printed out, manually signed by the qualified doctor and sent to the transfusion department together with the specimens. Of the 40,591 transfusion requests, 40,291 transfusion requests were complete, with a completeness rate of 99.3%, indicating a significant improvement after implementation of the BTMIS (Table 1, $p < 0.0001$).

Prior to BTMIS implementation, the major missing items or errors in the request forms were lack of pre-transfusion laboratory test results (4.6%), unqualified doctor's signature (4.2%), incorrect or missing blood group (2.5%), lack of transfusion or pregnancy history (1.6%) and wrong purpose of transfusion (1.5%). After implementing BTMIS, due to the control of the system, the items of gender, age, department, ward, bed number and blood group in request forms were complete and correct. The unqualified doctor's signature was the main incomplete item (0.45%). The frequency of other incomplete items was less than 0.1% (Table 1, $p < 0.0001$). In summary, the patients' information, blood component requirements, laboratory tests, transfusion or pregnancy history and doctors' signatures on the transfusion request forms had significantly improved.

BTMIS increased the appropriate transfusion rate and decreased RBCs and FP utilization in surgical departments

Our BTMIS could automatically generate a pre-transfusion evaluation form according to the newest laboratory results. If no laboratory results were available, the doctors needed to fill in the patient's condition or blood loss. This evaluation form was more accurate than the previous handwritten one. After implementing BTMIS, the appropriate transfusion rate increased from 87% to 99.4% (Table 1, $p < 0.0001$).

We also analysed the characteristics of the included cases. During the implementation of BTMIS, diabetes, hypertension and problems with the liver, lung, kidney, heart and other important organs increased significantly, except for obesity. Hb triggers (latest Hb before transfusion) and targets (Hb upon discharge) decreased from 7.3 ± 2.7 g/dl and 8.2 ± 2.1 g/dl to 6.8 ± 2.5 g/dl and 7.8 ± 1.7 g/dl, respectively (Table 2, $p < 0.05$). PT and APTT triggers increased, PLT and fibrinogen triggers had no obvious changes (Table 2). Although there was no statistical difference between PT and APTT triggers, it was shown that the laboratory indications for blood transfusion were stricter after implementing BTMIS. This was reflected in the decrease in average RBCs and FP utilization in most surgical departments, except for the RBC utilization in gastrointestinal surgery and the FP utilization in gastrointestinal surgery and obstetrics and gynaecology. The RBCs and FP utilization in non-surgical departments did not change much, except for the significant increase in plasma utilization in the infectious disease department (Table 3). This may be related to the relief of blood shortages, meaning more patients could perform plasma exchange.

BTMIS potentially improved the reporting practices for ATRs

Reporting ATRs in BTMIS was more convenient than the previous handwritten reporting method. The doctors became more active in reporting these reactions. The reporting rate increased from 0.22% to 0.49%, particularly for allergic reaction and FNHTR, which increased significantly (Table 1, $p < 0.0001$ and $p < 0.05$, respectively). However, during the implementation of BTMIS, changes in hospital transfusion regulations may have somewhat promoted the reporting of ATRs, and increases might be partly due to these. We also found two cases of wrong blood in tubes using the twice blood type comparison function of the BTMIS (Table 1), which showed that our system was also helpful in improving blood transfusion safety.

TABLE 2 Patient characteristics[a]

Variable	No BTMIS ($n = 151,375$)	BTMIS ($n = 182,654$)	p value[b]
Male	66,259 (43.1)	80,488 (44.1)	<0.0001
Age (years)	54.1 ± 18.8	54.9 ± 18.3	<0.0001
Hospital stay (days)	11.1 ± 20.9	9.5 ± 13.1	<0.0001
Hypertension	42,801 (28.3)	58,178 (31.9)	<0.0001
Diabetes mellitus	25,828 (17.1)	38,220 (20.9)	<0.0001
Hepatic disease	33,998 (22.5)	50,980 (27.9)	<0.0001
Pulmonary disease	28,614 (18.9)	42,118 (23.1)	<0.0001
Renal disease	17,908 (11.8)	31,349 (17.2)	<0.0001
Peripheral vascular disease	4217 (2.8)	5900 (3.2)	<0.0001
Congestive heart failure	4179 (2.8)	1829 (1.0)	<0.0001
Coagulopathy	97 (0.06)	307 (0.17)	<0.0001
Obesity	13 (0.00)	27 (0.01)	0.1034
Hb (g/dl)			
Triggers	7.3 ± 2.7	6.8 ± 2.5	0.0064
Targets	8.2 ± 2.1	7.8 ± 1.7	0.0046
PT (s)	20 ± 13.8	22.5 ± 12.5	0.0680
APTT (s)	48.4 ± 26.6	51.8 ± 19	0.1580
Platelets ($\times 10^9$/L)	11.8 ± 15.9	11.9 ± 9.0	0.9686
Fibrinogen (g/L)	1.4 ± 1.0	1.3 ± 0.9	0.7547

Abbreviations: APTT, active partial thromboplastin time; BTMIS, blood transfusion management information system; Hb, haemoglobin; PT, prothrombin time.

[a]Data were reported as number (%), or mean ± SD.

[b]Unpaired t test was performed using GraphPad Prism. A value of $p < 0.05$ was considered statistically significant.

BTMIS increased the quality of transfusion medical records

Transfusion medical records were important legal documents; auditing transfusion medical records was routine work in China. After implementing BTMIS, the adequacy rate of transfusion medical records increased from 74.8% to 90.4%. The inadequacy rates of informed consent for transfusion, pre-transfusion laboratory tests and documentation of the transfusion process were reduced from 6.4%, 6.2% and 12.6% to 1.7%, 2.0% and 5.9%, respectively (Table 1, $p < 0.0001$), but inadequate documentation of the transfusion process remained relatively high. Further steps must be taken to improve this quality indicator.

DISCUSSION

Nowadays, human mistakes have become the greatest transfusion risks [11]. The frequency of these mistakes can be reduced by strengthening process management and using information technology [12]. Although information technology plays a positive role in reducing human mistakes, its role in the quality management of transfusion practice still requires further study.

In this study, we designed the twice blood type model in BTMIS. One sample was used to identify the blood type, another sample was used to perform a compatibility test and re-test the blood type. When both blood types were consistent, we could perform further cross-matching. After implementing BTMIS, we found two cases of wrong blood in tubes, indicating that the BTMIS could improve the safety of blood transfusion.

TABLE 3 Erythrocytes and plasma utilization during the study periods[a]

Departments	Red blood cells (units/patient)			Frozen plasma (ml/patient)		
	No BTMIS	BTMIS	p value[b]	No BTMIS	BTMIS	p value[b]
Infectious Diseases	0.22 ± 0.06	0.21 ± 0.03	0.7466	95.71 ± 14.53	168.92 ± 20.64	0.0151
Haematology	1.19 ± 0.17	1.22 ± 0.03	0.9776	18.15 ± 5.24	23.68 ± 6.56	0.4629
Gastroenterology	0.37 ± 0.08	0.31 ± 0.02	0.3404	25.95 ± 0.91	27.04 ± 1.54	0.4516
Cardiovascular Medicine	0.04 ± 0.02	0.02 ± 0.00	0.1581	0.30 ± 0.06	0.32 ± 0.29	0.9456
Nephrology	0.08 ± 0.03	0.08 ± 0.01	0.9664	0.47 ± 0.34	0.44 ± 0.32	0.9477
General Medicine	0.05 ± 0.01	0.04 ± 0.01	0.3783	0.11 ± 0.03	0.10 ± 0.09	0.8510
Oncology	0.23 ± 0.07	0.17 ± 0.03	0.3015	6.05 ± 2.30	7.99 ± 1.76	0.3501
Critical Care Medicine	3.91 ± 0.94	3.83 ± 0.27	0.6406	449.15 ± 148.29	452.77 ± 65.66	0.7749
Cardio-Thoracic Surgery	0.69 ± 0.20	0.36 ± 0.05	0.0788	57.16 ± 18.90	27.46 ± 4.93	0.1037
Hepatobiliary Surgery	0.30 ± 0.10	0.22 ± 0.06	0.3700	26.48 ± 7.01	15.77 ± 3.28	0.1137
Neurosurgery	0.41 ± 0.10	0.21 ± 0.04	0.0576	35.09 ± 7.67	16.17 ± 4.51	0.0376
Orthopaedics	0.37 ± 0.06	0.22 ± 0.01	0.0215	26.70 ± 8.60	13.36 ± 1.06	0.0866
Vascular Surgery	0.09 ± 0.05	0.06 ± 0.00	0.3216	2.02 ± 1.25	1.75 ± 0.19	0.7523
Urology Surgery	0.09 ± 0.04	0.06 ± 0.01	0.2218	4.69 ± 1.82	2.69 ± 0.62	0.2020
Gastrointestinal Surgery	0.17 ± 0.01	0.22 ± 0.02	0.0209	6.66 ± 3.17	7.45 ± 1.82	0.8156
Gynaecology and Obstetrics	0.07 ± 0.02	0.06 ± 0.01	0.8222	2.52 ± 0.66	2.88 ± 0.36	0.5793

Abbreviation: BTMIS, blood transfusion management information system.

[a]Data were reported as mean ± SD.

[b]Unpaired t test was performed using GraphPad Prism. A value of $p < 0.05$ was considered statistically significant.

Our software could extract patient information from HIS, thus avoiding omissions and errors caused by doctors' manual completion. After using BTMIS, the completeness of patient information in the transfusion request forms reached 100%. The BTMIS also automatically extracted laboratory test results from the LIS. The rate of request forms lacking pre-transfusion laboratory test results reduced from 4.6% to 0.08%. An unqualified doctor's signature represented the most common incomplete indicator (0.45%), accounting for 61.1% of all incomplete items. This was because the electronic handwritten signature had not been approved in China, doctors needed to print the electronic request form, manually sign it and send it to the transfusion department. Sometimes there were missing or incorrect signatures, so this needed to be strengthened outside the system.

Overall, after using BTMIS, the completeness rate of transfusion request forms increased from 81.5% to 99.3%. The completeness rate of our request forms was higher than the rates of 80.2% reported by Jain et al. [13], 81.2% reported in a study from Nigeria [14] and 76.8% reported by Pandey et al. [15]. It indicated that BTMIS was effective in improving the completeness of request forms.

Blood resources in China were extremely short, and the highest target of appropriate transfusion rate set by the Evaluation Criteria of Tertiary Hospitals in China was 100% [16]. So most hospitals had to adopt restrictive blood transfusion strategies and strictly controlled the indications of blood transfusion. Before implementing BTMIS, our appropriate transfusion rate (87%) was similar to the rates of about 90% reported by Chinese different grade hospitals [17–19]. After implementing BTMIS, the appropriate transfusion rate increased from 87% to 99.4%. Hb triggers and targets reported in other studies were significantly higher than our data, those studies showed that the role of information system in reducing blood utilization was limited [20–22]. However, our data showed that the role of information systems was substantial. We manually reviewed the pre-transfusion evaluation forms that could not be automatically judged by the system, randomly audited the discharged medical records and evaluated doctors' appropriate transfusion rates monthly. These measures may have a synergistic effect on BTMIS.

Our system also included a reporting function for ATRs. The doctors reported the ATRs to the medical affairs office, and then these ATRs were reported to the Chinese Haemovigilance Network, apart from those reported reactions with incomplete information. After implementing BTMIS, the rate of reported ATRs increased from 0.22% to 0.49%, therefore potentially reflecting improved reporting practices.

Because of the limitations of our system in the informed consent of transfusion and examination of pre-transfusion infectious diseases, after implementing BTMIS, the adequacy rate of transfusion medical records had increased from 74.8% to 90.4%. Although our adequacy rate of transfusion medical records was higher than the rates of 10.6% reported in a study from Germany and 80% reported by Skodlar et al. [23, 24], other measures should be taken to further improve the adequacy.

In conclusion, the use of information technology could improve the quality of transfusion practice. This indicated that information technology could be a useful tool for improving the management quality of blood transfusion in regions with poor information construction. However, the use of information technology alone was usually insufficient, medical education, regular audit and other methods of cooperation will be helpful.

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H.L. and G.P.X. designed the experiments. X.M.X. and H.L. developed the information system. J.J.L. and Q.G. collected and analysed the data. L.F.W. and G.P.X. wrote the paper. G.P.X. reviewed and edited the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

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Differential enzymatic deglycosylation reveals attachment of red cell B antigen onto the carbohydrate moiety of glycophorin A and glycophorin B

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Abstract

Background and Objectives: Early studies indicate that red cell A and B antigens are attached primarily onto band 3 and GLUT1 on the erythrocyte membrane and little onto glycophorin A (GPA) and glycophorin B (GPB). But as GPA and band 3 form stable protein complexes and GPA is much more heavily glycosylated than band 3, this study re-examined the association between ABO antigens and GPA/GPB.

Materials and Methods: Band 3/GPA-associated protein complexes were first immunoprecipitated, followed by differential enzymatic deglycosylation that removed sialic acids, *N*-glycans and *O*-glycans. Serological anti-A (BIRMA 1) and anti-B IgM (GAMA 110) could be used for western blot (WB); however, only the anti-B IgM showed significant reactivity for the immunoprecipitates isolated by anti-band 3. The expression of the B antigen in un-deglycosylated and differentially deglycosylated band 3 immunoprecipitates was thus compared.

Results: Besides attachment to band 3, red cell B antigen expressed substantially on GPA monomer and homodimer, GPA*GPB heterodimer, and GPB monomer and dimer via attachments through the *N*- and *O*-glycans.

Conclusion: Immunoprecipitation (IP), as a means of protein separation and concentration, was used in combination with a WB to differentiate glycosylation on different proteins and oligomers. This study implemented differential enzymatic deglycosylation during IP of the band 3 complexes. This combined approach allowed separate identification of the B antigen on GPA/GPB monomer and dimer and GPA*GPB heterodimer, and band 3 on the WB and verified non-trivial expression of the B antigen on GPA and GPB on the erythrocyte surface.

Keywords

ABO blood group, B antigen, band 3 (anion exchanger-1; AE1), erythrocyte (red blood cell; RBC), glycocalyx, glycophorin A (GPA), glycophorin B (GPB), glycoprotein

Highlights

- A novel method that combined immunoprecipitation–western blot and enzymatic deglycosylation was developed to differentiate glycosylation in individual components of the band 3 complexes.
- Previous glycochemical studies report that 80% of red cell ABH determinants are capped on band 3 and few on glycophorin A (GPA) or glycophorin B (GPB).

- This protein-based study identifies nontrivial expression of red cell B antigen on the *N*- and *O*-glycans of GPA/GPB oligomers.

INTRODUCTION

Red cell A and B antigens are synthesized from H antigen by Golgi membrane-bound *N*-acetylgalactosaminyltransferase plus the UDP-GalNAC donor and galactosyltransferase plus the UDP-Gal donor, respectively. Also, in the trans-Golgi network, H antigen is synthesized by fucosyltransferase 1 preferentially onto the polylactosamine structure of specific *N*- and *O*-glycosylated membrane proteins and glycosphingolipids [1, 2]; hence, it has been suggested that only specific red cell membrane proteins could be conjugated with ABO antigens [1–3]. Red cell A and B antigens have been reported to attach primarily to the *N*-glycan structure of the GLUT1 glucose transporter and band 3 [3–5].

GLUT1 and band 3 are both large transporter proteins, each with multiple transmembrane spans and a *N*-glycosylation site [6–8]. Each human erythrocyte expresses 200,000–700,000 copies of GLUT1 protein [9]. Recent super-resolution imaging reveals that *N*-glycosylation of GLUT1 could induce and modulate GLUT1 clustering and raft formation on the red cell surface [10]. On the other hand, band 3 and glycophorin A (GPA) are the two most abundant proteins on the red cell membrane (each with ~1 million protein molecules on an erythrocyte). GPA and homologue glycophorin B (GPB) are type I membrane proteins with the most extensive glycosylation (their carbohydrate contents constituting over half of their molecular weights) [3, 11–13]. Paradoxically, the carbohydrate moiety of GPA is rich in polylactosamines but was reported to have few ABO antigens [3–5, 14–16].

Recent glycan release studies using chemical means, such as β-elimination and GC/MS show a definite but small presence of ABO antigens on the *N*- and *O*-glycans of GPA [14–16]. GPA is a 'mucin-like' sialoglycoprotein with one *N*-glycosylation and 16 *O*-glycosylation sites [17]. GPB, a homologue of GPA with 95% sequence similarity, is also covered with mucin *O*-glycans like GPA but lacks the *N*-glycosylation site [18]. This study re-evaluated the association between ABO antigen and GPA/GPB. By combining antibody-based immunoprecipitation (IP) and differential enzymatic deglycosylation, we found nontrivial expression of red cell B antigen on GPA/GPB.

MATERIALS AND METHODS

Samples

Mackay Memorial Hospital Institutional Review Board (MMH-IRB) approved the study of red blood cell (RBC) samples from consenting healthy subjects (MMH-IRB registration: 09MMHIS170). ABO serology was performed with anti-A IgM (BIRMA 1, IMMUCOR GAMMA) and anti-B IgM (GAMA 110, IMMUCOR GAMMA) using the standard tube method.

SDS-PAGE and western blot

The membrane fraction (ghost) of RBC samples was isolated by hypotonic burst, followed by at least five phsophsate-buffer saline (PBS) washes to remove haemoglobin that could interfere with chemiluminescent detection in WB. Ghost samples were then solubilized in a PBS-based lysis buffer that contained 0.5% CHAPS, 0.5% NP-40 and cOmplete™ protease inhibitor (PI) cocktails (Roche, Indianapolis, IN, USA), with constant rotator-mixing at 4°C. The concentration of protein lysates was determined by Lowry assay. Prior to SDS-PAGE, protein lysates were denatured at 37°C for 40 min in the lithium dodecyl sulfate (LDS) sample buffer (Invitrogen, Carlsbad, CA, USA) supplemented with 20 mM dithiothreitol (DTT). In each WB experiment, equal quantities of the protein lysates (minimal 40 μg/lane) were loaded per lane and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 4%–12% precast Bis-Tris gel (Novex gel electrophoresis system, Invitrogen). After SDS-PAGE, the separated proteins were transferred onto a polyvinylidene fluoride (PVDF) membrane by conventional electrophoretic transfer. The transferred membrane was blocked with 5% non-fat milk for 30 min prior to WB.

The primary monoclonal antibodies (mAbs) used in WB were anti-A IgM (BIRMA 1; 1:200 dilution), anti-B IgM (GAMA 110; 1:1000 dilution), anti-GPA/GPB IgG (R1.3 [19], International Blood Group Reference Laboratory [IBGRL], UK; 1:200–1:1000 dilution) and anti-band 3 AE12 IgG (Alpha Diagnostic, San Antonio, TX, USA; 1:20,000 dilution) and BRIC 170 (IBGRL; 1:2000 dilution). The signals from primary mAb binding were amplified using goat anti-mouse IgG + IgM conjugated with horseradish peroxidase (HRP 1:10,000 or higher dilution; Jackson ImmunoResearch Laboratories, West Grove, PA, USA), and then detected using the SuperSignal West Pico Chemiluminescent Substrate (Invitrogen-Pierce).

IP combining enzymatic deglycosylation

Anti-band 3 mAb (clone AE12: targeting the intracellular *N*-terminal domain of band 3) was used to immunoprecipitate RBC proteins bound directly and indirectly to band 3. For each IP reaction, 2 μl of AE12 mAb was first rotator-mixed with 40 μl of anti-IgG-coated M280 dynabeads (Dynal, Oslo, Norway) for 3 h. Ab-bead binding was then chemically crosslinked in freshly prepared 20-mM dimethyl pimelimidate dihydrochloride in 0.2 M triethanolamine, pH 8.2, on a rotator at room temperature for 30 min, followed by 15-min quenching in 50 mM Tris, pH 7.5 and three washes with 0.1% Tween-20/PBS, as suggested by the Dynal's product manual [20, 21]. The IP control 'IgG bead-only' underwent the same treatment but without the addition of AE12 mAb. Ghost lysate samples were

solubilized in a PBS-based lysis buffer that contained 0.3% CHAPS, 0.3% NP-40 and 2× cOmplete™ PI cocktails for at least 30 min at 4°C. For each IP reaction, the Ab-bound beads were mixed with an equal protein quantity of the ghost lysates (at least 1-mg proteins per sample reaction) on a low-speed rotator at 4°C overnight. After overnight binding, the beads were then washed six times with a IP-wash buffer comprised of 0.1% CHAPS, 0.1% NP-40, 50 mM Tris, pH 8.0 and 150 ~ 300 mM KCl (the salt concentration increased from 150 to 300 mM in the middle two washes and then returned to 150 mM). Band 3-associated proteins were eluted in 100 µl of the 2× LDS sample buffer (Invitrogen) supplemented with 20 mM DTT and 0.5% SDS at 70°C for 20 min.

For enzymatic deglycosylation combining band 3 co-immunoprecipitation, the sample-bound Dynabeads, after overnight mixing, were washed twice with an IP-wash buffer (0.1% CHAPS, 0.1% NP-40, 150 mM KCl and 50 mM Tris, pH 8.0), followed by two PBS washes. After removal of the last wash buffer, each IP tube was added with 17.8 µl of the deglycosylation buffer provided by the Prozyme Glyko's Enzymatic Deglycosylation kit (including 1 µl denaturation solution [2% SDS and 1 M β-mercaptoethanol], 1 µl detergent solution [15% NP-40], 4 µl 5× incubation buffer [0.25 M Na-Phosphate, pH 7.0] and 11.8 µl ddH_2O). The sample-bound beads in this buffer were rotator-mixed for 1 h with occasional sonication before adding specific cocktails of the deglycosylation enzymes. We performed two enzymatic deglycosylation treatments: the first cocktail included 0.4 µl neuraminidase (50,000 units/ml; molecular weight [MW] ~43 kDa; New England Biolabs, Ipswich, MA, USA), 0.8 µl *N*-glycanase PNGase F (>5000 units/ml; MW ~35 kDa; Prozyme, San Leandro, CA, USA) and 1 µl ddH_2O; the second cocktail included 0.4 µl neuraminidase, 0.8 µl PNGase F and 1 µl *O*-glycanase (>1250 units/ml; MW ~18 kDa; Prozyme). Both deglycosylation treatments required overnight incubation at 37°C. After overnight deglycosylation, the dynabeads were washed six times using the IP wash buffer (0.1% NP-40, 50 mM Tris, pH 8.0 and 150 ~ 300 mM KCl), followed by elution in 100 µl of the elution buffer (2% Invitrogen's LDS sample buffer supplemented with 0.5% SDS and 20 mM DTT), at 70°C for 20 min. To quantitatively compare the yields of IP, one tenth of each IP product (10-µl eluent) was loaded per lane on a 4%–12% NuPAGE Bis-Tris gel for SDS-PAGE and analysed by western blot (WB), as well as by Colloidal Coomassie blue staining (Invitrogen-Pierce).

RESULTS

WB revealed attachment of A and B antigens to erythrocyte membrane proteins

To examine the expression levels of ABO antigens on RBC membrane proteins, this study used a combined approach of antibody-based detection and differential enzymatic deglycosylation. We found that the anti-A (BIRMA 1) and anti-B (GAMA 110) IgM, widely used in red cell serological typing, showed specificity for WB analyses of ghost lysates from subjects with different ABO types (Figure 1), although

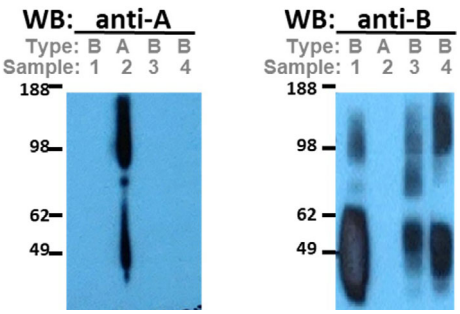

FIGURE 1 Western blot (WB) revealed distribution of A and B antigens on erythrocyte membrane proteins. The membrane fraction of each red blood cell sample was extracted by hypotonic lysis and then detergent-solubilized. Equal quantities of the protein lysate samples were separated by SDS-PAGE, followed by WB using anti-A IgM (left: BIRMA 1) and anti-B IgM (right: GAMA 110). Molecular weights (in kilodaltons) were compared to that from the SeeBlue Plus2 pre-stained standard (Invitrogen).

both anti-A and anti-B IgM showed smear band patterns on WB. The distribution patterns of A and B antigens on the WB were slightly different. WB by anti-B showed stronger band intensities at MWs 40–65 kDa than at ≥95 kDa, but WB by anti-A showed stronger band intensities at regions ≥95 kDa.

Currently, GLUT1 and band 3 are the two known erythroid membrane proteins that the A and B antigens are attached to. GLUT1, with 492 AA and a *N*-glycosylation site, shows 'smear-like' or diffusive bands on immunoblot (monomers: ranging 35–70 kDa; dimers: 80–100 kDa), which resulted from its different degrees of *N*-glycosylation or *N*-glycoforms [22–25]. Band 3, with 911 amino acids (AA) and one *N*-glycosylation site [26, 27], is associated with heavily glycosylated GPA and homologous GPB. GPA and GPB form several stable oligomers (distinct band sizes ranging from ~20 to 100 kDa) that could withstand denaturation by 2% LDS (or SDS). To examine whether the A and B antigens could be attached to GPA/GPB oligomers, we extracted band 3/GPA-associated complexes from RBCs; this approach aimed to reduce interference from the smear bands of GLUT1 on WB. We compared IP by R1.3 (a mAb that recognizes GPA/GPB on immunoblot), by E4 (a mAb that recognizes GPA on immunoblot) and by AE12 (a potent anti-band-3 mAb). Among the three IP approaches, IP by AE12 was most effective in capturing GPA, GPB and band 3 (Figure S1).

The B antigen attached to the *N*- and *O*-glycans of GPA/GPB

Anti-B IgM (GAMA 110) bound to several distinctive bands in the WB of the band 3 immunoprecipitates (Figure 2). Anti-A IgM (BIRMA 1), however, showed faint signals at ~51–55 kDa in the WB of band 3 co-immunoprecipitates (Figure 2). The faint bands recognized by BIRMA 1 were present in the band 3 immunoprecipitates from type A and type B samples (Figure S2); they likely resulted from weak reactivity to a structural epitope shared by the A and the B antigens. Because

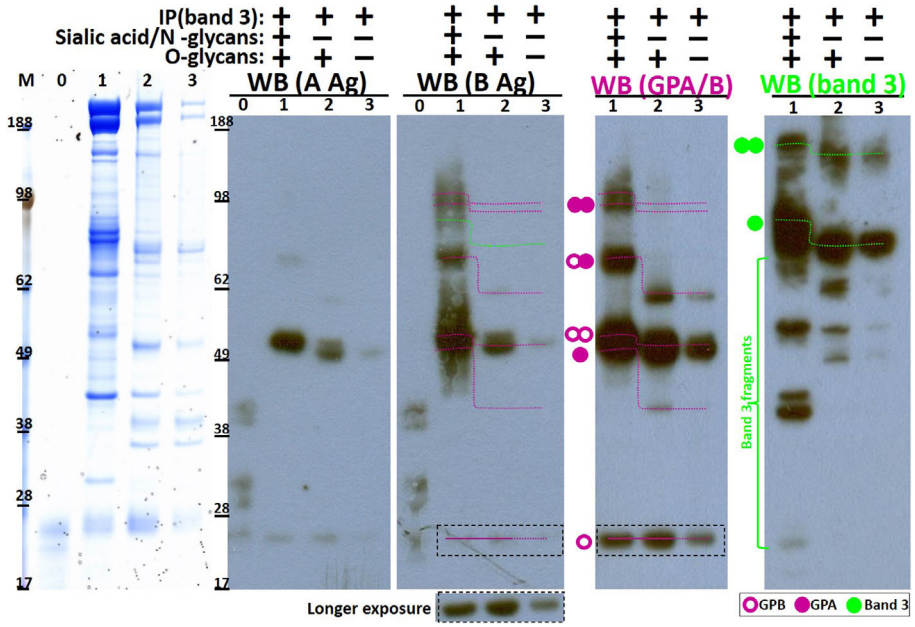

FIGURE 2 Differential enzymatic deglycosylation revealed the presence of B antigen (Ag) on the carbohydrate moiety of glycophorin A (GPA)/glycophorin B (GPB) complexes extracted from a type B subject. For each immunoprecipitation (IP) reaction with or without deglycosylation, one tenth of the reaction products (v/v) was loaded per lane for SDS-PAGE and western blot (WB). Lane '1' contained band 3-associated complex proteins extracted from the erythrocytes of a type B subject by anti-band 3 IP. Lanes '2' and '3' contained differentially deglycosylated band 3 co-immunoprecipitates (lane '2': Treatments with sialidase A and PNGase F; lane '3': Treatments with sialidase A, PNGase F and *O*-glycanase). Lane '0' contained no lysates or IP samples but only the deglycosylation enzymes (sialidase A, PNGase F and *O*-glycanase) as in the third set. The differentially deglycosylated IP products were separated by SDS-PAGE, followed by Coomassie blue staining (left panel) and WB using monoclonal antibodies against the A antigen (BIRMA 1 clone), the B antigen (GAMA 110), both GPA and GPB, or 'GPA/B' (R1.3) and band 3 (AE12). Protein monomer and dimer are indicated by single and double balls, respectively. Monomeric GPB (~24 kDa) is symbolized by an empty pink ball, and monomeric GPA (~50–55 kDa) by a filled pink ball. The GPB homodimer (~55 kDa) is symbolized by two empty pink balls; the GPA-GPB heterodimer (~74 kDa) is symbolized by an empty pink ball bound to a filled pink ball; the GPA homodimers (~92 and 96 kDa) are symbolized by two bound filled pink balls. Monomeric band 3 is symbolized by a filled green ball. Molecular weight changes by the differential deglycosylation treatments were marked by pink (for GPA/GPB) or green dotted lines (for band 3) on the WB.

IP is a concentrating process (GPA/GPB and other band 3-bound proteins were concentrated in this case), IP could enhance signals that were not obvious in WB of the lysates. Compared to anti-A BIRMA 1, anti-B GAMA 110 is a more potent antibody and showed much stronger reactivity toward ghost lysates (Figure 1) and toward the immunoprecipitates by anti-band 3 (Figure S2). We thus used GAMA 110 for the following IP experiments that combined differential enzymatic deglycosylation.

In our combined protocol of IP and deglycosylation, anti-band 3 IgG and magnet dynabeads were first chemically crosslinked to prevent anti-band 3 IgG from being eluted with the immunoprecipitates at the final step. After overnight mixing anti-band-3-coated dynabeads and ghost lysates from a type B subject, we performed two sets of enzymatic deglycosylation 'on beads'. In one set of the deglycosylation using sialidase and PNGase F, the signals of the B antigen were substantially reduced on GPA monomer and homodimer, and GPA*GPB heterodimer (Figure 2: comparing 'lane 1' vs. 'lane 2' in WB [B Ag] and WB [GPA/B]). Removal of the *N*-glycan in band 3 also diminished the signal of the B antigen on monomeric band 3 (Figure 2: comparing 'lane 1' vs. 'lane 2' in WB [B Ag] and WB [band 3]). Despite that band 3 dimer and GPA dimers showed similar MWs (90–120 kDa) on WB, GPA dimers was slightly higher in MW than the band-3 monomer (comparing the two right panels in Figure 2); this IP–WB band pattern was similar to Figure 3b in our previous publication [25]. Careful alignment of the three WBs (against the B Ag, GPA/B and band 3 in Figure 2) verified that the B antigen was conjugated to the *N*-glycans of band 3 and dimeric GPA.

The other set of enzymatic deglycosylation 'on beads' used sialidase, PNGase F and *O*-glycanase. Although this enzymatic cocktail treatment probably only removed some *O*-glycans and not the complex *O*-glycans, it further reduced the intensities of GPB and GPA (on WB [GPA/B]), as well as the intensity of the B antigen on GPB/GPA monomers and dimers (on WB [B Ag]), when comparing 'lane 2' versus 'lane 3' in Figure 2. Thus, red cell B antigen was also conjugated onto the *O*-glycans of GPB and GPA.

Although the B antigen was identified on the immunoprecipitated GPB, it was not observed in the WB of the ghost lysates from type B samples (Figure 1). As GPB is a less abundant and smaller protein (91 AA; 250,000 protein copies/RBC), compared to GPA (150 AA; 1,000,000 protein copies/RBC), GLUT 1 (492 AA; 200,000–700,000 protein copies/RBC), or band 3 (911 AA; 1,000,000 protein copies/RBC) [6], it is possible that the B antigen on GPB might not be as

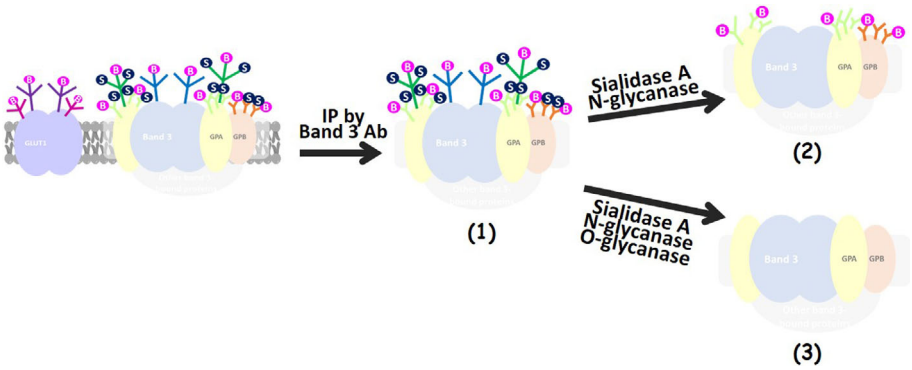

FIGURE 3 A diagram illustrates the study design and the main findings that the B antigen was found on glycophorin A (GPA)/glycophorin B (GPB), in addition to band 3. For simplicity, only GPA (yellow) and GPB (light orange) were shown in the band 3-associated complex; the rest of the complex components were represented by a light grey block. During immunoprecipitation (IP) by anti-band 3 mAb, membrane proteins and complexes were solubilized, and most GLUT1 (purple blocks) was dissociated from the band 3 complex. GLUT1, band 3 and GPA each have an *N*-glycosylation site, and their *N*-glycans were symbolized by the large tree shapes. GPA and GPB each have multiple *O*-glycosylation sites, and their *O*-glycans were symbolized by the small Y shapes. Sialic acids (symbolized by the 'S' balls) are abundantly decorated on GPA and GPB. The B antigen (symbolized by the 'B' balls) was found to be attached to the *O*- and *N*-glycans in the band 3/glycophorin complex. The numbers 1 ∼ 3 corresponded to lanes 1 ∼ 3 in Figure 2.

abundant and only by IP (concentration of selected proteins) could this be revealed.

DISCUSSION

To differentiate glycosylation on individual protein components of the band 3 complexes, a novel method that combined IP and differential enzymatic deglycosylation was developed in this study. By comparing un-deglycosylated and differentially deglycosylated band 3-associated proteins, we identified B antigen on GPA/GPB monomers and dimers and GPA*GPB heterodimer and band 3. Our results verified the nontrivial expression of the B antigen on GPA and GPB on the erythrocyte surface, as previously suggested [15, 16]. Our finding is contrary to previous glycochemical findings that suggested about 1–2 million (80%) of red cell ABH determinants capped on band 3 [28]. The discrepancies are likely due to the different experimental approaches: our Ab/enzyme-based versus previous glycochemical methods. This study was also, however, limited by the lack of an effective anti-A mAb for IP–WB experiments; thus, we could only demonstrate the association between GPA/GPB and red cell B antigen here (Figure 2).

N-linked glycosylation generally begins in the endoplasmic reticulum (ER) and continues in the Golgi network; this co-translational and post-translational process supports protein folding and complex assembly [29]. An important function of GPA is to assist or chaperone the folding and assembly of band 3 complexes in the ER and the Golgi apparatus. GPA begins protein–protein interaction with band 3 in the ER, and at least some subpopulations of GPA and GPB are recruited into band-3-associated protein complexes on the erythrocyte surface [30–32]. In the absence of GPA and GPB (e.g., on the membrane of M^kM^k RBCs), the size of the *N*-glycans on band 3 is larger, whereas the size of the *N*-glycans on GLUT1 is smaller [22]. This indicates that the absence of GPA (chaperone of band 3) delays band 3 biosynthesis and trafficking through the ER and Golgi apparatus, resulting in longer residence of band 3 in the ER/Golgi and more extensive *N*-glycosylation on band 3. From the M^kM^k study, the delayed biosynthesis of band 3 and the larger extent of *N*-glycosylation on band 3 conceivably affect the rate of protein synthesis and the extent of *N*-glycosylation of GLUT1 [22]. One of the three known populations of GLUT1 oligomers has been shown to interact with band 3 on the human erythrocyte surface [30].

Thus, GLUT1, band 3 and GPA/GPB are capped with ABO antigens and conceivably undergo glycosylation in an inter-dependent fashion in the ER and the Golgi network. The inter-dependence between band 3 and GPA/GPB stems during protein biosynthesis and has important functional consequences as the stable band 3/GPA complexes on the RBC membrane are required for optimal anion transport activities of band 3 [22]. Besides supporting the anion transport function of band 3, GPA is decorated with sialic acids on its carbohydrate moiety, which are the main contributors of red cell negative charges (glycocalyx) that prevent agglutination during blood circulation. Although the red cell ABO antigen is functionally unclear, numerous reports continue to show its effects on individual health [3] (e.g., the risk associated with the early onset of ischaemic stroke [33]). The finding of ABO expression on glycophorin glycocalyx may open the door to potential roles of ABO antigen in red cell circulation.

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CONFLICT OF INTEREST

The author declares no conflict of interests or competing financial interests.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Prevalence of adsorbed A antigen onto donor-derived group O red cells in children following stem cell transplantation: A single-centre evaluation

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Abstract

Background and Objectives: A group AB D-positive child presented 1 year after haematopoietic stem cell transplant (HSCT) from a group O D-negative donor as group A D-negative. Engraftment remained at 100% in white cell lineages. The reason for the unusual result was explored, and the scarcely reported phenomenon of adsorption of secreted antigen was considered. This study also investigated the prevalence of secreted antigen adsorbed onto donor-derived group O red blood cells (RBCs) in children after HSCT and defined a process for laboratory management.

Materials and Methods: Retrospective data analysis of HSCTs carried out over 19 months at Great Ormond Street Hospital was conducted to identify cases of adsorbed A antigen after HSCT. Investigation of RBC reactions with different clones of anti-A and in vitro experiments was performed to recreate adsorption.

Results: Nineteen A to O HSCTs were conducted over 19 months, of which six (31%) displayed weak A antigen on RBCs despite full myeloid engraftment. Negative reactions with anti-A were obtained when run on an alternative clone. Laboratory protocols for the future management of these cases have been developed.

Conclusion: Passive adsorption of secreted antigen is responsible for these results and is more widespread than previously reported, as a third of A to O HSCTs at our centre demonstrated this phenomenon. A process has been implemented into the laboratory to manage this cohort, ensuring component groups compatible with both donor and recipient are given, and the shared care centres are aware of these requirements.

Keywords

blood groups, haematopoietic stem cell, immunohaematology, RBC antigens and antibodies, serological testing, transplantation

Highlights

- Passive adsorption of secreted antigen is prevalent in A to O haematopoietic stem cell transplants and characterized by weak reactions with anti-A antisera.
- In this paper, we make recommendations for laboratory handling and management of these patients.
- Identification of these patients is key to appropriate ABO selection of blood groups.

INTRODUCTION

In July 2020, a 5-year-old boy of group AB D positive underwent a group O D-negative-matched unrelated donor haematopoietic stem cell transplant (HSCT) at Great Ormond Street Hospital (GOSH) for severe congenital neutropenia. The procedure was uneventful, with 100% donor engraftment 1 month after HSCT. Twelve months after HSCT, engraftment and blood group were tested as per institutional protocol. Although he continued to be 100% donor engrafted and have normal neutrophil counts, the group was surprisingly found to be A D-negative.

The absence of B and D antigens is unusual in that A and B antigens are co-expressed on red blood cells (RBCs). If a mix of patient and donor cells were present, dual populations across the ABD forward group wells would be observed, signifying both donor and recipient groups.

As the grouping pattern was not explainable by the transplant it was initially assumed that a wrong blood in tube (WBIT) had occurred. WBIT incidents are a cause for concern in transfusion practice and can occur when a sample is collected from the wrong person or labelled with another patient's details. They remain one of the largest categories reported to serious hazards of transfusion (SHOT), an independent haemovigilance scheme for the United Kingdom [1]. The patient was recalled, and the repeat sample was also grouped as A D-negative. Of note, the reaction strengths with anti-A were weaker than would usually be seen in a patient with A antigen at 2+ in each sample.

The patient was well known to the laboratory and had many previous samples grouped as AB D positive, so a WBIT on the pre-HSCT samples was ruled out.

Despite the absence of a dual population, the laboratory contacted the referring/local hospital to check the patient's blood transfusion history. No transfusion was recorded in the last year, and the referring hospital had not grouped the patient recently.

The risk of a typographical error on the patient protocol was ruled out with confirmation from the transplanting team that a group O D-negative donor had been selected, and so the focus moved to confirm the donor group. The site performing the original grouping of the donor was contacted, and it was confirmed that the intended donor was O D-negative. A donor WBIT was discussed and ruled out as the donor had been tested more than twice.

At this point, the possibility that unintended donor cells had been transplanted was considered. DNA from the donor cells had been extracted and stored on site before infusion for follow-up engraftment and chimerism studies, as described by Kricke et al. [2]. Discussions with the International Blood Group Reference Laboratory (IBGRL) concurred that error was a likely explanation, and they agreed to perform ABO grouping on the stored DNA. The donor DNA grouped as O D-negative.

Finally, the possibility of delayed erythrocyte engraftment was explored, although it would be expected to see AB D positive and not A D-negative. Delayed erythrocyte engraftment is usually a risk of a major mismatched transplant where the donor has isohaemagglutinins against donor RBCs [3]. The case described here was a minor mismatch, where the donor has antibodies directed at the recipient. With a minor mismatch and no dual populations present, delayed erythrocyte engraftment was unlikely to be the cause of the unusual result. Furthermore, erythrocyte engraftment has been shown to be faster in children than in adults [4].

Engraftment studies showed that the patient had 100% myeloid and whole blood engraftment confirming that the transplanted cells were what was detected in the patient. Erythroid engraftment can only be directly assessed by separating erythroid precursors from bone marrow samples (such as CD235a-positive selection) [5]; however, bone marrow samples were not available for this patient. Myeloid engraftment has been described as a surrogate marker for erythroid engraftment [5], and given that the patient is fully engrafted in whole blood and myeloid without evidence of any low-level mixed chimerism, it can be concluded that the patient's erythroid cell lineage is also fully engrafted.

Following the evidence, including engraftment and donor DNA analysis, it was confirmed that the cells the patient received were from the intended O D-negative donor and were fully engrafted into the patient.

A literature review found a small number of reported cases of persistent A antigen after HSCT [6–8], always in cases where the donor was group O and the reactions with anti-A were weak. Literature is not vast in this area, and there are not many reported cases.

In 1962, Renton and Hancock [9] reported that certain group O sera completely agglutinated the RBCs of a group A patient transfused with seven bottles of group O blood. Usually, a dual population would be present. They observed the same in an AB patient transfused with a large volume of group O. In the course of their experiments, they demonstrated that this phenomenon of adsorption had occurred in vivo in a number of patients, causing a loose, weak reaction with antisera.

In 1987 Needs et al. [10] described recipient ABH substance on donor-derived group O cells in secretors but not in non-secretors. A, B and H antigens are not only found on the surface of red cells but can be secreted into fluids, such as plasma [8, 11, 12]. Secretor status is decided by the gene *FUT2* and is independent of ABO and *Hh* inheritance, having no influence on haematopoietic tissue [12]. Approximately 85% of the population display secretor phenotype [9]. HSCT recipients who are also secretors will continue to secrete their original group antigen; after transplant by this mechanism, secreted blood group antigens can attach to H chains of the donated/transfused group O cells. Once this has taken place, A antigen on the donor-derived group O RBCs reacts with anti-A antisera and agglutinates RBCs, thus giving the appearance of a group A (or AB if B is also present on RBCs).

In 2012 Vooght et al. [8] wrote an editorial for the American Journal of Haematology where they linked this phenomenon of adsorbed antigen to HSCT patients with persistent detectable A and or B antigens many years after transplant. They also noted the lack of research in the modification of erythrocyte antigens. They discussed the cases of two patients in receipt of HSCT, both displaying persistent A antigen despite 100% white cell chimerism, comparable to the cases in this paper.

Another case was reported by Grey et al. in 2017 [6]. Here they noted a patient presenting as group A D positive 28 months after HSCT with an O D-positive donor. They had persistent weak reactions with monoclonal anti-A despite full donor engraftment at 8 months after HSCT; however, this was assessed using the lymphocyte chimerism only, so it should be interpreted with caution. No chimerism analysis is available at 28 months. They were able to test the sample against different clones of anti-A and found negative reactions could be obtained using the clones LM 297/628 (LA-2) (DiaClon ABO/Rh for newborns) and very weak agglutination with ES131 (ES-15), Birma-1, ES-4 (DiaClon ABO/Rh for newborns). Using adsorption/elution studies, they were able to confirm the presence of A antigen on the patient red cells. They were also able to demonstrate that the patient was typed as Le(a−b−), of which around 85% are secretors in a Caucasian population [9], showing consistency with the theory of adsorption.

Flow cytometric analysis was performed on various transplanted and transfused patients by Hult et al., in 2017 [7]. They demonstrated a higher proportion of acquired antigens expressed by secretors than non-secreting individuals. This was demonstrated for both transfused and transplanted individuals. A raised mean fluorescent intensity (MFI) in line with a higher level of A antigen was observed when cells were treated with anti-A secretor plasma compared to non-secretor. No increase in MFI was detected when tested with anti-B.

Adsorption of antigen onto red cells has not only been described in terms of transfused and transplanted cells [6–8, 10, 11] but can also occur in the rare para-Bombay phenotype [13, 14]. Unlike the traditional Bombay phenotype where ABH is neither secreted nor expressed on RBCs, in para-Bombay, an active secretor gene can be present and ABH substance secreted (but not expressed on red cells) [15, 16]. It is reported that this secreted ABH can adsorb onto red cells resulting in weak antigen expression [13, 14]. When Banerjee et al. [17] reported two para-Bombay B secretor phenotypes using elution studies, they reported that one patient had a reaction with anti-B (signifying the presence of weak B antigen on RBCs) and one did not. If this adsorption can occur for para-Bombay phenotypes and for transfused RBCs, then it can also happen in donor-derived group O cells.

Upon discussion of the case discussed here and the research findings with the multi-disciplinary team (MDT) at GOSH, it became apparent that there was another patient known to the clinical team showing the same results. It was decided to undertake a three-part evaluation.

1. A review of all HSCT recipient patients over a defined period of time to see how far widespread this phenomenon might be.
2. Studies to investigate reactions with available cell lines on a larger patient group (7) than those done by Grey et al. in 2017 (1).
3. Reproduction of the phenomenon in vitro using plasma from the affected patients against group O blood donor RBCs.

Finally, the laboratory will define and introduce a process for managing these patients on the Laboratory Information Management System, ensuring their group is reported safely and that appropriate component groups are selected for the safe management of blood component administration.

TABLE 1 Anti-A cell lines available for investigation via Bio-Rad blood grouping card, and column technology, at Great Ormond Street Hospital

Card	Anti-A clone (s)	Anti-AB
Bio-Rad ID full group	A5	N/A
Bio-Rad ABO/Rh for newborns	LM 297/628 (LA-2)	ES131 (ES-15), Birma-1, ES-4

METHODS

Data review

To ascertain the prevalence of these cases, an extract of all patients undergoing an HSCT at GOSH within the 19 month time frame from May 2019 to November 2020 was obtained. This date range ensured that assessment was made at around 1 year after HSCT, the normal timepoint (at GOSH) for investigating blood group change.

Patients with A or B red cell antigens present prior to HSCT (A, B, AB) that received a group O donor were focused on, as these were the cases with potential to uptake soluble antigen onto donor-derived RBCs. Information was expanded to include the following: most recent grouping pattern, engraftment status, date of most recent sample, immunosuppressive therapy status, transfusion history and diagnosis. This information enabled the identification and separation of the HSCT patients by donor information and current grouping reactions to identify potential cases of adsorbed antigens.

Clones

ABO typing was performed on six of the affected patients using Bio-Rad column agglutination technology and commercially available NHS Blood and Transplant (NHSBT) reagents. All analyses were performed in accordance with manufacturer's instructions via automation, using Bio-Rad IH500 analysers. The cell lines of anti-A contained within the columns of the cards are displayed in Table 1. These are the cards in routine use within the GOSH transfusion laboratory.

Adsorption studies in vitro

Plasma from four of the affected individuals was incubated with group O donated packed RBCs (at a ratio of five parts plasma to one part packed RBCs) to recreate adsorption of secreted A antigen in plasma onto H chains of group O donated RBCs. Plasma from an HSCT patient transformed from group A to O (presumed to be a non-secretor) was used alongside as a control. Samples were incubated at 37°C and read at 1, 24 and 48 h.

TABLE 2 Summary of clinical information for each of the seven identified patients

Patient	Age at HSCT (years)	Indication for HSCT	Days since last clinical follow-up	Status at last follow-up
1	3	Congenital neutropenia	251	Normal neutrophil count. Alive
2	13	Congenital neutropenia	236	Normal neutrophil count. Alive
3	2	HR AML	272	In remission. Alive
4	6 months	*TCIRG* deficient osteopetrosis	256	Normal blood counts and vision. Alive
5	2	SAA	404	Normal blood counts. Alive
6	8	*DOCK 8* deficiency	320	Normal immune function. Alive
7	7	Relapsed JMML		Deceased

Note: Days since follow-up calculated at the time of writing.
Abbreviations: HR AML, high-risk acute myeloid leukaemia; HSCT, haematopoietic stem cell transplant; JMML, Juvenile myelomonocytic leukaemia; SAA, severe aplastic anaemia.

TABLE 3 Summary of patient results, including grouping pattern, at the time of investigation

			Current grouping pattern							
			Forward group	Reverse group	Engraftment (%)					
Patient	Recipient group	Donor group	ABDC	A_1B	BM	CD34	WB	Myeloid	Days since HSCT	Days since last transfusion
1	AB D positive	O D negative	200 0	0 0	NT	NT	100	100	956	937
2	A D positive	O D positive	204 0	0 0	NT	NT	100	100	435	526
3	A D positive	O D negative	200 0	0 3	100	100	100	100	381	557
4	AB D positive	O D negative	200 0	0 0	NT	NT	100	100	351	390
5	A D positive	O D positive	203 0	0 3	NT	NT	>99	100	426	344
6	A D positive	O D positive	203 0	0 2	NT	NT	100	NT	358	350

Note: The clone of anti-A used here was A5 (Bio-Rad ID full group card). Number of days calculated from the time of sample assessment.
Abbreviations: BM, bone marrow; C, control; HSCT, haematopoietic stem cell transplant; WB, whole blood; NT, not tested.

Secretor status

It was not possible to assess the secretor status of the patients as the required reagent (Le^a and Le^b anti-sera) was not available from the supplier at the time the samples were viable.

RESULTS

Data review

A total of 109 HSCTs took place during the 19 months analysed; of these, 19 were group A or AB recipients with group O donors.

Ten recipients expressed B antigen prior to transplant. None of them retained a weak B antigen after transplant. This is in agreement with the flow cytometric analysis of Hult et al.

Of the 19 patients, 8 had transformed blood group; 2 had not engrafted; 1 had no available follow-up data and 1 was after the second transplant, and therefore, on the extract twice.

Of the remaining seven patients, all had weak agglutination with anti-A in their most recent sample. Their clinical information can be viewed in Table 2 and results (at the time of investigation) are displayed in Table 3. Patient 7 met the criteria for inclusion but had received a third HSCT at the time of assessment, and consequently relapsed and so was excluded from further investigation.

Clones

The six patient samples were available for further testing, and the results are displayed in Table 4. All patients displayed weak agglutination with clone A5. Conversely, all were negative against clone LM 297/628 (LA-2). Weak to negative reactions were seen when the samples were run against clone ES131 (ES-15), Birma-1 and ES-4, which is present in the AB column of the Bio-Rad newborn grouping card. Examples of analyser images can be found in Figure 1.

Adsorption in vitro

Despite attempts to recreate the adsorption of antigen onto H chains of group O cells in vitro, sample quality was not viable due to haemolysis; therefore, it was not possible to proceed with this stage of the study.

DISCUSSION

During this study, attempts to replicate this in vitro incubating O cells with group A plasma were unsuccessful, and no measurable uptake of antigen took place. Renton and Hancock [9] linked this to the slowness

TABLE 4 Reactions of each patient against the three available clones of anti-A

Patient	Recipient group	Donor group	Anti-A cell lines			Days since HSCT	Days since most recent transfusion
			A5	LM 297/628 (LA-2)	ES131 (ES-15), Birma-1, ES-4		
1	AB D positive	O D negative	2	0	0	956	937
2	A D positive	O D positive	2	0	1	435	526
3	A D positive	O D negative	2	0	W	381	557
4	AB D positive	O D negative	2	0	0	351	390
5	A D positive	O D positive	2	0	1	426	344
6	A D positive	O D positive	2	0	0	358	350

Note: Clone A5 (Bio-Rad ID full group card), LM 297/628 (LA-2) (Bio-Rad ABO/Rh for newborns), ES131 (ES-15), Birma-1, ES-4 (Bio-Rad ABD confirm card). Number of days calculated from the time of sample assessment.
Abbreviations: HSCT, haematopoietic stem cell transplant; W, weak.

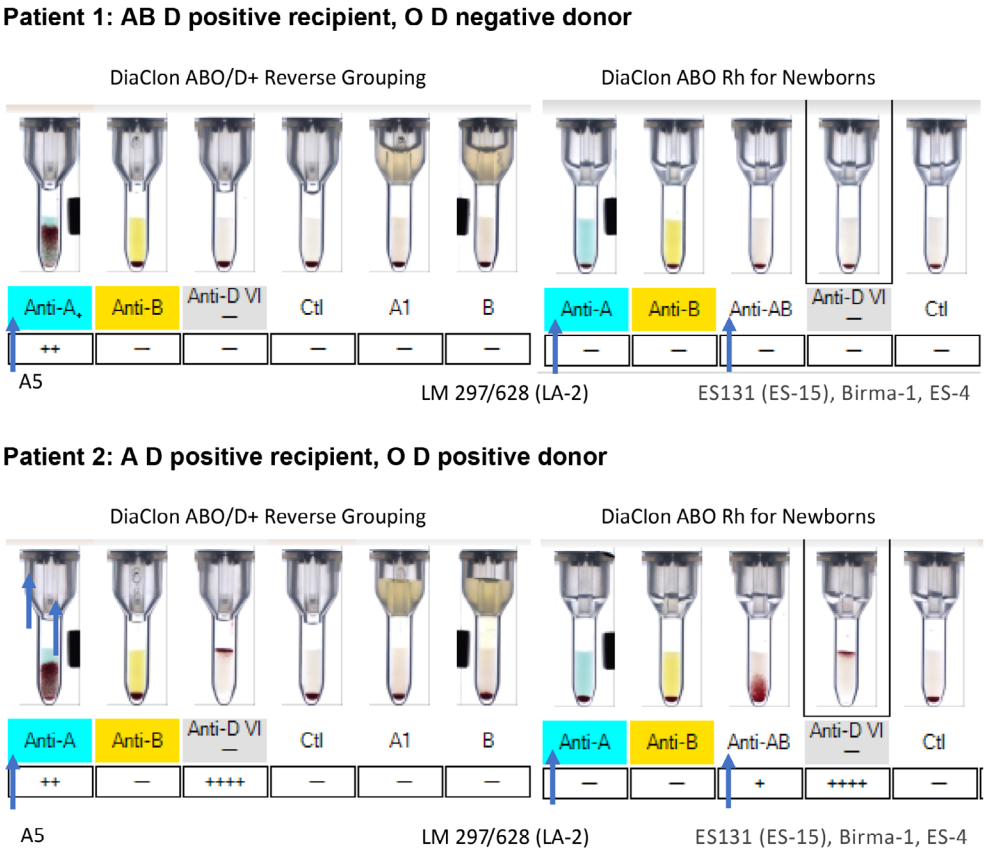

FIGURE 1 Grouping results from patients 1 and 2 using Bio-Rad DiaClon ABO/RH reverse grouping card and Bio-Rad DiaClon ABO/Rh for newborns. Note the different results obtained with the different clones of anti-A

(2 weeks) of adsorption in vivo and the inability to keep cells incubated in vitro for long periods without degradation. They concluded that group O cells could acquire small amounts of A or B substances in circulation. It is this small amount of uptake that is responsible for the weak results and it is postulated that uptake is not replicable in vitro as the available free antigen in the plasma had already saturated patient RBCs in vivo.

In this study, negative reactions were obtained when using monoclonal anti-A clone LM 297/628 (LA-2), weak reactions with clone ES131 (ES-15), Birma-1, ES-4 and weak reactions with the laboratory standard clone A5. This is despite all clones reporting 100% sensitivity and specificity. This has been performed on a larger scale than previously performed by Grey et al. in 2017 [6], and the results support their findings. While the different results from the antibody clones were not researched, further studies are needed to investigate if the results obtained are due to differences in the epitopes recognized by the monoclonal antibodies used or whether owing to a different H

chain conversion mechanism, there are differences in epitope availability on the A antigen. A further point of interest is the clone that yields the strongest reaction, A5, is the clone that is used in the routine forward grouping. In normal circumstances, the other available clones would not be used. Clone LM 297/628 (LA-2) is used in the Bio-Rad confirm card, but this is only used the first time that a patient's sample presents to the laboratory. Clone ES131 (ES-15), Birma-1, ES-4 is found on the Bio-Rad newborn card and is only used for patients under 4 months of age.

In terms of prevalence amongst secretors, Banerjee et al. [17] reported two para-Bombay secretor phenotypes. Not only did they observe adsorbed antigen with a group B secretor, in contrast to the studies and cases reported in this paper, but this adsorption only occurred for one of the two patients. In line with this study data, this also shows that although 85% are said to be secretors, the phenomenon does not occur in 85% of people.

TABLE 5 Criteria that need to be met in order to change a patients group to donor group on the LIMs in the cases of adsorbed A antigen

Criteria for blood group change in patients displaying adsorbed A antigen
Weak A with clone A5
Negative reaction with clone LM 297/628 (LA-2)
Weak or negative reaction with clone ES131 (ES-15), Birma-1, ES-4
Full engraftment by chimerism analysis (use myeloid engraftment as a surrogate where erythroid engraftment is not available)
No dual populations of red blood cells
No antibodies to donor type
No history of blood transfusion within 3 months
DAT negative

This study has shown that around one third of the A to O HSCTs (6/19) were displaying weak A antigen 1 year or more after transplant. This is despite full engraftment in all patients and the absence of immunosuppressive therapy. There was no correlation with diagnosis and no interfering transfusion history. See Table 2.

This phenomenon is far more widespread than previously reported, as six cases have been identified in a single paediatric centre within 19 months, as described in this study, and more cases have been found as time progresses. With no published guidance on management, it is crucial to consider which ABO group components to select for these patients and how best to report their group on the LIMS. As the GOSH LIMS must have an ABO group assigned, the patients will be assigned as the donor group. The basis of this is that giving an A transfusion if the patient were to make anti-A is riskier than giving O plasma/platelets to group A/AB red cells. As a result, the laboratory at GOSH has devised a protocol for handling these patients whereby the group can be amended to a donor group if the criteria in Table 5 are met.

In terms of transfusion, these patients will remain on biocompatible component groups as described in Table 6, and information will be sent on to the respective shared care hospitals. It is difficult to meaningfully assign a blood group, and there is a danger that a new hospital could assign the patient to group A. When a weak reaction is found, standard practice would be to investigate fully before issuing group-specific components, and default components would be suitable for these patients (O RBCs, AB plasma/platelets). While the reverse group does not always correlate in cases of minor mismatch [18], long term follow-up of these patients will show if anti-A isohaemagglutinins are eventually made, although often this is not the case as a lack of anti-recipient antibodies gives way to long-term tolerance of the original blood group [19]. If it were to appear it may mop up the soluble A antigens and the patient could group as an O.

While the secretor status of the six patients could not be determined due to the unavailability of the required reagent, through the

TABLE 6 Appropriate groups to select for patients displaying adsorbed antigen post HCST [20]

				Platelets		FFP/Octaplas	
	Recipient	Donor	Red cells	First choice	Second choice[a]	First choice	Second choice
Major ABO incompatibility	O	A	O	A	AB[a], B, O	A	AB
	O	B	O	B	AB[a], A, O	B	AB
	O	AB	O	AB[a]	A, B, O	AB	-
	A	AB	A, O	AB[a]	A, B, O	AB	-
	B	AB	B, O	AB[a]	B, A, O	AB	-
Minor ABO incompatibility	A	O	O	A	AB[a], B, O	A	AB
	B	O	O	B	AB[a], A, O	B	AB
	AB	O	O	A	A, B, O	AB	-
	AB	A	A, O	A	A, B, O	AB	-
	AB	B	B, O	B	B, A, O	AB	-
Bi-directional ABO incompatibility	B	A	O	B	B, A, O	AB	-
	A	B	O	A	A, B, O	AB	-

Note: Recipients should receive Rh D-negative red cells and platelets except where both donor and recipient are Rh D positive.
Abbreviation: FFP, fresh frozen plasma; HSCT, haematopoietic stem cell transplant.
[a]Choices are listed in order of preference, but stock levels may determine the choice. High Titre -negative platelets should be selected.

comparison of the patient results and clinical picture to those of the authors referenced it can be concluded that passive adsorption of secreted antigen is responsible for these anomalous grouping results. The adsorption is of no consequence as a marker of disease or relapse as all patients remain clinically well and chimerism remains stable. The adsorption is more widespread than previously reported, with at 6 of 19 transplant patients affected and an awareness needs to be raised in the wider HSCT and Transfusion communities. A process has been implemented into the laboratory to manage these patients on the LIMS and ensure component groups compatible with both donor and recipient are given, and shared care centres are aware of the requirements. Along with an awareness of this possibility within laboratories, the presence of defined handling protocols, good communication channels with clinical and other laboratory teams, and the Transfusion Medicine team's participation at multi-disciplinary meetings (MDTs) remain crucial to identifying and managing these complex but not so unusual cases.

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K.C. investigated the anomalous groups, performed data analysis, analysed samples, presented to clinicians and wrote the manuscript. S.K. tracked and provided Molecular Haematology results and reviewed and edited manuscript. A.H. provided data extracts, facilitated meetings between K.C. and clinicians, assisted K.C. in obtaining patient samples and organized training for the clinical team on this subject. K.M. provided clinical opinion and information and reviewed and edited the manuscript. R.M. facilitated meetings between teams involved and the laboratory and edited and reviewed the manuscript.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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SHORT REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

Medication use in Canadian blood donors

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Abstract

Background and Objectives: Policies regarding medication use vary between blood centres. We evaluated medication use in eligible Canadian Blood Services whole blood donors to inform possible process improvements and allow comparisons between donors and the general population.

Materials and Methods: All donors are asked about medication use in the last 3 days, and medications and their reason for use are documented in our donor computer system. Donor computer records were reviewed from January 1, 2020, to March 31, 2022 to extract information on medications by donor age and sex; medications were grouped into therapeutic classes. Stability of medication use over time was determined in a random sample of 100 donors who made at least two donations in the study period.

Results: One-third of successful (eligible) donors were taking medications; of these, 80% were on one or two medications. Five classes of medication accounted for 72% of medication use, and 13 classes account for 93% of use. Use remains relatively stable over time.

Conclusion: Medication use is common, with a few classes accounting for most use. Drop-down lists and storage of information from one donation to the next may enhance efficiency.

Keywords

blood donors, donor criteria, medication use

Highlights

- One-third of successful whole blood donors at Canadian Blood Services are taking at least one medication.
- Of these medications, 72% fell into five main therapeutic classes. If we extend the classes to 13, then 93% of the medications were included. Medications were mainly used to treat chronic conditions or cardiovascular risk factors.
- Drop-down lists and storage of information from one donation to the next may enhance efficiency.

INTRODUCTION

Assessment of medication use and determination of eligibility criteria for donors on medications varies widely among blood centres [1, 2]. As with other donor criteria, the primary reason for determining medication use is to ensure that donors are not taking any medications that could increase their risk of adverse reactions or affect the quality or safety of their blood components. At Canadian Blood Services, donors are asked about certain high-teratogenicity medications on the donor questionnaire (DHQ). Additionally, donors are asked 'In

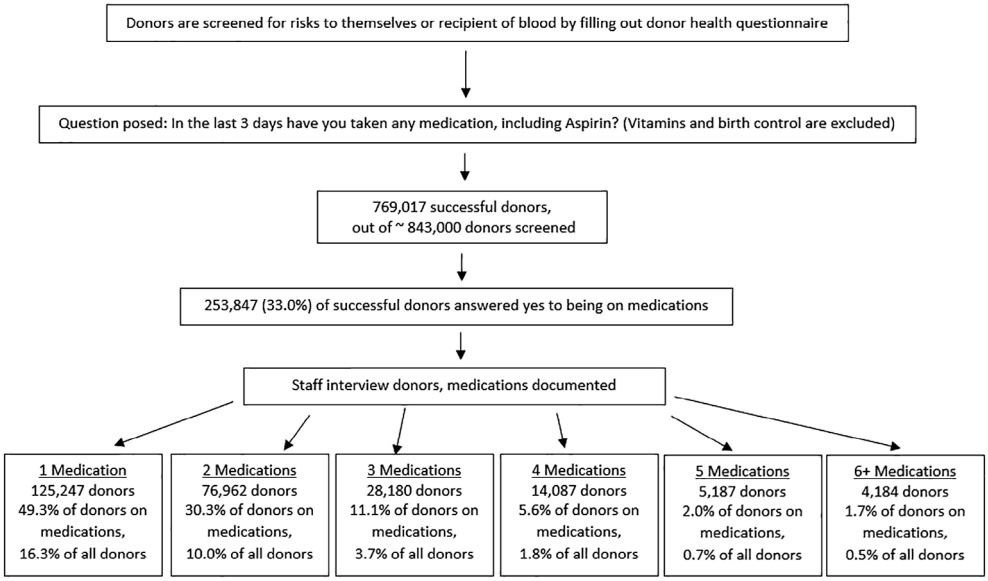

FIGURE 1 Medication use in successful (eligible) blood donors on their first donation between January 1, 2020, and March 31, 2022

the last 3 days have you taken any medication, including aspirin? (Vitamins and birth control are excluded)'. Staff document the medication taken and the reason for use at each donation.

We assessed medication use in donors who were acceptable to donate (termed successful donors) and the stability of medication use over time. These data can suggest possible operational improvements to the way information about medication use is obtained. It also allows some comparison of medication use and underlying health conditions in donors compared with the general population.

MATERIALS AND METHODS

Denominator data on blood donors and data on donors answering yes to taking medications were extracted from The National Epidemiology Donor Database (NEDD), which contains DHQ information from eProgesa (MAK systems) for donations between 1 January 2020 and 31 March 2022 (28 months).

Data on the spelling of medications were corrected (90% accuracy) using R (R Project, Tidyverse), primarily 'string replace all', which allows the user to enter all erroneous spellings of a specific text pattern and replace them with the correct spelling. Only the first donation for each donor in the 28 months period was considered for this portion of the study.

Medications were grouped based on their indications and compared between male and female donors and age groups using the two-proportion *Z*-test. Results were considered statistically significant if $p < 0.05$.

To evaluate the consistency of medication use, a random sample of 100 donors who donated more than once during the study period was generated using R. Medication use of each of these donors was analysed for all donations in the study time frame, and R was used to correct the spelling of all donations.

Approval of our Research and Ethics Board was not required since no additional information was collected from donors and all data are aggregate.

RESULTS

One-third of over three-quarters of a million donors who were eligible to donate answered yes to medication use, with most taking one or two medications (Figure 1). The proportion of donors reporting at least one medication increased with age from 18% in 17- to 25-year-olds, 23% in 26- to 44-year-olds, 40% in 45- to 64-year-olds and 56% in donors 65 and older ($p < 0.001$).

Figure 2 shows the five most common classes of medications in females, males and all donors (usually sex assigned at birth) overall in different age groups. Antidepressants, antihypertensive agents, antilipemic agents and non-steroidal anti-inflammatory agents (NSAIDs) were the four most common classes in males and females; the fifth most common class was thyroid hormones for females and proton pump inhibitors for males. Antidepressant use remains stable, while the use of other medication classes increases with age. The five common classes account for approximately 72% of total medication use.

Figure 3 shows the prevalence of the 13 most common classes of medications, accounting for approximately 93% of overall medication use, by sex. Females used antidepressants, NSAIDs and thyroid hormones more frequently than males (12.2%, 10.4% and 5.8% in females vs. 4.9%, 6.1% and 1.8% in males, respectively). Antihypertensive and antilipemic medication use was more frequent in males (11.2% and 8.6% in males, compared with 6.7% and 3.8% in females, respectively). All differences were statistically significant.

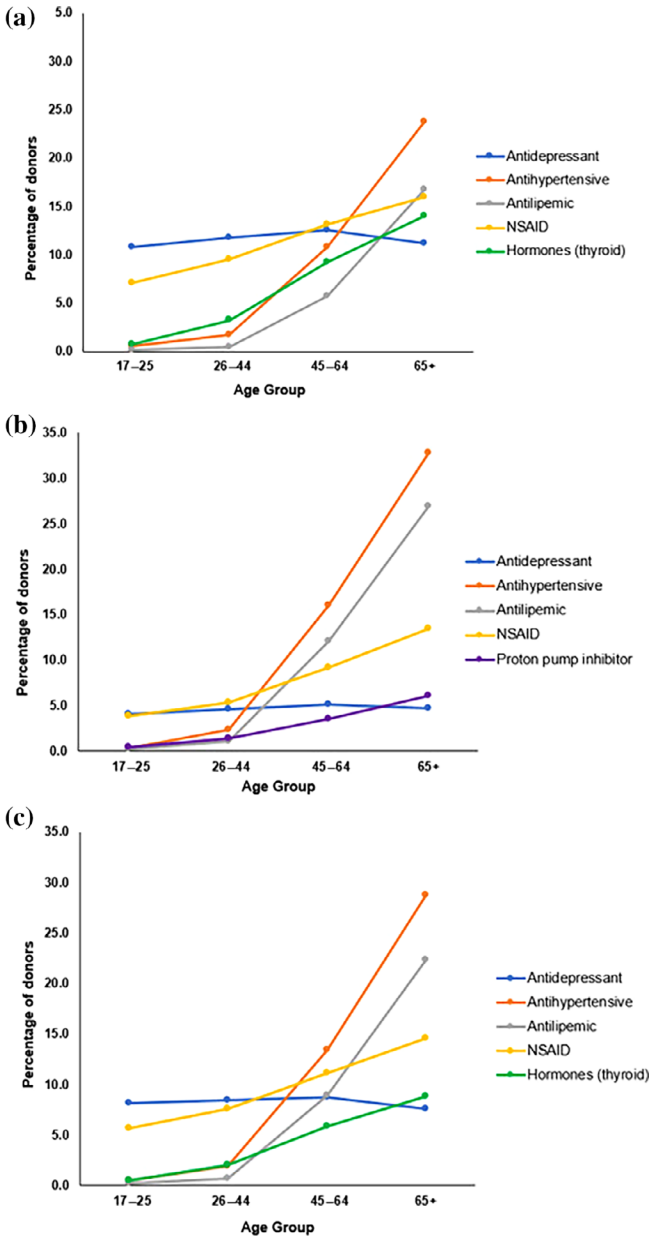

FIGURE 2 Percentage of all successful donors' use of the five most common classes of medications in females (a), males (b) and overall (c) by age group

A total of 3356 donors (0.43%) reported taking iron supplements. Iron use varied from 0.03% in first-time male donors to 0.63% in repeat female donors. This does not include donors taking multivitamin tablets that include iron.

Consistency of medication use on the random sample of 100 donors who donated at least twice in the study period demonstrated that 23 were on identical medications for all donations in the study period, 26 were on the same medications except for addition or subtraction of an over-the-counter NSAID or antihistamine, seven had a change to a different medication in the same class (such as a different antihypertensive medication) and 44 had one or more medications added (35 donors) or subtracted (9 donors) in a new class.

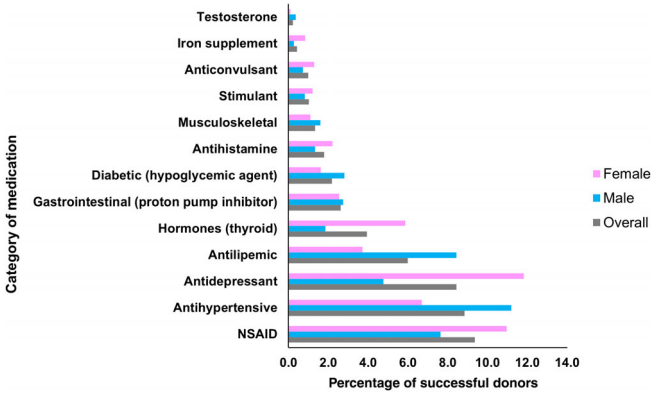

FIGURE 3 Use of the most frequently reported classes of medications in females, males and donors overall. There was a statistical difference between sexes with a significance level of 1%, for all medication classes

The average number of donations per donor in the study period in each of these groups was six.

DISCUSSION

Blood centres vary in their approach to screening donors for medication use. For example, while the United States has a medication deferral list, the United Kingdom, Canada and Australia ask about medication use in the last day, last 3 days or last 12 months before donation, respectively, in addition to questions about the use of specific medications [3–5]. Asking donors about all medication use may on occasion provide some useful information about deferrable underlying conditions missed on screening about health issues. However, as shown in our study, this approach results in a large amount of information to document that is not relevant to eligibility, with one-third of eligible donors taking at least one medication. The relative constancy of medication use over several donations suggests that storing information in the computer system and presenting the donor with the list of medications taken on their last donation would result in a significant improvement in efficiency. Drop-down lists with the most common medications or classes might also enhance efficiency, since most donors are taking medications in a small number of medication classes.

Not surprisingly, most medication use in successful donors is for treatment of chronic common conditions such as depression, hypothyroidism, gastritis and type 2 diabetes, or for cardiovascular risk factors such as hypertension and hyperlipidaemia. Hypertension and hyperlipidaemia are common in the older Canadian population and practice guidelines specify medication use. We hope to obtain data on medication use in the general population from the Canadian Health Measures Survey to determine how closely donors compare with the general population or a selected subset of the general population [6]. This would be useful in assessing how easily studies performed on donors reflect the general population [7].

Iron use is of particular interest. Since iron is lost when donating blood, Canadian Blood Services recommends that donors at highest risk of iron deficiency (donors under the age of 25 years, menstruating females and donors donating frequently) consider taking iron supplements. It is therefore disappointing that less than 0.5% of donors mentioned take iron supplements, considerably lower than levels found in US studies. For example, a study by Cable et al. found that 4% of blood donors in Connecticut were taking iron supplements [8]. It is possible that donors considered supplements to be vitamins, and therefore did not mention them, while in studies focused on donor iron, donors are specifically asked about any iron that they are taking. Implementation of selective ferritin testing in female donors in early 2023 at every tenth donation will provide an opportunity to enhance messaging about iron supplements.

Testosterone therapy may result in polycythaemia and erythrocytosis, mitigated by frequent phlebotomy. Physicians may encourage patients to become blood donors, and in the United States, some centres allow these individuals to donate more frequently. In Canada, these donors are not permitted to donate more frequently than other donors (every 56 days for males), and only 0.4% of male donors were taking testosterone, considerably lower than the proportion found in a US study of 16 blood centres, where 1.6% of the entire donor population was on testosterone therapy [9]. This may be related to lower prescription frequency in males, or a differential donation rate in Canada of donors on testosterone compared with these US centres.

A weakness of our study is that we did not assess medication use in deferred donors. This is often difficult to do, since most unacceptable medications in Canada are given for medical conditions that are themselves reasons for deferral, such as cardiac medications given for unstable angina. A strength of our study is the very large denominator, as well as the operational requirement to document all medications and the reason for their use.

In summary, about one-third of eligible donors are on medication, with most taking one or two medications that are fairly stable over time; a small number of medication classes are responsible for most medication use. These data are useful in considering possible enhancements to donor screening that would save donor and staff time and improve efficiency. Comparisons of medication use in donors and the general population may provide insights on the generalizability of public health studies conducted in the donor population.

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M.G. and S.F.O. conceived the study; O.M. performed data extraction and statistics; M.G., O.M. and S.F.O. wrote the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

Climate change impacts blood supply resilience

As Dr. Lozano recognizes [1], the increasing frequency of climate-related disasters highlights the need for proactive efforts to address the current and future impacts of climate change and promote environmental sustainability. Lessons from the COVID-19 pandemic, which has impacted key blood supplies and blood donations, likewise highlight the importance of additional planning and preparedness for the health impacts of climate change [2].

Two important steps can support the blood sector's efforts to mitigate impacts on systems, providers and patients. First, more attention should be given to blood and blood product supply chains. Such steps as stockpiling key supplies before disasters and emergencies, identifying vulnerable areas and populations, ensuring a resilient and 'diverse' network of suppliers and conducting emergency preparedness training and exercises at the facility and public health systems levels can help to ensure emergency and disaster preparedness. Healthcare systems and blood establishments can work to assess their risks and develop plans and responses for climate-change-related and other emergencies [3, 4].

Second, interdependencies within the blood sector, broader public health system and other critical infrastructure areas (e.g., electricity/energy, water, communications) require collaboration and partnership within the blood sector and with other health and public health professionals and organizations, private businesses, emergency management, national and international environmental organizations, regulatory agencies and others to further assess climate change impacts and prevention, preparedness, mitigation and response activities. The World Health Organization and others rightly emphasize an all-hazards, whole-of-society/whole-of-government approach as common actions and partnerships can bolster preparedness for a wide range of potential emergency and disaster situations [5].

Many resources exist to aid blood sector professionals in their understanding of climate change and potential steps to mitigate how these changes ultimately affect the supply of blood and blood products (see e.g., https://www.lancetcountdown.org/). Dr. Lozano's call for additional steps by blood sector professionals to address the climate crisis and its impacts hopefully will not pass unheeded.

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The author declares that there is no conflict of interest.

DISCLAIMER

The author previously worked on blood policy issues at the US Food and Drug Administration. The opinions expressed are solely those of the author and should not be imputed to any public or private entities.

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GUIDELINES

Vox Sanguinis ISBT International Society of Blood Transfusion

Recommendations for in vitro evaluation of blood components collected, prepared and stored in non-DEHP medical devices

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Abstract

Background and Objectives: DEHP, di(2-ethylhexyl) phthalate, is the most common member of the class of ortho-phthalates, which are used as plasticizers. The Medical Device Regulation has restricted the use of phthalates in medical devices. Also DEHP has been added to the Annex XIV of REACH, "Registration, Evaluation, Authorisation and Restriction of Chemicals" due to its endocrine disrupting properties to the environment. As such, the sunset date for commercialisation of DEHP-containing blood bags is May 27th 2025. There are major concerns in meeting this deadline as these systems have not yet been fully validated and/or CE-marked. Also, since DEHP is known to affect red cell quality during storage, it is imperative to transit to non-DEHP without affecting blood product quality. Here, EBA members aim to establish common grounds on the evaluation and assessment of blood components collected, prepared and stored in non-DEHP devices.

[Correction added on 23 December 2022, after first online publication: The Abstract section was corrected in this version.]

Materials and Methods: Based on data as well as the input of relevant stakeholders a rationale for the validation of each component was composed.

Results: The red cell components will require the most extensive validation as their quality is directly affected by the absence of DEHP, as opposed to platelet and plasma components.

Conclusion: Studies in the scope of evaluating the quality of blood products obtained with non-DEHP devices, under the condition that they are carried out according to these recommendations, could be used by all members of the EBA to serve as scientific support in the authorization process specific to their jurisdiction or for their internal validation use.

Keywords
blood collection, blood components, blood safety, plasma, platelet components, red cell components

INTRODUCTION

Di(2-ethylhexyl) phthalate (DEHP) has been used in blood bags since 1955 to make PVC blood bag systems flexible to allow processing of the drawn donor blood in a closed system into various blood components for the treatment of patients. Concerns about the health effects of plasticizers that could lead to endocrine disruptive consequences have resulted in European legislation aiming to diminish or ban the use of phthalate plasticizers. The new Medical Device Regulation of the European Union (EU) (EU 2017/745 MDR) defines the restrictions relating to the presence of phthalates and other endocrine-disrupting substances in medical devices, such as blood bag collecting systems.

For new devices, the application date for CE marking under the new MDR was due on 21 May 2021, whereas current CE certificates are valid until 26 May 2024. In case the benefit risk assessment (BRA) demonstrates that alternatives would be more hazardous to health, or could be a threat, then an exemption for DEHP may be provided for specific applications.

The European Chemical Agency (ECHA) has, however, recently submitted (10 July 2019, into force 23 November 2021) a recommendation to the European Commission to amend the Authorization List (Annex XIV of REACH, 'Registration, Evaluation, Authorization and Restriction of Chemicals') entries by adding the endocrine-disrupting properties of four phthalates, including DEHP, meaning that some previously exempted uses will require (market) authorization. ECHA indicates that for three of the four phthalates, the endocrine-disrupting properties concern human health only, and so, for blood bags, REACH defers to the medical device legislation for these. Concerning DEHP, however, the endocrine disrupting properties relate to human health and the environment, and therefore, REACH does not defer to the medical device legislation. Consequently, this results in a sunset date for the commercialization of DEHP-containing products after 27 May 2025 (as opposed to 26 May 2024 as per MDR). The European Commission, in collaboration with the Member States and the European Parliament, will make the actual amendment of the entries; companies must apply before 27 November 2023 for authorization to ECHA on exemptions of uses.

In light of this, various DEHP alternatives are being explored that are capable of maintaining similar physical characteristics of the blood bag set, as well as maintaining similar blood component quality (see Appendix S1 for extra background information). Although the various plasticizers that may both derive from the collection, as well as storage bag, do not seem to have a different impact on platelet or plasma quality during storage, they do have a considerably variable impact on the red cell storage lesion. DEHP is lipophilic, allowing DEHP to leach into the storage medium, mainly associating with plasma and red cells, albeit to a lesser degree. Moreover, DEHP incorporates into the red cell membrane [1], resulting in its stabilization [2], and surprisingly, leading to a strong reduction of haemolysis during storage. Also, DEHP was found to reduce microvesicle formation while favourably impacting osmotic resistance and morphology. These changes were found to result in increased survival after transfusion [3]. Replacing legacy storage solutions (for Europe, mainly SAGM) with other additive solutions (Table 1) has been shown to mitigate increased haemolysis levels to varying degrees [4, 5] with some evidence for complete mitigation.

In the last decade, with the upcoming DEHP ban, a range of plasticizer alternatives have been explored (Table 2), with promising candidates in bold. The European Pharmacopoeia of November 2017 has already incorporated four suitable alternatives: BTHC, DINCH, DEHT and TOTM/TEHTM to be used in containers meant for plasma for fractionation. The proposed DEHP alternatives confer satisfactory physical characteristics towards the PVC bags while simultaneously leaching into blood products to a lesser degree. Also, the toxicity of these plasticizers in rodents is decreased by tens to hundreds of folds (SCENIHR report on the safety of medical devices containing DEHP plasticized PVC, 2015). The consequence, however, of the absence of the membrane stabilizing plasticizer DEHP is that RBC haemolysis levels increase during storage (Table 3).

Importantly, and in parallel to the ECHA recommendations, blood bag systems will most likely upgrade to MDR (Medical Device Regulations) Class III, implying that a clinical evaluation will be required for any new blood bag system (from 21 May 2021). In April 2019, EBA answered a consultation by the EU Scientific Committee on Health,

TABLE 1 Additive solutions

Constituents	SAG-M	AS-1	AS-3	PAGGS-M	AS-5	AS-7 (SOL-X)
NaCl (mmol/L)	150	154	70	72	150	
Na_2HPO_4 (mmol/L)				16		12
$NaHCO_3$ (mmol/L)						26
NaH_2PO_4 (mmol/L)			15.5	8		
Citric acid (mmol/L)			2			
Na-citrate (mmol/L)			20			
Adenine (mmol/L)	1.25	2	2	1.4	2.2	2
Guanosine (mmol/L)				1.4		
Glucose (mmol/L)		111	61	47		80
Dextrose anhydrous (mmol/L)	41				41	
Na-gluconate (mmol/L)						
Mannitol (mmol/L)	30	41		55	45.5	55
pH	5.7	5.5	5.5	5.7	5.5	8.5
Osmolarity (MOsm/L)	376	462	291	345	372	237

Environmental and Emerging Risks (SCHEER) on 'Preliminary guidelines on benefit-risk assessment of phthalates' and in June, EBA presented its position at a European Commission Stakeholders meeting in Brussels, as follows:

- There are currently no commercially available validated non-DEHP devices for red blood cell collection and storage with comparable quality to DEHP-containing devices.
- Both recipients and donors (only in case of aphaeresis) are exposed to DEHP.
- An orderly (validation of the whole chain) transition to non-DEHP blood bag systems is needed to guarantee the sufficiency and safety of blood transfusion and transplantation.
- A concerted action between manufacturers, blood establishments and users is needed.
- Time is needed for the proper assessment of alternatives and validation of the blood supply chain from the donor to the final recipient.

In this context, and as there are major concerns in meeting the sunset date, some of the European manufacturers plan to apply for authorization for the continued use of DEHP beyond the sunset date. The intention is to push prolonged authorization for the use of blood bag sets towards the end of this decade in order to allow for an orderly transition to a European non-DEHP portfolio and prevent risks of supply gaps. The authorization strategy has been presented successfully to ECHA, as well as to the European Commission and was well received by both parties.

NON-DEHP BLOOD COMPONENT EVALUATION PHASES

Evaluation of blood components prepared using non-DEHP medical devices can be run through three successive phases.

A **Phase 1** study should be carried out based on positive Phase 0 data (where the manufacturer tests for ISO 3826 compliance and also obtains some [biochemical] quality data) and pertains an initial investigation of the blood components (in vitro evaluation), which should be carried out on a certain minimum number of components (see chapter 4.2). CE-certification of the blood bag system is usually not yet required at this stage. The study should include at least a worst-case scenario to validate biochemical quality parameters and maximum shelf life (which might be combined with a best-case scenario). Components produced during this stage should not be used for transfusion. A Phase 1 study may be restricted with regards to study size and number of quality parameters tested based on the existence of extensive Phase 1 studies from other blood establishments testing the same blood bag system of the same manufacturer (or from Phase 0 data, including biochemical quality data; see Table 4). A restricted Phase 1 study may, for example, be suitable in case of differences between jurisdictions in the maximum allowed overnight hold (48 h vs. 72 h), cold versus warm filtration, and so forth, or when jurisdiction-specific quality requirements are not covered by the adopted results. A restricted Phase 1 study, and the extent of tests performed, may depend on the differences in processing, and as such, comprises a restricted selection of tests as listed in chapter 5.

In a **Phase 2** study, an operational validation should be carried out on a larger number of blood units. CE certification in this stage is usually required in most jurisdictions. Data under routine conditions are gathered. Standard routine quality tests, as required by Guidelines (EDQM Guide and/or local guidelines) are performed. It is advised to already start collecting data on, for example, leakage issues and ease of handling in this phase, although a much larger study size is most likely to be required to be able to statistically compare the incident frequency of the new with current blood bag systems. Components produced during this stage can be used for transfusion if they comply with the routine quality parameters (depending on jurisdiction regulation or authorization process). Usually, Phase 2 is started with

TABLE 2 DEHP alternatives

Plasticizer short name	Full name	Leaching potential	Toxicity	Comments
ATBC	Acetyl tri-*n*-butyl citrate	Higher leaching observed compared to DEHP in medical devices.	Rapidly metabolized. No obvious toxic effects noted in animal models, and no human studies available.	Used in cosmetics and as a plasticizer for PVC. Used in food applications. Has been used in medical devices, including blood bags and tubing.
BTHC	*n*-Butyryl-tri-*n*-hexyl citrate	Slightly lower leaching rates than DEHP (limited data)	Rapidly metabolized. Low toxicity in animal models, and no human studies available.	Uses similar to ATBC, in use in commercially available platelet storage bags.
COMGHA	Castor-oil-mono-, glycerides, hydrogenated, acetates	Slightly lower leaching rates than DEHP	Not completely metabolized, possibly due to limited absorption in the GI tract. Low toxicity in animal models, and no human studies available.	Similar use as DEHP. Approved in EU for food packaging. Listed in the European List of Notified Substances (ELINCS) as no. 451-530-8
DEHA	Di(2-ethylhexyl) adipate	Slightly higher leaching rates than DEHP (limited data)	Reproductive toxicity noted	Has been put on the Community rolling action plan by REACH based on concerns of toxicity
DINCH	1,2-Cyclohexane dicarboxylic acid diisononyl ester	Lower than DEHP	Low toxicity in animal models, limited human studies available. Adverse effects of DINCH metabolites on Human reproductive health. GreenScreen classification is Moderate due to endocrine activity	Shows potential. Similar use as DEHP. Approved in EU for food packaging. Currently in use in commercially available RCC and platelet storage bags. CE certified medical devices for paediatric use
DINP	Di-iso-nonyl phthalate	No data	Similar to DEHP toxicity in animal models, and no human studies available.	Not used in medical devices. Listed in Regulation (EU) No 1907/2006 Annex XVII, 52 and 10/2011. Restrictions on use in toys.
DEHT	Di(2-ethylhexyl) terephthalate	Lower than DEHP	Possibly lower than DEHP in animal models. Only two skin irritation and sensitization studies in humans with no evidence found. GreenScreen classification is low	Shows potential. Similar use as DEHP. Used in toys and other consumer products. No information on use in medical devices, other than some explorative studies
TOTM/TEHTM	Trioctyltrimellitate	Unclear leaching rates compared to DEHP (limited data)	Poorly absorbed and metabolized. Low toxicity in animal models. Some skin sensitization in human skin studies.	Used as plasticizer in electrical wires but also on commercially available platelet and plasma blood bags. Not approved in Europe for food contact.

TABLE 3 Blood bag system considerations

Collection/processing step	Requirements[a]
Blood collection	• Compatibility with mixers, sampling with vacutainer tubes/corresponding sampling
Blood bag systems	• Bottom-and-top and top-top systems • 2-component (whole blood filtration) and 3-component (generation of buffy coat, filtration of separated components) • Apheresis and whole blood systems
Post-collection, pre-processing rest/transport	• Cooling down (37–20°C, butane-1,4-diol plates or similar) • Transport from collection site to processing site • Same-day processing or pre-processing overnight hold in RT
Centrifugation	• Speed up to ~5000 × *g* without leakage: ○ RT for whole blood systems and platelet components ○ Cold (4°C; certain plasma/cryoprecipitate/serum components)
Component separation	• Separation on automated, semi-automated and manual blood component extractors, including compatibility with features such as clamping, sealing, RBC detectors, RBC mixers, hanging/mounting of bag, pressure on bag, breaking of breakaway cannulas • Whole blood separation and manual platelet concentrate processing
Filtration	• RBC • Whole blood • Whole blood/platelet sparing • Plasma • Platelets
Tube sealers	• Sterile closing and easy, ergonomic separation of tube ends, weld integrity
Sterile docking devices	• Sterile docking/welding and easy, ergonomic opening of weld of ○ Same plasticizer tubes ○ Different plasticizer tubes (all combinations of plasticizers in use)
Storage equipment	The material should be suitable for storage in the below-mentioned temperatures, including withstanding relatively fast temperature changes, frozen transport or RT agitation without breaking/leakage etc. • Plunge freezing devices (from RT to −40°C or below within 60 min or similar) • Freezers, <−30°C, FFP storage • Freezers, from −60 to −80°C, cryopreservation of RBC • Refrigerators 2–6°C, RBC storage • Platelet agitators 20–24°C, platelet storage • Plasma thawers (from −70°C to liquid [RT] FFP)
Labelling	• BE labels and manufacturer labels should be easy to attach and stick to the material during the above-mentioned temperature changes.
Secondary processes and sampling	• Compatibility with connection to sets for automated platelet production, pathogen reduction, cell wash and so forth, which may have connection tubes of different plasticizers (see also Sterile docking devices). • Overall quality of secondary processing after storage in new plasticizers/additive solution combinations.
Transfusion	• Compatibility with spikes of transfusion sets, blood warmers and so forth (according to ISO 1135)

[a]Results for the various aspects mentioned in this table should be obtained by manufacturers. Some have to be, however, confirmed in a Phase 2 study (operational validation), such as the ease of use and leakage frequency.

increased frequency of quality control, which can be decreased to standard frequency if the results are satisfactory.

A **Phase 3** study may comprise passive or active haemovigilance surveillance, which depends on the criticality of the change. Also, a Phase 3 study might entail a recovery study. For critical changes, such as a change of plasticizer, active haemovigilance may be required in which a patient population size, monitoring points and alert and termination levels are defined such that unexpected severe increases in adverse event frequency will be detected as early as possible.

Upon implementation of a non-DEHP blood bag system, the question is whether all components are to be validated (i.e., be subjected to Phase 1 and 2 studies) or whether validating a subset of components within each group (i.e., within the RBC, platelet or plasma group) is sufficient. In Table 4, suggestions for the evaluation and validation of the components, as well as the underlying rationale, are provided.

Number of assays and statistical analysis

Currently, the EDQM guide defines no requirement on the minimum number of components to be tested for quality during storage other than suggesting an 'appropriate' number to be defined by the institution itself. One of the reasons why it is difficult to propose a certain minimum number is that based on the variability of certain parameters, as well as study design (paired/unpaired), different study group sizes are required. As such, the minimum number of components to

TABLE 4 Extent of validation per blood component

Red blood cells	
Standard RBCs, leucoreduced from BC-removed WB (RBC leucofiltration) or from WB (WB leucofiltration).	An extensive study is required (i.e., full phase 1–3) per combination of plasticizer and storage medium per manufacturer, preferentially with two different process variables (e.g., processing within 8 h and processing after overnight hold). Based hereon, other blood establishments from other EU countries may adopt the results and internally perform a restricted qualification (Phase 1), taking into account the impact of, for example, possible variation in processing operations between blood establishments.
• Standard RBCs, irradiated (x-ray and gamma) • Standard RBCs, irradiated (x-ray and gamma) and washed	Given acceptable data from a Phase 1 study on the standard RBC component (i.e., similar quality), in which the new and current blood bag sets are compared side by side in a worst-case scenario, a restricted Phase 1 qualification can be performed with a lower number of units (Section 3) and analysis of fewer biochemical parameters (chapter 5.1). Although data is available showing that irradiation of DEHT/PAGGSM RCC leads to a comparable increase of haemolysis levels as compared to its DEHP/SAGM control counterpart [9], little data is available on other combinations, warranting a restricted Phase 1 qualification.
Derived from standard RBCs/other • RBCs, split (paediatric) units • RBCs, washed • RBCs, cryopreserved • RBCs, for intrauterine transfusion • RBCs, for neonatal exchange transfusion • RBCs, from apheresis	Given acceptable Phase 1 data on standard RBCs and Standard RBCs, irradiated (x-ray and gamma; i.e., similar quality), and provided the foil of the bags are identical, an operational change control (Phase 2 study) only as there is no indication these components will be affected.
Platelets	
• Platelets, leucoreduced, pooled from BC • Platelets, leucoreduced, apheresis	Platelets are currently already being stored in non-DEHP storage bags; as such, only DEHP from the collection bag and tubing may leach into the final platelet product. Taking into consideration previous evidence on the minor effect of DEHP plasticizer on platelet components [4, 5], only an operational validation (Phase 2 study) is required unless unconventional DEHP alternatives or storage solutions are used. In such a case, Phase 1 validation is required, as specified in Tables 7 and 8.
Derived from the platelet products above • Platelets, leucoreduced, pooled irradiated • Platelets, leucoreduced, pooled, pathogen reduced • Platelets, leucoreduced, apheresis, pathogen reduced • Platelets, leucoreduced, apheresis, irradiated • Platelets, split (paediatric) units • Platelets, washed • Platelets, cryopreserved	Taking into consideration the argumentation with regards to the standard platelet products from pooled BCs and apheresis, only an operational change control with underlying argumentation is required, followed by routine data collection (Phase 2).
Plasma	
• Plasma, fresh frozen, (leucoreduced), from WB • Plasma, fresh frozen, from apheresis	Plasma factor content stability is not influenced by the plasticizer. As such, various DEHP alternatives are endorsed in the Ph. Eur. An operational validation (Phase 2 study) is required to ensure bag characteristics and processing compliance in the absence of DEHP.
Derived from the plasma products above • Plasma, fresh frozen, pathogen reduced • Plasma, fresh frozen, irradiated • Cryoprecipitate • Cryoprecipitate, irradiated • Cryoprecipitate, pathogen reduced	Taking into consideration the argumentation with regard to the standard plasma product, only routine testing is required (Phase 2).
Whole blood	
• Whole blood (Leucoreduced, platelet reduced) • Whole blood (Leucoreduced, platelet sparing filter)	Whole blood storage for transfusion purposes is gaining renewed attention. Little is known about the effect of alternative plasticizers on the individual components during prolonged contact with other components. Also, little is known of the effect of, for example, lipaemia in the absence of DEHP on whole blood component quality. These uncertainties warrant a thorough approach through a full phase 1–3 validation.

(Continues)

TABLE 4 (Continued)

Red blood cells	
Granulocytes	
• Granulocyte concentrate	Considering the absence of any level of proof of a beneficial effect of DEHP on granulocyte concentrates, we propose that the change of plasticizer of the collection devices (whole blood or apheresis) be assessed according to routine testing (Phase 2, chapter 5.6).

be tested differs somewhat between blood establishments and has become the norm based on historical experience. From an EBA survey among member states, it has become clear that a Phase 1 study containing at least 15 units is acceptable for all participating blood establishments, although some require less. As such, it is suggested to include at least 15 units in a Phase 1 study. Pairing is advised when possible (in case of, e.g., comparing best/worst case scenarios). Based on the results of this study, other blood establishments aiming to use the same non-DEHP blood bag system (i.e., same plasticizer, storage solution and manufacturer) can perform an operational Phase 2 validation (with some destructive testing to check shelf life) and a Phase 3 study.

RATIONALE FOR REQUIRED QUALITY AND PROCESS PARAMETER ANALYSIS PER BLOOD COMPONENT

RBC component quality and process parameters

Haemolysis rates have been reported to increase during storage in DINCH, DEHT and BTHC. Interestingly, for red cells stored in DINCH-PVC and DEHT-PVC in the storage medium SAGM, although resulting in increased haemolysis rates, this was not found to be associated with enhanced potassium leakage and ATP reduction [4, 5]. In another study using the storage medium SAGM, similar haemolysis levels were found when comparing DEHP and DINCH, while BTHC haemolysis rates doubled or tripled [6]. This study also reported a higher rate of osmotic fragility, as well as decreased deformability when storing in BTHC and DINCH. Yet another study [7], also using SAGM, found no differences when comparing haemolysis between DEHP and a di (2-ethylhexyl) 4-cyclohexene-1,2-dicarboxylate (DOTH)/DINCH combination. In this study, baseline haemolysis rate ($t = 0$) was, however, already substantial (0.4%), while some units were close to exceeding 0.8% haemolysis in the third week of storage, which is the maximum amount of haemolysis that European countries adhere to in their guidelines when storing up to 6 weeks (Guide to the preparation, use and quality assurance of blood components, EDQM, 2020). This study also reported comparable ATP, glucose, sodium and potassium levels. These are but a few of the studies that have been conducted, but it exemplifies the heterogeneous results even with the same storage medium. What seems to be clear although is that haemolysis rates are affected slightly to substantially affected in most cases. This seems to be, however, independent of a loss of intracellular homeostasis but mainly a consequence of the absence of membrane stabilization that used to be fulfilled by DEHP. Indeed, osmotic fragility may increase, and deformability may decrease slightly, but glucose consumption, lactate production, ATP levels, 2,3-DPG concentration and sodium/potassium levels are largely unaffected. Although ATP levels may be similar when stored in SAGM-DEHP, SAGM-DINCH, SAGM-DEHT or SAGM-BTHC, it remains that they decrease during storage.

That changing storage media may compensate for the increased haemolysis levels due to the absence of DEHP is exemplified well in a study performed by Graminske et al. [8]. In this study, a substantial increase in haemolysis upon storage in AS-1-DEHT as compared to AS-1-DEHP was reported, while storing in PAGGSM-DEHT resulted in mean haemolysis close to AS-1-DEHP control (0.38% vs. 0.32%) [8]. Although these results seem promising, this study used a DEHP-containing collection set. As collection set-derived DEHP leaches into the whole blood to a certain degree, this will contaminate the produced red cell concentrate, which may affect haemolysis levels during storage. Similar results were reported in two studies by Larsson et al. in which non-DEHP collection sets were used to compare DEHP/DEHT and SAGM/PAGGSM combinations in a regular setting [5] and a corresponding irradiation setting [9]. They reported promising results with haemolysis only slightly higher in PAGGSM-DEHT (0.27 ± 0.03) as compared to 0.23 ± 0.04 in its SAGM-DEHP counterpart 49 days post-collection in the non-irradiated setting. A slightly larger difference was found after irradiation, with 0.35 ± 0.07 in PAGGSM-DEHT as compared to 0.28 ± 0.04 in SAGM-DEHP after a total of 28 days of storage. Last, a recent study by Vermeulen et al. showed very promising results, with PAGGSM-BTHC haemolysis levels being comparable to SAGM-DEHP (0.38 ± 0.12 vs. 0.36 ± 0.17, respectively) [10]. In the same study, a haemovigilance surveillance was performed to track adverse event frequency. No indication for higher rates of adverse event frequency was found in patients receiving non-DEHP RCC transfusions as compared to patients receiving the standard DEHP-containing product.

Clearly, the number of studies that are performed on this subject are numerous but many more combinations of blood bag system, plasticizers and storage media are to be explored. Ongoing research is required to identify and optimize the best possible combination of plasticizer and storage solution. It is critical that the non-DEHP era will not come at the cost of an RBC product that is more rapidly degraded, possibly more harmful for the patient or will lead to reduced storage times and as a consequence, results in more outdating. This would, overall, negatively affect the blood supply. A reduced RBC shelf life could jeopardy the continuous blood supply, especially during crisis, such as the COVID-19 pandemic [11].

TABLE 5 RBC quality parameters

Parameter	Novel additive solution	Novel plastic/ plasticizer
Unit volume (ml)	Required	Required
Haematocrit (L/L) or %	Required	Required
Haemoglobin (Hb) (g/unit)	Required	Required
MCV (Mean corpuscular volume)	Required	Required
Residual WBC (10^6/Unit)	Recommended	Required
Supernatant K^+ (mmol/L)	Required	Required
Haemolysis (%)	Required	Required
ATP	Required	Required
2,3-DPG[a]	Recommended	Recommended
pH	Required	Required
Lactate (mmol/L)	Required	Required
Glucose (mmol/L)	Required	Required
pCO_2 (kPa)	Recommended	Recommended
pO_2 (kPa)	Recommended	Recommended
RMV: RBC microvesicle	Required	Required
Erythrocyte morphology	Recommended	Required
Leachables from plastic film in supernatant and cells[b]	/	Required
Osmotic fragility	Recommended	Recommended
Deformability	Recommended	Recommended
Oxidative haemolysis	Optional	Optional

[a]2,3-DPG analysis kits are currently unavailable.
[b]To be carried out by the manufacturer.

Key considerations

- The absence of DEHP may severely impact RBC quality during storage.
- Many plasticizer and storage solution combinations are available that may affect a range of storage parameters.

Proposal

Considering these elements, we recommend for each unique combination of plasticizer and storage solution to analyse and assess the critical quality and process parameters listed in Tables 5 and 6, respectively, in a Phase 1 study for the standard RBC components listed in Table 4. The quality parameters of RBC are verified throughout the storage period with four control steps between the day of preparation of the final product and the expiry date (date of preparation of the final product (T1) and four checks during storage; i.e., D14, D28, D35 and D42). As mixing due to sampling may, however, potentially affect haemolysis, it is advised to minimize sampling frequency. Also, other control points may be suitable in the case of, for example, irradiated RBC.

TABLE 6 RBC process parameters

Parameter	Novel additive solution	Novel plastic/ plasticizer
Collection time and volume	Required	Required
Storage temperature between collection and processing	Required	Required
Time delay between collection and processing into BC	Required	Required
Temperature during processing steps (collections, transportation, use of cooling plates, etc.)	Required	Required
Centrifugal force (RCF)	Recommended	Required
Centrifugation time	Required	Required
Centrifugal temperature	Required	Required
Time for separation	Required	Required
Temperature at filtration	Required	Required
Height of filtration	Required	Required
Filtration time	Recommended	Recommended
Storage temperature	Required	Required
Mixing during storage (no, yes, number of mixing)	Required	Required
Storage time	Required	Required

PC component quality and process parameters

Platelet concentrates have been stored for decades in devices providing optimum gas permeability, a characteristic for which PVC-DEHP is not suitable. The devices currently used for the preparation and storage of platelets are therefore already devoid of DEHP: PVC-BTHC, PVC-TOTM and polyolefin plastic films. To date, platelet concentrates are likely to contain only a very low concentration of DEHP, its origin being reduced to the transitional phase of collection and storage of the collections. The contact time with the collection device is, in all cases, less than 24 h and equal to the time of collection for apheresis platelets (less than 2 h). For the apheresis process, it can be considered, given the extremely short timeframe of the apheresis procedure, that the contact time is insufficient to represent a significant concentration of DEHP in the final PC, although significant amounts of DEHP from the tubing may still leach into the product.

Many studies published in the 1980s focused on the preservation of non-DEHP PVC platelet concentrates (PVC-TOTM, PVC-BTHC), but with the objective of demonstrating that the increased permeability to gas (O_2, CO_2) of these plastic films represented a real benefit in maintaining the homeostasis, metabolism and functionality of platelets. Even if an effect of drastically reducing DEHP concentration in concentrates was to be observed, it would have been largely masked by the effect of increased gas exchange.

To the best of our knowledge, no study has supported any benefit of DEHP in maintaining platelet homeostasis, or in reducing the effects of aging or in platelet functionality. A first study published by

TABLE 7 Platelet quality parameters

Parameter	Novel plastic/ plasticizer
Volume (dl)	Required
Platelet concentration (g/L)	Required
Platelet content ($\times 10^{11}$/unit)	Required
pH	Required
Mean platelets volume (MPV; fl or μm^3)	Required
Residual WBC (10^6/unit) (dl)	Required
Morphology, for example, Swirl score	Required
Activation/apoptosis, for example, beta thromboglobulin, CD62P (expression or soluble), phosphatidylserine exposure (Annexin V)	Required
Lysis, for example, LDH	Required
Residual red cell count (dl)	Recommended
Plasma/PAS ratio (dl)	Recommended
Metabolic activity: ATP, pH, lactate, glucose, pCO_2, pO_2	Recommended
Function, for example, aggregation thromboelastography/thromboelastometry	Recommended
Cytokines/chemokines	Recommended
Platelet microvesicles	Recommended
Residual content of 'added substances' (e.g., pathogen reduction agent)	Recommended
Leachables from plastic film in supernatant and cells[a]	Required

[a]To be determined by the manufacturer.

Valeri [12] in 1973 shows that the accumulation of DEHP in platelet concentrates has no benefit on platelet viability.

The few studies available show that in the presence of DEHP, the generation of aggregates appears to be reduced [13–15]. The presence of DEHP has no influence on the development of LDH or resistance to hypotonic shock. Labow [16] observed, at the start of the storage period, a difference in the morphological index of platelets in the presence of DEHP, but this observation could not be linked to an effect on functionality. Lagerberg [4] found no significant effect on platelet concentrates prepared from whole blood collected and processed in 100% DINCH devices. That DEHP alternatives do not impact platelet quality was supported by L. Larsson [5], who monitored platelet quality prepared from whole blood collected and processed in a DEHT device. The study did not show any significant difference in quality between platelet concentrates produced with the DEHP device and concentrates obtained with the DEHT device.

Key considerations

- The current contact duration during which the diffusion of DEHP is possible is relatively low for PCs, resulting in only a trace of DEHP in the current platelet components.
- There is a lack of documented benefit provided by the DEHP on the quality and conservation of the PCs.

TABLE 8 Platelet process parameters

Parameter	Apheresis platelets novel plasticizer	Pooled WB platelets novel plasticizer
Collection time	Recommended	/
Storage temperature between collection and processing	Recommended	Recommended
Time delay between collection and processing	Recommended	Recommended
Centrifugal force (RCF)	Required	Required
Centrifugation time	Required	Required
Centrifugal temperature	Required	Required
Temperature at filtration	Recommended	Recommended
Height of filtration (if applicable)	Recommended	Recommended
Filtration time (if applicable)	Recommended	Recommended
Total platelet/storage bag volume ratio	Required	Required
Storage temperature	Required	Required
Storage time	Required	Required

Proposal

Considering these elements, as well as the considerations listed in Table 4 for this product group (i.e., the use of unconventional plasticizer/storage medium), only an operational validation (Phase 2 study) is required unless unconventional DEHP-alternatives or storage solutions are used. Platelets are currently already often being stored in BTHC or DINCH. As such, only when unconventional DEHP alternatives are being used do we recommend for each unique combination of plasticizer and resuspension medium of platelets to analyse and assess the critical quality and process parameters listed in Tables 7 and 8, respectively, in a Phase 1 study for the platelet components listed in Table 4. The quality parameters of PCs are verified throughout the storage period with two control steps between the day of preparation of the final product and the expiry date (i.e., D2, D5, D7). Other control points can be added.

Plasma component quality and process parameters

Plasma, either as FFP by quarantine or for fractionation (PDMPs) is stored frozen in a PVC-DEHP bag. An exception is the plasma treated with amotosalen, as the final storage bag is made of EVA. It is described that the diffusion of DEHP is much higher in plasma than in other components due to the presence of lipoproteins and triglycerides. DEHP is degraded in liquid plasma to MEHP, which is, in fact, a toxic compound. To the best of our knowledge, no study supports any benefit of the presence of DEHP or MEHP during the freezing stages, during storage in frozen form, or in its liquid form after thawing on the in vitro properties of plasma.

TABLE 9 Plasma quality parameters

Parameter	Novel plastic/ plasticizer
Volume (ml)	Required
Residual WBC (dl)	Recommended
Protein after thawing (g/L)	Required
Immunoglobulin (G, M, A) (g/L)	Required
FVIII:C (IU/ml)	Required
Residual platelets (dl)	Recommended
Residual red cells (dl)	Recommended
PT ratio (prothrombin time)	Recommended
Thromboelastography/ thromboelastometry	Recommended
APTT ratio	Required
Fibrinogen (g/L)	Required
FII, V, VII, IX, X, XI (UI/ml)	Recommended
vWf:Ag	Recommended
vWf:RiCof	Recommended
AT III (Antithrombin), Protein C, Protein S	Recommended
TAT/Frag1.2/FPA + FXIIa	Recommended
C3a (mg/L) and C5a (µg/L)	Recommended
C1 inhibitor	Recommended
Alpha-2 anti-plasmin	Recommended
Plasminogen	Recommended
ADAMTS13	Optional
Leachables from plastic film[a]	Required

[a]To be determined by the manufacturer.

Lagerberg concludes that there is no significant effect on the haemostasis parameters of plasma derived from whole blood collected and processed in a DINCH device [4]. In addition, the available in vitro data show that amotosalen-treated plasma stored in a bag devoid of DEHP is fully compliant in terms of regulatory requirements, as well as for in vitro attributes. Larsson monitored the quality of plasma derived from whole blood collected and processed in a DEHT device after preparation on D0 and on D0 and D7 after frozen storage and thawing in a DEHT device [5]. The study did not show any significant difference in haemostasis and biochemistry parameters between the DEHP and the DEHT devices.

Key considerations

- There is a lack of documented benefit provided by the DEHP on the quality and conservation of plasma and its factors.
- Some DEHP alternatives are already authorized in the Ph. Eur.

Proposal

As mentioned in Table 4, no effect of DEHP on plasma factor stability has been documented, and various DEHP alternatives are already listed in the Ph. Eur. As such, only an operational validation (Phase 2 study) is required. If regulatory bodies require Phase 1 validation data, we, however, recommend the below parameters to be tested. The below parameters should be tested throughout the storage period (four control steps between the day of preparation of the final product) and the expiry date (date of the application of a new process [T1] and three checks after frozen storage-thawing on D1–14, at 6 months and at 12 months, respectively T2, T3 and T4). Other control points can be added for the validation of the extension of storage. Tables 9 and 10 list the required quality and process parameters to be assessed for plasma. For each parameter, mean, SD, min, max and median values should be reported.

TABLE 10 Plasma process parameters

Parameter	Apheresis plasma	Whole blood plasma
Collection time and volume	Required	/
Storage temperature between collection and processing	Required	Required
Time delay between collection and processing	Recommended	Recommended
Delay between collection and filtration if applicable	Required	Required
Height of filtration	Recommended	Recommended
Filtration time	Recommended	Recommended
Time between collection and freezing	Required	Required
Storage temperature	Required	Required
Storage time	Required	Required
Time of thawing	Required	Required

WB component quality and process parameters

There is growing interest in the use of whole blood for the treatment of major haemorrhages. In reality, this component is either (a) a unit of whole blood that has been leucoreduced (also removing most of the platelets) resulting in a unit of red cells in plasma, or (b) a unit of whole blood that has been leucoreduced using a platelet-sparing filter so that the majority (>70%) of platelets remain in the final component along with the red cells and plasma. The requirements for validation of WB containers with novel plasticizers will, therefore, be similar to those outlined for red cells, plasma and platelets above. However, the shelf life of WB components is a compromise of the conditions for each component when stored separately. For WB, the shelf life is commonly 14–21 days of refrigerated storage, which is shorter than usual for red cell storage but longer than usual for plasma and longer than usual for platelets but at a lower temperature. It would therefore be appropriate to study the quality parameters of each component within the stored WB, as it is not known whether there is an additive effect of the plasticizer on the components during longer storage or through prolonged contact with other components.

Key considerations

- The effect of DEHP is thought only to have a significant impact on RBCs.
- The storage of whole blood components may be of the duration when the effects of DEHP start to be observed on the reduction of haemolysis of RBCs (14–21 days).
- The presence of a high proportion of plasma in the suspension media of the red cells may allow significant diffusion of DEHP.
- It is possible that a change in plasticizer may have a significant effect on the quality of red blood cells within a whole blood component.

Proposal

Considering these elements, we propose that the effect of changing the plasticizer for whole blood components be assessed in accordance with the tables for the individual components therein.

Cryoprecipitate component quality and process parameters

Cryoprecipitate is manufactured from FFP by slowly thawing plasma at 1–6°C and centrifugation to collect the precipitate. Excess liquid (cryopoor plasma) is removed, and the precipitate is resuspended in a small amount of residual plasma and is then re-frozen for storage. As with FFP, cryoprecipitate is currently stored frozen in a PVC-DEHP bag.

Key considerations

- To the best of our knowledge, no studies have been performed to study the effect of non-DEHP collection systems/storage bags on cryoprecipitate.
- It is assumed that cryoprecipitate will be produced from adequately validated FFP from non-DEHP collections. Therefore, investigators will be aware of any specific losses of clotting factors, which should be particularly considered.

Proposal

Considering these elements and the absence of any level of proof of a beneficial effect of DEHP on cryoprecipitate, we propose that the change of plasticizer of the collection devices (whole blood or apheresis) be assessed as a simple verification of conformance only (Phase 2). Tables 11 and 12 specify the required quality and process parameters for cryoprecipitate. For each parameter, mean, SD, min, max and median values should be reported.

TABLE 11 Cryoprecipitate quality parameters

Parameters	Novel plastic/plasticizer
Volume (ml)	Required
FVIII:C (IU/ml)	Required
Fibrinogen (g/L)	Required
Leachables from plastic film[a]	Required

[a]To be determined by the manufacturer.

TABLE 12 Cryoprecipitate process parameters

Parameters	Novel plastic/plasticizer
Collection time	/
Storage temperature between collection and processing	Required
Centrifugal force[a]	Required
Centrifugal time[a]	Required
Centrifugal temperature[a]	Required
Time delay between collection and processing	Recommended
Time between collection and freezing	Required
Storage temperature	Required
Storage time	Required
Time of thawing	Optional
Time of storage at +1°C	Required

[a]If applicable, that is, siphon or centrifugal method.

Granulocyte component quality and process parameters

Granulocyte components/concentrates are blood components that are currently only produced by a relatively small number of blood establishments. They can be manufactured from whole blood or collected specifically by apheresis and are currently stored in either standard DEHP plasticized PVC bags or in bags designed for the storage of platelets, of which the latter are already devoid of DEHP. These components are stored for 24–48 h due to a decrease in activity and viability.

Granulocyte components stored in non-DEHP platelet bags manufactured from whole blood are likely to contain a relatively low concentration of DEHP, its origin being reduced to the collection and intermediate storage of whole blood/buffy coats. The contact time with the collection device/intermediate storage is likely less than 24 h. For granulocyte components collected by apheresis, the amount of DEHP is also likely to be relatively low, as processing and transfusion are performed as quickly as possible after collection. To the best of our knowledge, no study has supported any benefit of DEHP in maintaining granulocyte activity or viability. In a controlled study, Miyamoto and Sasakawa reported that DEHP decreased both chemotaxis and bactericidal activity, including a comparison to TOTM-PVC [17, 18]. In contrast, Drewnaik et al. described that G-CSF mobilized

TABLE 13 Granulocyte concentrate quality parameters

Parameters	Novel plastic/plasticizer
Volume (ml)	Required
Granulocyte content (10^{10})	Required
Haemoglobin content (g/U)	Recommended
Haematocrit (%)	Required
Platelet content (10^{11})	Recommended
Leachables from plastic film	Recommended
Granulocytes viability	Required

TABLE 14 Granulocyte process parameters

Parameters	Apheresis granulocyte	WB pooled granulocyte
Collection time	Required	/
Storage temperature between collection and processing	Required	Required
Centrifugal force	/	Required
Centrifugal time	/	Required
Centrifugal temperature	/	Required
Time delay between collection and processing	Recommended	Recommended
Time between collection and pooling	/	Required
Added solutions (PAS/Plasma)	Required	Required
Time delay between processing and irradiation	Recommended	Recommended
Storage time	Required	Required
Storage temperature	Required	Required

neutrophils stored for 24 h, have a normal function when tested in assays of respiratory burst, chemiluminescence, phagocytosis, chemotaxis and superoxide anion production [19]. This suggests no harmful effect of DEHP, although this study and others like it were not designed to specifically look at the effect of DEHP. It must, however, be acknowledged that granulocyte components can contain a significant number of both red cells and platelets; however, given the short shelf life of these components (≤48 h), it is not considered that the presence of either red cells or platelets in these components require any special consideration.

Key considerations

- The effect of DEHP is thought to have a significant impact on RBCs only.
- The storage of granulocyte concentrates is of short duration, ≤48 h, and much less than the period from which the effects of DEHP start to be observed on the reduction of haemolysis of RBCs (14–21 days).
- The current contact duration during which the diffusion of DEHP is possible should result in only small amounts of DEHP in granulocyte-containing components.
- There is a lack of documented benefit of DEHP on the activity and viability of granulocytes, with one paper suggesting a negative effect.

Proposal

Considering these elements and the absence of any level of proof of a beneficial effect of DEHP on granulocyte concentrates, we propose that the change of plasticizer of the collection devices (whole blood or apheresis) be assessed as a simple verification of conformance only (Phase 2). Tables 13 and 14 specify the required quality and process criteria for Granulocyte Concentrates. For each parameter, mean, SD, min, max and median values should be reported.

CONCLUDING REMARKS

The deadlines for the transition to DEHP-free blood components are rapidly approaching. A multitude of studies has been performed aiming to address the impact of DEHP absence on the quality of the various blood components. It is clear that mainly red cell components are affected by this change, while platelet and plasma components are seemingly unaffected. It is clear that red cell component quality reduction in the absence of DEHP may be dampened through the use of next-generation storage solutions. Still, many combinations of plasticizer and storage solution alternatives are to be tested to ensure a transition to an optimal non-DEHP product. So far, studies have mainly focused on the major blood components, with little data on the successive impact of, for example, irradiation, pathogen reduction and so forth. As such, in this article, we have aimed to propose a framework, which allows blood establishments to share and rely on extensive international investigations to more efficiently enrol the same non-DEHP blood bag sets across countries. Also, we have aimed to provide a rationale, as well as guidance for the requirement of studying sub-component quality and process parameters so that most jurisdictions' quality requirements are covered and so that they may adopt internationally obtained results. We have refrained from defining quality criteria thresholds, as it is up to the blood establishments themselves to judge whether potential increments in critical blood quality criteria that are still within the ranges specified in the EDQM Guide are acceptable. International adoption of blood product quality data would significantly reduce time constraints in view of the DEHP deadlines, as well as reduce the cost associated with this change. It is clear that the main effort has to be focused on erythrocyte-containing products and that not all sub-components require extensive validation, especially those derived from platelet or plasma components. Even so, ensuring that all blood and subcomponents remain of quality is of prime importance during this large-scale transition. We propose that through extensive international collaboration, the best possible result is to be obtained.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUPPORTING INFORMATION

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

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