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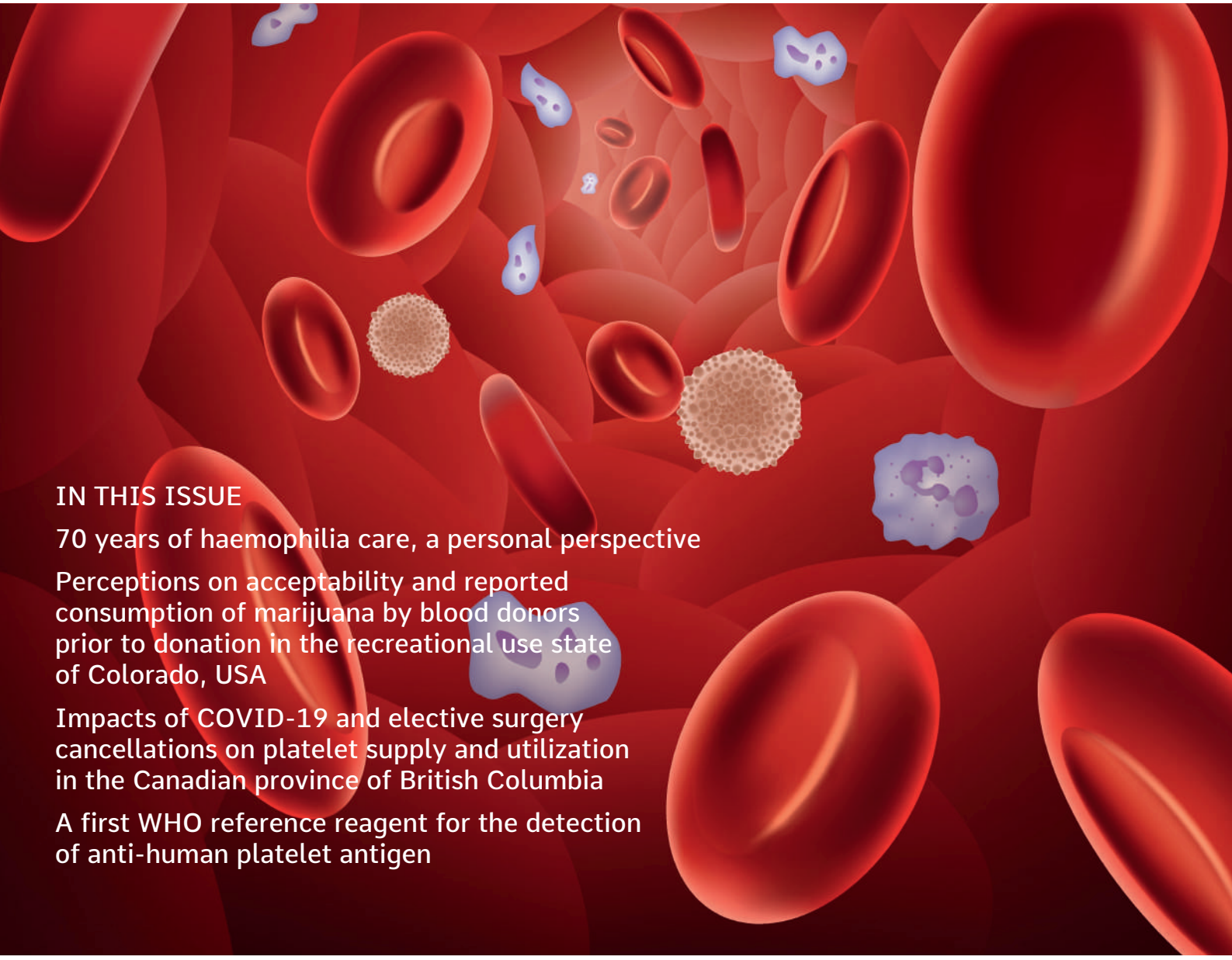

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Aims and Scope

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

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

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

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REVIEW ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Recommendation for validation and quality assurance of non-invasive prenatal testing for foetal blood groups and implications for IVD risk classification according to EU regulations

Frederik Banch Clausen[1,2] | Åsa Hellberg[2,3] | Gregor Bein[4] | Peter Bugert[5] | Dieter Schwartz[6] | Tadeja Dovc Drnovsek[7] | Kirstin Finning[8] | Katarzyna Guz[9] | Katri Haimila[10] | Christine Henny[11] | Helen O'Brien[12] | Agnieszka Orzinska[9] | Kirsten Sørensen[13] | Steinunn Thorlacius[14] | Agneta Wikman[15] | Gregory Andrew Denomme[2,16] | Willy Albert Flegel[2,17] | Christoph Gassner[2,18] | Masja de Haas[2,19,20] | Catherine Hyland[2,12,21] | Yanli Ji[2,22] | William J. Lane[2,23,24] | Núria Nogués[2,25] | Martin L. Olsson[2,3,26] | Thierry Peyrard[2,27] | C. Ellen van der Schoot[2,28] | Christof Weinstock[2,29] | Tobias Legler[30]

[1]Laboratory of Blood Genetics, Department of Clinical Immunology, Copenhagen University Hospital, Copenhagen, Denmark

[2]cfDNA subgroup from the International Society of Blood Transfusion (ISBT) Working Party on Red Cell Immunogenetics and Blood Group Terminology (RCIBGT), Amsterdam, The Netherlands

[3]Department of Clinical Immunology and Transfusion Medicine, Office for Medical Services, Region Skåne, Sweden

[4]Institute for Clinical Immunology and Transfusion Medicine, Justus-Liebig-University, Giessen, Germany

[5]Institute of Transfusion Medicine and Immunology, Heidelberg University, Medical Faculty Mannheim, German Red Cross Blood Service Baden Württemberg – Hessen, Mannheim, Germany

[6]Department of Blood Group Serology and Transfusion Medicine, Medical University of Vienna, Vienna, Austria

[7]Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia

[8]National Health Service Blood and Transplant, International Blood Group Reference Laboratory, UK

[9]Institute of Hematology and Transfusion Medicine, Warsaw, Poland

[10]Finnish Red Cross Blood Service, Helsinki, Finland

[11]Interregional Blood Transfusion SRC, Berne, Switzerland

[12]Clinical Services and Research, Australian Red Cross Lifeblood, Brisbane, Australia

[13]Department of Immunology and Transfusion Medicine, Oslo University Hospital, Oslo, Norway

[14]The Blood Bank, Landspitali University Hospital, Reykjavik, Iceland

[15]Clinical Immunology and Transfusion Medicine Karolinska University Hospital and CLINTEC Karolinska Institutet, Stockholm, Sweden

[16]Versiti Blood Research Institute and Diagnostic Laboratories, Milwaukee, Wisconsin, USA

[17]Department of Transfusion Medicine, NIH Clinical Center, National Institutes of Health, Bethesda, Maryland, USA

[18]Institute for Translational Medicine, Private University in the Principality of Liechtenstein, Triesen, Liechtenstein

[19]Department of Immunohaematology Diagnostic Services, Sanquin Diagnostic Services and Sanquin Research, Amsterdam, The Netherlands

[20]Department of Haematology, Leiden University Medical Center, Leiden, The Netherlands

[21]School of Biomedical Sciences, Queensland University of Technology, Brisbane, Queensland, Australia

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[22]Guangzhou Blood Center, Institute of Clinical Blood Transfusion, Guangzhou, China

[23]Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts, USA

[24]Harvard Medical School, Boston, Massachusetts, USA

[25]Immunohematology Laboratory, Blood and Tissue Bank, Barcelona, Spain

[26]Department of Laboratory Medicine, Lund University, Lund, Sweden

[27]Institut National de la Transfusion Sanguine, Centre National de Référence pour les Groupes Sanguins, Paris, France

[28]Department of Experimental Immunohematology, Sanquin Research, Amsterdam, The Netherlands

[29]Institute of Clinical Transfusion Medicine and Immunogenetics Ulm, German Red Cross Blood Service Baden-Württemberg-Hessen, and Institute of Transfusion Medicine, University of Ulm, Ulm, Germany

[30]Department of Transfusion Medicine, University Medical Center Göttingen, Göttingen, Germany

Correspondence
Frederik Banch Clausen, Laboratory of Blood Genetics, Department of Clinical Immunology, Copenhagen University Hospital, Copenhagen, Denmark.
Email: frederik.banch.clausen@regionh.dk

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Abstract

Background and Objectives: Non-invasive assays for predicting foetal blood group status in pregnancy serve as valuable clinical tools in the management of pregnancies at risk of detrimental consequences due to blood group antigen incompatibility. To secure clinical applicability, assays for non-invasive prenatal testing of foetal blood groups need to follow strict rules for validation and quality assurance. Here, we present a multi-national position paper with specific recommendations for validation and quality assurance for such assays and discuss their risk classification according to EU regulations.

Materials and Methods: We reviewed the literature covering validation for in-vitro diagnostic (IVD) assays in general and for non-invasive foetal RHD genotyping in particular. Recommendations were based on the result of discussions between co-authors.

Results: In relation to Annex VIII of the In-Vitro-Diagnostic Medical Device Regulation 2017/746 of the European Parliament and the Council, assays for non-invasive prenatal testing of foetal blood groups are risk class D devices. In our opinion, screening for targeted anti-D prophylaxis for non-immunized RhD negative women should be placed under risk class C. To ensure high quality of non-invasive foetal blood group assays within and beyond the European Union, we present specific recommendations for validation and quality assurance in terms of analytical detection limit, range and linearity, precision, robustness, pre-analytics and use of controls in routine testing. With respect to immunized women, different requirements for validation and IVD risk classification are discussed.

Conclusion: These recommendations should be followed to ensure appropriate assay performance and applicability for clinical use of both commercial and in-house assays.

KEYWORDS
blood group, cell-free DNA, EU, foetal RHD genotyping, HDFN, quality assurance, validation

INTRODUCTION

In the care and management of pregnancies at risk due to blood group incompatibility, non-invasive foetal blood group genotyping based on the analysis of cell-free foetal DNA from maternal plasma can be used to predict the foetal blood group status [1]. For any diagnostic assay used in medical laboratories, strict rules apply for validation, maintenance and quality assurance. Medical laboratories that offer clinical testing are obliged to assess the information about the performance of an in-vitro diagnostic (IVD) assay provided by the manufacturer or to validate their own *in-house* assay. In the European Union, IVD devices are classified on a scale of increasing risk to patients and the public (class A, B, C or D) [2]. Blood group typing assays for transfusion, transplantation and cell administration are split between the

highest risk class D (ABO, Rh, Kell, Kidd and Duffy systems) and class C (all other blood group systems) [2].

Non-invasive prenatal testing of foetal blood groups can have different clinical objectives. Pregnant women can become immunized against a specific foetal blood group antigen, where the woman can produce IgG antibodies that, either in the current or next pregnancy, can be transported across the placenta and facilitate the destruction of foetal red blood cells leading to haemolytic disease of the foetus and newborn (HDFN) [3]. Non-invasive prenatal testing of foetal blood groups can assist in the management of immunized women, where the determination of foetal antigens helps clinicians to verify the diagnosis and plan for timely treatment. A different objective exists for non-immunized RhD negative women, where antenatal and postnatal anti-D prophylaxis is administered to prevent the women from becoming immunized. In some countries, antenatal prophylaxis is administered to all RhD negative women as universal prophylaxis, despite having no effect or purpose when a woman carries an RhD negative foetus [4]. Rhesus immunoglobulin (RhIg) is regarded as compatible with foetal red blood cells; however, as a blood-derived product, it does possess a potential risk of carrying a contaminant or infectious agent and should therefore be used only when necessary [5]. Moreover, there is worldwide shortage of RhIg [6]. In addition, RhIg donors require active (hyper) immunization, which, from an ethical perspective, further promotes the use of RhIG only when necessary. A non-invasive prenatal test for foetal RhD (NIPT RhD) can identify those women who will benefit from prophylaxis, hereby guiding so-called targeted prophylaxis. As such, the prediction of the foetal RhD status helps by restricting the administration of prophylaxis, thus avoiding unnecessary treatment and unnecessary use of valuable RhIg [7, 8]. Consequently, we find that the clinical objectives as well as the inherent risks associated with the clinical applications of non-invasive prenatal testing of foetal blood groups can be highly different, which should be reflected in the risk classification.

This report considers recommendations for the classification of IVD assays specifically for non-invasive prediction of foetal *RHD* status, when applied as screening of non-immunized pregnant women to guide targeted prophylaxis. In relation to Annex VIII of the In-Vitro-Diagnostic Medical Device Regulation 2017/746 of the European Parliament and the Council [2], we recommend that this IVD should be placed in risk class C.

In addition, we present specific requirements for assay validation and quality assurance. This set of recommendations should be taken into consideration by those responsible for the introduction non-invasive prenatal testing of foetal blood groups in diagnostic laboratories.

METHODS

The authors, consisting of the leading experts in the field, reviewed the literature about IVD assay validation in general and publications about performance studies dealing primarily with foetal *RHD* assays. Recommendations were formulated after discussions. Pertinent issues were discussed among international experts at two conferences, first raised and discussed at the International Meeting of Cell-Free DNA, in Copenhagen 2019, and thereafter discussed by the cell-free DNA (cfDNA) subgroup from the International Society of Blood Transfusion (ISBT) Working Party on Red Cell Immunogenetics and Blood Group Terminology (RCIBGT), at the ISBT meeting in Basel in 2019. This multi-national position paper was circulated among all co-authors, whose comments were then addressed until this consensus was reached. The recommendations of the manuscript are formulated and endorsed by the cfDNA subgroup from the ISBT RCIBGT working party.

RESULTS

Legislation for the performance evaluation of IVD in the European Union

IVD assays for non-invasive prenatal testing of foetal blood groups are medical devices. In May 2017, the In-Vitro-Diagnostic Medical Device Regulation (IVDR, 2017/746) [2] of the European Parliament and the Council about IVDs came into force. After transition periods, this regulation is repealing the previous Medical Devices Directive 98/79/EC [9]. According to the new regulation, IVD is categorized into four risk classes A, B, C and D. In 2019, a corrigendum specified that devices used 'to determine foeto-maternal blood group incompatibility' are included with devices 'intended to be used for blood grouping,' thus placing non-invasive prenatal testing of foetal blood groups under risk class D [10].

Assays that are used for blood group determination in order to guarantee immunocompatibility of blood, blood components, cells, tissue or organs used for transfusion, transplantation or cellular therapy need to fulfil the highest standards for risk class D IVD. In our opinion, non-invasive foetal *RHD* screening belongs to a different category in terms of both severity and frequency of side effects due to unnecessarily given or withheld administration of universal RhIg prophylaxis, compared with side effects from incompatible transfusion due to incorrect blood group determination. It is therefore unnecessary to categorize reagents for the non-invasive determination of the foetal RhD status according to risk class D. In our opinion, these IVDs belong to risk class C. Similarly, as already listed in the IVDR, such reagents are equal to reagents with the intended use to screen for genetic diseases in embryo or foetus, which are placed under risk class C.

To establish recommendations for foeto-maternal blood group testing, published recommendations for the validation of molecular qualitative virus diagnostics tests can be used as reference points [11]. In addition, real-time polymerase chain reaction (PCR) details have been published for non-invasive testing of >50,000 cases of RhD negative pregnant women using several protocols [7, 12–25], demonstrating high assay performance, and from which the extensive experience can be applied to establish specific recommendations.

Reagents, including calibrator and control reagents released for the determination of the blood groups belonging to the ABO, Rh (C, c, D, E, e), and Kell systems, need to fulfil particular requirements. In 2002, common technical specifications (CTS) for IVD medical devices (CTS, Commission Decision 2002/364/EC) were laid down, which include detailed requirements for lot release tests and performance evaluation of antibodies distributed for the determination of blood group antigens [11]. In agreement with colleagues, Institut de Biotechnologies Jacques Boy (Reims, France) [26], Deutsche Gesellschaft für Transfusionsmedizin und Immunhämatologie (DGTI) [27] and the cfDNA subgroup of the ISBT RCIBGT working party, we find that reagents with the intended use 'Determination of the foetal *RHD* status from maternal plasma for targeted anti-D prophylaxis' are not comparable with antibodies used for the determination of the blood group antigen RhD (RH1), which precedes and aims to ensure an RhD compatible transfusion of red blood cells. Manufacturers of non-invasive foetal blood genotyping assays may consider CTS while planning a performance evaluation study; however, the requirements need to be different due to sample specification (plasma instead of whole blood), target molecules (nucleic acids instead of red cells), reagent properties (e.g., oligonucleotides instead of antibodies), methodology (nucleic acid amplification test instead of haemagglutination test) and a different purpose of the test as well as considerably different immunization risks (as further discussed below).

Non-invasive foetal RHD screening

Risk assessment

In the context of blood transfusion, high CTS demands are reasonable because a false-negative RhD determination in blood donors or a false-positive determination in recipients of red cell concentrates may result in anti-D immunization in 3%–70% of cases [28]. In contrast, a false-negative result for the foetal *RHD* status, when applied as screening to guide only targeted prophylaxis during pregnancy, entails the omission of an antenatal anti-D prophylaxis only. If RhD is determined in the newborn (e.g., from cord blood), this error is revealed, and postnatal anti-D prophylaxis is administered accordingly. This approach is associated with an increase of the immunization risk from about 0.2%–0.4% to about 0.7%–1% [29, 30]. Thus, this risk is considerably less than the immunization risk associated with a false-negative result determined with serological blood group reagents in the context of red blood cell transfusion. In some countries, a false-negative NIPT RhD result will cause the omission of both antenatal and postnatal prophylaxis, and consequently, the risk of immunization is higher, around 4%–16% [4, 31, 32], elsewhere stated as a prevalence of 3.5% [1]. In these countries, cord blood serology has been terminated on the basis of high performance of the foetal *RHD* genotyping assay, with diagnostic sensitivities around 99.9% [7]. Taken together, the immunization risk in the context of screening non-immunized RhD negative women is still much less than the anticipated risk in the context of red blood cell transfusion, where also a mismatched transfusion in an alloimmunized recipient can lead to severe haemolytic transfusion reactions. In this risk assessment, it should also be noted that non-invasive foetal *RHD* genotyping has been reported to identify more foetal RhD positive cases which were false negative by standard cord blood serology than causing false-negative results, effectively decreasing the overall risk of immunization [20].

Estimate of the number of specimens required for determining diagnostic accuracy

The total number to be investigated for the determination of diagnostic accuracy is an important figure of any performance evaluation. For the validation of new anti-RH1 (anti-D) reagents, CTS requires a sample of 3000 specimens for a new formulation; or if reagents have been clearly characterized, 1000 specimens still have to be investigated; and if the application field or the mode of use changes, 500 specimens need to be analysed. The CTS focusses on the safety of blood and organ donations and thus requires extremely accurate results when IVD is applied. For this reason, CTS confines to infectious disease tests for blood donation screening and to blood group serology assays. The requirements for the performance evaluation of anti-D sera/reagents also include tests that show that RhD variants are detected.

Based on the risk assessments presented above, a performance evaluation study with 1000 specimens is considered sufficient when a new technology for the determination of the foetal *RHD* status from maternal blood is used (Table 1). With a new technology, assay performance should be evaluated with diagnostic sensitivity and specificity. For such evaluation, it is recommended that the lower limit of the 95% confidence interval of the sensitivity should be ≥99% and that the lower limit of the 95% confidence interval of the specificity should be ≥96%. Cord blood genotyping can be applied to resolve discrepancies.

If a laboratory makes minor modifications to an existing published protocol, fewer specimens are needed. Based on the extensive literature about the application of real-time PCR for the determination of the foetal *RHD* status [7, 8, 12–25, 33–36], with real-time PCR details published for >50,000 cases from several protocols [7, 12–25], the investigation of 100 specimens is sufficient for a performance evaluation of an assay using this well-validated and published real-time PCR technology in pregnancies of at least 10 weeks of gestation. Oligonucleotides and probes for *RHD* exon 5 and 7 have been verified in a multicentre study [37]. The concentration of primer and probes has been previously published by Grootkerk-Tax et al. [12]. Other combinations of *RHD* exons have also been applied successfully [16–18, 21–23, 38–40]. It should be noted, however, that the majority of published studies have been done in predominantly Caucasian pregnancies, and that the accuracy in non-Caucasian, especially in African populations, has not been established yet. In order to minimize manual errors, nucleic acid extraction should be performed with an automated procedure, which has already been validated and published with at least 1000 specimens at another site or in a side-by-side comparison

TABLE 1 Summary of requirements for the validation of assays with the intended use to determine the foetal RHD status from maternal blood

Diagnostic sensitivity and specificity	1000 specimens for a new technology; at least 100 specimens for a well-validated and published technology
Requirement for specimen validation	Plasma from D negative pregnant women, 10–29 weeks, including a general documentation of collection tube type, transportation time and conditions in the validation report
Analytical sensitivity	Dilution series (1-in-2) WHO reference material (NIBSC 07/222) in four replicates, the dilution 1-in-2 must test positive in four of four replicates
Measuring range and linearity	A dilution series (0.5 log) from 100 ng/ml *RHD* positive DNA spiked into *RHD* negative plasma, tested in three replicates, the concentration ≤500 pg/ml plasma must test positive in three of three replicates
Intra-assay precision	Eight replicates from a plasma pool, obtained from *RHD* negative pregnant women with an *RHD* positive foetus, tested in one run
Inter-assay precision	Nine replicates from a plasma pool, obtained from *RHD* negative pregnant women with an *RHD* positive foetus, tested in at least three runs on three different days The tests should be performed by different technicians
Robustness	Three runs, 12 samples per run, six *RHD* positive and six *RHD* negative samples, respectively, from patients or blood donors

with such a validated, automated procedure with at least 100 specimens [13, 14, 16–23, 25]. General pre-analytical issues should be evaluated with great care to optimize sensitivity [15, 37, 41, 42]. The pre-analytical conditions including the gestational week of the validation specimens should resemble those of the clinical specimens that are to be tested after the validation phase.

For Europe, it can be expected that in about 60%–65% RhD negative pregnant women, a positive foetal *RHD* status is determined and, in about 35%–40%, a negative foetal *RHD* status is obtained [13, 18, 20, 22]. According to this distribution, it is acceptable that diagnostic sensitivity and specificity are investigated in any given cohort of RhD negative pregnant women with sufficient accuracy without specifying detailed numbers for *RHD* positive and *RHD* negative foetuses. Analytical sensitivity and specificity largely depend on the genomic target region. Assays that detect *RHD* exon 7 and at least one additional *RHD* exon allow for detection of most *RHD* variants [43, 44]. Other exon combinations have also been applied successfully, and selection of exons may vary for the population. In Europeans, it is not required to test for *RHD* variants, for example, if the woman carries an aberrant *RHD* allele, which is associated with an RhD negative phenotype or a very weak RhD variant (e.g., DEL). In these cases, which are rare or of low frequency, it is still possible to recommend universal antenatal anti-D prophylaxis, and the low rates of variants do not impact on the effectiveness of foetal *RHD* screening programs [45]. However, the development and validation of assays with respect to these RhD variants are encouraged for non-Caucasian populations. In general, laboratories and manufactures should always describe the assay limitations related to variants.

Analytical detection limit

For the determination of the analytical detection limit of a new NIPT RhD assay, World Health Organization (WHO) reference material is commercially available (Product Number 07/222, National Institute for Biological Standards and Control [NIBSC], Potters Bar, Hertfordshire, United Kingdom) [46]. After DNA extraction, a dilution series (1-in-2) of the DNA should be tested with four replicates of each dilution. The dilution 1-in-2 must test positive in all four replicates; the results from higher dilutions are part of the validation dossier and further characterize the assay. Additional calculations of detection limit may be useful to assess a theoretical detection limit of the whole setup [47].

Measuring range and linearity

The WHO reference material is not suitable for the determination of measuring range and linearity because the concentration of *RHD* specific nucleic acids is too low for this purpose. Suitable material is *RHD* positive DNA (preferably from leukocytes from a hemizygous donor) spiked into *RHD* negative ethylenediaminetetraacetic acid (EDTA) plasma at a concentration of 100 ng/ml. A semilogarithmic dilution series is made with DNA in H_2O and then spiked into *RHD* negative plasma before DNA extraction. Each dilution point must be tested with three PCR replicates. A concentration of ≤500 pg/ml plasma must test positive in all three replicates.

Precision

The intra-assay precision should be analysed using a plasma-pool from *RHD* negative pregnant women with *RHD* positive foetuses. The intra-assay precision can be determined in one run with at least eight aliquots of this plasma pool. For real-time PCR, the coefficient of variation for intra-assay precision of cycle threshold (Ct)-values should be ≤5%.

In order to determine the inter-assay precision, a plasma pool as described above is required. In three different runs on three different days, three aliquots of this plasma pool must be tested (in total nine aliquots). Different technicians should perform these tests. For real-time PCR, the coefficient of variation for inter-assay precision of Ct-values should be ≤5%.

Robustness

There are different options to characterize the robustness of diagnostic assays. Therefore, the laboratory or the manufacturer must decide how this topic is addressed. However, experiments designed to evaluate the contamination risk are mandatory. Three subsequent runs with at least 12 samples each should be performed. Every run should comprise at least six plasma samples from *RHD* positive and six plasma samples from *RHD* negative patients or blood donors in a checkerboard pattern.

Quality assurance

A diagnostic laboratory offering the determination of the foetal *RHD* status from maternal blood requires a quality management (QM) system. As part of a total QM system, each shipment of a new lot of reagents must be tested with controls. Recommendations for the storage of reagents are published [15].

If IVD products issued for testing the foetal *RHD* status are assigned to Annex II of Directive 98/97/EG or risk class D of IVDR 2017/746, lot-specific testing and release by a notified body is required [2]. If placed under class C, lot-specific testing and release by a notified body is not required.

Pre-analytics in routine operation

In general, pre-analytic issues need to be carefully assessed as pre-analytical steps may affect the assay sensitivity. The interval of time between venipuncture and separation of plasma from blood cells should be as short as possible when using EDTA tubes and should preferably not exceed 5 days [41]. However, each laboratory should perform their own evaluation of the allowed time duration under the specific transportation conditions used by the individual laboratory. Each laboratory should also perform its own evaluation of storage conditions as well as sample tubes used. For haemolytic specimens, a reduced sensitivity has been described; therefore, plasma needs to be visually checked for haemolysis signs before nucleic acid extraction [41, 42].

Controls in routine operation

It is preferable to use a system for run control for monitoring the extraction efficiency as well as having a control system for amplification efficiency. Various solutions can be selected, and ultimately, each laboratory will have to decide upon a solution that is practical and efficient according to their setup.

For a run control, the recommendation is to collect the remaining plasma from blood tubes after testing for foetal *RHD* and produce a plasma pool in order to use aliquots of this pool, preferably in every run [20]. Due to the smaller fragment size of cfDNA [48], it is inappropriate to use plasma from RhD positive individuals or plasma from RhD negative individuals spiked with *RHD* positive leukocyte DNA. The trend of the run control's amplification signals can be assessed in order to recognize a decreasing analytical sensitivity. The extraction and amplification efficacy should be analysed in each sample by detecting a known target sequence. Maternal, foetal or artificial (added to the specimen or the first extraction buffer) target sequences are appropriate. Recommended measures for quality assurance in routine operation are presented in Table 2.

As a test of reproducibility, the laboratory should participate in an external quality assurance (EQA) scheme. An international scheme has been running as a workshop [49–51]. EQA is now offered from the Danish EQA organizer DEKS. If no EQA is offered on a national level, blinded samples can be exchanged with another laboratory at least once a year.

Assays for non-invasive prediction of foetal blood group status in immunized women

For immunized women, non-invasive prenatal genotyping of foetal gene targets can predict antigen positivity, assisting in the management of immunized pregnant women. The prediction of foetal antigens helps clinicians to verify the diagnosis and plan for timely treatment.

However, there is some degree of variation in how the knowledge of the foetal blood group status is used in the management of the immunized women. It may have a direct impact on indication of treatment and how the pregnant woman is further monitored, or it may have a less direct impact where knowledge of the foetal blood group status is merely a contributing component to the diagnosis, combined with other indications including antibody titres and ultrasound, and further monitoring of the pregnant woman will continue unchanged

TABLE 2 Quality assurance in routine operation

Transportation time	As short as possible, preferably ≤5 days from blood sampling until plasma separation
Test for confounding factors	Visual inspection for haemolysis before nucleic acid extraction
Positive run control	One plasma pool from *RHD* negative pregnant women with *RHD* positive foetuses per run[a]
Negative run control	One plasma from *RHD* negative individual/ plasma pool[b]
Extraction/ amplification control	Either a human housekeeping gene or a heterologous DNA fragment is present during DNA extraction and amplification/ detection

[a]The use of a plasma pool is recommended, not required. If not feasible, an *RHD* positive DNA control is mandatory, preferably diluted to a concentration of *RHD* positive DNA close to the detection limit.
[b]The use of a plasma pool is recommended, not required. If not feasible, an *RHD* negative DNA control is mandatory.

or only slightly decreased. Therefore, depending on each country's management regime, an overall risk assessment is difficult. Still, a false-negative result may convey the risk that a pregnancy is not properly monitored, and a potentially affected foetus may not be discovered and treated in a timely manner. Hence, the risk associated with immunized women concerns the health of the foetus rather than the risk of immunization of the woman as in the context of screening non-immunized pregnant RhD negative women to guide targeted prophylaxis.

The issue of risk classification for immunized women has been discussed by the author group. There are different opinions as to whether testing of immunized women should be placed under risk class C or D according to EU regulations. This relates to different opinions as to how these women should be monitored. Overall, two opinions are prevailing: One placing the testing under class C, where a high level of monitoring is continued despite predicting the foetus to be at no risk of HDFN, and one placing the testing under risk class D, where monitoring of the pregnant woman is substantially decreased or even discontinued when the foetus is predicted to be at no risk. In addition, some favour risk class D because the risk concerns the foetus, whereas others favour risk class C. Outside of Europe, in Australia, the risk classification is class 3, equivalent to the EU class C.

In addition, several different techniques are used for testing of immunized women, unlike the almost universally used real-time PCR for foetal *RHD* genotyping of non-immunized RhD negative women. These techniques include real-time PCR [52, 53], real-time PCR with various modifications [54, 55], matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) mass spectrometry [56], targeted DNA sequencing [57–60] and Droplet Digital PCR [61]. Furthermore, unlike foetal *RHD* genotyping of non-immunized women, the number of cases of immunized women are often much lower and quite rare for antigens other than RhD. Consequently, the number of published cases and protocols is small compared to foetal *RHD* screening as guide for targeted prophylaxis [1].

Therefore, validation of these assays is more challenging, although validation of assays for immunized women can be done using samples from non-immunized women when tested at equivalent gestational age as expected from clinical samples.

We recommend that the risk class decision for assays intended for non-invasive prenatal testing of foetal blood groups in immunized women should be taken locally depending on the jurisdiction of each country, relating to each country's regime for management of immunized women. The validation and quality assurance should generally follow the same principles as recommended for foetal *RHD* status. We recommended that manufacturers put specific claims on their NIPT products, with respect to the need of further monitoring of alloimmunized women if the foetus has been tested antigen negative.

For immunized women, it must be possible to convey a clinical test result from an assay that has been tested only with a low number of clinical cases. In a clinical setting, we recommend that clinicians should be informed of this low number of cases, so that results are warranted by the number of samples tested and clinicians made aware that caution should be taken for result interpretation. A strategy of re-testing can be applied for cases with a negative result to ensure two independent negative results as basis for concluding a foetal blood group status and, thus, no risk of HDFN. In addition, the use of foetal DNA controls to show the presence of foetal DNA in case of a negative blood group genotyping result is strongly recommended [62].

DISCUSSION AND CONCLUSION

Non-invasive foetal *RHD* genotyping has become a standard part of antenatal care for RhD negative women in many countries. Foetal *RHD* genotyping screening programs allow RhD negative women carrying a compatible (RhD negative) foetus to avoid unnecessary administration of prophylactic RhIg and thus avoid exposure to a blood-derived product as well as conserve the valuable RhIg for rational use. NIPT RhD represents one of the first clinical applications of cell-free foetal DNA and has been used as a clinical tool for almost 20 years [63]. High assay performance of real-time PCR assays has been extensively documented in the literature [7, 12–25], exemplifying the large experience with this assay. In contrast, assays for women immunized to blood group antigens other than RhD are reported in smaller numbers in the literature due to the rarity of cases [1].

The risk assessment of the immunization risk in the context of targeted routine antenatal anti-D prophylaxis programs is found to be in a different category when compared to the immunization risk in the context of blood transfusion. In addition, it can be argued that non-invasive foetal blood group genotyping serves different clinical purposes for immunized and non-immunized women, where the risk in immunized women concerns the foetus and the risk in non-immunized women concerns foremost the women (and only later the foetus, if the woman becomes immunized and initiates a new pregnancy). Concerning the potential risk associated with prophylaxis, demanding less strict requirements for assays intended for screening non-immunized RhD negative women will help advance the implementation of screening programs and targeted prophylaxis, thereby avoiding unnecessary treatment and thus overall decreasing the potential risk associated with prophylaxis.

In relation to the EU regulation, we, the leading experts in the field, do not agree that all assays for non-invasive prenatal testing of foetal blood groups should be placed under risk class D. We, thus, disagree specifically with the corrigendum of 2019 [10]. Alternatively, we recommend at least placing non-invasive foetal *RHD* genotyping under risk class C, when applied as a screening of RhD negative non-immunized pregnant women to guide targeted prophylaxis.

We have presented specific recommendations for assay validation in terms of performance evaluation, analytical detection limit, range and linearity, precision, robustness, quality assurance, pre-analytics and use of controls in routine testing. These recommendations are endorsed by expert colleagues and the cfDNA subgroup of the ISBT RCIBGT working party. The resulting recommendations are designed to ensure appropriate assay performance and applicability for safe clinical use.

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T.L. conceived the manuscript; T.L., Å.H. and F.B.C. wrote the draft. All authors contributed with statement discussion and formulation. All authors critically revised and approved the manuscript.

ORCID

Frederik Banch Clausen https://orcid.org/0000-0003-2487-5373
Åsa Hellberg https://orcid.org/0000-0002-7000-1899
Katri Haimila https://orcid.org/0000-0001-8904-666X
Agneta Wikman https://orcid.org/0000-0002-8810-7974
Gregory Andrew Denomme https://orcid.org/0000-0001-8727-1679
Willy Albert Flegel https://orcid.org/0000-0002-1631-7198
Yanli Ji https://orcid.org/0000-0001-6792-5612
William J. Lane https://orcid.org/0000-0002-1097-5229
Martin L. Olsson https://orcid.org/0000-0003-1647-9610

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COMMENTARY

Vox Sanguinis ISBT International Society of Blood Transfusion

Seventy years of haemophilia care: A personal perspective

INTRODUCTION

Having lived for over 70 years with severe haemophilia A, I have taken up the challenge to portray not only my life with haemophilia but also my career in haemophilia and patient advocacy. The result has been a book named, *Surviving Hemophilia, A Road Trip Through the World of Healthcare*. This commentary is an excerpt of the memories, emotions and experiences contained in the book.

MY EARLY HAEMOPHILIA YEARS

Luckily enough, soon after my birth in 1951, I was quickly diagnosed with a spontaneous form of severe haemophilia A. At the same time, my parents were told that I would not grow old, maybe I would reach the age of 20, maybe 30, as there was no effective treatment. Despite this perspective, my parents never patronized me and tried to give me a carefree youth. After a couple of life-threatening bleeds, my physician proposed to treat me with growth hormones. I was 6 years old. This turned out to be an experimental treatment with di-ethyl-stilbestrol (DES), introduced by the French doctor Raymond A. Turpin in the late 40s. At that time, DES was used as a medication for a variety of female reproductive problems and to inhibit growth in adolescent girls. DES caused dreadful anomalies in women and their offsprings; as for me, DES inhibited my linear growth at 1.45 m. This was one of the drastic experimental treatments for haemophilia in the days when there was no treatment at all. I also took part in a more innocent experiment, the use of peanuts or peanut extract, a therapy developed by H. Bruce Boudreaux who himself had haemophilia. Both therapies did not have any positive outcome.

A SPRING OF HOPE

The real turnaround moment for haemophilia treatment came when Judith Pool described how to isolate blood plasma concentrates that contained more Factor VIII on a volume basis than fresh plasma. In 1967, I received my first infusion with Factor VIII concentrates. This had an unexpected effect; I became stuffy and could hardly breathe. I thought I was going to die. I was lying in a single room with the door closed; I tried to scream, but that was impossible because of my thick throat. Luckily enough, a nurse entered my room, she saw what was going on, and I guess she reduced the infusion speed. It was an allergic reaction; I never again had one that serious.

PROPHYLAXIS AND HOME TREATMENT

Gradually, my physician thought that a regular dose of Factor VIII could avoid most of my spontaneous bleeds, and prophylaxis entered my life. It was soon to be followed by transfusions at home that solved my needle fear, one of my early anxieties. I got a normal, regular life and even sports came within reach. In the mid-70s, the Italian doctors Pier Mannucci and Zaverio Ruggeri introduced a winter sport holiday for haemophilia families in the Italian Dolomites. My Dutch haematologist Jan Wouter Ten Cate thought they were crazy, but they invited him to take part in such a holiday. Jan Wouter Ten Cate asked me instead and since then, I enjoyed cross-country skiing together with families of the Italian Haemophilia Society for almost 40 years. Winter sports had been unimaginable for me, for my parents and my doctors. The confidence everyone with haemophilia gained from this experience reflects the freedom and emancipation of the haemophilia community in those years.

THE WINTER OF DESPAIR

All feelings of optimism and freedom disappeared with the viral infections caused by hepatitis C virus (HCV) and human immunodeficiency virus (HIV) in the late 70s and 80s. In the end, these virus infections caused a winter of despair in the international haemophilia community with many thousands of HCV and HIV transmissions. Without an effective treatment for HIV, almost all HIV-infected people with haemophilia died. Early HCV treatments were little effective; only recently, there is a real therapy to treat HCV. For many people with haemophilia, this treatment came into existence too late. They either died or lived with severe liver damage.

COMORBIDITY IN OLDER PEOPLE WITH HAEMOPHILIA

Untreated bleeding episodes caused joint damage already in my early life, and experimental treatment limited my growth. I was infected

Cees Smit (1951) is the author of 'Surviving Hemophilia, A Road Trip Through the World of Healthcare,' Eburon Academic Publishers, Utrecht, 2020.

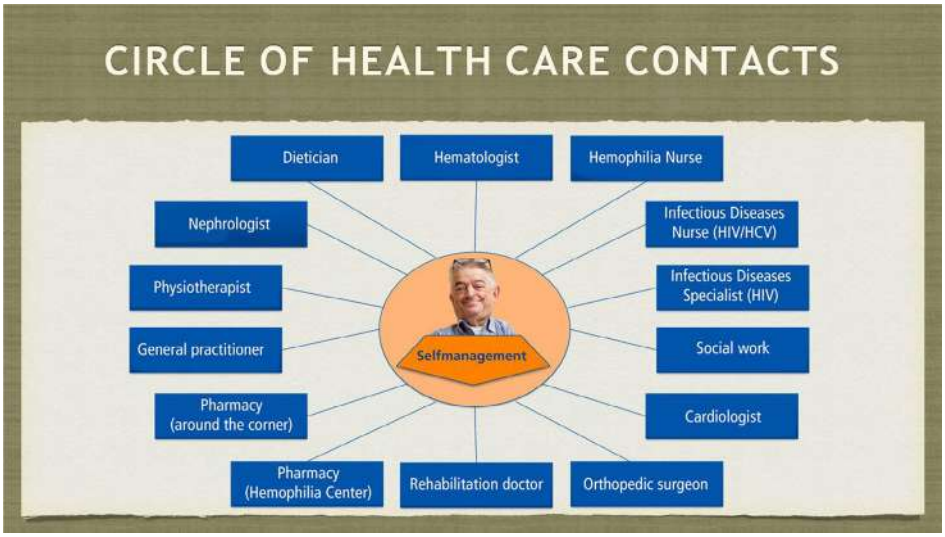

FIGURE 1 An example of healthcare contacts' network of an ageing man with haemophilia

with HCV and HIV, and side-effects of HIV treatment resulted in serious renal complications. I am now a typical example of an ageing man with haemophilia and complex comorbidity. This results in my extensive network of healthcare contacts (Figure 1). The combination of the needle phobia from my childhood, together with comorbidity and polypharmacy issues, creates for me the phenomenon of the 'fear factor', the lack of control when you are hospitalized and when you need medical treatment from physicians who are less familiar with haemophilia. An additional fear is related to my ageing process. I experience my health situation as fragile and easily unbalanced.

COMMERCIALISM OVER VOLUNTARISM

There are some less beautiful sides of haemophilia care and plasma supply. These are the focus of the pharmaceutical industry on profits and the negligence to create a safe blood policy. It is less known that Judith Pool wrote a letter to the Nixon administration about the new US national blood policy. She wrote in 1974: 'My concern [...] is that it in no way requires or even encourages the use of volunteer blood for this purpose but assumes a continuation of the dangerous, expensive, wasteful, and unethical purchase of plasma by pharmaceutical houses to provide such therapeutic material'. Another memorable moment in time can be found in the famous book *Journey*, published by Suzanne and Robert Massie in 1976. They described the battle between Hyland/Travenol (now Takeda) and the American Red Cross over the production of Factor VIII concentrate, a battle that was won by Hyland/Travenol. The Massies consider this as a great loss for the system of solidarity and the voluntary non-remunerated blood and plasma donation. A third striking and neglected fact in the 70s is that the plasma industry ignored that their own factory workers fell ill with hepatitis [1].

A FORESEEABLE DISASTER

The tainted blood history shows that somewhere on this road, people not only ignored an orange, but even a red light. Persons in charge within the international haemophilia community focussed on the availability of Factor VIII to meet demands, while people with haemophilia embraced the freedom they got from their medication. No one wanted to lose these acquired rights. In the 1970s, the plasma fractionation industry knew the dangers of hepatitis viruses in the plasma supply. Even some of their employees warned for these contaminations. This makes it unacceptable to speak of a 'tragic incident' in haemophilia history. Tainted blood was a foreseeable disaster. Numerous accusations have been made against national governments and are still being made in the Infected Blood Inquiry in the United Kingdom [2]. Until now, the international plasma industry has escaped an investigation into the causes of their plasma procurement process and its inadequacies in the 70s and 80s. People with haemophilia and their families still experience the burden of this tragedy. Many died and, those who survived live with ill health for more than 40 years.

A NEW SPRING OF HOPE

The voluntary blood and plasma sector never succeeded in getting control over the blood and plasma supply. On the contrary, the haemophilia market is now a booming business with more than 60 companies active in a billion-dollar market. Plasma concentrates for haemophilia have been replaced by recombinant products, extended half-life products, factor-replacement therapies and gene therapies. For people with haemophilia, new therapies cause uncertainties, while existing products have its relatively unease like intravenous access. Unfortunately, people in three quarters of the world still have no access to haemophilia therapy because of the price of the haemophilia products and its relative scarcity.

GLOBALISM OVER SELF-RELIANCE

For other plasma products, especially immunoglobulins, the world is dependent on the large paid plasma donor pool in the United States. This plasma pool is no longer owned by US-based plasma collection centres but by large international pharma companies. National blood transfusion services gradually lost their share on this market. European countries permitted the selling of their plasma factories, BPL UK and Biotest in Germany, to China's Creat. It shows that globalism has become a dominant factor in the blood and plasma industry and supersedes nation's historical principle of self-sufficiency. At present, dealing with COVID-19, we experience the impact of globalism on our health care. It is a situation that was to be foreseen when we look at the history of haemophilia treatment. History does not repeat itself, but it often rhymes.

COROLLARY

I am glad I have survived these 70 years living with haemophilia, and I must acknowledge that I could have never come this far without support. A large group of professionals, doctors, nurses, my friends, my parents, my family, my partner and her children have always been at my side. I am also grateful to the many donors in the Netherlands who voluntarily donate their blood plasma.

CONFLICT OF INTEREST

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Cees Smit, Patient Expert

Department of epidemiology, Leiden University Medical Center (LUMC), Hoofddorp, The Netherland

Correspondence
Cees Smit, Graan voor Visch 15715, 2132 EM Hoofddorp, The Netherlands.
Email: info@smitvisch.nl

Cees Smit (1951) is the author of 'Surviving Hemophilia, A Road Trip Through the World of Healthcare,' Eburon Academic Publishers, Utrecht, 2020.

ORCID

Cees Smit https://orcid.org/0000-0002-0735-7067

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ORIGINAL ARTICLE

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Determinants of the intention to donate umbilical cord blood in pregnant women

Mariana Fernandes | Guido Alessandri | Rubi Abbad | Caterina Grano

Department of Psychology, Sapienza University of Rome, Rome, Italy

Correspondence
Caterina Grano, Department of Psychology, Sapienza University of Rome, Via dei Marsi, 78, 00185 Rome, Italy.
Email: caterina.grano@uniroma1.it

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Abstract

Background and Objectives: Umbilical cord blood (UCB) donation is a behaviour promoted by many countries' health systems. However, UCB donation is not a widespread behaviour among expectant mothers, and little is known about the reasons that may lead to it. The aim of the present study was to analyse the contribution of Theory of Planned Behaviour (TPB) variables among both primiparous and multiparous women in predicting intention to donate UCB.

Materials and Methods: Three hundred seventy-six expectant mothers completed questionnaires that captured sociodemographic data, parity, previous donation, attitudes, subjective norms, perceived behavioural control (PBC) and intention to donate UCB. Multigroup analysis structural equation modelling was conducted using Mplus (version 8.02).

Results: Multigroup path analyses showed that intentions were strongly predicted by subjective norms and moderately predicted by positive attitudes and PBC in both primiparous and multiparous women. TPB constructs explained 71% of the variance in intentions for both groups.

Conclusions: Future interventions to increase intention to donate among primiparous and multiparous women could primarily consider the influence of partner and significant others in determining positive intentions and secondarily target increasing positive attitudes and perceptions of control.

KEYWORDS
donor motivation, donors, embryonic stem cell

INTRODUCTION

Umbilical cord blood (UCB) banking consists in the collection and storage of the UCB from the placenta and umbilical cord, soon after childbirth [1] to preserve potentially life-saving cells that are usually considered medical waste [2, 3]. Cord blood transplantation is considered a valid alternative treatment in haematologic diseases (malignant and non-malignant), primary immunodeficiencies and metabolic disorders [4, 5], especially in the case of scarce probability of having a compatible donor [5]. Moreover, clinical studies have proved the pluripotent nature of cord blood cells [6, 7], highlighting a wide range of possible clinical applications in neonatology [6, 8], regenerative medicine and immune modulation [9]. UCB can thus be used in the treatment of various life-threatening diseases such as leukaemia, cerebral palsy, neonatal hypoxic–ischaemic encephalopathy and diabetes.

UCB can be stored either in a public bank for allogeneic use (available to anyone who might be compatible) or in private banks for autologous or family use [1]. Several European and US professional

organizations and institutions [10] support and promote the donation of UCB to public institutions for altruistic and solidarity purposes (e.g., patients needing transplantation will be able to rapidly find a matching donor), since the use of cord blood stored in private cord blood banks for autologous use rarely occurs [11]. Although in the previous two decades, many public banks for allogeneic use have been established in Italy, actual donations remain very low, with only 2.2% of parents donating the cord blood to a public cord blood bank [12]. There are several possible reasons for the current low cord blood donation rates. For instance, although women and/or couples are aware of the possibility of donating UCB, they may either have little knowledge of donation possibilities or the procedures to be followed or even be unaware of the uses of UCB [13, 14]. In the view of these premises, it seems particularly relevant to understand the factors that influence expectant parents' decisions to donate cord blood, as this may be essential for encouraging donation.

Considering demographic factors, results of previous studies indicated that educational level and age are positively associated with UCB donation [5, 15, 16], while mixed results are reported on what concerns household income [4, 16].

Although a growing number of studies highlighted the role of psychosocial factors in explaining, predicting and promoting different donation behaviours [17, 18], research on UCB donation is still very limited with only a few studies highlighting a positive association between attitudes and UCB donation [19, 20].

One of the most frequently applied models for predicting donation behaviours and social behaviours, in general, is the Theory of Planned Behaviour (TPB) [21]. In the TPB model, the most proximal determinant of behaviour is considered intention to perform that behaviour [21]. In turn, intentions are determined by attitudes (i.e., an evaluation or appraisal of the behaviour as favourable or unfavourable), subjective norms (i.e., perceived social pressure and expectations from significant others to perform the behaviour) and perceived behavioural control (PBC) (i.e., how much control individuals believe they have over the performance of the behaviour) [21, 22].

Considering the TPB in the more general context of blood donation, a systematic review reported that the model explained between 31% and 72% of the variance in blood donation intentions and between 54% and 56% in blood donation behaviour [17]. Across the studies, attitudes towards blood donation, subjective norms and PBC significantly predicted intention to donate blood, being the influence of subjective norms on intentions less consistent [17]. Furthermore, PBC had the strongest effect in predicting intentions to donate blood [17]. Similarly, in a meta-analytic review [18] including 37 studies on blood donation intentions and 24 predictive studies on blood donation behaviour, intentions had the strongest associations with donation behaviour. In addition, large positive effects were found for attitudes and PBC in predicting intentions, while a medium positive association was observed for subjective norms [18]. Past donations were also positively associated with both intention to donate and donation behaviour [18].

Despite the successful use of the TPB in the context of blood donation [17, 23], to the best of our knowledge, only one study investigated the prediction of cord blood donation within this framework [24]. In this study, attitudes, normative beliefs, subjective norms, awareness and behaviour control were all considered predictors of women's intention to donate cord blood, explaining together 52% of the variance for intention to donate. However, it is not possible to infer the contribution of each of these constructs in this study, as the regression coefficients were not reported by the authors and the results were only summarily described [24]. Other studies have considered only some of the TPB variables [19, 20]. For example, Kim and colleagues [19] examined the knowledge and attitudes of early post-partum women about storage, donation and disposal of their cord blood, as well as sociodemographic factors influencing cord blood donation, and found that knowledge, attitudes, income and source of information influenced cord blood donation. None of these studies has been conducted in the Italian context.

Based on these premises, the present study aims at extending previous research on the predictors of the intention to donate UCB considering primiparous and multiparous Italian women using the well-established theoretical framework of the TPB.

METHODS

Participants

Three hundred seventy-six pregnant women in the third trimester of pregnancy participated in the study. To be included, women had to be in the last semester of pregnancy and be fluent in Italian. Eleven participants with pathologies precluding the donation of UCB were excluded from the study. A total of 365 participants remained.

Procedure

Expectant mothers were recruited through a variety of means (e.g., information centres for maternity and birth, family associations, cultural and sports associations). Participation in the study was anonymous and voluntary, and no incentives or payments were offered. The survey was administrated as an online questionnaire. First, participants read and signed a consent form and then were asked to provide their email address. Afterwards, participants received an email containing a personal code and the web link to access the online questionnaire, to which they had access only through that same personal code. The study was approved by the Ethical Committee of the Department of Psychology of 'Sapienza' University of Rome (Prot. 001066).

Measures

The questionnaire, in addition to sociodemographic information (age, parity, gestation month, current relationship, education level of the expectant mother), included a question evaluating potential illnesses precluding UCB donation (e.g., autoimmune diseases, infectious diseases, etc.). Before starting to answer the questionnaire, women were

asked if they were aware of the possibility of donating or conserving UCB. All these questions were answered on a dichotomous scale (yes/no).

Theory of planned behaviour

Regarding the formulation and scaling of the TPB variables, we followed the questionnaire construction recommendations of Fishbein and Ajzen (2010) [25]. The items used to measure the TPB constructs are listed in Appendix S1.

Attitudes towards UCB donation were assessed using a combination of outcome beliefs measured by 10 semantic differentials: 'Donate the blood cord of my baby would be for me…' 'useless–useful,' 'difficult–easy,' 'disappointing–rewarding,' 'senseless–sensible,' 'disadvantageous–advantageous,' 'unsatisfactory–satisfactory,' 'wrong–right,' 'stupid–wise,' 'unnerving–reassuring' and 'expensive–economical.' Items were scored on a 7-point Likert scale. Cronbach's alpha resulted in 0.91.

Subjective norms regarding UCB donation were measured using a combination of normative belief measures and motivation to comply measures. Subjective norms were measured through five items rated on a 7-point Likert scale, ranging from completely disagree (1) to completely agree (7). Example of items were as follows: 'Most people who are important to me think that I should donate the cord blood of my baby' and 'My partner would approve….' Higher scores correspond to higher perceived social approval. Cronbach's alpha resulted in 0.90.

PBC over UCB donation was measured using a combination of control belief measures and power items. PBC was assessed with three items: 'Suppose you decide to donate your baby cord blood. How easy or difficult do you think it will be?', 'very difficult' (1) to 'very easy' (7). 'Deciding to donate the cord blood of your baby is…' 'not at all up to me' (1) to 'completely up to me' (7) and 'How much control do you feel you have over your decision to donate your baby cord blood?' 'not at all under my control' (1) to 'completely under my control' (7). Items were scored on a 7-point Likert scale with higher scores representing greater PBC over cord blood donation. Cronbach's alpha resulted in 0.95.

Mothers' intention to donate cord blood was measured through five items. An example of an item was 'Do you intend to donate your baby cord blood?' 'I definitely do not' (1) to 'I definitely do' (7). The remaining items asked participants how strongly they wanted to donate, how likely it was for them to though about donate and how much they would like to donate. Items were scored on a 7-point Likert scale with higher scores indicating stronger intentions to donate. Cronbach's alpha resulted in 0.95.

Past donation/storage for primiparous/multiparous woman was evaluated through a dichotomous question (yes/no).

Data analysis

Descriptive analysis was conducted with the statistical program SPSS for Windows version 25.0 (IBM Corp, Armonk, NY). Data are reported as mean ± standard deviation (SD). Categorical data are reported as counts and percentages. To estimate the hypothesized model, we used M*plus* (version 8.02, Muthén & Muthén, Los Angeles, CA) [26] with the Satorra and Bentler [27] scaled chi-square statistic ($SB\chi^2$) and robust standard errors, which takes

TABLE 1 Participants sociodemographic characteristics ($N = 365$)

	Primiparous ($n = 275$)		Multiparous ($n = 90$)	
	N	%	*N*	%
Months of gestation				
Seventh	85	30.9	18	20.0
Eighth	101	36.7	42	46.7
Ninth	88	32.0	28	31.1
Tenth	1	0.4	2	2.2
Education level				
Primary school	–	–	1	1.1
Middle school	30	10.9	17	18.9
Higher school	122	44.4	50	55.6
Degree	123	44.7	20	22.1
Missing	–	–	0.5	2.2
Current relationship				
Married	142	51.6	59	65.6
Cohabitant	119	43.3	30	33.3
Have a partner (not cohabitant)	14	5.1	1	1.1
Mean age (SD)	31.22	5.24	33.09	5.63

Abbreviation: SD, standard deviation.

into account the non-normal distribution of the data (Mplus estimator = MLM). According to a multifaceted approach to the assessment of model fit [28], the following criteria were employed to evaluate the goodness of tested models: the Satorra–Bentler χ^2 likelihood ratio statistic supplemented by the comparative fit index (CFI) and the root mean square error of approximation (RMSEA), with associated confidence intervals. The significance value of χ^2 is sensitive to large sample sizes and easily produces a statistically significant result [29]. Values of RMSEA < .08 and CFI > .90 were considered acceptable [30].

Multiple-group structural equation modelling was used to investigate measurement invariance across the two groups (primiparous and multiparous) [26]. In our approach, the equivalence across the two groups was tested as follows: after evaluating the configural model, each level of invariance was evaluated separately for groups and then simultaneously. The complete sequence of models is shown in Table 3. Overall, configural, metric, scalar and strong invariance [31] were evaluated. The plausibility of the equality constraints implied by the different models was examined with the Satorra–Bentler SB$\Delta\chi^2$ difference test between nested models (i.e., the sex constrained model vs. the unconstrained model [32]).

RESULTS

The final sample was composed of 365 participants, with a mean age of 31.67 (SD = 5.39; age range = 18–44). Primiparous constituted 75.3% (n = 275) of the sample. Sociodemographic characteristics for the entire sample are reported in Table 1. The vast majority of respondents reported being aware of the possibility of donating or conserving UCB (97.5%). Considering past UCB donation, only 2.7% had previously donated UCB (n = 10).

Correlations

Table 2 presents the descriptive statistics and the zero-order correlations among the mean scores on the overall measures of attitudes, subjective norm, PBC, past behaviour and behavioural intention. All correlations resulted significant and in the expected direction, except for those involving past behaviour in the multiparous group. Indeed, past behaviour resulted uncorrelated with all of the variables included in the model. It was, thus, decided to remove this variable from all subsequent structural equation models.

TABLE 2 Zero-order correlations among study variables

Multiparous	ATT	NS	PBC	INT	PB	Mean	SD
ATT	1					6.20	1.42
NS	0.56**	1				4.52	2.28
PBC	0.19*	0.30**	1			5.57	1.99
INT	0.52**	0.66**	0.44**	1		4.62	2.18
PB	0.05	0.05	−0.03	0.04	1	0.87	0.38
Primiparous	**ATT**	**NS**	**PBC**	**INT**	**PB**	**Mean**	**SD**
ATT	1					6.07	1.66
NS	0.66**	1				4.68	2.32
PBC	0.39**	0.45**	1			5.43	1.99
INT	0.69**	0.87**	0.52**	1		4.95	2.32
PB	–	–	–	–	–	–	

Note: **p < 0.01; *p < 0.05.
Abbreviations: ATT, attitudes; INT, behavioural intention; NS, subjective norms; PB, past behaviour; PBC, perceived behavioural control; SD, standard deviation.

TABLE 3 Model fit and comparisons

Title	SBχ2	*scr*	*df*	CFI	RMSEA (95%CI)
M1. Unconstrained model	862.416	1.20	448	0.926	0.074 (0.067, 0.082)
M2. Equal loadings	881.717	1.21	467	0.926	0.073 (0.065, 0.080)
M4. Equal structural paths	885.133	1.21	470	0.926	0.073 (0.065, 0.080)
	SB$\Delta\chi$2		***Δdf***	***p***	
M2 versus M1	22.12		19	0.28	
M3 versus M2	3.42		3	0.31	

Abbreviations: CFI, comparative fit index; CI, confidence intervals; RMSEA, root mean square error of approximation.

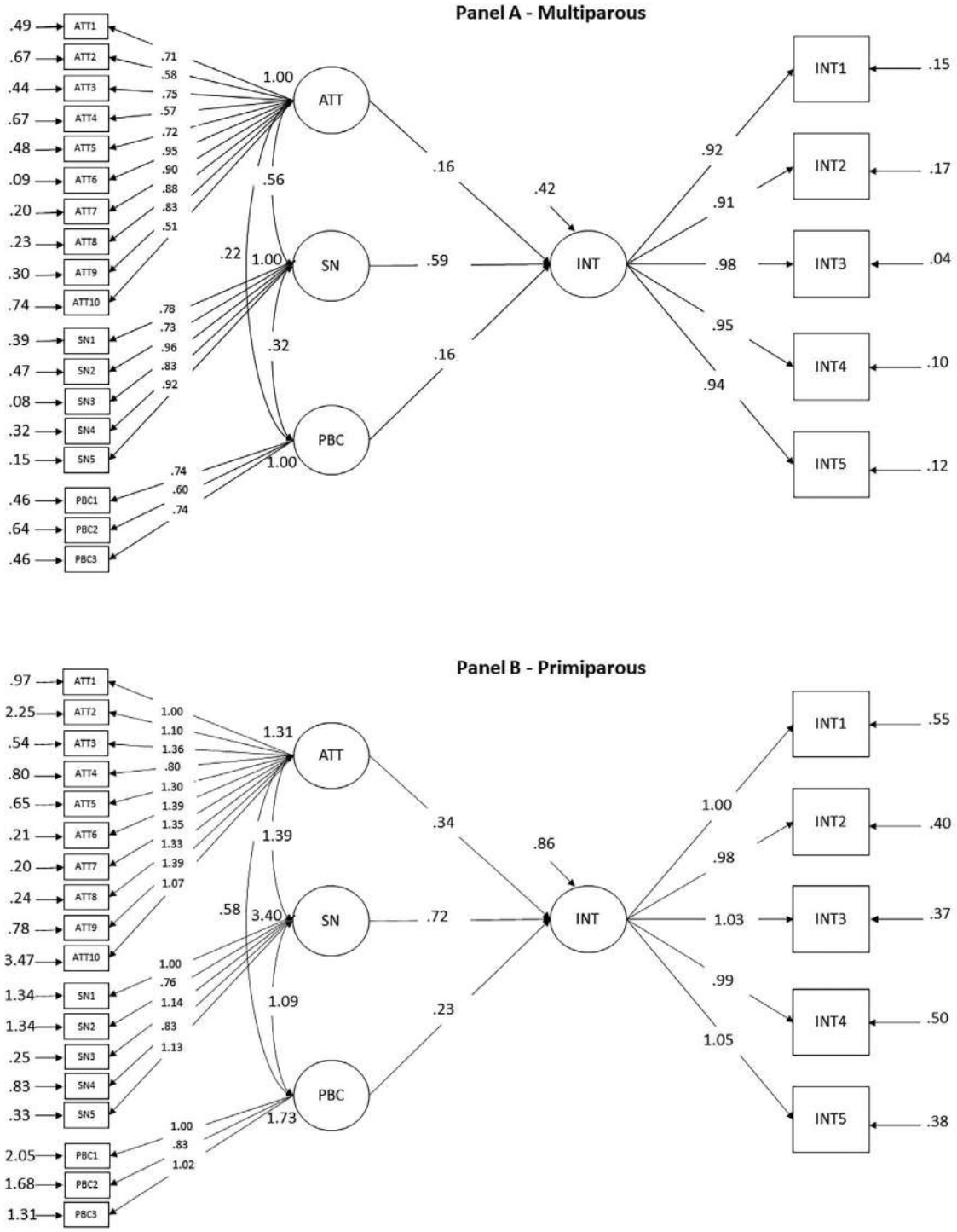

FIGURE 1 The hypothesized model with completely standardized estimates for (a) multiparous and (b) primiparous
Note: ATT, attitudes; PBC, perceived behavioural control; INT, behavioural intention; SN, subjective norms

Structural equation analysis

Our theoretical model was tested as a multiple-group structural equation model simultaneously on primiparous and multiparous groups. In this model, attitudes, subjective norms, PBC and behavioural intention were specified as latent variables saturated, respectively, by 10, 5, 3 and 5 items. Parameters were all freely estimated across groups with no restriction imposed. As reported in Table 3, this model fitted the data very well. Following standard procedures, first, across-group invariance was imposed on factor loadings and then on structural regression paths. Overall, these constraints did not change the fit of the model (See Table 3, bottom part). The final model with all restriction included is represented in Figure 1, panels (a) and (b). Overall, behavioural intention was strongly predicted by subjective norms,

moderately predicted by attitudes and PBC. Subjective norms and attitudes were strongly correlated, whereas there were moderate correlations between subjective norms and PBC and between attitudes and PBC. Explained variance was high ($R^2 = 0.71$ in both groups).

DISCUSSION

Stemming from the TPB, the aim of the present study was to explore the extent to which attitudes, subjective norms and PBC influence the intention to donate UCB in Italian primiparous and multiparous pregnant women [21].

For decades, TPB has been applied to effectively explain, predict and promote blood donation behaviour [17, 18], but, surprisingly, only one study used this framework to predict blood cord donation [24]. The present study findings extend previous research in the Italian context supporting the robustness of the TPB model in explaining blood cord donation intentions. The fit indices across the cross-sectional analyses confirmed the model structure both in primiparous and multiparous women and the invariance across the two groups. Together, the variables included in the TPB model explained 71% of the variance in intentions to donate UCB in both samples, with subjective norms being the strongest determinant of intentions and attitudes and PBC significantly contributing to explaining intentions both in primiparous and multiparous women. This amount of explained variance is consistent with the results reported in other studies on blood donation [17]. Unfortunately, regarding cord blood donation in specific, further comparisons are limited, since Natan's study [24] suffers from a few methodological issues, namely because items were ill-defined and regression results were only partially reported.

Differently from previous studies on blood donation [17, 18], it seems that subjective norms play a stronger role in determining intentions in the present study. The direction of the path from subjective norms to intention was positive, implying that higher perceived social approval for cord blood donation results in higher intention for UCB donation. This finding is not surprising considering that blood cord donation is generally a shared decision within a couple and that subjective norms also encompassed a partners' approval measure. In fact, previous studies showed that pregnant women consider their husband/spouse to have a key role in the decision about cord blood donation [4, 33]. Based on these findings, it would be desirable that future studies should also include the perspective of the partner and partner–actor interactions in determining blood cord donation intentions. Other explanations for this finding are also possible, such as the fact that although cord blood donation is well known, the women underline the need of knowing more clear procedures on cord blood banking processes [34] and turn to relevant others to form their intentions. Moreover, consistently with other studies on blood donation [17, 18], attitudes and PBC were also found to have a predictive role on intended behaviour donation.

Some practical implications can be drawn based on the present findings. Interventions directed towards raising UCB donation intentions may need to target relevant others (partner, families and health professionals) as their opinion may influence expectant mothers intentions. Interventions should also consider increasing positive attitudes towards cord blood donation and enhance women's perceptions of control over this behaviour. Targeting these constructs may be particularly relevant, given that cognition-based interventions have been shown to be effective in other contexts, such as blood donation [35]. Although the present study did not consider whether expectant mothers were counselled by health care providers to donate their cord blood, future studies could examine whether perceptions of control and social pressure change as a result of the counselling sessions received.

For example, educational interventions and motivational interviews to decrease ambivalence and resistance towards UCB donation may be considered as means to increase intentions to donate.

The present study has some limitations that need to be acknowledged. First, despite the fact that assessing UCB donation behaviour would have been optimal, it is important to note that only 2.2% of women donates UCB in Italy donate UCB [12], similar to other countries [36, 37]. Therefore, a much larger initial sample would have been necessary to estimate the intention–behaviour pathway. Nevertheless, we know from previous studies and meta-analyses on TPB that intentions generally range from 13.8% to 29% for different health behaviours (e.g., physical activity, diet, breastfeeding, safe sex, abstinence from drugs) [38, 39] and from 19% to 38% for blood donation behaviour [18]. Future population studies are needed to evaluate how behavioural intentions will determine actual donations in the specific context of UCB donation. Secondly, the study considered a convenience sample. Participants were expectant mothers who volunteered to be part of the study, and they may differ from mothers who were not available (i.e., a general tendency to be altruistic). Therefore, caution is needed in generalizing. Thirdly, the present research focussed on testing the prediction of intentions within one model, the TPB. Thus, the structural paths examined were those specified when following this framework. Evaluating the contribution of other models, as well as integrating the TPB model with other constructs (self-efficacy, self-identity, etc.) could hypothetically generate distinct results. Finally, although previous studies in the blood donation context reported that past behaviour was predictive of intentions, this variable could not be used in the present study because only 2.7% of participants had donated UCB in the past.

In conclusion, the results of the present study demonstrate the usefulness of TPB in predicting intentions to donate UCB and highlight the importance of partner and significant others in forming positive intentions to donate UCB. Unlike other studies on blood and organ donation [17, 18, 40], it appears that subjective norms play a stronger role in determining intentions in the context of UCB. This is consistent with other research on bone marrow donation [41]. These aspects, along with positive attitudes and perceived control over cord blood donation, should be considered when planning future interventions to increase donation intentions.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ORCID

Mariana Fernandes https://orcid.org/0000-0002-7005-2805
Guido Alessandri https://orcid.org/0000-0003-4731-5248
Caterina Grano https://orcid.org/0000-0002-1899-0773

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Perceptions on acceptability and reported consumption of marijuana by blood donors prior to donation in the recreational use state of Colorado, USA

Kyle Annen[1,2] | Melkon G. DomBourian[1,2]

[1]Department of Pathology, University of Colorado School of Medicine, Aurora, Colorado, USA

[2]Department of Pathology and Laboratory Medicine, Children's Hospital Colorado, Aurora, Colorado, USA

Correspondence
Kyle Annen, Department of Pathology and Laboratory Medicine, Children's Hospital Colorado, 13123 East 16th Avenue, Aurora, CO 80045, USA.
Email: kyle.annen@childrenscolorado.org

Abstract

Background and Objectives: Blood donor opinions and behaviours regarding marijuana use are not well known nor is the potential impact to the blood supply. We sought to assess opinions and frequency of marijuana use in proximity to blood donation via a survey of blood donors at a hospital-based blood collection site in a state where recreational marijuana use has been legal since 2012.

Materials and Methods: Blood donors at least 18 years of age who donated between 2014 and 2019 were surveyed electronically, with all responses kept anonymous to encourage engagement and accurate reporting.

Results: Overall response rate was 8.03% (12,186 surveys sent with 979 responses). Of responding donors, 23.5% indicated that they felt that consuming various forms of marijuana was acceptable prior to blood donation. Marijuana use <72 h prior to blood donation was reported in all demographic groups surveyed except age 18–24 years. Of donors who reported daily marijuana use, 47.4% indicated >20 donations and 52.6% indicated apheresis platelet donation.

Conclusion: Nearly one quarter of responding blood donors feel that marijuana use is acceptable prior to blood donation, and nearly every demographic group surveyed indicated use of marijuana <72 h prior to donation. These results suggest the need for additional research to determine if marijuana-related metabolites in collected blood products negatively impact recipients, particularly vulnerable populations such as children and pregnant women. These results may inform whether changes in donor screening or testing for marijuana use are warranted.

KEYWORDS
blood donors, cannabis, marijuana, THC

INTRODUCTION

It is currently unknown whether blood donors in the United States use marijuana prior to blood donation and if so, in what frequency. It is also unknown if the legalization of marijuana for medical or recreational use has impacted the perception by blood donors that marijuana use is safe in proximity to blood donation. In 2012, two states, Colorado and Washington, became the first to legalize recreational marijuana use [1, 2]. Since then, marijuana legalization for recreational purposes has expanded to 15 states in total, with many other states having passed related legislation [3]. In addition, legislation to decriminalize marijuana at the federal level was passed by The US House of Representatives in 2020 and awaits review in the Senate as of this writing [4]. In a household survey conducted in the states of

Washington, Colorado, Oregon and New Mexico in 2017, 41% of respondents endorsed having used marijuana in their lifetime and 9% endorsed use in the past month [5]. In a cross-sectional US survey from 2002 to 2014, the perception that marijuana presented no risk to the user increased from 5.6% to 15.1% [6]. As this trend of expanded legalization continues, the prevalence of marijuana use is expected to increase as public opinion is trending towards acceptance [7]. This raises the question of whether blood donors who reside in states or countries where marijuana is legal for recreational use may perceive that the drug is harmless and feel that it is acceptable to donate after recent marijuana consumption.

The US Food and Drug Administration (FDA) requires screening questions for specific medications with a set deferral period, such as those that are known to cause teratogenicity (vitamin A derivatives, 5-alpha-reductase inhibitors); that impact the effectiveness of the product (anticoagulants) or indicate a possible risk of bacterial contamination (antibiotics). Additionally, the FDA requires questions that screen for intravenous drug use (IVDU) but does not screen for prescription opioids or recreational drugs that may be consumed orally or by inhalation [8]. In addition, the Universal Donor Health Questionnaire (DHQ) does not ask donors about marijuana use and commonly used legal drugs such as tobacco or alcohol are also not specifically addressed [8, 9]. This is despite nicotine and its metabolites, as well as prescription drugs such as opiates, benzodiazepines, stimulants, or barbiturates having been proven to be present in whole blood units [9, 10]. In 2019, the FDA held a public hearing to obtain stakeholder views on marijuana and marijuana-containing products [11]. This hearing did not extend to blood donation issues, and currently, the FDA does not require the testing of blood donors or blood products for marijuana or any illicit drugs.

Despite recreational marijuana consumption being legal in several countries around the world, there is a paucity of international literature on marijuana use specifically among blood donors. International guidance documents regarding blood and blood components such as the European Parliament Commission Directive 2004/33/EC mirror guidance in the United States and does not specifically address marijuana use, but does mention non-prescribed drugs in the context of infectious disease risk from intravenous/intramuscular (IV/IM) route [12]. Through formal correspondence with colleagues within the International Society of Blood Transfusion (ISBT) Haemovigilance Working Group, we were able to identify policies in countries where medical marijuana use has been legalized and recreational use has been decriminalized or legalized including: the Netherlands, Denmark, Norway, Finland and Canada. Deferral periods for the European countries listed ranged from 24 h post single use, 4 weeks for habitual use and up to 12 months for any use. Canadian marijuana deferral policy focusses on whether a potential donor is intoxicated and would be unable to understand screening questions and therefore be unable to provide informed consent, but does not specify a specific period of time for deferral (personal correspondence, ISBT Haemovigilance Working Group, 16 June 2021).

The potential impact of Δ9-tetrahydrocannabinol (THC), the psychoactive component of marijuana, or its metabolites in donated blood products on a recipient is currently unknown. THC has been found in plasma products [13, 14], and approximately, 10% is bound to red cells [15, 16]. While the large volume of distribution of THC would seem to make significant transfusion-associated marijuana exposure seem less likely in adults, the potential impact of THC on vulnerable populations through a blood transfusion, such as pregnant women, premature infants, or the foetus during intrauterine transfusion, has not been established. THC rapidly crosses the placenta, and maternal marijuana use has been shown to negatively impact foetal brain development and lead to pregnancy complications such as spontaneous pre-term birth, although these studies are based on more than a single THC exposure [17–19].

Children's Hospital Colorado Blood Donor Center is a hospital-based collection centre in the US state of Colorado, which collects approximately 8000 units of whole blood and 5000 units of apheresis platelets annually for hospital use in neonatal, paediatric and high-risk pregnancy care. Our blood donor centre supports a wide range of services including intrauterine transfusion; heart, liver, and kidney transplant; as well as haematology and oncology including bone marrow transplant. Given both the changes in societal perspectives on marijuana use, and unknown impact of possible THC transmission by blood transfusion into the vulnerable populations that our institution regularly serves, we wanted to better understand donor attitudes and behaviour related to marijuana use. Here, we report a survey of blood donors at our collection centre to define and quantify both the perspectives regarding, and actual usage of, marijuana in proximity to blood product donation.

MATERIALS AND METHODS

Study design

A cross-sectional study was conducted from August 2019 through December 2019 via an 18-question survey delivered via email (surveymonkey.com, San Mateo, CA). Responses were anonymous to encourage truthful reporting. The survey questions addressed perceptions of marijuana use in relation to blood donation and transfusion, personal use of marijuana products, demographic information and donation history. An optional comments section was available for most non-demographic questions. The option to skip any question was available, thus a respondent could indicate marijuana use but decline to answer gender, and so forth. Institutional review board approval was obtained from the University of Colorado. Participants who had donated between 1 January 2014 and 9 October 2019 were sent the survey. Responses were collected for 3 months.

Study participants

Eligibility criteria included Children's Hospital Colorado blood donors 18 years of age and older who qualified for blood donation under the

regulatory requirements set by the FDA. Donors are qualified based on a brief physical exam with established acceptable vital signs, minimum haematocrit level and successful completion of the DHQ, which includes screening questions regarding medications (e.g., antibiotics or medications with known teratogenicity), IVDU or other blood exposure, travel history (e.g., malaria or variant Creutzfeldt Jakob exposure) and high-risk sexual practices (e.g., sex with a prostitute) [8, 20]. All donors had donated at least once during the survey time period. Donors who did not supply an email address or provided incomplete responses were excluded from the survey. Participants were informed that the survey was anonymous to encourage truthful reporting, thus responses were not tracked for follow-up.

Analysis

Results were analysed based on the proportion of respondents who answered the question, using SurveyMonkey (San Mateo, CA) and Microsoft Excel (Redmond, WA). Results are reported using descriptive statistics.

RESULTS

Demographics and donation history

A total of 12,186 donors met inclusion criteria and were sent surveys, from which 979 individual donors took part, for a response rate of 8.03%. The majority of respondents identified as female (65.4%) and one respondent identified as 'other'. Age ranges were grouped in 10-year increments up to age 65 years (see Table 1), with the majority of respondents in the 35- to 44-year-old category (25%). This is generally comparable to the demographic distribution at our centre, with a female preponderance. For comparison, of 935 blood donors at our center between 2019 and 2020, 56% were female. Majority age group was 35–44 (24%), and donations represented 72% whole blood and 22% apheresis platelets. The most common donation setting in our survey responses was the main hospital donor centre (66.8%) with the remaining donors presenting to mobile drives. Product donations were primarily whole blood units (90.6%) with the highest reported number of individual donations was 1–5 units (32.6%), followed by greater than 20 units (30.1%). Donors were able to indicate that they had donated more than one type of product (e.g., select both 'whole blood' and 'platelets on machine').

TABLE 1 Demographic distribution of survey respondents with proportion who indicated use of marijuana since legalization in Colorado

Demographics	Response (%)	Indicated marijuana use <72 h prior to blood donation (% of total respondents)
Gender		
Male	336 (34.4%)	16 (4.8%)
Female	640 (65.4%)	20 (3.1%)
Other	1 (<1%)	1 (100%)
Age (years)		
18–24	32 (3.27%)	0
25–34	200 (20.4%)	14 (7%)
35–44	246 (25.2%)	11 (4.5%)
45–54	219 (22.4%)	3 (1.4%)
55–64	165 (16.9%)	3 (1.8%)
65+	116 (11.9%)	6 (5.1%)
Donation site (multiple option)		
Mobile drive	460 (47%)	21 (4.6%)
Donor centre	654 (66.8%)	23 (3.5%)
Frequency of donation in units		
0	5 (0.5%)	0 (0%)
1–5	319 (32.6%)	10 (3.1%)
6–10	207 (21.2%)	6 (2.9%)
11–15	88 (9%)	5 (5.7%)
16–20	59 (6.1%)	2 (3.4%)
Greater than 20	295 (30.1%)	14 (4.7%)
Type of products donated (multiple option)		
Whole blood	887 (90.6%)	30 (3.4%)
Apheresis platelets	262 (26.8%)	15 (5.7%)
Apheresis plasma	114 (11.6%)	9 (7.9%)
Apheresis red cells	81 (8.3%)	9 (11.1%)

Marijuana use reported by blood donors

When questioning if a donor had used marijuana (not including cannabidiold (CBD) products) since legalization, 32.6% of respondents stated they had tried it at least once. Of these, 2.9% of the donors reported using marijuana prescribed by a physician, with migraines, anxiety and pain being common reasons for treatment. Most donors reported frequency of using marijuana (edibles, smoke and/or vape) as less than once a month (40.3%) with a minority reporting using it daily (6.8%) or a few times a week (5.8%). For donors who reported marijuana use, 43 of 312 respondents (13.8%) stated they have used marijuana within 72 h of blood donation. Within this user subgroup of respondents, 32.5% reported donating more than 20 units of blood products, 34.8% were platelet donors and 16.2% indicated donating both. When looking at those who reported daily use, 47.4% (9/19) had donated >20 units; 52.6% (10/19) were platelet donors and 26.3% (5/19) indicated donating both (see Figure 1).

Concerns about reporting use

Several questions were designed to directly address marijuana use by donors (see Table 2). Donors were queried 'If you were asked, at the

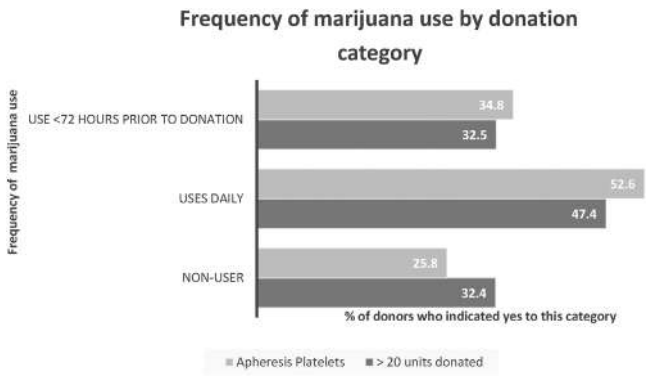

FIGURE 1 Blood donors who used marijuana frequently reported a proportionally higher rate of repeated donations and apheresis platelet donations than non-users

time of donation, if you had used a marijuana product recently (<72 h), would you feel comfortable answering honestly?' Most respondents indicated 'Yes'. Some reasons cited included that they do not use it (67.8%), and because it is legal (13.1%) or because they do not believe it affects the blood donation (3.5%). Responses for 'No' to this question included being concerned about that information being part of the donor record (1.4%); feeling it should not matter (1.9%) and that the question was too personal (0.7%). Comments for this question highlighted concerns for potential legal or financial risk, such as one respondent who stated 'I would be concerned about it being on record, especially not knowing the confidentiality standards for donor records off the top of my head. It is legal in Colorado, but not nationally, and many employers have different standards, and I don't know what my insurance company's reaction would be.' Another respondent pointed out that workplace drives at locations where the employer does not allow marijuana use when off-duty could be a motivation to lie about recent use: 'My donation was mobile and made at my place of work. I would be concerned that this information could be shared or accidentally seen.' Several comments also raised concerns about social judgement, such as 'I wouldn't think my use has any bearing on my ability to give blood at all and therefore might be less likely to answer honestly. Also, the stigma.'

Perceptions on marijuana use in blood donation

To help identify the changes in cultural perception on acceptability of marijuana use in relation to blood transfusion, we designed several questions to address this issue (see Table 3). Most respondents felt that it was unacceptable to use marijuana prior to blood donation; however, 23.5% of respondents indicated it was acceptable. Of note, three donors who indicated that they have used marijuana <72 h prior to a blood donation responded that they felt it was not acceptable to use marijuana prior to blood donation; two indicated they would not feel comfortable answering the question honestly if they were asked in screening and one of these donors indicated daily

TABLE 2 Survey responses: Self-reported marijuana use and frequency of marijuana by blood donors

Survey question	Response choices	Answer (%)
Remember this survey is anonymous: Have you personally used marijuana (not including CBD oil) at any time since it became legal in Colorado (medical or recreational)? $N = 728$	Yes, edibles	256 (26.1)
	Yes, smoke or vape	200 (20.4)
	No	272 (62.6)
You indicated that have used marijuana (edibles, smoke or vape), how often do you use it? $N = 310$	Just tried it once	101 (32.6)
	Less than once a month	125 (40.3)
	A few times a month	45 (14.5)
	A few times a week	18 (5.8)
	Daily	21 (6.8)
Have you used marijuana prior to blood donation (72 h or less)? $N = 312$	Yes, edibles	19 (6.1)
Remember this survey is anonymous: Have you personally used marijuana (not including CBD oil) at any time since it became legal in Colorado (medical or recreational)? $N = 728$	Yes, smoke or vape	24 (7.7)
	No	272 (87.2)

use of marijuana. When asked 'how would you feel if you knew that you or someone you cared about could receive a unit of blood from someone who had recently consumed a marijuana product?' the majority (43.5%) of respondents answered that they would 'be concerned but accept the transfusion if it was really needed' with 22.9% of respondents saying 'it wouldn't matter' and 5% saying they would 'refuse the blood product even if it could negatively affect their health.' Comments generally reflected that the impact was unknown and the respondent would trust their medical professional, although some had concerns. One respondent commented 'This would be a grand travesty and complete breach of confidence in the medical world. We come to hospitals and clinics to receive care that is SAFE, EFFECTIVE, and most importantly PROVEN. Marijuana is very far from being proven. It has no place when it comes to emergency medicine.' Several others were concerned about allergic reactions, hypersensitivity to THC or the impact on their job if it were to cause a positive test.

Questioning the timing of marijuana use prior to donation showed 13.2% respondents thought timing did not matter and no deferral was needed. The majority (30.3%) thought donors should wait at least 72 h. Other time periods included at least 1 day (14.1%), more than a week (14.4%) and more than a month (12.4%). Some respondents (3.8%) thought no length of time would be sufficient and that people who use marijuana should be permanently deferred.

TABLE 3 Survey responses: Donor perceptions of marijuana use in proximity to blood donations

Survey question	Response choices	Answer (%)	Uses marijuana <72 h? (N = 37)
Do you think it is acceptable for a blood donor to consume or smoke marijuana prior to a blood donation?	Yes	230 (23.5)	31
	No	684 (69.9)	3
If you were asked, at the time of donation, if you had used a marijuana product recently (<72 h), would you feel comfortable answering honestly?	Yes, because I don't use it	664 (67.8)	0 (0)
	Yes, because it is legal in Colorado	128 (13.1)	11
	Yes, because I don't believe it affects the blood donation.	34 (3.5)	11
	No, because that question is too personal	7 (0.7)	1
	No, because I'd be concerned about that being in my donor record	14 (1.4)	5
	No, because it isn't a question that should matter for blood donation	19 (1.9)	7
	Yes, other	56 (5.7)	2
	No, other	8 (0.8)	0
If it were considered acceptable for someone to consume marijuana prior to blood donation, how long should a person wait to donate after consuming?	Anytime, I don't think it matters	129 (13.2)	21
	They should wait at least a day	138 (14.1)	8
	They should wait at least 72 h	297 (30.3)	7
	They should wait more than a week	141 (14.4)	0
	They should wait more than a month	124 (12.4)	0
	People who use marijuana should be permanently deferred (never allowed to donate)	37 (3.8)	0
How would you feel if you knew that you or someone you cared about could receive a unit of blood from someone who had recently consumed a marijuana product?	It wouldn't matter	225 (22.9)	30
	I would be concerned but accept the transfusion if it was really needed	426 (43.5)	6
	I don't know	204 (20.8)	1
	I would refuse the blood product even if it could negatively affect my health	49 (5)	0

Note: Donors had the option to skip questions.

DISCUSSION

The objective of this study was to determine the opinions and behaviours of blood donors related to marijuana use both in general and in proximity to blood donation in a state with legalized recreational use. Through our anonymous survey, we found that among donors respondents almost a quarter found marijuana use acceptable prior to blood donation. We also saw that among donors that use marijuana daily, almost half had donated greater than 20 times, and many of them were apheresis platelet donors.

Although 15 states have legalized recreational marijuana [3], as of this writing, it is still illegal at the federal level in the United States, and many employers consider a positive drug test grounds for termination. This is true in the state of Colorado currently; although a House Bill was introduced in January of 2020 that would prohibit firing an employee for using marijuana when off-duty, it was postponed indefinitely [21]. Countries with nationally legalized recreational marijuana use such as Canada are also addressing this dilemma [22]. This issue is reflected in the small proportion of blood donors (1.4%) who stated they would be concerned about marijuana use being in the blood donor record.

Honesty, when answering the DHQ is critical to the safety of the blood supply. Although many highly personal questions are asked about sexual activity, it is known that blood donors are not always honest or comfortable with providing responses. A German study relevant to blood donor truthfulness in reporting recreational drug use found that of 186 donors surveyed, all denied using recreational drugs. However, hair and urine samples collected at the time of donation from these same surveyed donors showed that 10 donors tested positive for recreational drugs with six positives for cannabinoids [23]. In our survey, while the majority of donors indicated they would be honest in answering a question about marijuana use, some did state they would not be honest, feeling that the question should not be in the donor record (1.4%), is too personal (0.7%) or that it should not matter for blood donation (1.9%).

The legal and potential employment implications for a recipient must also be considered. Although confidentiality measures are in place to enable blood donors to securely and honestly answer the DHQ, privacy breaches may occur. Lawsuits have been brought alleging negligence both in screening donors and failure to inform recipients of risks. Cases related to confidentiality breaches regarding

human immunodeficiency virus in the blood supply provide guidance for informed consent and record-keeping policies that would be applicable to donor marijuana use status [24].

Although transfusion services and blood collection centres have generally not been found liable for harm if they were following standard practices, some lawsuit cases ruled that the blood supplier or trade group should have enacted screening or provided recommendations when the industry became aware of the risks to the recipient [25, 26]. Transmission of small amounts of drugs – from prescriptions or recreational use – is possible but is not generally disclosed as part of the blood transfusion consent [10]. Because we do not know the transmissibility of marijuana in blood products, it has not been proven that there would be no impact should a recipient receive a blood or platelet transfusion on an outpatient basis and then have workplace or school drug testing, a traffic accident or other incident that could incorrectly implicate them as a marijuana user. The legal risk is further complicated by the interstate transfer of blood products, where a unit could be collected in a legal state and transfused in one where marijuana is still illegal. Although the timing of a positive drug test after a receiving a THC-positive unit may seem unlikely, a chronic marijuana user may have a higher baseline plasma level of THC due to high lipophilic binding, with subsequent redistribution, resulting in a potentially higher dose of THC in the donated unit [16]. Knowing the transmissibility of THC in the blood supply may be helpful for patients in this unfortunate predicament and may also support deferring for marijuana usage.

There were several weaknesses of the study design. A survey sent prior to legalization was not conducted; therefore, it is unclear if use of marijuana, when illegal, significantly varied when compared to use after legalization. As this was an electronically administered survey, there is possible bias in the response group. In an effort to encourage truthful reporting, the survey was not linked to respondent's email addresses, thus, we could not send reminders to participants who did not respond and could not follow-up with respondents to verify their responses. We chose not to re-send the survey to all donors who met inclusion criteria to avoid receiving repeat responses. We also do not know if our sample was diluted by surveying a 5-year range of donors, as many may no longer be active donors and thus not responsive to communication from our centre. This likely means that our respondents were those who had a high likelihood to respond to surveys or were highly interested in the topic and possibly recent donors. This may also account for the high percentage of donors who reported both donating more than 20 units and frequent marijuana use, skewing our results towards a higher percentage than is representative of the overall donor population. The converse is also possible: the topic of marijuana use may have dissuaded some donors from responding. We also note that donor respondents were a female majority; a review of the literature shows that sex differences in cannabis use do exist. Males are found to use marijuana more frequently and in higher doses when compared to females, which may imply that our results underestimate marijuana use in our donor population [27]. In addition, the non-incentivized participation rate of 8.03% was lower than anticipated. For comparison, Children's Hospital Colorado blood donors were sent a routine Donor Experience Survey during the same time period as our survey, with a response rate of 15.7%.

We may also have unintentionally introduced a bias with the question by choosing '72 h' as our cutoff for several questions. The timeframe of 72 h was chosen for the survey as there is evidence that that is the approximate amount of time in which THC will become undetectable in urine [28]. One question: 'If it were considered acceptable for someone to consume marijuana prior to blood donation, how long should a person wait to donate after consuming?' had the majority (30.3%) of responses indicate the 72 h was the ideal window. Due to our use of '72 h' as a timeframe for marijuana use in this and several other questions, we may have inadvertently signalled that this was a preferred response. This question did receive a wide range of responses, with 14.1% choosing 'they should wait at least a day' and 3.8% stating that 'people who use marijuana should be permanently deferred (never allowed to donate).'

One of the reasons that little attention has been paid to the amount of marijuana that may be transfusion-transmitted is that it is believed to be an insignificant amount for an adult. Whether marijuana metabolites can be transmitted by blood transfusion in a quantity significant enough to cause harm to a vulnerable patient such as a neonate is unknown. Studies on marijuana use during pregnancy have reported increased likelihood of low birth weight and require care in the neonatal intensive care unit [29–31]. In addition, there is no 'minimum safe dose' established for marijuana use in pregnancy. If it is determined that any significant amount of THC is present in donated units, a deferral may be needed until the safety limit of marijuana can be established. The FDA seeks to protect vulnerable populations from blood transfusion risks – users of teratogenic mediations such as finasteride are deferred for 30 days, even though the ingested dose is minimally transmitted through a blood transfusion after only a 3-day deferral [32]. Comparatively, although it has not been proven or studied, there is a perception by the blood industry that a single exposure of marijuana, diluted into a recipient, would be essentially harmless, as discussed by CBS-Hema-Quebec Donor Selection Criteria Working Group, an advisory group that includes medical, scientific, quality and regulatory affairs, and operational experts from both organizations, as well as a pharmacist and a donor representative (personal correspondence, Mindy Goldman, 4 June 2021).

The findings in this study shows that, among our population of blood donors, marijuana use in proximity to donation does occur. Marijuana use less than 72 h prior to blood donation was reported in all demographic groups surveyed except for the 18–24 age group. Overall, of donors who indicated they had used marijuana less than 72 h prior to blood transfusion, 32.5% indicated that they had donated more than 20 times, and although the raw numbers for this group are very small, it is of interest that frequent marijuana users appear to be frequent donors as noted in Figure 1: within those who reported daily use, 52.6% indicated they were apheresis platelet donors and 47.4% indicated they had donated greater than 20 products, with 26.3% indicating both. It is possible that this number represents a small group of marijuana users who are highly motivated donors and also motivated survey responders; as this is one of the

most desirable populations for blood donation, this represents a concern for the blood supply, should these individuals ever need to be deferred from donating apheresis platelets.

Platelets, as a plasma-based product, may have more potential for transmission of THC to vulnerable populations [15]. If this is the case, then a question on the DHQ with a related deferral period may need to be established. In 2014, Booth and Gehrie proposed that a 2-week deferral would be appropriate to allow for complete metabolism of any marijuana use [33]. As we found a dedicated population of repeat platelet donors who use marijuana in our survey, these results, if applicable to the other states or countries that allow recreational marijuana use, suggest that a 2-week deferral would have a significant impact on the donor blood supply. If blood product testing for THC and its related metabolites was used in place of screening questions, this would create additional delay and cost as detection in whole blood would utilize the high complexity methodology of liquid chromatography tandem mass spectrometry [34].

Colorado was one of the first states to legalize recreational marijuana; the results we have reported here may be a harbinger for the trends of use by blood donors as recreational marijuana use becomes more socially acceptable both nationally and internationally. Based on our results, with a proportion of nearly every demographic group in our survey indicating they had used marijuana less than 72 h prior to blood donation, there is no doubt that some blood donors are using marijuana. Now it is up to the stewards of the blood supply to determine whether or not there is enough THC transmitted via blood transfusion to have a negative impact on the recipient, whether it be physiological or present legal or financial ramifications. Further studies on the impact of THC transmitted by blood transfusion, especially platelet products, on the recipient are warranted to determine if additional action such as screening or deferral is needed.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ORCID

Kyle Annen https://orcid.org/0000-0002-7192-5057

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Qualifying coronavirus disease 2019 convalescent plasma donors in Israel

Marina Izak[1] | Veronica Gendelman[1] | Sharron Bransburg-Zabary[1] | Evgeniy Stoyanov[1] | Roni Gat[2,3] | Daniel Cohen[4] | Jacob Chen[5,6,7] | Yasmin Maor[7,8] | Avi Benov[5,9] | Boaz Lev[10] | Oren Zimhony[11] | Eilat Shinar[1,2]

[1]National Blood Services, Magen David Adom, Ramat Gan, Israel

[2]Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel

[3]Clinical Research Center, Soroka University Medical Center, Beer Sheva, Israel

[4]School of Public Health, Tel Aviv University, Tel Aviv, Israel

[5]Trauma and Combat Medicine Branch, Israel Defense Forces Medical Corps, Ramat Gan, Israel

[6]Hospital Management, Meir Medical Center, KfarSaba, Israel

[7]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

[8]Infectious Disease Unit, Wolfson Medical Center, Holon, Israel

[9]The Azrieli Faculty of Medicine, Bar-Ilan University, Safed, Israel

[10]Epidemic Unit, Ministry of Health, Jerusalem, Israel

[11]Infectious Disease Unit, Kaplan Medical Center, Rehovot, Israel

Correspondence
Marina Izak, Faculty of Health Sciences, Magen David Adom, Ramat Gan, Israel.
Email: marinai@mda.org.il

Abstract

Background and Objectives: Passive immunization using investigational COVID-19 convalescent plasma (CCP) is a promising therapeutic strategy and could improve outcome if transfused early and contain high levels of anti-SARS-CoV-2 antibodies. We report the management of a national CCP collection and distribution program in Israel.

Materials and Methods: From 1 April 2020 to 15 January 2021, 4020 volunteer donors donated 5221 CCP units and 837 (20.8%) donors donated more than once. Anti-nucleocapsid IgG antibodies were determined using chemiluminescent immunoassay method (Abbott). A statistical model based on repeated IgG tests in sequential donations was created to predict the time of antibody decline below sample/cut-off (S/CO) level of 4.0.

Results: Ninety-six percent of CCP donors suffered a mild disease or were asymptomatic. Older donors had higher antibody levels. Higher antibody levels (S/CO ≥4) were detected in 35.2% of the donors. Low positive (S/CO ≥1.4–3.99) were found in 37%, and 27.8% had undetectable antibodies (S/CO ≤1.4). The model predicted decrease antibody thresholds of 0.55%/day since the first CCP donation, providing guidance for the effective timing of future collections from donors with high antibody levels.

Conclusions: An efficient CCP collection and distribution program was achieved, based on performing initial and repeated plasma collections, preferably from donors with higher antibody levels, and only antibody-rich units were supplied for therapeutic use. The inventory met the quantity and quality standards of the authorities, enabled to respond to the growing demand of the medical system and provide a product that may contribute to improve prognosis in patients with COVID-19.

KEYWORDS
antibodies, convalescent plasma, donors

INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe respiratory syndrome coronavirus 2 (SARS-CoV-2), is one of the biggest global health threats of the last century.

At the time of this writing, a year into the pandemic, specific treatment remains elusive [1]. Although the available vaccines may become a principal game changer in the prevention of new infection, passive immunization by transfusion of COVID-19 convalescent plasma (CCP) is still used widely. This strategy is based on century-old reports that describe the efficacy of treating patients during the 1918 influenza A pandemic by transfusions of CCP [2–4] and from small reports, showing encouraging clinical benefit of CCP in patients with severe COVID-19 [5–7].

Based on these reports, the Israeli Ministry of Health (MOH) requested Magen David Adom National Blood Services in Israel (MDANBS) to establish an investigational CCP program as a part of a national COVID-19 treatment protocol.

As of today, data accumulated worldwide suggest that transfusion of CCP is safe and effective [8, 9]. Recent data from matched controlled studies [10, 11], from randomized clinical trial [12] and from retrospective analysis [13] showed benefit of CCP in patients treated early with CCP containing high-titre antibodies (Ab), while others did not show decrease in mortality [14, 15]. Based on these data, U.S. Food and Drug Administration (FDA) issued on 4 February 2021 a revision of the Emergency Use Authorization (EUA) for CCP and limited the authorization to the use of high-titre CCP only [16]. Several trials are ongoing, investigating clinical benefit of CCP [17] and standardization of serological and neutralization assays [18].

In Israel, transfusing CCP is currently an integral component of the early treatment of COVID-19, as a part of a national investigational program. All aspects of CCP collection, processing, testing and distribution to hospitals nationwide are centrally performed by Magen David Adom National Blood Services (MDANBS), to assure standardization, quality and impartiality. The treatment protocol was based on transfusion of two CCP units (200 ml each) 24 h apart, to patients approved by the MOH research committee. The results of treating the first group of COVID-19 patients have been previously reported [19], and the correlation of clinical benefit with higher anti-SARS-CoV-2 Ab in transfused CCP was shown.

A key question for every CCP collection and distribution centre is how to select the right plasma donors. In this article, we report our experience accumulated since 1 April 2020, in recruiting CCP donors and in inventory management, as our aim is to qualify and supply for transfusion CCP units with highest anti-SARS-CoV-2 antibody levels.

MATERIALS AND METHODS

Donor population

The Ethics Committee of the MOH approved an Institutional Review Board (IRB) protocol to recruit individuals who recovered from COVID-19 as potential CCP donors and conduct laboratory tests to qualify and supply CCP units for treatment. CCP collections were initiated on 1 April 2020, according to the first FDA protocol [20], with local modification to comply with the MOH regulations and the IRB protocol [21], similar to programs established simultaneously around the world [22].

Potential CCP donors required evidence of COVID-19 by molecular tests and two consecutive negative test results after symptomatic recovery, thereafter 14-day deferral needed before plasma collection.

Recovered COVID-19 patients were referred to the MDANBS by various sources, including MOH's database, the Israeli Defense Forces, cohorts in closed ethnic communities and social media. Donors gave their consent for transfer of personal data to MDANBS.

All CCP donors were non-remunerated volunteers whose health histories complied with MOH and MDANBS criteria for blood donations. Only males or nulliparous females were recruited to mitigate the risk of transfusion-associated acute lung injury (TRALI). A Donor Recruitment Call Center was established and operated by trained MDANBS personnel, who conducted telephone interviews with potential donors to assure compliance with requirements. Pre-donation screening included evaluation of potential donors' records in the MDANBS computer database (Progesa, MAK-system) to identify prior disqualifying deferral.

Plasma collection

CCP collections were initially performed at the main MDANBS plasmapheresis donation centre that routinely performs apheresis plasma collections and had been involved in similar projects previously [23]. To respond to the rapidly growing demands for CCP, four additional donation sites were opened, additional mobile apheresis equipment (MCS+, Haemonetics, Covina, CA) was purchased and extra apheresis operators were trained.

In addition to the standard MDANBS Donor Health Questionnaire, every donor signed an informed consent for the CCP collection.

Apheresis collections of 600 ml of CCP were obtained, divided into three 200-ml units, frozen at −30°C within 22 h and labelled as Apheresis Convalescent Plasma according to ISBT-128 standards (ISBT code E9743). As anti-SARS-CoV-2Ab testing was not available during the first 2 weeks of CCP collections, we saved archive samples for further studies.

Donors' testing

Blood samples from each donation were tested according to MOH and MDANBS standards and the IRB protocol, including ABO/Rh, *Treponema pallidum* haemagglutinin assay (PK7300 Beckman Coulter, Brea, CA), red blood cells (RBC) antibody screening (Erythra, Grifols, Spain), serological tests for human immunodeficiency virus I/II (HIV-I/II), hepatitis B virus (HBV), hepatitis C virus (HCV), human T-lymphotropic virus I/II (HTLV-I/II) (Alinity S, Abbott, Green Oaks, IL) and individual donor nucleic acid testing (ID-NAT) for HIV-I/II, HCV, HBV and West Nile virus (WNV) (Panther, Grifols, Spain).

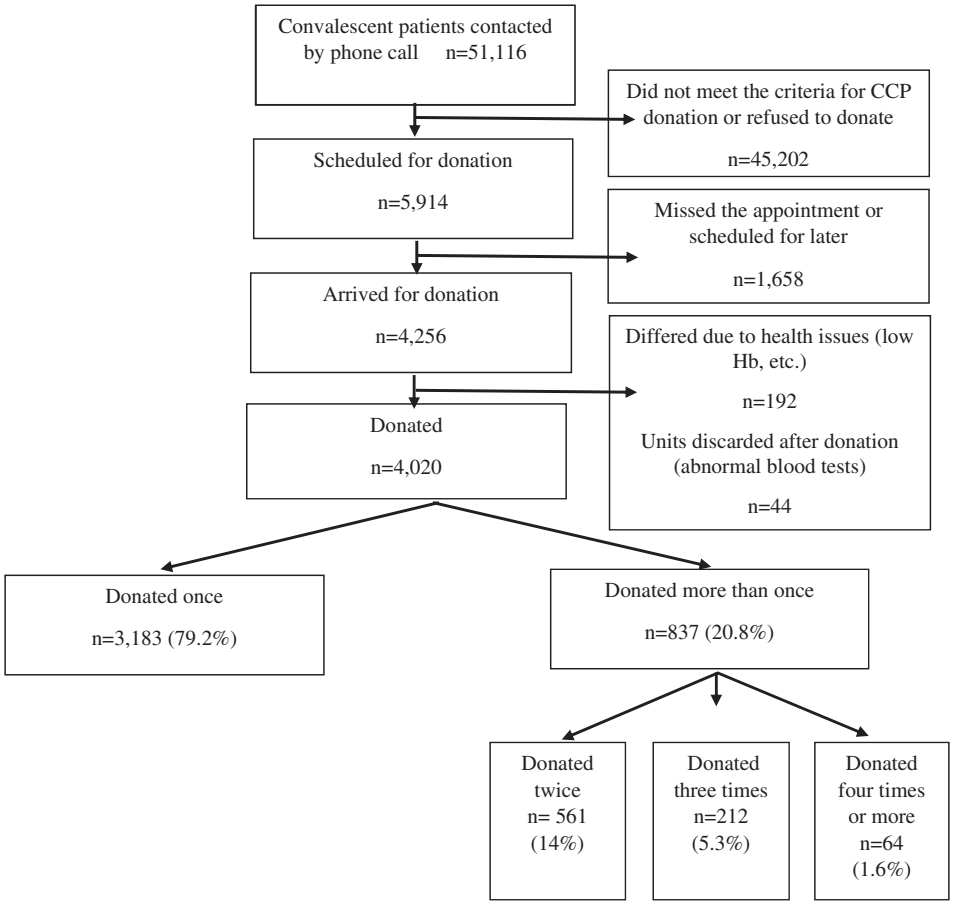

FIGURE 1 Recruitment of convalescent plasma donors (1 April–15 January, 2021). CCP, COVID-19 convalescent plasma;Hb, haemoglobin

Anti-SARS-CoV-2 antibodies

Commercially available assays for anti-SARS-CoV-2 Ab differ by the Ab subclass (IgM, IgA, IgG or total antibody), the targeted antigen (subunit 1[S1] of the spike protein, nucleocapsid protein [N] or the receptor-binding domain [RBD]) and by assay method, that is, lateral flow assay (LFA) [24, 25], neutralizing Ab assay (nAb) [26, 27], enzyme-linked immunosorbent assay (ELISA) [28] and chemiluminescent immunoassay (CLIA) [29, 30]. For this project, we used multiple laboratory methods to test the presence of different anti-SARS-CoV-2 Ab.

1. Anti-S (S1 subunit) SARS-CoV-2 Ab
   Serum samples were tested for anti-S IgG and IgA, using ELISA (EUROIMMUN AG, Germany), performed in the Research Laboratories of the School of Public Health, Tel Aviv University during the first month of the project (April, 2020). A positive result was defined as a sample to calibrator absorbance (S/CO) ratio ≥ 1.1 [28].
2. Anti-N (nucleocapsid protein) SARS-CoV-2 Ab
   Starting 1 May 2020, all CCP collections were tested for anti-N by CLIA, performed on the Architect i2000 SR (Abbott, Green Oaks, IL) automated immunoassay analyser [29]. Testing also included samples retained from the first month's apheresis collections. Positive result was defined as S/CO≥1.4 [29, 30]. Having accumulated a sufficient CCP inventory (since 1 October 2020), we qualified for transfusion CCP units by S/CO: one unit had an Ab level of S/CO ≥7.0 and another– S/CO ≥4.0, thus an average S/CO≥4.5 was provided, in line with the later decision of FDA, issued on 4 February 2021 [16].
3. Viral neutralization assay
   As initial reports indicated a positive correlation between anti-S and anti-N IgG values and nAb activity [22, 26], we compared our results of anti-S by ELISA (EUROIMMUN) and anti-S by CLIA (Abbott) with results of neutralization studies for the first 53 CCP units. The Israeli Institute for Biological Research team performed the test, using a modified plaque reduction neutralization test (PRNT) with Vero E6 cells (ATCC® CRL-1586™), as described previously [19].
4. Pre-donation anti-S SARS-CoV-2 rapid point of care (POC) assay
   We evaluated anti-S BELTEST-IT COV-2 Rapid Test (PharmAct AG, Germany) LFA [31], as a part of the pre-donation screening on a capillary blood sample, to avoid collections of plasma from donors with undetectable Ab. Results were obtained within 15 min. Only potential donors with positive IgG by POC results proceeded to the apheresis collection. Venous blood samples were obtained as well for all potential donors (including first and subsequent donations) and tested for anti-N by CLIA.

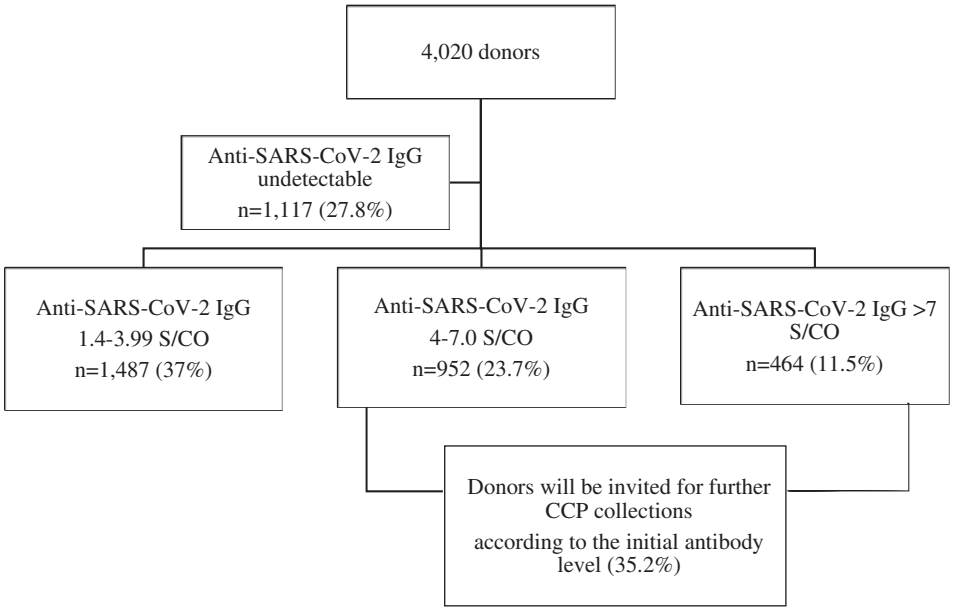

FIGURE 2 Identification of convalescent plasma donors suitable for further donations, based on anti-nucleocapsid antibody IgG results. CCP, COVID-19 convalescent plasma;S/CO, sample/cutoff

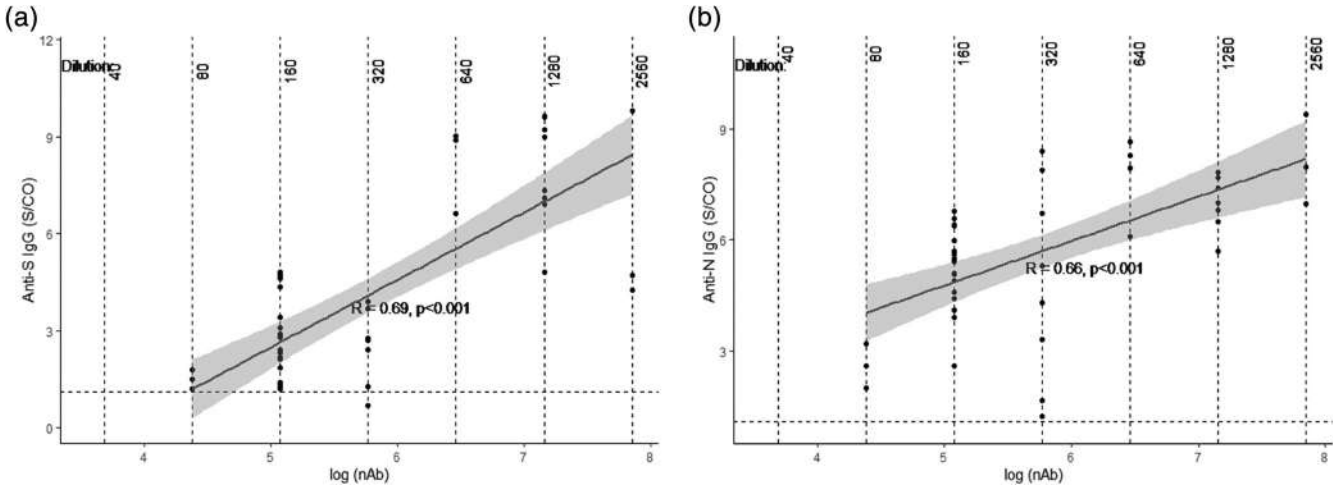

FIGURE 3 Correlation between neutralizing antibody (nAb) activity (*x*-axis) and two serological assays (*y*-axis): (a) anti-S, EUROIMMUN and (b) anti-nucleocapsid (anti-N), CLIA; Abbott. Vertical dotted lines represent the cutoff for nAb positivity at the indicated titre. The dashed horizontal lines represent the cutoff for serological assays positivity. *R* is calculated by Spearman correlation test

Statistical analysis

Descriptive statistics are presented as mean ± SD for continuous variables and compared using independent *t*-test or Mann–Whitney test. Categorical variables presented as number of observations and percentage and compared using Pearson $\chi 2$ test. To assess correlations between Ab levels measured by EUROIMMUN and CLIA tests, we used Spearman's correlation test.

A statistical prediction model of decline of anti-N Ab in subsequent CCP donations was created by generalized linear mixed models (GLMM) with a random intercept for each participant. GLMM was used to account for clustering with link function fitted to distributions. The last detectable Ab is the dependent variable, a function of: (1) time between first donation to subsequent donation and (2) initial antibody level. Data were analysed using R software (Version 3.5.1).

RESULTS

Donors' demographics and eligibility

From 1 April 2020, until 15 January 2021, the CCP Call Center performed 51,116 telephone interviews (217/day), resulting in 5914 (11.6%) scheduled appointments. Most of the donors referred were

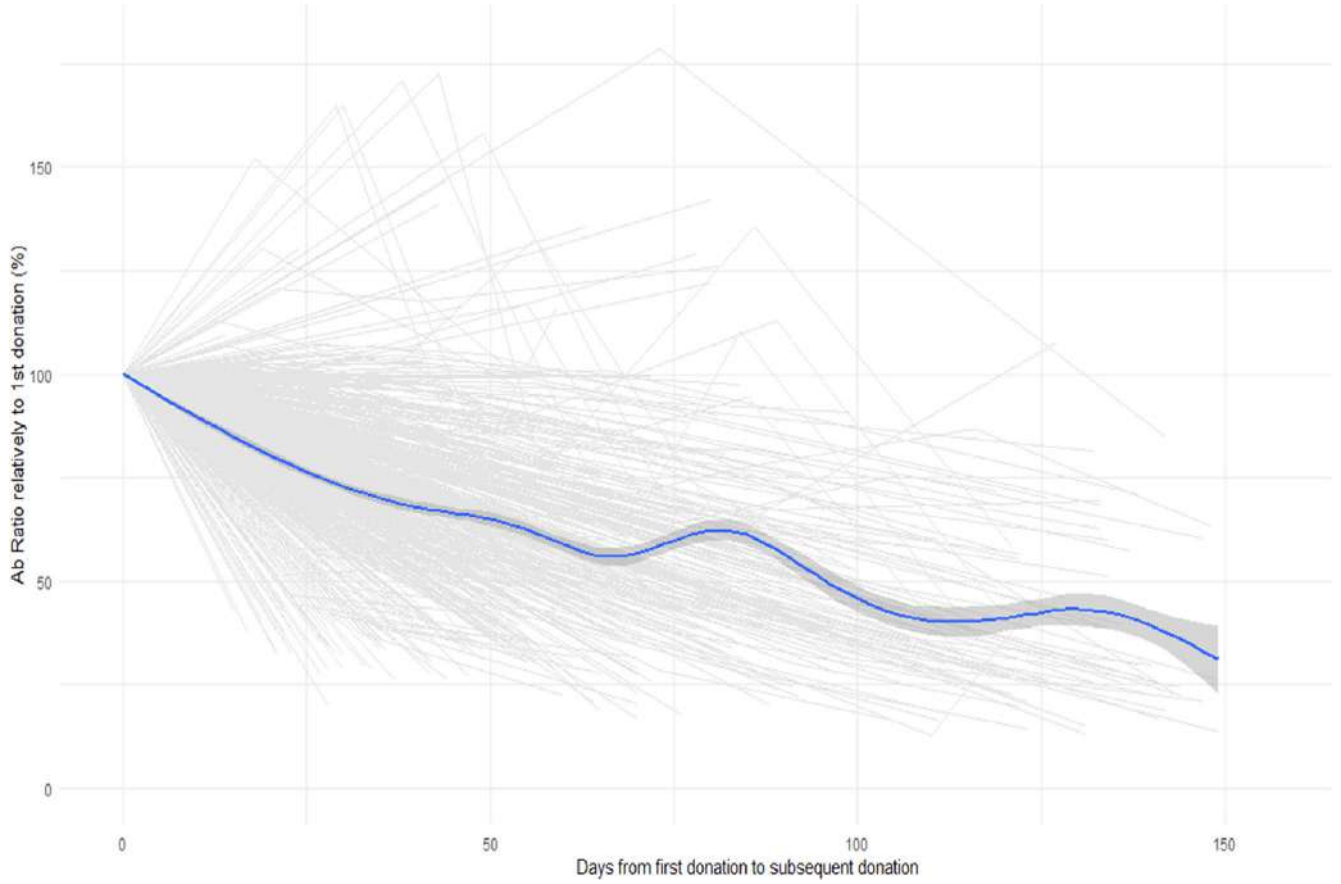

FIGURE 4 Gradual decrease of anti-nucleocapsid (anti-N) antibody levels (%) in convalescent plasma donors through 150 days since first donation. Anti-N anti-SARS-CoV-2 IgG antibodies detected by chemiluminescent microparticle immunoassay (CLIA; Abbott, Green Oaks, IL). Donors with sample/cut-off ratio (S/CO ≥1.4) were analysed. Ab level in the first donation for each donor was defined as 100% and, in subsequent donations, was calculated (%) relative to his/her first donation. Light grey lines represent donors' anti-N levels (in %) through subsequent donations; trend-line represents the mixed model regression analysis using R software. Ab, antibodies [Colour figure can be viewed at wileyonlinelibrary.com]

ineligible for donation due to health reasons or unwilling to donate plasma. Only 72% (4256/5914) of the scheduled donors arrived to the Apheresis collection centres, of whom 4064 donated CCP and 192 (4.5%) were further deferred on site due to health reasons (i.e., low haemoglobin, abnormal blood pressure or other health conditions). The percentage of deferrals on site for CCP donors was lower than for regular blood donors: 11.3% blood donors deferred in 2019 and 10.5% in 2020 due to health reasons. Disqualifying laboratory test results for transfusion-transmitted diseases, abnormal blood count or presence of clinically significant Ab to blood group antigens were found in blood samples of 1.08% CCP donors (44/4064), comparing to 0.53% among blood donors in 2019 and 0.58% in 2020. Final analyses were performed on 4020 CCP donors (Table S1, Figure 1), their mean age was 32.6 ± 12.9 years and 736/4020 (18%) were female and 36% were first-time donors. About 96% of the CCP donors (3859/4020) were asymptomatic or had a mild COVID-19; 161/4020 (4%) had moderate disease. Analysis of the first 726 CCP donors' self-reports revealed that the mean time from the onset of symptoms to the first CCP collection was 45.6 ± 14.5 days.

Anti-SARS-CoV-2 antibodies

First 230 CCP collections were tested by EUROIMMUN. Anti-S1 IgG Ab were undetectable in 17% (39/230). As the results of the test were not available at the time of CCP release, 19 of these CCP units were transfused. Two-weeks' follow-up was available for 15 patients that received at least one unit of CCP with undetectable anti-S1 IgGAb levels, and as discussed previously [19], the lower mean antibody level in transfused plasma was a predictor of worse outcome in the group of 49 patients analysed in the study [19].

All 5221 donations from the 4020 donors were tested for anti-N by CLIA; 1117/4020 (27.8%) had an anti-N S/CO <1.4 (negative); 1487/4020 (37.0%) had S/CO of 1.4–3.99 (low positive); 952/4020 (23.7%) had S/CO 4.0–7.0 (positive) and 464/4020 (11.5%) had S/CO >7.0 (high positive) (Figure2). Antibody levels were higher in older donors:96 individuals older than 60 years had a mean ± SD S/CO of 5.74 ± 2.65, while the youngest 506 donors (age 17–20 years) had a mean ± SD S/CO of 3.74 ± 1.79 ($p < 0.001$) (Table S2). In stratification to age strata, there was no difference in antibody levels between genders.

Results of 199 rapid point-of-care test (POC) were compared to the anti-N results: 171/199 (85.9%) showed concordant results, with 143 positive and 28 negative by both assays. Disagreement of POC with anti-N CLIA was observed in 28/199 samples. Of them, 27/199 were positive by the POC assay and negative by the anti-N assay and 1/199 negative by the POC assay and positive by the anti-N assay. The positive predictive value (PPV) for POC was 0.84 and the negative predictive value (NPV) was 0.97, with sensitivity of 99.3% and specificity of 50.9%. Comparison of anti-S by EUROIMMUN and anti-N by CLIA was performed on 139 samples; PPV of EUROIMMUN was 0.96 and NPV was 0.63 (sensitivity of 92.4% and specificity of 79.2%).

NAb activity was determined in 53 CCP units from 29 donors, as described previously [19]. The median nAb titre was 1:160 (interquartile range [IQR] 1:160–1:640, range 1:20–1:2560). NAb titre was <1:160 in eight CCP units (15.1%) and ≥ 1:160 in 84.9%. Taken as a continuous variable, the IgG anti-S by EUROIMMUN yielded positive correlation $r = 0.69$ ($p < 0.001$) with nAb after logarithmic transformation of both variables. IgG anti-N by CLIA showed a positive correlation of $r = 0.66$ ($p < 0.001$) with nAb, both by Spearman's rank correlation (Figure3).

Gradual decrease of antibody level over time

Of 4020 apheresis donors, 837 (20.8%) had more than one CCP collection; 561 donated twice, 212 donated three times, 44 donated four times, 16 donated five donations and 4 had six donations (Table S1). As we aimed to deliver only antibody-rich CCP to COVID-19 patients, only donors with initial anti-N IgG level of S/CO ≥ 4.0 were invited for subsequent donations, and two CCP units were delivered to patients: one unit with IgG S/CO ≥ 7.0 and one with S/CO ≥ 4.0, providing an average S/CO ≥ 4.5.

The timeframe of Ab decrease was predicted by statistical model as 0.55%/day (Table S3, Figure4) and could be calculated relatively to the level at the first collection, for example, if initial S/CO was 10.0, the decrease to S/CO of 4.0 will take 92.65 days (Table S4).

Blood groups in CCP donors

The prevalence of ABO/Rh blood groups in CCP donors was similar to that of blood donors in the general Israeli population, according to the MDANBS database from 2019. Higher prevalence of blood group A and a lower prevalence of group O among COVID-19 patients reported previously [32, 33] was not seen in CCP donors in Israel; however, higher percentage of AB group in convalescents (9%) comparing to blood donors (8%) was statistically significant ($p = 0.002$).

DISCUSSION

This report describes the steps taken to rapidly establish a program for the recruitment of volunteer CCP donors, qualifying their plasma by a multi-assay laboratory protocol and supplying it to COVID-19 patients in Israel. Over 2300 COVID-19 patients treated until 15 January 2021, as a part of an investigational, multi-institutional national program.

Patients recovered from COVID-19 were referred by the MOH or responded to calls in the social media to become CCP donors. To facilitate recruitment of eligible individuals, personnel of CCP Call Center conducted health-screening interviews. Although only 11.6% of the calls yielded appointments, of which 72% of the donors showed up, remarkable low percentage of donors (4.5%) were deferred on site, compared to a deferral rate of 11% in our regular blood drives. The low percentage of deferrals on site was probably a result of pre-donation telephone interviews with potential donors to assure compliance with requirements, the tactics that were not accepted for regular blood donations. The relatively high percentage of CCP units discarded due to abnormal blood tests (1.08% for CCP vs. 0.42% for blood donations) could be a result of high percentage of first-time donors among CCP donors comparing to regular blood donors (36% vs. 20%, respectively).

Almost all our CCP donors were asymptomatic or had mild COVID-19 disease, as most of potential donors with moderate or severe disease were not qualified for CCP collection, usually due to persistent symptoms. The addition of pre-donation screening method enabled to collect CCP only from donors who showed the presence of antibodies, thus, saving time and resources of both the donors and the blood services.

Based on published data on efficacy of antibody-rich CCP and according to recently approved FDA policy [16], we used a statistical prediction model to optimize our CCP inventory of units with higher IgG antibody levels. Since the model predicted that anti-N Ab decreased at 0.55%/day from the time of the first donation (Tables S3 and S4 and Figure 4), only donors with higher antibody levels were re-scheduled for further plasma donations, keeping short periods between collections (2 weeks). All donated units were retested for anti-N antibodies in subsequent donations.

Our study has few limitations. One is the fact that 88.4% of individuals who were referred to the MDANBS were found to be non-eligible due to health issues, parity in women or refused to donate. Consistently low compliance rate of COVID-19 convalescents to donate plasma was recently described by our colleagues [34]. We need to further study this phenomenon. Secondly, we used anti-N Ab as a surrogate marker of anti-viral neutralizing activity; however, follow-up of repeated nAb or anti-S IgG was not available during the study period. Thirdly, no anti-N IgGAb were found in high proportion of our CCP donors, in agreement with published data on lower Ab levels anticipated in cohort of asymptomatic individuals [35] and in patients with mild symptoms [36, 37], with rapid decline of anti-N and nAb[38]. Anti-S antibodies were undetectable in 17% of our donors by EUROIMMUN test and in 14% by the rapid POC lateral flow test. We are awaiting for follow-up results of nAb tests in a larger group of CCP donors that will be performed by the Israeli Institute for Biological Research; they will be helpful to understand better the relationship between anti-N and nAb.

Another limitation was unknown period between recovery and donation in many donors. It is clear that early CCP donation soon after recovery is associated with higher antibody levels, but unfortunately, the donors' information provided from the sites to MDABS was uneven and sometimes incomplete.

Currently, all expectations are concentrated on the results of vaccination programs. Although over 3 million individuals were already vaccinated in Israel by the Pfizer-BioNTech vaccine [39], urgent requests for CCP units for new COVID-19 patients are being added daily. Gaps in knowledge still exist for the timeframe for SARS-CoV-2 antibody formation and decline, the relationship between antibody levels and the severity of COVID-19 disease and the protective effect of antibodies against re-infection with the SARS-CoV-2 wild type or variants [40, 41].

In this ongoing project, we focussed on rapid creation of sufficient CCP inventory, by collection of CCP with higher antibody levels, as we believe that a better outcome for COVID-19 patients can be achieved by providing CCP transfusion early during the course of the disease. This challenging task was achievable and maybe less complicated in Israel, where the country's blood collection processing and supply is concentrated in a centralized Blood Services Establishment. Building a CCP inventory was achieved by being part of an integral, multi-disciplinary program involving community stakeholders (hospitals), governmental regulators (MOH) and research laboratories, all supporting the national program for donor recruitment and laboratory qualification of CCP. We encourage additional programs to share their experiences to support a timely determination of best practices for CCP programs during the current pandemic.

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M.I. wrote the first draft of manuscript, S.B.-Z. analysed the data, V.G., E.S. and D.C. coordinated the testing, R.G. performed statistical analysis, J.C., Y.M., A.B., B.L. and O.Z. contributed to the design of project and reviewed the final manuscript, E.S. devised the project, planned experiments and wrote the final manuscript.

CONFLICT OF INTEREST

No conflict of interest to disclose.

ORCID

Marina Izak https://orcid.org/0000-0002-4297-6373

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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DEHT is a suitable plasticizer option for phthalate-free storage of irradiated red blood cells

Linda Larsson[1,2] | Sara Ohlsson[2] | Julia Derving[2] | Beatrice Diedrich[1,2] | Per Sandgren[1,2] | Stella Larsson[2] | Michael Uhlin[1,2]

[1]Department of Clinical Science, Intervention and Technology (CLINTEC), Karolinska Institutet, Stockholm, Sweden

[2]Department of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden

Correspondence
Linda Larsson, Department of Clinical Science, Intervention and Technology (CLINTEC), ANA Futura 8, Karolinska Institutet, SE-171 77 Stockholm, Sweden.
Email: linda.larsson@ki.se

Abstract

Background and Objectives: Due to increasing concerns about possible endocrine-disrupting properties, the use of the plasticizer di(2-ethylhexyl) phthalate (DEHP) will be banned in future blood storage. Di(2-ethylhexyl) terephthalate (DEHT) provides sufficient red blood cell (RBC) quality during conventional blood bank storage. It is important that a new plasticizer also maintains acceptable quality during exposure to high cell stress, such as irradiation, which is commonly used to prevent graft-versus-host disease.

Materials and Methods: A total of 59 RBC units were collected and processed in polyvinyl chloride (PVC)-DEHT or PVC-DEHP blood bags combined with either saline-adenine-glucose-mannitol (SAGM) or phosphate-adenine-glucose-guanosine-saline-mannitol (PAGGSM) additive solution. All units were X-ray irradiated on day 2 post-collection. Sampling for assessment of parameters of storage lesion was performed on day 2 pre-irradiation and day 14 and 28 post-irradiation.

Results: Though irradiation increased cell stress, DEHT/PAGGSM and current common European preference DEHP/SAGM were equally affected up to 14 days post-irradiation for all measured parameters. At day 28, haemolysis and microvesicle count were slightly increased in DEHT, whereas extracellular potassium ions, glucose, lactate, pH, mean corpuscular volume and microvesicle phosphatidylserine remained unaffected by plasticizer choice throughout storage. No individual unit exceeded 0.8% haemolysis, not even in DEHT/SAGM, the combination overall most affected by irradiation. Of the four combinations, membrane stability was least impacted in DEHP/PAGGSM.

Conclusion: We demonstrate that DEHT is a suitable plasticizer for storage of RBCs after X-ray irradiation cell stress. This strengthens the option of DEHT as a viable non-phthalate substitute for DEHP.

KEYWORDS
DEHP, DEHT, irradiation, phthalate, plasticizer, red blood cells

INTRODUCTION

The normal lifespan of a red blood cell (RBC) is around 120 days in circulation, but maximum storage time of a refrigerated RBC concentrate (RCC) is usually limited to five to seven weeks due to a degenerative process called RBC storage lesion. This is a descriptive name for the large number of metabolic, oxidative and morphological changes that RBCs undergo during blood bank storage conditions, which ultimately leads to cell rupture: haemolysis [1]. When RBCs lyse, haemoglobin is released. Haemoglobin is a strong inducer of reactive oxygen species (ROS) that, just like RBC microvesicles (RMV), are linked to pro-inflammatory and pro-thrombotic processes [1, 2]. Therefore, it is crucial to keep the number of haemolysed cells in an RCC as low as possible up until the point of transfusion. The upper limit for haemolysis has been set to 0.8% of red cell mass in the Council of Europe member states [3].

The haemolysis generation is affected by exposure to different manufacturing and storage stressors [4–6]. One of the most extreme stressors is irradiation. Irradiation, usually through X-ray or gamma methods [7], is an important measure to inhibit proliferation of residual white blood cells (WBCs) and thereby prevent transfusion-associated graft-versus-host disease [3, 8, 9]. However, irradiation stimulates the creation of ROS which, in turn, damages the RBC membrane through induction of lipid peroxidation and oxidation of membrane-bound proteins, including inhibition of Na^+/K^+ ATPase [10–12]. This premature loss of membrane integrity increases the rate of both haemolysis and RMV generation [6, 10–14] as well as the accumulation rate of extracellular potassium ions (K^+), associated with hyperkalaemia-linked cardiac arrest [10, 15, 16]. Consequently, the shelf-life of irradiated RCCs is frequently reduced between 24 h and 2 weeks. Among the more stringent practises is the '14 + 14 days' rule (maximum 14 days old RCCs at time of irradiation, maximum 14 days storage post-irradiation), which is advocated in for instance Sweden and the UK. [3, 17, 18].

The use of polyvinyl chloride (PVC) storage containers plasticized with di(2-ethylhexyl) phthalate (DEHP) has, in combination with suitable additive solution (AS), long been a cornerstone to maintain low haemolysis levels throughout storage. DEHP leaches from the PVC matrix and incorporates into the RBC membrane phospholipid bilayer, thereby stabilizing it and prolonging the time to lysis [19, 20]. Due to increasing concerns about possible endocrine-disrupting properties, the regulatory restrictions for medical devices have recently been updated with the aim to abolish the use of DEHP in blood storage [21]. Therefore, it is urgent to remove DEHP without compromising the blood component quality [22], a conundrum that has delayed the replacement process for a long time.

Although no other plasticizer has, so far, shown identical RBC storage quality to DEHP, there are a few promising candidates. In a previous study [23], we demonstrated that a structural isomer to DEHP, di(2-ethylhexyl) terephthalate (DEHT), provides adequate RBC quality during seven weeks of storage in both saline-adenine-glucose-mannitol (SAGM; primary AS in Europe) and phosphate-adenine-glucose-guanosine-saline-mannitol (PAGGSM). Satisfying quality of DEHT combined with AS-1 has also been confirmed [24].

It is important that a new plasticizer is versatile enough to maintain acceptable RBC quality not only at ideal storage conditions, but also during exposure to common blood bank stressors. Therefore, we wanted to subject RBCs stored in PVC-DEHT bags, combined with SAGM or PAGGSM, respectively, to X-ray irradiation and compare the results to corresponding DEHP storage. This study sheds light on the characteristics of DEHT for RBC storage during the condition of irradiation cell stress.

MATERIALS AND METHODS

Blood collection and component processing

A total of 59 RCCs were produced through collection and separation of whole blood (WB; 450 ml ± 10% in 63 ml citrate-phosphate-dextrose) from regular, voluntary, non-remunerated blood donors, following Karolinska standard operating procedures [23, 25]. Briefly, these include separation with Macospin (3130 × *g*, 11 min) and MacoPress Smart Revo (both Macopharma, Mouvaux, France) on the collection day (no overnight WB hold). Blood bag systems corresponding to the commercially available bottom-and-top NPT reference (Macopharma), including identical needle and leucoreduction filters, were manufactured completely of PVC-DEHT or PVC-DEHP, respectively. These bag systems contained either 100 ml SAGM or PAGGSM, respectively, for RBC resuspension. This set-up allowed four study arms to be created: DEHT/SAGM, DEHT/PAGGSM, DEHP/SAGM and DEHP/PAGGSM (blood type A; DEHP/SAGM $n = 14$, all other arms $n = 15$). The donors were chosen randomly for each arm; no pool-and-split strategy was applied. The RCCs were cold stored (2–6°C) within 8 h of donation. Tube sealers from the Qseal range (Conroy Medical AB, Upplands Väsby, Sweden) and sterile docking device TSCDII (TerumoBCT, Lakewood, CO) were used for both the DEHT and DEHP material.

On day (d) 2 post-collection, the RCCs were X-ray irradiated (canister center dose 37.85 Gy, maximum 38.53 Gy, minimum 26.42 Gy; Raycell Mk2, Best Theratronics, Ottawa, Canada) following guideline specifications [3]. The RCCs were sampled on d2 pre-irradiation for baseline values, then on d14 and d28 post-irradiation. Sampling was performed through sterile connection of 40 ml sampling bags (also made of PVC-DEHT or PVC-DEHP, respectively) to the RCCs, very gentle mixing during sampling, followed by immediate analysis.

The RBCs were exposed to the same plasticizer (DEHT or DEHP, respectively) at all time points, from collection until study end. Furthermore, the RCCs of different plasticizers were handled and stored separately to avoid accidental cross-contamination, as were the bag systems during the manufacturing process.

Assessment of RBC quality

The impact of X-ray irradiation on the different plasticizer/AS combinations was determined through collective assessment of membrane

effects and metabolic effects during storage. The RCCs were analysed for haemoglobin (Hb_{RCC}; g/l), haematocrit (Hct) and mean corpuscular volume (MCV; fl) through Swelab Alfa Plus Basic hematology analyzer (Boule Diagnostics AB, Spånga, Sweden). After centrifugation of the samples twice (1450 × *g*, 10 min, 20°C), free supernatant haemoglobin ($Hb_{supernatant}$; g/l) was measured with HemoCue plasma/low haemoglobin photometer (Radiometer Medical ApS). Haemolysis (%) was calculated as (100-Hct) × $Hb_{supernatant}$ (g/l) / Hb_{RCC} (g/l). Extracellular K^+ (mmol/l), pH, glucose (mmol/l) and lactate (mmol/l) were assessed with ABL 800 Flex blood gas analyser (Radiometer Medical ApS, Brønshøj, Denmark). Measurements of RMV count and RMV phosphatidylserine externalization (Annexin V positive RMVs) were performed by flow cytometry (CytoFLEX, Beckman Coulter, Brea, CA), protocols identical to previous study [23].

In addition, residual WBCs were counted with cell counter ADAM-rWBC (NanoEnTek, Seoul, South Korea) at storage start. All donors were screened for required serological markers [3], and all RCCs were bacteriologically tested at storage end (Karolinska University Laboratory, Clinical Chemistry and Clinical Microbiology departments).

Statistical analysis

After verifying a Gaussian distribution (D'Agostino-Pearson normality test), mean ± standard deviation (SD) was computed (GraphPad Prism v.8.2 for Windows; GraphPad Software Inc., La Jolla, CA). To test statistical significance between the study arms, two-way analysis of variance (ANOVA) was applied with Holm-Sidak's correction as post-hoc test, while differences between time points within a single study arm were compared using repeated measures one-way ANOVA.

Ethics statement

As all used material was fully anonymised, the Stockholm Regional Ethical Review Board did not consider an ethical application necessary.

RESULTS

All RBC units fulfilled European and national quality criteria in Hb_{RCC}, Hct and WBC count [3, 17]. Screenings for bacteria and infectious markers were negative.

Haemolysis was similar for DEHT/PAGGSM and DEHP/SAGM at d14 post-irradiation. However, during the second half of storage, it increased faster in DEHT, especially in combination with SAGM, and at d28, all four study arms differed ($p < 0.001$; Figure 1a, Table 1). The highest individual unit (DEHT/SAGM) reached 0.65% haemolysis, meaning all units remained safely below 0.8% at storage end.

Elevated extracellular K^+ signifies membrane leakage and/or suppressed metabolism. Extracellular K^+ increased sharply after irradiation in all study arms, independent of plasticizer/AS combination. DEHT/PAGGSM stayed similar to both DEHP arms throughout storage, whereas storage in DEHT/SAGM resulted in slightly higher concentration than both PAGGSM arms (d14: $p < 0.001$, d28: $p < 0.05$; Figure 1b, Table 1). The two SAGM arms (DEHT/SAGM vs. DEHP/SAGM) remained similar throughout storage. The difference in concentration between the study arms stayed within 2.4 mmol/l (min-max range of the d28 means: 57.2–59.6 mmol/l).

Increased RMV count signifies loss of membrane integrity. The RMV count was similar for all study arms at d14 post-irradiation. However, at d28, DEHT/SAGM had the highest observed count, followed by DEHT/PAGGSM (Figure 1c, Table 1). Although the overall percentage of RMVs exposing the apoptosis marker phosphatidylserine increased after irradiation ($p < 0.05$), there was no difference between the study arms at the separate time points (Table 1).

Glucose, lactate and pH are markers of metabolic capacity. The glucose concentration and pH were both initially higher in SAGM storage than in PAGGSM (glucose: $p < 0.05$, pH: $p < 0.001$), but the differences ceased with storage time. Correspondingly, the lactate generation was faster and indicated an overall higher metabolism during SAGM storage. MCV is sensitive to osmolality. A distinct increase in MCV was detectable from d14 onwards in both SAGM arms, whereas in PAGGSM, MCV decreased compared to d2 ($p < 0.001$; Figures 2a–c, Table 1). No distinct differences could be related to plasticizer for glucose, lactate, pH or MCV.

DISCUSSION

This study demonstrates that DEHT is a suitable plasticizer for storage of RBCs after applying severe cell stress in the form of X-ray irradiation.

The observed haemolysis in irradiated RBCs stored in DEHT in combination with either SAGM or PAGGSM (Figure 1(a)) was in accordance with the previously shown pattern of non-irradiated RBC units stored in DEHT [23], however, expectedly amplified. The non-irradiated d49 haemolysis values were exceeded somewhere between d14 and d28 post-irradiation. This similarity suggests that irradiation itself does not interfere with the DEHP removal and/or DEHT addition in other ways than the accelerated speed in haemolysis generation observed also after irradiation of conventional DEHP-stored RBCs [6, 11, 13, 14]. Even with DEHP, the possibility of individual units exceeding 0.8% haemolysis during post-irradiation storage remains a health risk, in particular to the more fragile patient categories such as, for instance, paediatric patients. The literature for irradiation of non-DEHP storage containers is scarce, likely because removal of DEHP has been linked to critical haemolysis levels in non-irradiated units [26]. This study indicates that PVC-DEHT containers provide sufficient RBC storage environment with existing commercial ASs for at least 14 days, but even up to 28 days post-irradiation, when irradiation is carried out early after donation.

The extracellular K^+ results were also encouraging. The minor concentration differences between the four plasticizer/AS

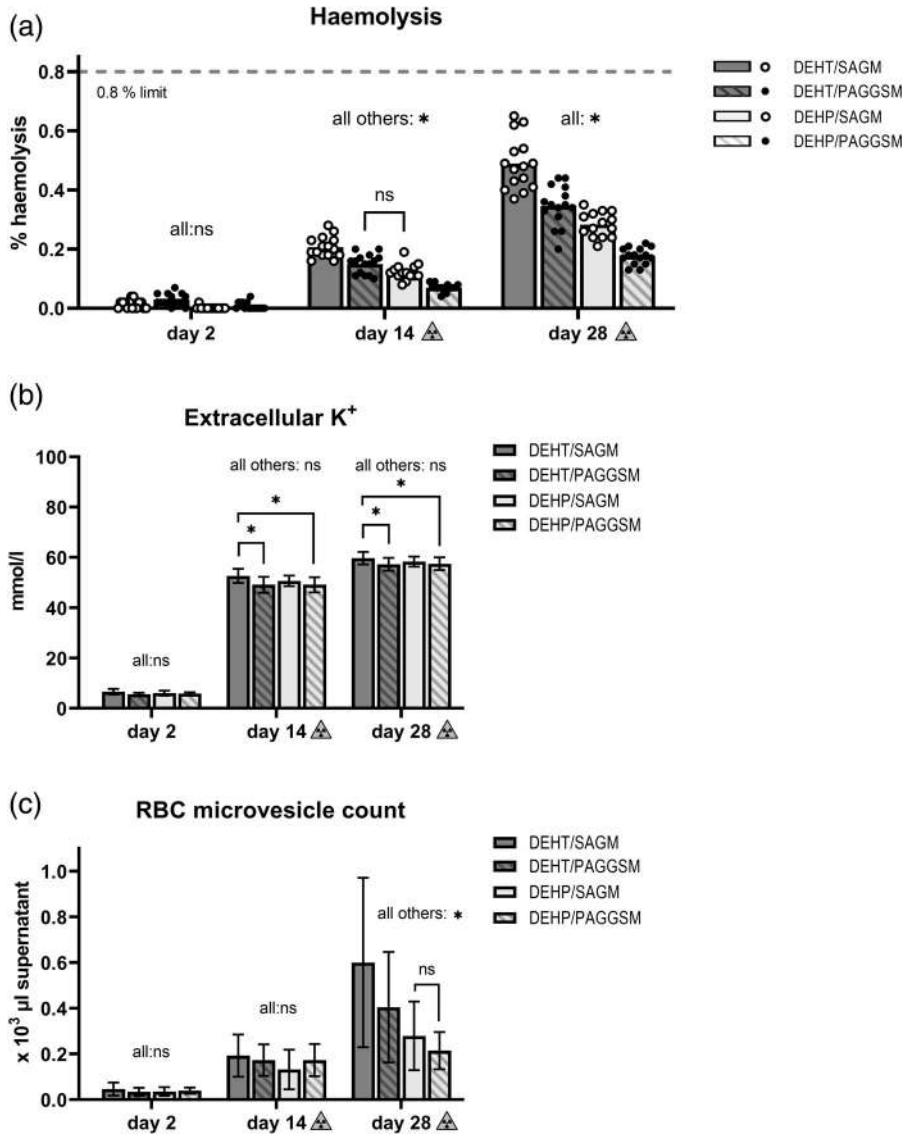

FIGURE 1 (a) RBCs stored in DEHT had acceptable haemolysis throughout storage, especially when combined with PAGGSM additive solution. No units exceeded 0.8%. The bars show mean with a scatter dot plot representing the individual values. (b) Irradiation implicated sharp increases in extracellular K^+ concentration in all study arms. DEHT/SAGM was marginally higher than both PAGGSM arms post-irradiation but similar (ns) to DEHP/SAGM. (c) RBC microvesicle count was similar in both plasticizers at d14 post irradiation but higher in DEHT at d28. PAGGSM detained the microvesicle generation compared to SAGM. Values in (b) and (c) are displayed as mean ± standard deviation. Significance levels: *$p < 0.05$, no significance (ns). Radiation symbols represent post-irradiation. DEHT/SAGM, DEHT/PAGGSM, DEHP/PAGGSM: $n = 15$, DEHP/SAGM: $n = 14$. DEHT, di(2-ethylhexyl) terephthalate; DEHP, di(2-ethylhexyl) phthalate; SAGM, saline-adenine-glucose-mannitol; PAGGSM, phosphate-adenine-glucose-guanosine-saline-mannitol; RBC, red blood cell

combinations were likely not of clinical significance and suggest that the irradiation itself had substantially more impact than the possible theoretical influence of plasticizer or AS (Figure 1b).

RMVs are shed from the RBC membrane as part of the storage lesion process, as ROS increases and antioxidants and ATP are depleted [2]. In this study, the generation of RMVs had similar but earlier onset than in non-irradiated units [23]. This is likely attributed to increased ROS generation caused by irradiation, both directly and through the positive feedback loop provided by the haemolysis-induced increase of free haemoglobin [11, 12]. A relationship was demonstrated between RMV count and both plasticizer and AS, the latter likely related to the ATP dependency of the RMV generation; the lower RMV count in PAGGSM-stored units suggests a slower reduction of ATP. Though ATP was not analysed in this study, the associated markers pH, lactate and glucose all trended towards a higher metabolic rate in SAGM storage (Figure 2a,b).

Phosphatidylserine is a phospholipid normally located in the inner leaflet of the RBC bilayer membrane. Membrane proteins of ATPase character control the asymmetry between the inner and outer leaflet,

TABLE 1 Red blood cell analysis results pre-irradiation (day 2) and post-irradiation (day 14 and 28).

	Day 2 (pre irradiation)				Day 14 (post irradiation)				Day 28 (post irradiation)			
Analysis parameter	DEHT/SAGM	DEHT/PAGGSM	DEHP/SAGM	DEHP/PAGGSM	DEHT/SAGM	DEHT/PAGGSM	DEHP/SAGM	DEHP/PAGGSM	DEHT/SAGM	DEHT/PAGGSM	DEHP/SAGM	DEHP/PAGGSM
Haemolysis (%)	0.01 ± 0.01	0.03 ± 0.02	<0.01	0.01 ± 0.01	0.21 ± 0.04[a,b,c]	0.15 ± 0.03[a,e]	0.12 ± 0.03[b,f]	0.07 ± 0.01[c,e,f]	0.49 ± 0.09[a,b,c]	0.35 ± 0.07[a,d,e]	0.28 ± 0.04[b,d,f]	0.18 ± 0.03[c,e,f]
RMV, count ($\times 10^3$/µl supernatant)	0.05 ± 0.03	0.03 ± 0.02	0.04 ± 0.02	0.04 ± 0.01	0.19 ± 0.09	0.17 ± 0.07	0.13 ± 0.09	0.17 ± 0.07	0.60 ± 0.37[a,b,c]	0.40 ± 0.24[a,d,e]	0.28 ± 0.15[b,d]	0.21 ± 0.08[c,e]
RMV, externalized phosphatidylserine (%)	57.0 ± 18.8[c]	51.3 ± 25.1[e]	48.3 ± 22.4[f]	32.4 ± 14.7[c,e,f]	56.7 ± 11.8	47.0 ± 10.9	53.8 ± 13.8	47.1 ± 10.9	71.7 ± 7.6	63.1 ± 10.7	66.4 ± 12.7	58.3 ± 9.7
Extracellular K^+ (mmol/l)	6.6 ± 1.1	5.6 ± 0.5	6.0 ± 1.0	5.9 ± 0.5	52.6 ± 2.8[a,c]	49.1 ± 3.1[a]	50.6 ± 2.1	49.1 ± 3.0[c]	59.6 ± 2.5[a,c]	57.2 ± 2.6[a]	58.2 ± 2.0	57.4 ± 2.5[c]
pH 37°C	6.950 ± 0.017[a,b,c]	6.811 ± 0.032[a,d]	6.916 ± 0.028[b,d,f]	6.804 ± 0.031[c,f]	6.606 ± 0.019[a,c]	6.569 ± 0.028[a]	6.580 ± 0.032	6.573 ± 0.030[c]	6.432 ± 0.031	6.403 ± 0.044	6.414 ± 0.041	6.415 ± 0.034
Glucose (mmol/l)	29.1 ± 1.5[a,c]	27.7 ± 1.7[a,d]	29.0 ± 0.8[d,f]	27.0 ± 1.3[c,f]	22.0 ± 0.8	21.3 ± 1.2	21.8 ± 1.5	21.3 ± 1.1	18.0 ± 1.5	17.1 ± 1.9[d]	18.7 ± 1.9[d]	17.7 ± 1.4
Lactate (mmol/l)	5.2 ± 0.8	4.3 ± 0.4	5.3 ± 0.7	4.5 ± 0.5	19.5 ± 1.9[c]	17.4 ± 1.6	18.7 ± 1.9	16.8 ± 1.7[c]	26.4 ± 3.3[c]	24.5 ± 3.9	24.9 ± 3.9	22.9 ± 3.0[c]
MCV (fl)	93.3 ± 3.0	92.6 ± 5.5	94.0 ± 3.3	93.4 ± 4.2	100.0 ± 3.6[a,c]	89.7 ± 5.0[a,d]	99.3 ± 3.3[d,f]	89.9 ± 3.7[c,f]	105.0 ± 3.8[a,c]	90.9 ± 4.8[a,d]	103.0 ± 3.5[d,f]	90.4 ± 3.5[c,f]

Note: Data are presented as mean ± standard deviation. DEHT/SAGM, DEHT/PAGGSM, DEHP/PAGGSM: $n = 15$, DEHP/SAGM: $n = 14$. Significant differences ($p < 0.05$) are shown by letters a-f.
Abbreviations: DEHT, di(2-ethylhexyl) terephthalate; DEHP, di(2-ethylhexyl) phthalate; SAGM, saline-adenine-glucose-mannitol; PAGGSM, phosphate-adenine-glucose-guanosine-saline-mannitol; RMV, red blood cell microvesicles; MCV, mean corpuscular volume.
[a]DEHT/SAGM versus DEHT/PAGGSM.
[b]DEHT/SAGM versus DEHP/SAGM.
[c]DEHT/SAGM versus DEHP/PAGGSM.
[d]DEHT/PAGGSM versus DEHP/SAGM.
[e]DEHT/PAGGSM versus DEHP/PAGGSM.
[f]DEHP/SAGM versus DEHP/PAGGSM.

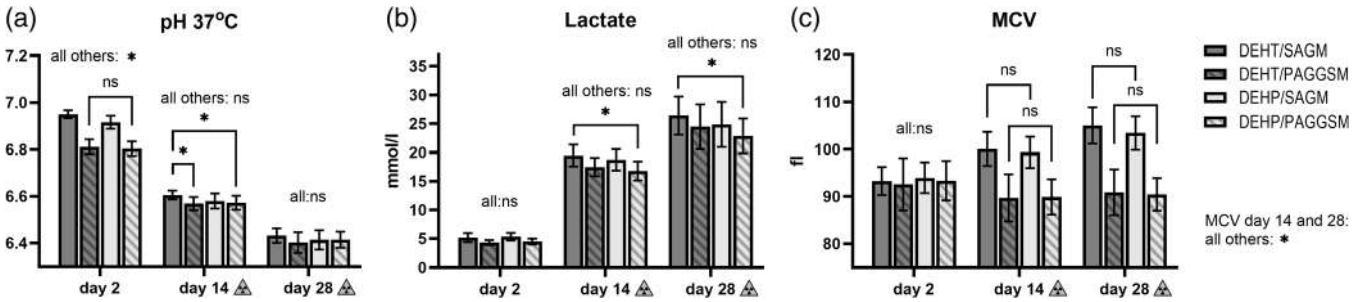

FIGURE 2 (a) pH, (b) lactate and (c) MCV were unaffected by plasticizers. Discernible differences could be linked to additive solution composition, which influence (a) decreased or (b, c) increased with time. Values are displayed as mean ± standard deviation. Significance levels: $^*p < 0.05$, no significance (ns). Radiation symbols represent post-irradiation. DEHT/SAGM, DEHT/PAGGSM, DEHP/PAGGSM: $n = 15$, DEHP/SAGM: $n = 14$. DEHT, di(2-ethylhexyl) terephthalate; DEHP, di(2-ethylhexyl) phthalate; SAGM, saline-adenine-glucose-mannitol; PAGGSM, phosphate-adenine-glucose-guanosine-saline-mannitol; MCV, mean corpuscular volume

and when ATP levels decrease, there is a progressive translocation of phosphatidylserine to the outer leaflet [27], where it plays a signalling role for phagocytosis [28]. The literature is ambiguous as to whether the percentage of phosphatidylserine positive RMVs from non-irradiated RBCs increases during storage or not; several studies, our own previous study included, seem to suggest that it does not [23, 29, 30]. However, as irradiation induces increased oxidizing damage to membrane-bound proteins [11, 12], it may be hypothesized that the observed increase in the frequency of RMVs exposing phosphatidylserine may be a consequence of irradiation damage. Possibly, irradiation targets ATPase the same way as for extracellular K^+. Phosphatidylserine externalization could not be related to AS the way it could for non-irradiated units. The irradiation damage to the ATPase may have outweighed the potential later effects of AS- and storage time-dependent metabolic depletion, especially as the RBCs were irradiated as early as d2 post-collection.

Irradiation appears to primarily impact the storage lesion parameters connected to the stability of the RBC membrane. Expectedly, the same parameters were affected when the membrane-stabilizing plasticizer DEHP was exchanged for DEHT. However, there seems to be no negative physical impact by irradiation on the DEHT plasticizer itself, as parameters that are unaffected by choice of plasticizer in non-irradiated RBCs remained unaffected also in this study. The AS-dependent differences for glucose, lactate, pH and MCV were well in accordance with previous findings for non-irradiated units [23] without any detectable dissimilarities that could be ascribed to potential irradiation damage to the plasticizer itself. Furthermore, sealing, sterile docking and general bag handling were performed without remark throughout the study.

This study has both strengths and limitations. One limitation is that irradiation was performed on d2, while it is allowed up until d28 post-collection in many countries [3], and there is evidence connecting irradiation of older RCCs to higher haemolysis rates [6, 13]. This study gives a good first indication, but a similar follow-up study with older units would be a necessary complement for further conclusions. Moreover, the irradiation process was performed by X-ray technique. Although proven to be equal to gamma technique for RBCs stored in DEHP [7], gamma irradiation of DEHT should be verified. Furthermore, it was not possible to directly measure ATP. Instead, indirect markers such as pH, glucose, lactate and K^+ had to be used for conclusions about the metabolism. Yet another limitation is the impact of donor variability as, for logistical reasons, no pool-and-split strategy was applied during processing. This is a weakness when comparing different study arms; however, it is simultaneously an important asset for demonstrating that despite donor variation, no individual units exceeded the limit for haemolysis, independent of the plasticizer or AS they were stored in (Figure 1a).

Numerous processing conditions affect the RBC storage lesion, which complicates inter-blood establishment comparisons [31, 32]. A strength of this study is that the irradiated RCCs were processed in parallel with the non-irradiated RCCs of our previous study about DEHT storage, using identical processing specifications, disposable materials, equipment, sampling procedure, laboratory staff and facilities. This optimizes the use of that study for comparison and reference [23].

This study demonstrates an encouraging quality of irradiated RBCs stored in PVC-DEHT blood bags combined with the European primary AS choice SAGM. The membrane integrity loss caused by DEHP removal can, however, be counteracted to some extent by instead choosing PAGGSM. DEHT/PAGGSM differed very little, if at all, to the current DEHP/SAGM storage. It is well established that SAGM is a suboptimal storage solution for RBCs and that, quality-wise, more favourable options exist [26, 33–35]. However, it does remain the first-hand choice in most European countries, likely due to its long-standing regulatory approval, cost, and the extensive evaluation efforts a transition would require. Perhaps, when the new medical device regulation is taken into effect and a new plasticizer will be mandatory, it may be a good opportunity to simultaneously consider a change of AS.

It could also be a good time to revise the guidelines for irradiation, as has been previously suggested [13], and opt for a more conservative post-irradiation timeframe. An important observation of this study was that neither the haemolysis levels nor the RMV count of RBCs stored in DEHT/PAGGSM differed statistically to DEHP/SAGM at 14 days post-irradiation. A maximum of 14 days post-irradiation storage time is already routine practice in Sweden and the UK [17, 18]. Such a revision would further facilitate the transition to DEHP-free materials.

In summary, this work investigates the additional stress of irradiation on red blood cells stored in PVC bags plasticized with DEHT. A satisfactory post-irradiation storage capacity of DEHT with either SAGM or PAGGSM additive solution is demonstrated. This adds further weight to the suitability of DEHT as a non-phthalate replacement option for DEHP.

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L.L. provided the research idea, study design, laboratory work, data analysis and manuscript composition; S.O. and J.D. assisted on laborations; B.D. managed the blood collection; P.S. and S.L. provided technical and medical expertise; M.U. supervised the study. All authors revised the manuscript and contributed with valuable input.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Linda Larsson https://orcid.org/0000-0002-0196-1773
Michael Uhlin https://orcid.org/0000-0002-2177-6727

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ORIGINAL ARTICLE

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HIV residual risk in Canada for apheresis source plasma donation without deferral for men who have sex with men

Eliana Aubé[1,2] | Antoine Lewin[2,3] | Sheila F. O'Brien[4] | Yves Grégoire[5] | Josiane Pillonel[6] | Whitney R. Steele[7] | Brian Custer[8,9] | Katy L. Davison[10] | Marc Germain[5] | Clive R. Seed[11] | Félix Camirand Lemyre[1,12] | the Surveillance, Risk Assessment and Policy Subgroup of the ISBT Transfusion Transmitted Infectious Diseases Working Party

[1]Department of Mathematics, Université de Sherbrooke, Sherbrooke, Quebec, Canada

[2]Medical Affairs and Innovation, Héma-Québec, Montreal, Quebec, Canada

[3]Faculty of Medicine and Health Science, Université de Sherbrooke, Sherbrooke, Quebec, Canada

[4]Epidemiology & Surveillance, Canadian Blood Services, Ottawa, Ontario, Canada

[5]Medical Affairs and Innovation, Héma-Québec, Quebec City, Quebec, Canada

[6]Direction des Maladies Infectieuses, Santé Publique France, St. Maurice, France

[7]Scientific Affairs, American Red Cross, Rockville, Maryland, USA

[8]Epidemiology and Health Policy Science, Vitalant Research Institute, San Francisco, California, USA

[9]Department of Laboratory Medicine, UCSF, San Francisco, California, USA

[10]Immunisation, Hepatitis & Blood Safety, Health Protection Agency, London, UK

[11]Donor and Product Safety (DAPS) Policy Unit, Australian Red Cross Lifeblood, Perth, Western Australia, Australia

[12]Centre de recherche, Centre Hospitalier Universitaire de Sherbrooke, Sherbrooke, Quebec, Canada

Correspondence
Félix Camirand Lemyre, Faculté des sciences, Université de Sherbrooke, 2500 boulevard de l'Université, Sherbrooke, c1/QC J1K 2R1, Canada.
Email: felix.camirand.lemyre@usherbrooke.ca

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Abstract

Background and Objectives: In Canada, men having sex with men (MSM) are deferred for 3 months from last sexual contact to reduce human immunodeficiency virus (HIV) risk to recipients. The aim of this paper was to model the Canadian residual risk of HIV-positive source plasma incorporating pathogen inactivation (PI) under no MSM deferral scenarios for apheresis plasma donations.

Materials and Methods: A combined Bayesian network (BN) and Monte Carlo approach were implemented to estimate the HIV residual risk under 3-month deferral compared with no deferral without quarantine scenarios for MSM donors. Models involve the stochastic generation of donation and its infection status based on its corresponding simulated donor profile. Viral load reduction conferred by PI used by source plasma fractionators was simulated. Model parameters were derived from Héma-Québec and Canadian Blood Services data, viral loads in a large sample of HIV-positive US blood donors, CSL Behring documentation and from published data.

Results: In the most likely scenario for the 3-month deferral model, there were 2.71 positive donations per 1,000,000 donations (95% confidence interval [CI] 2.63–2.78). For the no-deferral model, there were 3.01 positive donations per 1,000,000 donations (95% CI 2.94–3.09). For both scenarios, the risk of having an infectious pool was 0 in 300,000 pools (95% CI 0–0.0000123) after consideration of PI.

Conclusion: Based on simulation results, there would be a negligible HIV residual risk associated with the removal of a time-based MSM deferral without quarantine for source plasma incorporating PI.

KEYWORDS
Canada, deferral, HIV, MSM, pathogen inactivation technology, residual risk

INTRODUCTION

Since human immunodeficiency virus (HIV) emerged in the early 1980s, major efforts have been made to ensure blood products' safety and quality in order to avoid transmission of infectious agents to recipients [1]. One approach has been to exclude from donation those at higher risk for HIV. In North America, beginning in the early 1980s, men having sex with men (MSM) was identified as a very-high-risk group for acquired immunodeficiency syndrome (AIDS). Although little was known about HIV as the causative agent of AIDS at the time, MSM were permanently deferred from blood donation if they had sex with another man even once, since 1977. Since then, scientific knowledge concerning HIV infection, diagnostics and transmission modes have considerably expanded. Sensitive assays have reduced the window period (WP) of infection and computer systems have all but eliminated the chance of releasing a product in error. Internationally, MSM deferral from blood donation was initially permanent, but numerous countries have changed the deferral from a lifetime to progressively shorter periods [2]. Based on development of highly accurate testing methods and introduction of pathogen inactivation (PI), completely lifting the ban on donation from sexually active MSM has been suggested [3]. In particular, it has been proposed that MSM could safely donate plasma intended for fractionation, given that each donation is screened by serology and nucleic acid testing (NAT), and that the fractionation process includes very effective PI steps. However, it is necessary to carefully assess risks associated with this change since this group of potential donors remains at higher risk of HIV infection in Western countries. For example, at the end of 2016, the Public Health Agency of Canada estimated that 49.1% of HIV-infected persons in Canada were MSM [4].

In Canada, the lifetime exclusion for MSM blood donors was abolished in 2013 and replaced by a 5-year deferral. In 2016, deferral changed from 5 years to 12 months, and, in 2019, Canada authorized MSM blood donations after 3 months since last sexual contact with another man [5]. These policy changes were supported by mathematical models simulating various scenarios which allowed estimating the risk of releasing potentially infectious donations [5, 6]. Based on these estimations, simulated HIV residual risk remains extremely low, given that sensitivity of NAT has improved to the extent that the HIV WP for donation testing in Canada is 8–10 days [5].

One approach now being considered in Canada is the possible implementation of less restrictive MSM deferral for plasma intended for fractionation. In this context, we developed a stochastic BN model with Monte Carlo simulations to estimate the risk of HIV contamination of plasma-derived products if sexually active MSM were allowed to donate.

MATERIALS AND METHODS

Context

We define HIV residual risk as the probability of having an infectious pool of plasma after using PI processes. The infectious threshold is conservatively assumed to be one HIV RNA copy per pool. A pool designates the combination of a number of individual plasma donations required to reach a predefined volume of plasma. Individual donations are assumed to originate from donors who are considered safe to donate based on their answers to numerous risk questions, including those regarding sexual risks (see Data S1) and whose donation has tested negative for HIV-1/2 antibody and HIV-1 NAT. This allows an estimate of what the residual risk would be in a model where the MSM deferral for source plasma is 3 months (current) versus no MSM deferral (proposed).

Model

A two-stage Monte Carlo procedure was employed to estimate the rate of HIV-positive pools in $N = 300{,}000$ pools of fractionated plasma treated with one or more PI steps.

First stage

A BN is a probabilistic graphical model that uses a directed acyclic graph (DAG) to specify conditional relationships among a collection of variables. In the BN DAG, nodes represent variables, and edges reflect conditional dependencies. A joint probability distribution can then be constructed from the graphical structure by elicitation of the set of conditional probabilities of each node given its parents in the graph [7]. Figure 1 shows the BN DAG considered for our simulation models. It includes donor variables known to be associated with risk of infection: age at the time of donation, sex, donor status (new/repeat), MSM (yes/no), compliance with MSM deferral (yes/no, in the 3-month scenario only), level of risk (high/low, in the no-deferral scenario only), HIV infectious state (not infected/infected within the WP/infected and not in WP) and NAT (+/−). According to the BN graph in Figure 1, the joint probability over these variables factors as:

$$\begin{aligned} &P(\text{age, sex, MSM, status, compliance, HIV state NAT}) \\ &= P(\text{age})P(\text{sex}\,|\,\text{age})P(\text{MSM}\,|\,\text{sex})P(\text{status}\,|\,\text{age, sex, MSM})P(\text{compliance}\,|\,\text{MSM}) \\ &\quad \times P(\text{HIV state}\,|\,\text{sex, MSM, status, compliance})P(\text{NAT}\,|\,\text{HIV state}). \end{aligned}$$

The Elicitation section contains a description of how bodies of knowledge were used to model each factor above.

Second stage

The Monte Carlo algorithm performed simulations of each donation individually and independently from the joint distribution specified by the BN. To form plasma pools, simulated plasma donations (750 ml) were iteratively combined until a pooled volume ranging randomly from 4000 L to 6000 L was reached (personal communication with Rima Khalil, Héma-Quebec Stable products director). The HIV viral load of a pool was calculated as the sum of the viral load of each

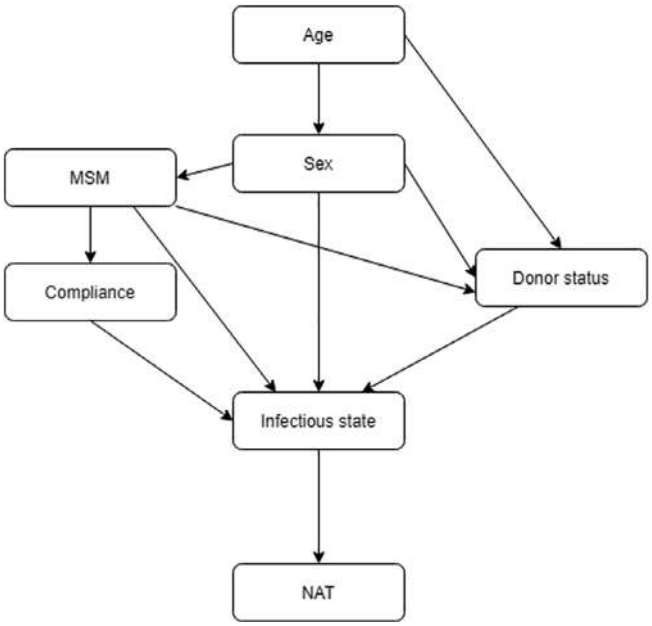

FIGURE 1 Directed acyclic graph (DAG) of human immunodeficiency virus (HIV) risk estimation for source plasma

positive donation adjusted for the volume of each plasma unit and the total volume of the pool. The effect of the PI process was simulated based on the viral load of each pool.

Elicitation

Simulation process flowchart

The BN DAG in Figure 1 was used as a process flowchart for donor profile simulations. First, age was generated, second, sex conditional on age, third, MSM status conditional on sex and so on. In the no-deferral model, rate of non-compliance was used as a proxy for the percentage of recently active high-risk MSM potential donors (calculations are shown in the Scenarios section). Although not explicitly shown in Figure 1, infectious state was also set to depend on prevalence and incidence rates. These were made specific to sex, donor status, MSM status and compliance variables. For newly positive donations, an extra simulation step was carried to consider WP risk.

Parameters used in the first stage

Donor age and sex

Distribution of donors by age group ($P(\text{age})$) was set to match sample proportions calculated based on plasma donation data from Canadian Blood Services (CBS) and Héma-Québec collected in 2018 (January 1–December 31). The minimum age for donation is 17 at CBS and 18 at Héma-Québec. Three age groups were considered: 17/18–24, 25–44 and above 44. This stratification was made since it could be seen from the data that the proportion of new donors (which needed to be simulated in our model) varied significantly among these three groups. Distribution of sex within each age group ($P(\text{sex}|\text{age})$) was determined similarly.

MSM donor proportion

$P(\text{MSM} = \text{yes}|\text{sex} = \text{female})$ was set to 0, and $P(\text{MSM}|\text{sex} = \text{male})$ was adjusted based on the MSM male donor proportion for the 12-month deferral model described in O'Brien et al. [8], see Data S1.

Donor status

The distribution of new and repeat donors ($P(\text{status}|\text{age},\text{sex},\text{MSM})$) was elicitated by matching sample proportions from Héma-Québec and CBS internal data.

MSM donor non-compliance with MSM deferral criteria

The rate of MSM failing to comply with deferral criteria was determined based on male non-compliance rate retrieved from the most likely scenario of O'Brien et al. [5]. Non-compliance in MSM ($P(\text{compliance} = \text{no}|\text{MSM} = \text{yes})$) was estimated using the non-compliance rate in men and MSM proportion:

$$P(\text{compliance} = \text{no}|\text{MSM} = \text{yes}) = \frac{\text{P(non-compliance in men)}}{\text{P(MSM} = \text{yes)}}$$

Further, $P(\text{compliance} = \text{no}|\text{MSM} = \text{no})$ *was set to* 0.

HIV state

For the conditional distribution of HIV state, parameters associated to HIV incidence/prevalence and WP probability were considered.

HIV prevalence/incidence: Here, prevalence is defined as the proportion of positive donations in a donation pool, whereas the incidence rate is defined as the proportion of new HIV cases in a donation pool over a year. Given the limited number of HIV-positive donations in our datasets (three positive donations in 5 years), prevalence and incidence rate estimations were not categorized by age group and were determined for all groups of donors $G = G_{(\text{sex,MSM,status,compliance})}$ corresponding to a plausible choice for donor profile variables.

To determine group-wise HIV prevalence and incidence rates, donations in Canada (CBS and Héma-Québec) from 1 July 2014 to 30 June 2019 were considered. For groups matching status = repeat, prevalence was elicitated according to the formula

$$\text{Prevalence in } G_{\text{status=repeat}} = \frac{\text{number of HIV infected donations in } G_{\text{status=repeat}}}{\text{number of donations tested in } G_{\text{status=repeat}}}$$

Prevalence in groups with status = new was considered 10 times higher than the one of their matched analogue with status = repeat (i.e., same characteristics except for status) obtained from the above Equation [5].

To determine group-wise HIV incidence, an annual mean of 2.15 donations per donor was used to estimate the number of annual

donations. Group-specific incident cases rate was determined from HIV-positive donations made by repeat donors who had made an HIV-negative donation in the 12 months preceding their positive donation. For groups associated with *status = repeat*, incidence rate was calculated as:

$$\text{Incidence rate in } G_{\text{status=repeat}} = \frac{\text{number of incident cases in } G_{\text{status=repeat}}}{\text{number of donations tested in } G_{\text{status=repeat}}}.$$

Incidence rate in groups with status = new was elicitated following the approach of Davison et al. and set equal to the one of their matched analogues with status = repeat multiplied by a truncated-at-zero normal random variable with mean of 1.65 (SD 1.0334). This adjustment factor was put forward following the analysis of first time and repeat English donor data during 2001–2007 [6].

For cases where MSM = yes, prevalence and incidence proportions were set equal to the number of HIV-positive donations from non-compliant MSM donors (current criteria), and the estimated number of non-compliant MSM donors was based on post-donation interviews and compliance survey.

WP donations: WP is the stage from infection to time at which pathogen becomes detectable by a given diagnostic procedure. The probability of having an incident donation being made during the WP was considered independent of the donor's profile given their 'newly infected' status, and set equal to:

$$P(\text{WP donation}|\text{incident case} = \text{yes}) = \frac{\text{WP length}}{\text{Median interdonation interval length}}.$$

The median interdonation interval length was calculated based on 2018 CBS and Héma-Québec data for plasma donation.

Parameters used in the second stage

Viral load

For WP donations, Weusten et al. model [9] was used to specify the viral load distribution. The maximum WP viral load was set to 14.5 copies/ml. Viral loads post WP were simulated based on U.S. HIV-positive blood donation database (1156 positive blood donations from the period of 2010 to 2018). Complete modelling details regarding viral load distribution are given in Data S1.

HIV serological and NAT

Performance, limit of detection and system failure parameters were elicitated based on routinely used procedures in Canadian blood operators involving Cobas multiplex and Procleix Ultrio Plus package inserts [10, 11], see Data S1 for details.

PI process

Donor screening and testing of donations and fractionation pools are the first and second steps in product safety, while virus reduction using PI is the third and last step applied to pooled plasma destined for plasma-derived medical products [12] (see also Data S1). The log reduction factor (LRF) is used to quantify this reduction, for instance, one LRF corresponds to inactivating 90% of the pathogen with the viral load being reduced by a factor of 10 (i.e., 1 log). HIV LRF for PDMD ranges from 15.3 (Privigen, CSL Behring, Bern, Switzerland) to 9.6 (RiaSTAP, CSL Behring, Marburg, Germany) (Personal communication with CSL Behring). For modelling purposes, the lowest mean LRF for HIV was used.

Model parameter values related to NAT performance and PI log reduction are detailed in Data S1.

Scenarios

Simulations were conducted according to a 3-month MSM deferral model and to a model with no MSM deferral without plasma unit quarantine. Both were simulated under four scenarios: most likely, optimistic and pessimistic i and ii. Values for the parameters are shown in Tables 1–3.

1. **Most likely scenario**: HIV incidence, prevalence, MSM donor proportion and non-compliance rates were taken as described in the Elicitation section. For the no-deferral model, proportion of males with high-risk MSM behaviour was determined using the proportion of non-compliant male donors as proxy for high-risk MSM behaviour as follows: $\frac{\text{non-compliant male donor proportion (3-month)} * 2}{1 + \text{non-compliant male donor proportion (3-month)}}$.

The 3-month non-compliant male donor proportion was multiplied by 2 for the no-deferral model since MSM donors who were non-compliant before lifting of the deferral were considered to remain blood donors after the lifting, and the number of high-risk newly eligible MSM donors was considered to be proportional to that number.

2. **Optimistic scenario**: HIV incidence, prevalence and MSM donor proportion were set as in the most likely scenario. Non-

TABLE 1 MSM parameter values according to deferral model

		Three-month deferral (95% CI)	No deferral (95% CI)
Proportion of MSM among donor population		0.0162 (0.0099–0.0254)	0.0172 (0.0110–0.0264)
Proportion of non-compliant MSM		0.0010 (0.0003–0.0020)	–
Proportion of men at high risk of HIV		–	0.0020 (0.0006–0.0040)
Proportion of MSM among new donors by age group	17/18–24	0.0522 (0.0348–0.0843)	0.0562 (0.0398–0.0864)
	25–44	0.0279 (0.0225–0.0324)	0.0332 (0.0282–0.0375)
	>44	0.0158 (0.0135–0.0186)	0.0218 (0.0197–0.0245)

Abbreviations: HIV, human immunodeficiency syndrome; MSM, men having sex with men.

TABLE 2 Human immunodeficiency virus (HIV) prevalence and incidence according to deferral models

HIV prevalence in the donor population		
Sex	**Donor type**	**HIV prevalence per 100,000 donations**
Men	First time	0.724
	Repeat	0.117
Women	First time	0.613
	Repeat	0.306
Probability that donation is an incident case for HIV in donor population		
Sex		**HIV incident donation rate per 100,000 donations**
Men		0.039
Women		0.102
MSM HIV prevalence		
Donor type	**Compliance**	**HIV prevalence per 100,000 donations**
First time	Non-compliant or high risk	155.40
	MSM donor population	65.28
Repeat	Non-compliant or high risk	15.54
	MSM donor population	6.53
MSM HIV incident donation probability		
Donor type	**Compliance**	**HIV incident rate per 100,000 donations**
Repeat	Non-compliant or high risk	15.54
	MSM donor population	2.7

Abbreviation: MSM, men having sex with men.

TABLE 3 Parameter values for window period and interdonation interval

Parameter	Value	Reference
Window period (mean [SD])[a]	9 (0.6) days	O'Brien et al. [5]
Interdonation interval (median [IQR; Q1, Q3])	14 days [7–34]	HQ and CBS

[a]Variability modelled by a normal distribution.
Abbreviations: CBS, Canadian Blood Services; HQ, Héma-Québec; IQR, interquartile range; SD, standard deviation.

compliance in male donors for 3-month deferral and high-risk MSM proportion for no-deferral model were reduced by half.

3. **Pessimistic scenarios**:

i. Compared to the most likely scenario, incidence, prevalence, non-compliance and/or high-risk MSM proportion were doubled, and MSM donor proportion was increased by a factor of 1.5.
ii. Parameters were set as in pessimistic i, except for MSM donor proportion. For the latter, the estimation of MSM proportion within the general population in Canada of 2.9% (95% confidence

TABLE 4 Human immunodeficiency virus (HIV) risk estimate

	Deferral model	Most likely	Optimistic	Pessimistic i	Pessimistic ii	Worst-case
HIV positive donations per 1,000,000 donations	3-month deferral	2.79	2.71	5.10	5.68	–
	No deferral	3.01	2.86	5.96	6.32	57.4
Number of pools with a viral load (in 300,000 pools)	3-month deferral	1323	1259	2885	3136	–
	No deferral	1483	1335	3719	3994	8617
Mean viral concentration per pool after NAT and PI (RNA copies/ml)	3-month deferral	1.93×10^{-14}	1.82×10^{-14}	1.95×10^{-13}	1.97×10^{-14}	–
	No deferral	1.95×10^{-14}	1.66×10^{-14}	1.95×10^{-14}	2.01×10^{-14}	2.81×10^{-13}
Probability of getting a pool with a viral load	3-month deferral	0.00441	0.00420	0.00962	0.01045	–
	No deferral	0.00494	0.00445	0.01240	0.01331	0.02872
Mean copies per pool after NAT and PI (RNA copies/pool)	3-month deferral	9.76×10^{-8}	9.17×10^{-8}	9.75×10^{-8}	9.73×10^{-8}	–
	No deferral	9.79×10^{-8}	8.16×10^{-8}	9.70×10^{-8}	1.00×10^{-7}	1.63×10^{-6}
Median copies per pool after NAT and PI (RNA copies/pool)	3-month deferral	1.75×10^{-8}	1.55×10^{-8}	1.65×10^{-8}	1.72×10^{-8}	–
	No deferral	1.47×10^{-8}	1.49×10^{-8}	1.59×10^{-8}	1.64×10^{-8}	1.63×10^{-8}
Maximum copies per pool after NAT and PI (RNA copies/pool)	3-month deferral	1.77×10^{-6}	1.15×10^{-6}	2.05×10^{-6}	1.99×10^{-6}	–
	No deferral	2.20×10^{-6}	1.26×10^{-6}	2.00×10^{-6}	2.28×10^{-6}	0.01041

Abbreviations: NAT, nucleic acid testing; PI, pathogen inactivation.

interval [CI] 1.8–3.9) was used, combined with the donor rate calculated for the general population [13].

In addition, a worst-case scenario was simulated for the no-deferral model using MSM donor proportion and MSM HIV incidence and prevalence rates based on the MSM general population (i.e., 443 per 100,000 MSM persons and 9.60%, respectively) [14, 15].

RESULTS

In the most likely scenario for the 3-month deferral model, the probability of having a pool with HIV RNA was 0.00441. An average number of HIV copies per pool after use of PI of 9.7606×10^{-8} (95% CI 9.7598×10^{-8}–9.7615×10^{-8}) was obtained, with a median of 1.7536×10^{-8} copies and a maximum of 1.7672×10^{-6} copies. For the no-deferral scenario, probability of having a pool with HIV RNA was 0.00494. The number of HIV copies per pool was 9.7856×10^{-8} (95% CI 9.7847×10^{-8}–9.7865×10^{-8}), along with a median number of 1.4700×10^{-8} and a maximum number of 2.2000×10^{-6} copies per pool. The mean number of HIV copies per billion litres ranges from 0.019334 to 0.019511 corresponding to a difference of 0.000177 copies per billion litres between the averages of the two deferral models.

For the worst-case scenario, the probability of having a pool with HIV RNA was 0.02872. The average number of HIV copies per pool after use of PI was of 1.6294×10^{-6} (95% CI 1.6244×10^{-6}–1.6346×10^{-6}), with a median of 1.6300×10^{-8} copies and a maximum of 0.010409. Results are presented in Table 4. There, absolute differences between mean viral concentrations are smaller than 1.59×10^{-15} copies/ml and are thus not clinically significant.

Finally, under the one HIV RNA copy per pool assumed threshold, none of the 300,000 pools simulation rounds, each round representing around two billion donations, would have been considered infectious (i.e., no manufactured product would have contained one virion of HIV after the use of PI process). A sensitivity analysis was conducted to highlight variables that are likely to affect risk estimates (Data S2).

DISCUSSION

Our model allowed to simulate HIV contamination risk in pools of plasma with a change from 3 months to no deferral without quarantine for MSM, considering the use of PI steps in the fractionation process. The results suggest that the latter risk of HIV transmission in plasma-derived products is negligible. According to our model, allowing sexually active MSM to donate would not significantly increase the risk of occurrence of an HIV RNA-positive pool. Our results also suggest that, while the vast majority of pools would not contain HIV at all, the average viral load within the HIV RNA-positive pools would only be slightly higher under the no-deferral model compared to the one under the 3-month deferral model and would be well under the one-per-pool HIV RNA copy threshold. Therefore, the safety profile of plasma-manufactured products, with respect to HIV transmission, would likely remain unaffected. Of note, our assumed threshold is arguably conservative, see, for example, Zanetti et al. [16], which states, regarding plasma for transfusion, that one human infectious dose corresponds to approximately 500–1000 HIV RNA copies. See also the study of HIV acquisition in Kleinman et al. [17].

In the worst-case scenario, only three infectious donations made outside the WP ended up in a pool, none of these, due to an NAT failure. This corresponds to three procedural errors in every 10,000 years in Canada.

Each source of data employed to estimate the model parameters has its limitations, and some parameters were more challenging to estimate. For instance, a response bias might have been present in surveys used to estimate the non-compliance rate among men for the MSM deferral criteria [18]. Non-compliance in Canada is low and may be different in other countries [19]. Our estimation of these rates was based on the ones presented by O'Brien et al. [8], obtained from a Canadian survey, to reflect Canadian donor behaviour with greater accuracy. Another limitation is that this simulation study did not consider the pre-exposure prophylaxis (PrEP) medication used to reduce the burden of HIV. PrEP use could interfere with HIV screening among blood donors and may result in undetectable RNA by nucleic acid/viral load assays for blood component qualification [20]. PrEP medication use is a deferral criterion for blood donors in Canada, and hence, its inclusion in the model should arguably not significantly increase the proportion of donations with HIV viral loads below the NAT detection limit under a realistic compliance rate assumption.

Despite the need to generate evidence-based data supporting policy changes regarding MSM ensuring the safety of recipients, few studies have evaluated intention to donate plasma among MSM. A qualitative study performed by Grace et al. indicated that time-based deferral would maintain the key issues regarding equity and fairness related to blood screening practices for MSM in Canada [21]. According to participants, policies regarding MSM blood donations should be more aligned with sound and up-to-date scientific evidence. Our results strongly suggest that allowing sexually active MSM to donate plasma for fractionation would be just as safe as the current approach of time-limited deferral, without having to impose a potentially inefficient and costly quarantine strategy that would be perceived as unduly burdensome and discriminatory.

Demand for plasma-derived medicinal products in Canada has increased over the past 10 years [22]. Our study supports the safety of expanding source plasma donation to include donors not subject to deferral for MSM. Based on the number of source plasma donations in Canada in 2018 (106,654 donations), no deferral model would lead to an increase of about 1119 donations (1.05%) if we added newly eligible donors according to pessimistic ii scenario.

Based on our modelling, there would be a negligible HIV contamination risk associated with a change from 3-month MSM deferral to no MSM deferral for source plasma donors in Canada. Given the aim of implementing less restrictive MSM deferral policies in Canada, our results support that it would be a safe next step to permit MSM to donate source plasma without a time-based deferral.

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AUTHOR CONTRIBUTIONS

All authors contributed to the design, reviewed model outputs and contributed to interpretation. E.A. performed the analysis and drafted the manuscript, and all authors revised it critically. All authors approved of the submitted version.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest identified.

ORCID

Antoine Lewin https://orcid.org/0000-0003-1748-4198
Sheila F. O'Brien https://orcid.org/0000-0002-5332-2789
Yves Grégoire https://orcid.org/0000-0002-1096-698X
Whitney R. Steele https://orcid.org/0000-0001-7955-3115
Katy L. Davison https://orcid.org/0000-0002-6337-892X
Clive R. Seed https://orcid.org/0000-0002-0234-4507
Félix Camirand Lemyre https://orcid.org/0000-0003-3277-2729

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Estimation of the latent therapeutic demand for immunoglobulin therapies in autoimmune neuropathies in the United States

Albert Farrugia[1] | Megha Bansal[2] | Ivan Marjanovic[3]

[1]Faculty of Medicine and Health Sciences, University of Western Australia, Perth, Western Australia, Australia

[2]ACIST Medical Systems, Eden Prairie, Minnesota, USA

[3]Private Consultant in Neurology, Milan, Italy

Correspondence
Albert Farrugia, Faculty of Medicine and Health Sciences, University of Western Australia, Perth, Western Australia 6009, Australia.
Email: albert.farrugia@uwa.edu.au

Abstract

Background and Objectives: The use of immunoglobulin (IG) solutions as an immunomodulatory therapy in certain neurological conditions has become an established modality and represents a significant proportion of total IG use. The estimation of the evidence-based potential demand designated as the latent therapeutic demand (LTD) for IG in these diseases is required for adequate planning of the plasma supply required to manufacture the product.

Materials and Methods: The diseases studied included chronic inflammatory demyelinating polyneuropathy (CIDP), Guillain–Barré syndrome (GBS) and multifocal motor neuropathy (MMN). The LTD for IG was assessed using a decision analysis model, using Microsoft Excel. The model analysed the epidemiological and clinical factors contributing to IG usage. One-way sensitivity analysis and probabilistic sensitivity analysis derived the LTD in grams per 1000 inhabitants. The key variables included the treatment schedule and the prevalence of the disease.

Results: The model estimates that an average annual IG demand and standard deviation for CIDP, GBS and MMN in the United States is 83.05 ± 24.5, 6.1 ± 3.2 and 36.1 ± 25.5 g/1000 inhabitants, respectively.

Conclusion: Together with previous work on the LTD for IG in immunodeficiencies, these results indicate that current IG usage reflects the estimated LTD for the main indications for IG in the United States The wide range of LTD found in all these studies emphasizes the need for more precise assessment of the underlying variables, particularly disease prevalence and dosage. Further studies on other indications such as secondary immunodeficiencies will augment these results and will assist in guiding demand planning for IG use and plasma collection in the United States and inform blood policy in other countries.

KEYWORDS
immunoglobulins, IVIG, plasma derivatives

INTRODUCTION

The use of immunoglobulin (IG) solutions purified from human plasma has become increasingly established as a therapy in various neurological diseases in the past 20 years [1]. This group of indications has overtaken primary immune deficiency (PID) as the largest users of IG [2] and thus represents an important driver for the collection of plasma for fractionation. The United States is one of the few countries in the world which collects more than enough plasma for its use and exports a considerable volume of plasma products outside the country. Despite this, shortages of IG for different diseases have become increasingly visible in the past few years [3, 4]. Plasma for fractionation is a finite resource, and, in many countries, growth in its collection has lagged behind the demand for IG [5]. Estimating the potential demand for IG indications is important for adequate planning of plasma collection and management of the limited supply. In previous work, the concept of latent therapeutic demand (LTD) has been used to estimate the requirements for IG in PID [6, 7] in the hypothetical scenario when usage is unconstrained by supply and reimbursement. Given that the usage of IG in neurological conditions now constitutes the single largest group of indications, an estimate of the LTD for IG arising from these diseases is relevant.

Chronic inflammatory demyelinating polyneuropathy (CIDP) is a chronic neurological disorder characterized by progressive weakness and impaired sensory function in the legs and arms. Treatment for CIDP includes corticosteroids such as prednisone, which may be prescribed alone or in combination with immunosuppressant drugs. Plasmapheresis (plasma exchange) and intravenous immunoglobulin (IVIG) therapy are effective [8], but plasma exchange can cause a rebound in the condition [9] and causes substantial inconvenience to the patients . Following the first approval of IG for use in CIDP by the US Food and Drug Administration (FDA) 2008 [10], IG use for this indication has expanded greatly, despite the lack of specific licensure for most of the products on the U.S. market [11]. This reflects the acceptance by medical specialists that the efficacy of the product transcends particular brands, a situation which mirrors the regulatory oversight of IG product in Europe, where the CIDP indication is embedded in the summary of product characteristics (SmPC) for intravenous immunoglobulin (IVIG) products, allowing all brands to be prescribed for CIDP if they have shown efficacy and safety according to the relevant Guideline of the European Medicines Agency (EMA) [12, 13].

Guillain–Barré syndrome (GBS) is an acute neurological disorder characterized by presence of different autoantibodies against the peripheral nervous system. Treatments include plasmapheresis and IVIg therapy, modalities which have similar efficacy rates [14, 15]. The benefit of IVIG in children with GBS is poorly supported by evidence, but the modality forms part of clinical practice by specialists in the disease [16]. Despite IVIG being indicated on the basis of clinical association guidelines [16], no IVIG product in the United States is specifically licensed for GBS. In Europe, it is approved for use in all brands.

Multifocal motor neuropathy (MMN) is an asymmetric, progressive neuromuscular motor disorder characterized by muscle weakness in the hands (commonly), with differences from one side of the body to the other in the specific muscles involved. MMN is a chronic disease which responds well and uniquely to IVIG treatment [17] and is indicated on the basis of guidelines (EFNS 2006); nevertheless, only one IVIG product is licensed for this indication in the United States. In Europe, it is approved for all brands.

Table 1 from the U.S. Food and Drug Administration (FDA) summarizes the status of the current immunoglobulin products licensed by indication in the United States [11]. It is noted that several of these products do not carry the label for all the neurological indications which are the subject of this paper. While usage of these products for indications which have not been approved constitutes off-label use, it is known that clinicians and insurers support the prescribing of these products for the whole spectrum of neurological indications, basing their decisions on which indications are supported through the relevant medical guidelines. Although regulatory authorities do not subscribe to the concept that immunoglobulins are generic products, clinical practice indicates that the safety and efficacy profiles of all licensed products are similar.

Despite the relatively small number of products specifically licensed for the above diseases, IG consumption in the United States for these conditions is very high. In this study, the LTD for IG for these three indications has been estimated using a decision analysis model previously described for estimating LTD of Factor VIII in haemophilia [18] and IG in PID [6].

METHODS

Model structure

For modelling the LTD for IG in the three neuropathies described in the Introduction, we used the decision analysis methodology similar to the methods developed by Stonebraker et al [6, 7] for modelling the LTD of IVIG in PID. The variables used in the model are shown in Tables 1 and 2. The model is based on the relationships of the epidemiological and clinical factors, integrated as shown in the flow charts in Figure 1. Each disease follows a different treatment schedule and depending on the incidence/prevalence of the disease the demand in terms of grams per 1000 inhabitants is calculated. For each disease, variables included prevalence, dosage and variations in treatment patterns. For CIDP, a treatment schedule based on an initial treatment of 24 weeks followed by a maintenance period of 24 weeks at a lower dose and frequency [19]. Since the America Association of Neurology (AAN) does not specify dosage levels for IVIG [16], the dosage was varied over a wide range in the analysis to reflect dosages reported by a range of studies and organizations [19, 20]. In GBS, a dose for 3–6 days was followed by a second complete dose in case of relapse [15, 16]. In MMN, a loading dose was followed by a maintenance dose every 1–6 weeks depending on the clinical status of the patient [17, 21, 22].

The model was built in Microsoft Excel. The analysis was conducted on one-way sensitivity analysis and probabilistic sensitivity

TABLE 1 Immunoglobulin products licensed for use in the United States. From Reference [11]

Product	Manufacturer	Indications
Gammagard liquid	Baxter Healthcare Corporation	• Primary Humoral Immunodeficiency • Multifocal Motor Neuropathy
Gammagard S/D	Baxter Healthcare Corporation	• Primary Humoral Immunodeficiency • B-cell Chronic Lymphocytic Leukaemia • Immune Thrombocytopenic Purpura • Kawasaki syndrome
Gammaplex	Bio Products Laboratory	• Primary Humoral Immunodeficiency • Immune Thrombocytopenic Purpura
Bivigam	Biotest Pharmaceuticals Corporation	• Primary Humoral Immunodeficiency
Carimune NF	CSL Behring AG	• Primary Humoral Immunodeficiency • Immune Thrombocytopenic Purpura
Privigen	CSL Behring AG	• Primary Humoral Immunodeficiency • Immune Thrombocytopenic Purpura
Gamunex-C Gammaked (distributed by: Kedrion Biopharma)	Grifols Therapeutics, Inc.	• Primary Humoral Immunodeficiency • Immune Thrombocytopenic Purpura • Chronic Inflammatory Demyelinating Polyneuropathy
Flebogamma DIF 5% and 10%	Instituto Grifols, SA	• Primary Humoral Immunodeficiency
Octagam	Octapharma Pharmazeutika Produktionsges. m.b.H	• Primary Humoral Immunodeficiency

TABLE 2 : Base-case values for population variables in the model

Parameter	Value	Source
US population	317,297,938	Census 2014
Prevalence		
CIDP	7.7/100,000	Laughlin 2009 [27]
GBS	2.3/100,000	NIH 2011 Genetics Home reference[31]
MMN	1.65/100,000	Lawson 2014 [32]
Male:female		
CIDP	56.6:43.4%	Laughlin 2009 [27]
GBS	54.6:45.4%	HCUP 2012 [41]
MMN	67.5:32.5%	Berg 2014 [40]
Age groups (years)		
CIDP		
18–44	18.8%	Laughlin 2009 [27]
45–65	40.9%	
65+	40.9%	
GBS		
1–18	9.11%	HCUP 2012 [41]
18–44	27.58%	
45–64	35.57%	
65+	27.70%	
MMN		
18–44	60.50%	Azulay 1997 [42], Berg-Vos 2002 [17], Harbo 2009 [43]
45–64	36.80%	
65+	2.60%	
Weight (kg) – male		
1–17	44.5	CDC 2010 [44]
18–44	83.5	
45–65	88.7	
65+	83.8	
Weight (kg) – female		
1–17	39.4	
18–44	67.8	
45–65	73.7	
65+	68.3	

Abbreviations: CIDP, chronic inflammatory demyelinating polyneuropathy; GBS, Guillain–Barre syndrome; MMN, multifocal motor neuropathy.

analysis. We have used probability distributions around variables as appropriate. These distributions were generated using Monte Carlo simulations with Excel, as has been described [23]. Normal and uniform distributions were used for some model parameters. The following formulas were used within Excel:

$$\text{For the Uniform Distribution}: \min + (\max - \min) * \text{RAND}()$$

$$\text{For the Normal Distribution}: \text{NORM.INV}(\text{RAND}(), \text{mean}, \text{std deviation})$$

Tornado diagrams were used to describe the sensitivity analyses (Figure 2), while the probability distributions were used to generate histograms (Figure 3). Excel has the capability to generate both these products [24, 25].

The respective models for the three conditions have been made available [26] as a resource for researchers and for comment.

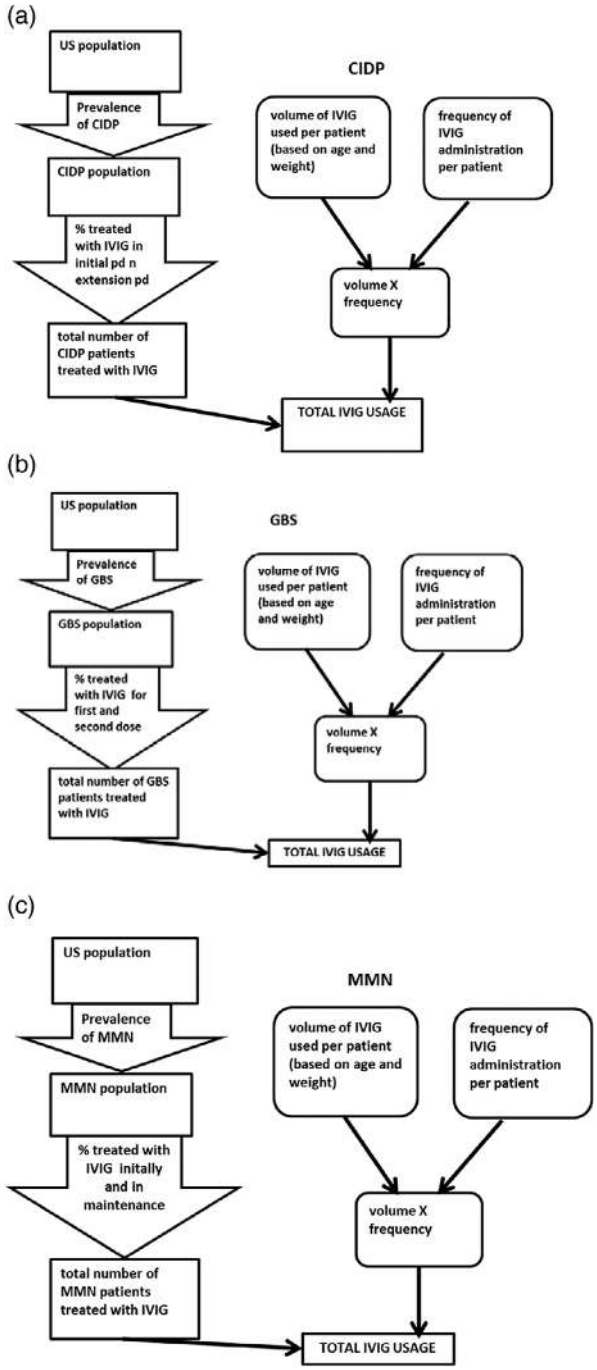

FIGURE 1 Flow charts showing the contribution of the relevant variables used to calculate latent therapeutic demand (LTD) for use of IVIG in chronic inflammatory demyelinating polyneuropathy (CIDP) (a), Guillain–Barre syndrome (GBS) (b) and multiple motor neuron neuropathy (MMN) (c)

Data sources

Prevalence

A review of medical records in the United States estimated the prevalence of CIDP as 8.9 per 100,000 [27]. Other reports provided a varied range of 1.9–7.7 per 100,000 worldwide [28–30]. Since the study done in the United States showed higher prevalence compared to the worldwide estimation, we assumed 7.7 per 100,000 as the base case and conducted one-way sensitivity analysis around prevalence of 1.9–8.9 per 100,000.

According to National Institutes of Health, the prevalence of GBS is quite varied, 0.6–4 cases per 10,000 [31]. We have taken an average of 2.3 cases per 100,000. The one-way sensitivity analysis utilizes 0.6 and 4 cases per 100,000 people as input values.

The prevalence of MMN is estimated from various sources to be 0.3-3 cases per 100,000 people [32]. The average of 1.65 cases per 100,000 people is used as the base case.

Patients treated with IVIG

CIDP is a rare disease and the review of medical records in the United States identified 23 cases treated for CIDP in the Rochester Epidemiology Project [27]. The prevalence computed in this project was 7.7 per 100,000 with a yearly incidence of 1.6 cases per 100,000, in the studied population of Olmstead County in Minnesota. The population of Olmstead County was 144,921 in the year in which the project was conducted. Among the cases identified with CIDP, 59% were treated with IVIG. Another retrospective study done in Massachusetts General Hospital identified 28.9% of patients being treated with IVIG in CIDP [33]. We used a uniform distribution to vary the patients treated in the initial period.

In the maintenance or extension period, the CIDP patients that responded well to IVIG were infused with lower dosage of IVIG (usually 1 g/kg) and at lower frequency (usually every 3–4 weeks) to avoid a relapse. Multiple studies and trials indicated that 50–70% patients responded well to IVIG [8, 34]. A retrospective study done in Spanish hospitals showed 45.3% of the patients responded well to the treatment and were assigned to the group with low frequency of infusions. This group was further divided among patients receiving drug at 6–8 week interval, 8–12 week and >12 weeks [35] .

A retrospective chart review study shows 86.9% of the patients suffering from GBS receive IVIG either alone or in combination with plasmapheresis [14]. Out of these, patients who relapse are provided with a second complete dose of IVIg. The evidence shows 8–16% of the patients relapse [15, 36].

A review study [22] for treatment of MMN reports 80% of the patients respond to IVIG. We used this figure (80%) as the number of people treated with IVIG in the United States due to lack of exact data. Since treatment of MMN is an ongoing treatment, we assumed 100% of these patients are treated with IVIG.

Dose of IVIG

We noted the administration of IVIG in CIDP for 1 year with 24 weeks on the initial period and 24 weeks in the maintenance period. Most of the trials and studies mentioned a loading dose of

TABLE 3 Base-case values for treatment patterns for CIDP, GBS and MMN

Parameter	Value	Source
Treatment of CIDP		
Patients treated with IVIG in the initial period	59%	Laughlin 2009 (Table 1) [27]
Patients treated with IVIG in the extension period	45.3%	Querol 2013 [35]
Time period of IVIG administration	24 weeks – initial period; 24 weeks – extension period	Hughes 2008 ICE study [19]
Volume of IVIG used per person in the initial period	2 g/kg over 2–4 days and then 1 g/kg over 1–2 days	Hughes 2008 ICE study [19]
Volume of IVIG used per person in the extension period	1 g/kg over 1–2 days	Hughes 2008 ICE study [19]
Frequency of dosage in the initial period	Every 3 weeks	Hughes 2008 ICE study [19]
Frequency of dosage in the initial period	Every 6–8 weeks or 8–12 weeks or >12 weeks	Querol 2013 [35]
Patients on low frequency of dosage in the extension period		
6–8 weeks	33.3%	Querol 2013 [35]
8–12 weeks	35.9%	
>12 weeks	30.8%	
Treatment of GBS		
% Treated with IVIG	86.9%	Oczko-Walker 2010 [14]
% Treated with IVIG for second dose (patients who relapse)	12%	va Doorn 2010, NBA 2012 Australian guidelines [39]
IVIG administration days	3.5 days	Patwa 2012 AAN guidelines [16]
Volume of IVIG used per person	1.2 g/kg	Patwa 2012 AAN guidelines [16]; NBA 2012 Australian guidelines [39]
Frequency of dosage	may be repeated in case of relapse or no response	van Doorn 2010 [15]
Treatment of MMN		
% Treated with IVIG	80.0%	Jinka 2014 [22]; 80% respond to IVIG treatment
% Treated with IVIG maintenance	100.0%	Aassumption
Interval between IVIG dose	Every 3.5 weeks	Berg-vos 2002 [17]
Volume of loading IVIG	2 g/kg	Jinka 2014 [22]
Volume of IVIG for maintenance	1.1 g/kg	Berg-vos 2002 [17]; Berg 2014 [40], NBA Australia 2012 guidelines [39]

Abbreviations: CIDP, chronic inflammatory demyelinating polyneuropathy; IVIG, intravenous immunoglobulin; GBS, Guillain–Barre syndrome; MMN, multifocal motor neuropathy.

2 g/kg of IVIg in the initial period over 2–4 days. For the remaining period, a range of 0.4–2 g/kg of IVIG is given every 3 weeks [19, 20, 37]. Most of the dosages recommended by guidelines such as the EFNS/PNS [38] and the Australian Guidelines [36] reflect the dosages used in the seminal clinical trial of Hughes et al [19] which we have used in our analysis. The American Academy of Neurology suggests the dosage, frequency and duration of treatment based on clinical assessment [16]. We varied the dosage using a uniform distribution. In the extension period, the same range of 0.4–2 g/kg of IVIG was used, but the frequency of infusion was reduced from 3 weeks to 6–24 weeks. The patients were divided in the groups of 6–8 weeks, 8–12 weeks and 12–24 weeks [35].

A randomized controlled trial for IVIG in GBS used 0.4 g/kg over 3–6 days [16]. The Australian guidelines recommended dosage to be 2 g/kg over 2–5 days [39]. We used an average of 1.2 g/kg over 4 days as the base case and varied the dose from 0.4–2 g/kg over 3–6 days in one-way sensitivity analysis.

The evidence shows an induction dose of 2 g/kg of IVIG is administered in MMN [22]. The maintenance dose varies between 0.1 and 2 g/kg every 1–6 weeks annually [17, 39, 40].

TABLE 4 Variables used in the sensitivity analysis

Parameter	Values for one-way SA	Probability distribution	Source
Treatment in CIDP			
Prevalence of CIDP	1.9–8.9/100,000		Laughlin 2009 [27]
Patients treated with IVIG in initial period	28.9–59%	Uniform ()	Darabi 2006 [33], Laughlin 2009 [27]
Patients treated with IVIG in extension period	35–70%	Uniform ()	Gorson 2012, Sarti 2009 [34]
Volume of IVIG used per person for maintenance (gm/kg)	0.4–2.0	Uniform ()	Australian 2012 guidelines [20] and Eftimov 2013 [37]
Frequency of dosage in the extension period			
1st group	6–8 weeks	Uniform ()	Querol 2013 [35]
2nd group	8–12 weeks	Uniform ()	
3rd group	>12 weeks	Uniform ()	
Treatment in GBS			
Prevalence of GBS	6–40/1000,000	Uniform ()	NIH 2011 Genetics Home reference[31]
% Treated with IVIG for second dose (patients who relapse)	8–16%	Uniform ()	Van Doorn 2010 pg S77 [15], NBA 2012 Australian guidelines [39]
IVIG administration days	3–6 days	Uniform ()	Patwa 2012 AAN guidelines [16]
volume of IVIG used per person	0.4–2 g/kg	Uniform ()	Patwa 2012 AAN guidelines [16]; NBA 2012 Australian guidelines [39]
Treatment in MMN			
Prevalence of MMN	0.3–3/100,000	Uniform ()	Lawson 2014 [32]
Interval between IVIG dose	every 1–6 weeks	Uniform ()	Berg-vos 2002 [17]
volume of IVIG for maintenance	0.1–2.0 g/kg	Uniform ()	Berg-vos 2002 [17]; Berg 2014 [40], NBA Australia 2012 guidelines [39]
Weight (kgs – 10th, 50th and 90th percentiles)	Male	Uniform ()	
1–17	27.95–44.5–74.10	Extended Swanson–Megill approximation	CDC 2010 [44]
18–44	62.60–83.5–112.75		
45–64	69.10–88.7–113.1		
65+	65.40–83.8–104.5		
	Female		
1–17	27.45–39.35–65.25		
18–44	52.5–67.8–102.6		
45–64	54.7–73.7–106.3		
65+	49.8–68.3–91.2		

Abbreviations: CIDP, chronic inflammatory demyelinating polyneuropathy; IVIG, intravenous immunoglobulin; GBS, Guillain–Barre syndrome; MMN, multifocal motor neuropathy.

Age, gender and weight of patients

In the cases where dose is administered per kg of the weight of patients, it is necessary to distinguish age and gender groups. The data for gender and age for CIDP were obtained from a retrospective study conducted in the United States. [27] The age groups are classified as 18–44, 45–64 and 65+ years of age. There was only one case of a 4-year-old in this study, which we excluded so that the population is aligned with the adult population in the clinical trial which we used [19] .

GBS inflicts both adults and children so we included another age group of 1–18 years of age to GBS. The proportions of patients in each age group were gathered from Healthcare Cost and Utilization Project (HCUP) of the U.S. Agency for Healthcare Research and Quality [41]. Several follow-up studies were used to populate the proportions in each age group in MMN [17, 42, 43].

The weight distribution was extracted from a report by the Centers for Disease Control [44], specified weight by age and gender. We used extended the Swanson–Megill (ESM) approximation [45], as described by Stonebraker et al [7] to obtain 10th,

TABLE 5 LTD for immunoglobulin in primary immunodeficiencies and neurological diseases. From Stonebraker et al [7] and this study

Condition	Mean LTD immunoglobulin g/10^3 population	References
Common variable immune deficiency (CVID)	65.4 ± 73.6	Stonebraker et al [7]
X-linked agammaglobulinemia (XLA)	25.5 ± 27.6	Stonebraker et al [7]
Severe combined immune deficiency (SCID)	13.4 ± 13.5	Stonebraker et al [7]
Wiskott–Aldrich syndrome (WAS)	0.5 ± 0.4	Stonebraker et al [7]
hyper IGM syndrome (HIGM)	0.3 ± 0.3	Stonebraker et al [7]
Chronic inflammatory demyelinating polyneuropathy (CIDP)	83.05 ± 24.5	This study
Guillain–Barré syndrome (GBS)	6.1 ± 3.2	This study
Multifocal motor neuropathy (MMN)	36.1 ± 25.5	This study
Total mean immunoglobulin consumption	230.35	

Abbreviation: LTD, latent therapeutic demand.

50th and 90th percentiles for use in the probabilistic sensitivity analysis.

RESULTS

Using the variables shown in Tables 3 and 4 for one-way sensitivity analysis, Tornado diagrams were generated which showed the dosage of immunoglobulin and the disease prevalence were the most important parameters influencing the decision analysis model in all the three conditions (Figure 2). Extension and second doses for CIDP and GBS respectively were less important in influencing demand. In MMN, the interval between doses was highly influential.

The model allowed the generation of a probabilistic distribution for LTD for each condition (Figure 3) which was skewed for all the three diseases. From this distribution, an average demand and standard deviation for CIDP, GBS and MMN of 83.05 ± 24.5, 6.1 ± 3.2 and 36.1 ± 25.5 g/1000 inhabitants, respectively, was estimated. The median extracted from the histograms of the probability distribution were 111, 9.5 and 65 g/1000 inhabitants, respectively.

DISCUSSION

This study augments previous work by Stonebraker and colleagues in which decision analysis methodology is used to estimate the unconstrained demand for plasma protein therapies [6, 7, 16]. The level of uncertainty and professional variation underpinning many of the key elements contributing to demand enable this methodology, through the sensitivity analyses which are a feature of the model, to synthesize the various values offered through clinical research and professional into an estimate for LTD. The relative contributions of the different variables into the final estimate are shown in the Tornado diagram generated by the one-way sensitivity analysis, allowing a rapid pictorial assessment of the ranking of these variables in the LTD (Figure 2). Unsurprisingly, the model identified dosage and disease prevalence as the highest contributors to the LTD for immunoglobulin for all the three disease studied, but the one-way sensitivity analysis also allowed generation of the range of values contributing to the final estimate.

This study has several limitations. Although we sought to assess the LTD of the neurological indications for which immunoglobulin is prescribed in the United States, these three conditions are not an exhaustive list of these indications in the medical literature [46]. Inclusion of these other rare conditions may be expected to augment the total LTD estimated in our study, and immunoglobulin has been used to treat exacerbations myasthenia gravis for many years [47], although the evidence base is poor [48], and new therapies are emerging for the disease [49]. While one product is approved for this indication in Europe, none are approved in the United States, and we were unable to access any data regarding its usage, possibly because of a lack of insurance for this condition.

Any study addressing the uncertainties underpinning LTD suffers from the limitations imposed by these uncertainties. As is shown by the sensitivity analysis, disease prevalence influences LTD greatly and is the subject of debate and controversy, particularly for CIDP, the indication associated with the highest LTD. In a recent meta-analysis, Broers et al [50] found a pooled prevalence of 2.81 per 100,000 over 11 studies included. Of note was this meta-analyses' exclusion of the widely cited study conducted by the Mayo Clinic for Olmstead County in the United States [27], which was used in our study to specify prevalence in the United States. The Mayo study was included in the studies considered by Broers et al, but data were not incorporated in the final analysis because Broers et al considered that the crude rates for the data were not visible. Broers et al comment that the inclusion of the Mayo Clinic study would have increased the pooled prevalence, and this study, which is the most widely cited for the prevalence of CIDP in the United States in the literature, reports a prevalence similar to a later study extracting prevalence data for the whole of the USA extracted from an insurance claims data base [51], increasing our confidence in the prevalence recorded by the Mayo Clinic .

Earlier studies in other geographies, for example, Japan [52] and Australia [29], and a more recent study from a single centre in Chile

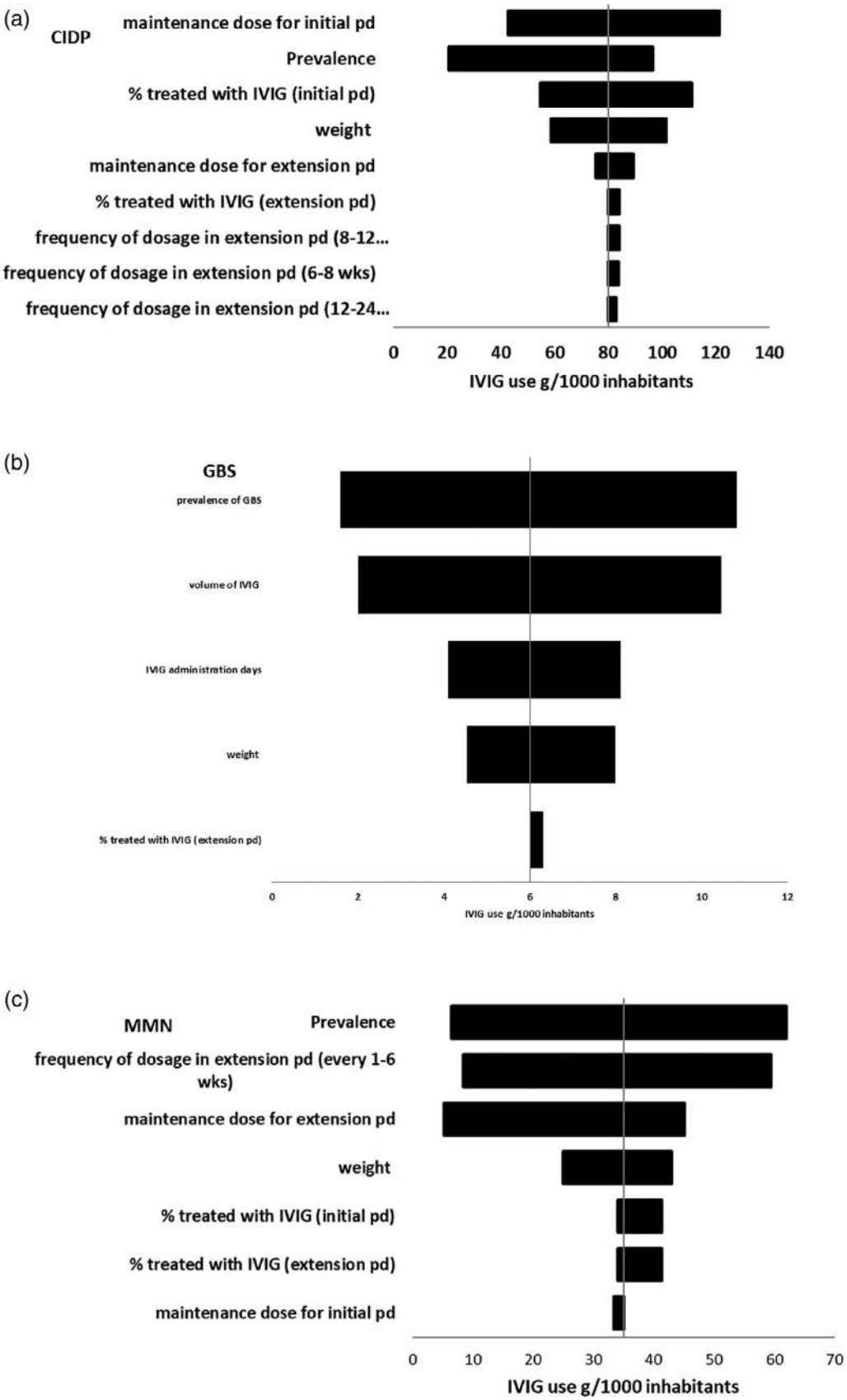

FIGURE 2 One-way sensitivity analysis (tornado diagram) – For variables included in the decision analysis model for CIDP (a), GBS (b) and MMN (c). CIDP, chronic inflammatory demyelinating polyneuropathy; GBS, Guillain–Barre syndrome; MMN, multifocal motor neuropathy

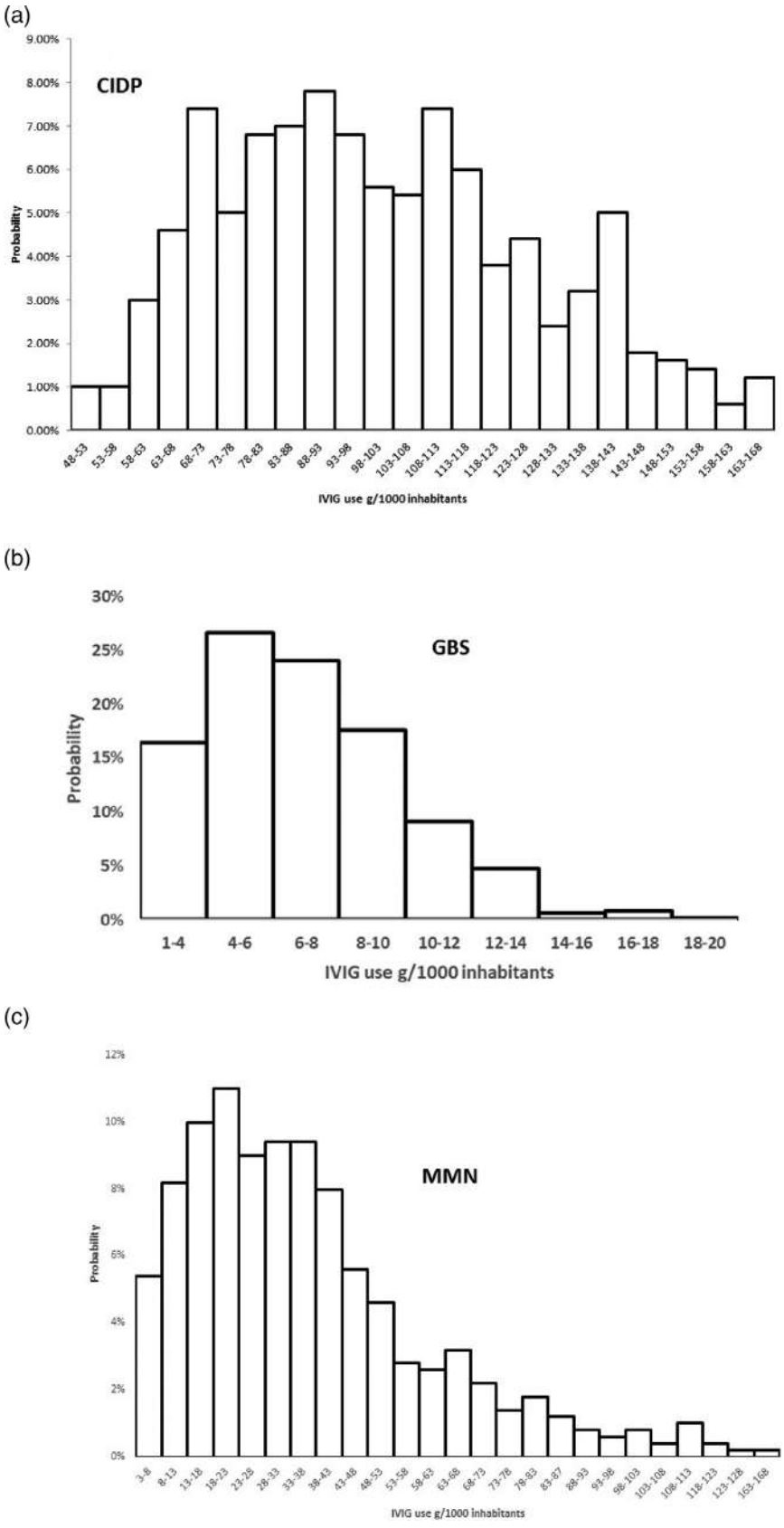

FIGURE 3 Distribution of IVIG use in the US, extracted from the model and estimated for patients with CIDP (a), GBS (b) and MMN (c). CIDP, chronic inflammatory demyelinating polyneuropathy; GBS, Guillain–Barre syndrome; IVIG, intravenous immunoglobulin; MMN, multifocal motor neuropathy

[53] have reported lower prevalence rates, while more recent studies in Australia [54] report prevalence rates of 10 per 100,000. In this latter study, a difference was noted between prevalence levels estimated using the diagnostic criteria of the European Federation of Neurological Societies/Peripheral Nerve Society (EFNS/PNS) [38], considered to be the most widely accepted criteria for the diagnosis of CIDP [50], and the patient numbers listed in a data base including patients prescribed immunoglobulin for CIDP.

Reported differences in disease prevalence can arise from a number of causes. In clinical studies, diagnostic alignment to different sets of criteria may result in these differences. In CIDP, the criteria of the EFNS/PNS are considered to have a higher diagnostic sensitivity than those previously published by the American Academy of Neurology (AAN) [55], which may underestimate prevalence and incidence of the disease [56]. A study in the UK reported a twofold difference between the prevalence of CIDP defined by the EFNS/PNS and that defined by the AAN [56], and this difference was also found in the meta-analysis of Broers et al. [50] However, the uncertainty generated by these different criteria is compounded by the fact that in more than 47% of cases, CIDP is misdiagnosed as other diseases and clinical adherence to diagnostic criteria is poor [57]. Differences in geographies and demographics may also contribute to these variations.

Overall, uncertainty in this key variable emphasizes the role of sensitivity analysis in defining and quantifying the extent to which findings such as those in the present study may be influenced by further research in the clinical and epidemiological definition of the disease. While meta-analyses such as those of Broers et al [50] reflect the uncertainty in prevalence through the prediction interval, this role is taken up by the Tornado Diagrams which are included in the present study.

In this study, we considered the sole administration route for immunoglobulin in treating these diseases to be intravenous. Although one product has been approved for subcutaneous administration in treating CIDP, there are still no data available on the extent to which this route has been adapted for CIDP [58]; furthermore, a European meta-analysis of studies conducted in Europe suggests that current practice involves similar doses with IVIG and SCIG in most cases of patients with neuropathies [59]. The US FDA's recommendation to correct the dosage of immunoglobulin when transitioning PID patients from IVIG to SCIG is not reflected in the case of CIPD [60].

Estimation of LTD for other groups of indications, notably conditions such as idiopathic thrombocytopenic purpura and immunodeficiency secondary to haematological malignancies, will increase insight in the factors influencing demand for these conditions. In the interim, our study suggests that the underlying demand for IG in the main neurological diseases approaches 125 g/1000 inhabitants. In Table 5, we list the LTD for immunoglobulin consumption for the indications where this has been studied [7] (this study). This suggests that approximately 75% of the amount of IG used currently in the countries using high amounts IG, including the United States, Canada and Australia [61], represents the consumption expected from decision analysis modelling for these two groups of indications. As diagnosis and treatment access improve in other, lower consuming countries, the actual usage is expected to reflect the levels estimated in these models. This scenario assumes that the underlying factors shaping demand are similar to those in high-usage countries. These factors assume, for example, dosage regimens such as those we have drawn from in influencing demand. Dosage in immunoglobulin therapies is a controversial topic [62], and the guideline-driven dosages underpinning much of current practice in the developed economies do not reflect possible individual variations in the required trough levels in PID patients [63], which, if used in determining dosages, may well influence the demand. Similarly, scrutiny of the doses used in the neuropathies may also result in altered regimens, as many of these doses were derived empirically from the initial clinical observations on the use of IVIG in idiopathic thrombocytopenic purpura (ITP) with minimal relevance to the different pathologies involved in the neuropathies. The global supply of IG is already under substantial stress [64], and increased effort is required to collect the volumes of plasma needed to generate these amounts of IG. These pressures should heighten efforts in understanding better the use of immunoglobulin in all the disease where it is present as a modality. They should also accelerate the search for alternatives to IG therapy, with some therapies already showing promise as candidates for treating the autoimmune neurological diseases assessed in the present study [65]. In the interim, we propose that decision analyses for estimating latent demand for therapies should be considered as useful tools in assisting policy makers and funders in planning access to medical interventions.

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A.F. designed the study, wrote the paper and provided critical comment regarding the provision of immunoglobulin. M.B. designed and executed the decision analysis model in Microsoft Excel. I.M. provided input in the clinical features and evidence based use of immunoglobulin in neurological diseases.

CONFLICT OF INTEREST

All three authors declare past provision of services to companies manufacturing immunoglobulin therapies. None of these conflicts was current at the time of writing this paper.

ORCID

Albert Farrugia https://orcid.org/0000-0003-0804-7463

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ORIGINAL ARTICLE

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Therapeutic granulocyte infusion for patients with severe neutropaenia and neutrophilic dysfunction: New Zealand experience

Shanee Chung[1] | Oliver Armstrong-Scott[2] | Richard Charlewood[1]

[1]New Zealand Blood Service, Auckland, New Zealand

[2]Department of General Medicine, Auckland District Health Board, Auckland, New Zealand

Correspondence
Shanee Chung, The Leukemia/BMT Program of B.C., 2775 Laurel Street, Floor 10, Vancouver, BC V5Z 1G1, Canada.
Email: shaneechung@gmail.com

Abstract

Background and Objectives: Studies have shown granulocyte transfusions (GTXs) may be beneficial in neutropaenic patients with severe systemic infections. New Zealand Blood Service has a policy for provision of granulocytes to New Zealand's District Health Boards. We set out to explore utilization of therapeutic granulocyte infusions in New Zealand.

Materials and Methods: Patients who received GTXs in the 16-year period between 2000 and 2016 were identified by the New Zealand electronic blood management system, eProgesa. Information pertaining to recipient demographics, disease-related factors, methods of granulocyte collection and clinical outcomes was obtained by the review of electronic transfusion and clinical records.

Results: Forty-five septic patients received granulocyte support for a total of 263 days. The median age of the recipients was 16 (range 0–74) years. Seventy-nine percent of the recipients had an underlying haematological malignancy with 50% having acute leukaemia. The median neutrophil count on the last day of GTX was 0.02×10^9/L (range 0–16.32). Sixty-three percent (27/43 patients with available data) had persisting severe neutropaenia when the GTXs were stopped. The median duration of support was 3 (range 1–32) days. Forty-six percent of granulocyte collections were performed via apheresis. Of the 44 patients, for whom survival outcome was available, 18 (41%) survived the acute illness.

Conclusion: GTXs were infrequently used, most commonly in the setting of an underlying haematological malignancy. This may be explained by the current weak evidence base supporting this therapeutic modality. Procuring a sufficiently large dose of granulocytes for infusion remains an issue for adult recipients.

KEYWORDS
apheresis – donation, blood components, granulocyte concentrate

INTRODUCTION

Cancer patients receiving intensive chemotherapy or those undergoing haematopoietic stem cell transplantation (SCT) often experience a prolonged period of severe neutropaenia, which predisposes them to invasive bacterial and fungal infections that are associated with high morbidity and mortality [1]. The strongest predictor of survival from these infections is improvement of the neutrophil count [2].

Granulocyte transfusion (GTX) has been around for many decades with the first documented GTX in 1934 [3]. Significant enthusiasm developed for GTX in the 1970s, but controlled trials in the 1970s and 1980s yielded mixed results [4], which were partially attributed to differences in qualities and doses of the granulocytes transfused. There was resurgence of interest in clinical application of GTXs in the 1990s with the advent of leukapheresis and clinical utilization of granulocyte colony stimulating factor (G-CSF). Granulocyte collection without stimulation of the donor may yield 0.1–1 $\times 10^{10}$ granulocytes, but this yield can increase to 4–8 $\times 10^{10}$ cells after stimulation with G-CSF and steroids [5]. A study has found that granulocytes obtained from donors stimulated by G-CSF retained immunological functions such as chemotaxis, respiratory burst, adhesion and bactericidal and antifungal activity, which remained unchanged even after storage of the granulocyte product for 24 hours [6].

The few published multicentre randomized controlled trials for GTX suffered from poor recruitment, and they were not able to show clinical benefit in the intervention arm [7, 8]. However, the RING study did show the subjects who received a higher dose of granulocytes (mean dose greater than 0.6 $\times 10^9$/kg per transfusion) had superior outcomes in a subgroup analysis [8]. The latest Cochrane review recommends regarding the use of GTX as investigational, given the lack of sufficient evidence from randomized controlled trials to support or refute its use in patients with neutropaenia and severe infection to reduce mortality [9]. In the recent years, several single centres published retrospective analyses of their real-life experience with GTX therapy. In one such study, providing GTX to severe neutropaenic patients with treatment-refractory, life-threatening infections resulted in 15/27 (56%) patients surviving to hospital discharge [10]. In another, 18/22 (81.8%) patients with neutropaenia due to haematological malignancy survived severe refractory abdominal infection when treated with GTX until recovery of absolute neutrophil count (ANC >1 $\times 10^9$/L) or significant clinical improvement. The patients achieving control of the infection within 7 days of the first GTX had significantly better overall survival ($p < 0.001$) [11]. Nguyen et al. described use of GTX as a bridge to an urgent allogeneic SCT in 19 severe neutropaenic patients with severe uncontrolled infection, where 90% of the GTX recipients were able to proceed to SCT with 80% continuing on GTX support until neutrophil engraftment. Following the SCT, 10% of the patients eventually succumbed to the initial infection, for which they received GTX. They showed an association between delay in provision of GTX and delay in proceeding to HCT ($p < 0.0001$), suggesting a potential role for GTX in facilitating an urgent SCT [12]. In the current multicentre retrospective observational study, we report on New Zealand's real-life experience in use of GTX for treating paediatric and adult patients with severe infection in the setting of neutropaenia or neutrophil dysfunction associated with wide ranging haematological and non-haematological disorders over 16 years. This project was carried out as a quality improvement audit for New Zealand Blood Service (NZBS), and an ethics application was not required as per the local guidelines.

New Zealand's policy and procedures for GTX

NZBS provides a 'vein-to-vein' transfusion service for the nation, and it is responsible for provision of all blood products. The national policy for provision of therapeutic granulocyte products states that GTX may be considered in patients with

1. persistent neutropaenia of less than 0.2 $\times 10^9$/L, which is expected to persist for longer than 5 days,
2. either septicaemia or life-threatening local infection that is not responding to 72 h of appropriate antimicrobial therapy or proven or probable fungal or yeast infection that is refractory to appropriate antifungal therapy,
3. good long-term prognosis from the underlying disorder and
4. a suitable donor.

Contraindications include poor long-term prognosis of the underlying disorder, known as human leukocyte antigen (HLA) alloimmunization (relative), severe respiratory compromise and requirement for ventilatory support.

The granulocyte products can be provisioned within 24 h of a request through the on-call Transfusion Medicine Specialist on 6 days of the week, from Monday to Saturday. A donor search is made on a list of ABO- and RhD-compatible NZBS apheresis donors. Family members of the patient, who meet requirements of the NZBS Collection Standards as evaluated by a NZBS Medical Officer, may become donors, but directed family donation is relatively contraindicated if allogeneic SCT is being considered due to risk of HLA alloimmunization. Given the limited size of the donor pool, granulocytes are only matched for ABO and RhD types, unless the recipient is known to have HLA antibodies.

Apheresis units from single donors expose the recipients to a limited number of HLA antigens compared to buffy coat units from several donors. Collection of an apheresis unit, however, requires priming of the donor with recombinant G-CSF (5–10 μg/kg) with or without dexamethasone (8 mg) in the evening prior to the collection date, whereas buffy coat units are routinely set aside for platelet pooling at NZBS collection sites. Consequently, buffy coat units are used for GTX if an apheresis donation cannot be arranged in a timely manner. For procurement of buffy coat units, whole blood donations, collected into 'top-and-bottom' bags, are centrifuged at 3616*g* (using a Heraeus 6000i centrifuged at 3300 rpm for 11 min) at room temperature, and the bottom red cell layer and the top plasma layer are removed using a Macopress automated blood component separator. The remaining buffy coat is transferred to a bag that is suitable for transfusion. For apheresis collection, NZBS currently uses Spectra Optia®, which utilizes continuous-flow centrifugation and optical detection technology. Other machines that had been in use over the preceding years include Haemonetics MCS+® and Fresenius Kabi's COM.TEC®.

All granulocyte products are irradiated prior to infusion to prevent transfusion-associated graft-versus-host disease. The requirements for an adequate buffy coat unit are volume of 35–65 ml and granulocyte and platelet contents of ≥1 $\times 10^9$ and ≥5 $\times 10^{10}$ per unit, respectively. The volume of an apheresis unit is defined locally, and it is between 200 and 500 ml in practice. Each apheresis unit should

contain ≥1 × 10^{10} of granulocytes. For quality control, each donation of granulocyte products is tested for volume and granulocyte content, and these data are entered into a statistical process control system, NWA, to identify trends and outliers. The collected granulocytes must be transfused as soon as possible but can be stored for up to 24 h from collection if they are kept at 20–24°C without agitation.

METHODS

An electronic search was performed on eProgesa, the electronic blood management system used by NZBS since year 2000, to identify all recipients of buffy coat and/or apheresis granulocyte units during the 16-year period between 2000 and 2016. We collected data related to the recipient's demographic features, the underlying disease status (primary diagnosis, indication for GTX), the intervention (method of procurement of granulocyte products, number of infusions given per patient, dose of granulocytes given per patient) and the clinical outcome (neutrophil count post-GTX, survival). The Transfusion Nurse Specialists working for the eight largest District Health Boards (DHBs) around New Zealand reviewed the electronic records of the GTX recipients belonging to their respective DHBs and other smaller DHBs supported by their DHBs and collected the pre-specified set of data. The authors analysed the data and prepared the manuscript.

STATISTICAL ANALYSIS

Pearson's chi-squared test with Yates' continuity correction was used for the correlation between increment in the neutrophil count during treatment and survival to the time of discharge from hospital. Kaplan–Meier analysis was used to estimate survival. The statistical software used was R (R Foundation for Statistical Computing, Vienna, 2018).

RESULTS

Between 2000 and 2016, 45 patients received GTX for a total of 263 days in New Zealand. Twenty-eight (62%) patients were females. The median age of the recipients was 16 with a range between zero and 74 years (Figure 1). Fifty-six percent of the recipients had underlying acute leukaemia, and another 22% had other haematological malignancies including large granulocytic leukaemia (two patients), chronic lymphocytic leukaemia (one patient), classic Hodgkin lymphoma (one patient), Burkitt lymphoma (one patient), chronic myeloid leukaemia (one patient), Juvenile myelomonocytic leukaemia (one patient) and T-cell lymphoproliferative disorder, NOS (one patient). Four patients had underlying aplastic anaemia. Four recipients did not have a primary haematological diagnosis. These included two paediatric patients with chronic granulomatous disease leading to granulocyte dysfunction and two patients with solid organ malignancy (Figure 2). All patients had documented bacterial or fungal infections that were refractory to conventional therapy. None received GTX as a secondary prophylaxis to prevent progression or recurrence of chronic infection.

Eleven out of the 20 DHBs in New Zealand have a dedicated Haematology Department, and the remaining DHBs are served by neighbouring DHBs with a Haematology Service. The majority of the GTXs (21 patients, 47%) were carried out by Starship Hospital, the largest tertiary paediatric service in New Zealand. Auckland City Hospital, the adult equivalent, was the second biggest user of GTX during the study period, but their usage was much less than that of Starship Hospital, with only six cases comprising 13% of the total number. This was slightly more than that seen in the rest of the country. Other haematology centres in both North and South Islands of New Zealand had one to four cases of GTX therapy in the 16-year period. There did not appear to be any apparent regional variation in the clinical practice; for example, Wellington Hospital Haematology

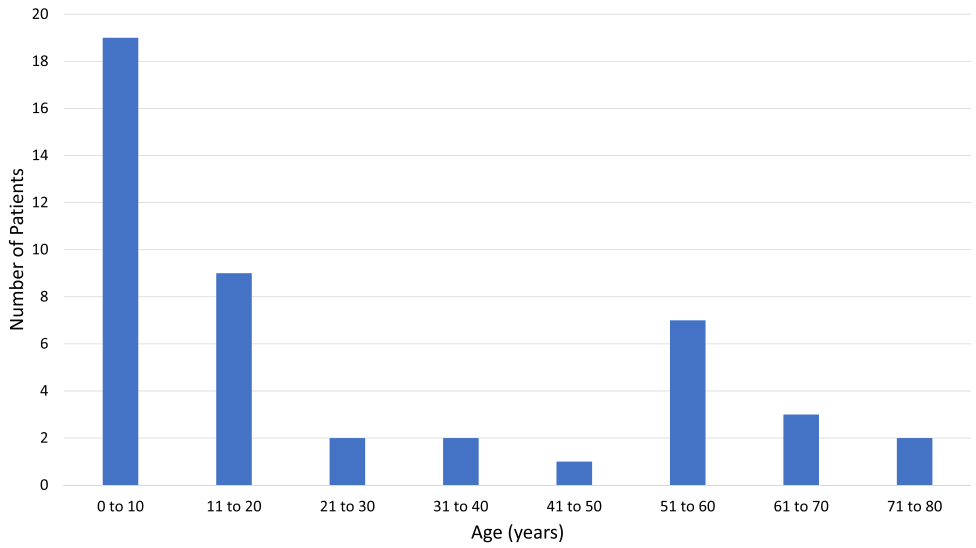

FIGURE 1 Age distribution of recipients. This graph illustrates the number of recipients of therapeutic granulocyte infusion in each age bracket [Colour figure can be viewed at wileyonlinelibrary.com]

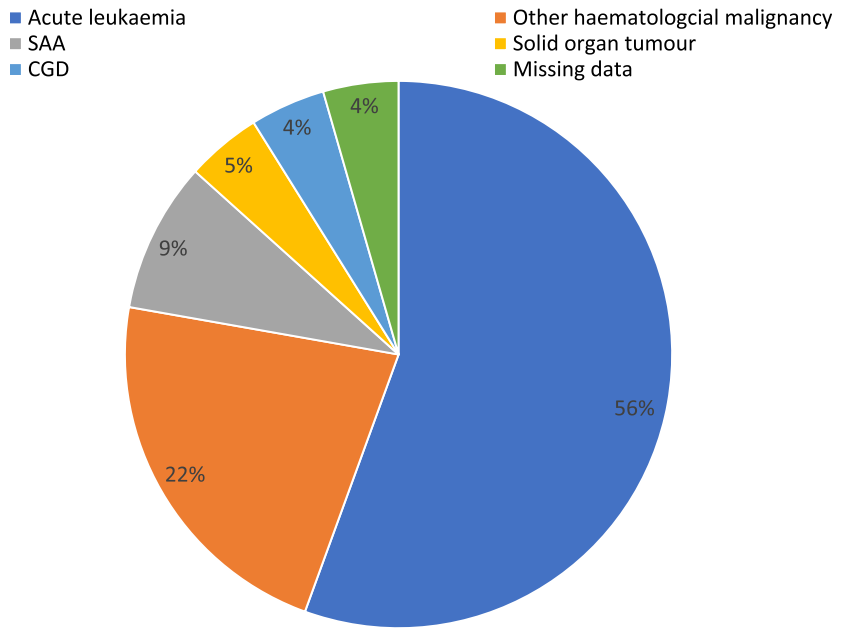

FIGURE 2 Underlying primary diagnoses of granulocyte transfusion (GTX) recipients. This graph illustrates the underlying primary diagnoses of the patients who received therapeutic granulocyte infusion for neutropaenia or neutrophil dysfunction in this series [Colour figure can be viewed at wileyonlinelibrary.com]

Centre, serving Capital and Coast, Hutt Valley and Wairarapa DHBs in the lower end of the North Island (a population of 525,910 people by the Ministry of Health 2020/21 estimate), used GTX on four occasions, whereas Dunedin Hospital Haematology Department, serving the lower end of the South Island (a population of 344,900 people by the same estimate) used it for three patients.

Thirty-four out of the 40 recipients with available data (85%) had severe neutropaenia (defined as a neutrophil count of less than 0.5×10^9/L) at baseline and three (7.5%) had moderate neutropaenia (defined as a neutrophil count between 0.5 and 1×10^9/L). Four of the five patients with missing data had only the total white blood cell (WBC) count without differentials reported on the day. Three of the 44 patients with available data (6.8%) had a neutrophil count above 1×10^9/L. Only one patient had a missing data for this latter category because all of the patients who had a WBC reported without differentials had a WBC of less than 1. The patients with a starting neutrophil count of 1×10^9/L or greater included a patient with an underlying chronic granulomatous disease (ANC 16.32×10^9/L), a paediatric acute leukaemia patient with typhlitis and abdominal wall cellulitis (ANC 3.29×10^9/L) and an adult patient with a bone marrow failure syndrome and *Escherichia coli* bacteraemia (ANC 1.1×10^9/L). The median neutrophil count at baseline was 0.02×10^9/L with a range between 0 and 16.32×10^9/L.

One hundred thirty-nine (139/303, 45.9%) therapeutic doses of granulocytes were collected via apheresis, and 164 (164/303, 54.1%) doses were derived from buffy coats (10–12 buffy coats per one therapeutic dose). The median number of GTXs given per patient was four with a range between one and 37 doses (Figure 3). The patient who received the highest cumulative dose of GTX was a 7-year-old female with a brain stem glioma, who had chemotherapy-induced neutropaenia and invasive pulmonary aspergillosis. Despite receiving 23 therapeutic doses of buffy coats and 14 apheresis units of GTX, she eventually succumbed to the acute infection. Her WBC at the commencement of GTX was 0.14×10^9/L, and the peak WBC during the course of GTX was 0.44×10^9/L (no differentials available).

The survival outcome data were available for all but one recipient. Twenty-six (58%) recipients were alive at the time of discharge from hospital. Eighteen (40%) died from severe infection.

The information on the granulocyte dose per kilogram body weight was available for a subset of 30 patients. Fifteen patients received $0–100 \times 10^8$/kg of granulocytes, and 40% of them survived the acute infection. Of the 11 patients who received $100–200 \times 10^8$/kg of granulocytes, 55% survived. All four patients who received greater than 200×10^8/kg of granulocytes were alive at the time of discharge from hospital ($p = 0.1014$).

Based on the available data, the post-GTX neutrophil count could be determined for a subset of 42 patients. During the time interval between the first GTX and the day after the last GXT, 27 (64%) patients had a rise in their neutrophil count of 0.2×10^9/L or greater, and 14 (52%) of these patients survived to hospital discharge. Out of the 15 patients who had less than 0.2×10^9/L increase in their neutrophil count, only five (33%) survived ($p = 0.4055$). All but six patients had an increment in their neutrophil count at one or more measurements while receiving GTX. Of the six patients who failed to have any rise in their neutrophil count during the treatment course, only one patient (17%) was alive at the time of discharge from hospital, in comparison to 18 out of the 36 patients (50%) who had at least a transient rise in their neutrophil count ($p = 0.282$).

Eight GTX incidents were associated with a reported transfusion reaction according to the NZBS haemovigilance record. The

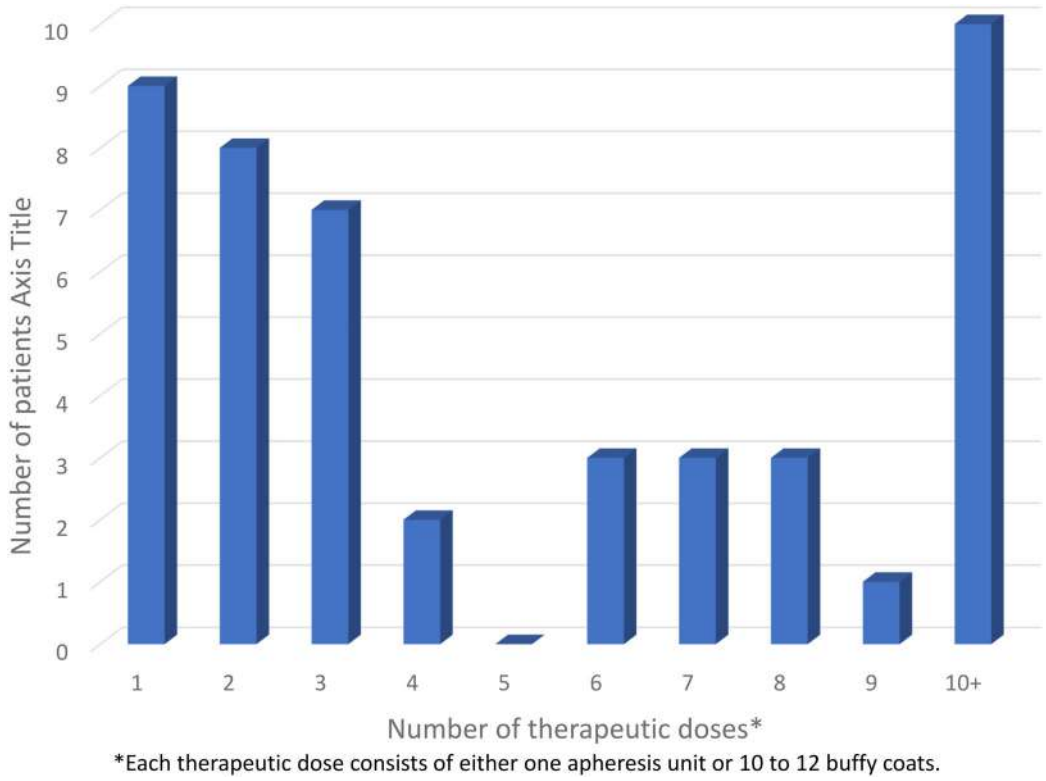

FIGURE 3 Number of therapeutic granulocyte doses given per patient. This graph illustrates distribution of the number of therapeutic doses of granulocytes given per patient. Each therapeutic dose of granulocytes consists of either one apheresis unit or 10–12 buffy coats [Colour figure can be viewed at wileyonlinelibrary.com]

commonest transfusion reaction was mild to moderate allergic reaction (four patients; two patients with buffy coat units and two patients with apheresis units). Two patients experienced non-haemolytic febrile transfusion reaction (one with buffy coat unit and one with apheresis unit) and one patient developed transfusion-associated circulatory overload after receiving an apheresis unit. There was one reported case of transfusion-associated polycythaemia in a paediatric patient who received a cumulative sum of 23 buffy coat units and 14 apheresis units over the treatment course.

DISCUSSION

In our retrospective study, we reviewed the GTX usage in New Zealand over a 16-year period between 2000 and 2016 and explored the associated clinical practice and patient outcomes. Although the NZBS protocol suggests GTX be given to patients with a neutrophil count of less than 0.2×10^9/L, 11 (24.4%) recipients had a neutrophil count of 0.2×10^9/L or greater at the time of treatment. This is because NZBS allows the requesting clinicians to make the decision to access GTX support for a septic patient after careful consideration of the patient's underlying disease, neutrophil function, anticipated trajectory of the neutrophil count and severity of the acute infection, in addition to the absolute neutrophil count.

There was little fluctuation in the demand for GTX over the years. The maximum number of cases was seen in 2008 and 2012, with five patients receiving GTX in each. The number of cases varied between one and four in other years, except for in 2002, when GTX was not given at all. This is not surprising as there has not been any groundbreaking improvement in the process of granulocyte collection or a major publication providing stronger evidence for benefit of GTX over this time. The majority of the recipients were paediatric patients located at Starship Hospital, the country's largest tertiary children's hospital. This may be partially because GTX is perceived to be more efficacious in paediatric patients, who are able to receive a higher number of granulocytes per kilogram body weight due to their smaller size. It may also be because doctors and the parents, who often are the surrogate decision-makers for paediatric patients, are more likely treat the very young patients aggressively and/or explore alternative therapeutic options when conventional measures are failing. Cost may also be an issue favouring paediatric recipients. Although all blood components are free of charge to the patient, NZBS operates on a cost recovery model, with the government-funded DHBs paying for each component. A single apheresis granulocyte component costs approximately €500, while a buffy coat from a single whole blood donation costs approximately €145.

New Zealand is a small country, hence, therapeutic granulocytes can generally be transported from a collection site to where a patient is situated by land or air in a timely manner. Provision of all blood products in New Zealand is coordinated by a single crown-owned, non-profit entity, NZBS, so there are insignificant variations in the cost or logistic complexities related to GTX provision around the country. Consequently, there does not appear to be any regional

variation in the usage of GTX. The overall number of cases has been low from all parts of the country. This is likely reflective of the clinicians' uncertainty or scepticism towards the therapeutic benefit of GTX in treating severe infections in immunocompromised patients, given the lack of clear evidence based on large well-conducted randomized controlled trials.

It is interesting to note that at 42 days (the RING trial's endpoint), our survival was over 70%, which is in keeping with the RING trial's expected indicator of success [8]. We have failed to demonstrate improved survival in patients who received a higher dose of granulocytes in the subgroup analysis, but it could be attributable to the small sample size and the incomplete data set. We were not able to determine the granulocyte dose per kilogram body weight for patients who received GTX prior to 2008 due to missing data on the granulocyte content in the infusion products in our current electronic quality assurance database. We also did not find statistically significant difference in survival in patients who had a rise in their neutrophil count of at least 0.2×10^9/L compared to those who did not ($p = 0.4055$), but in order to have a 95% chance of detecting a statistically significant difference, around 144 patients were needed in each group. Likewise, there was no statistically significant difference in survival between the patients who had any rise in the neutrophil count during the course of GTX and those who did not ($p = 0.282$), but we would have needed 24 patients in each arm to detect a statistically significant difference (50% vs. 16.7%) with 80% certainty.

GTX has a number of challenges. It is mandatory to have specialized expertise and facility, such as leukapheresis and HLA matching, to produce the granulocyte products and the manufactured granulocyte products need to reach the intended recipients within 24 hours. This may be particularly challenging in peripheral centres where the manufacturing expertise and facility are not available. The NZBS protocol considers a minimum therapeutic dose of granulocytes to be 1×10^{10}, although $>2 \times 10^{10}$ is desirable. Each bag of buffy coat unit should contain at least 1×10^9 of granulocytes, and at least 10 bags of buffy coat are given per infusion to achieve the therapeutic dose. Currently, there is no formal requirement to adjust the number of buffy coat units to be given based on the patient's weight although this does happen in practice. The recommended granulocyte dose from the 2009 Cochrane review is 3×10^8/kg, which equates to 2.1×10^{10} per infusion for a 70 kg individual. As the average weight of the population increases in the current obesity pandemic, provision of a sufficient dose of therapeutic granulocytes will likely become more and more challenging.

There are concerns over treatment-related adverse effects, particularly pulmonary events, CMV transmission and HLA alloimmunization. GTX appears safe in our study. During the 16-year period where 45 patients received a total of 303 therapeutic doses, only eight transfusion incidents were reported to have caused a transfusion reaction. The commonest transfusion reaction was an allergic reaction, and none was life-threatening. This is significantly less than what was reported in the RING study, where 41% of the 114 participants and 28% of the transfusions were associated with grade 1–2 transfusion reactions, the commonest of which were fever, chills and/or modest changes in the blood pressure [8]. In the same study, 20% of the recipients and less than 5% of the transfusions were associated with grade 3–4 reactions, such as hypoxia, tachycardia, hypotension and allergic reaction. There were no deaths attributable to GTX and no significant association between the granulocyte dose administered and the occurrence of a transfusion reaction. In New Zealand, reporting of transfusion reactions is voluntary, which may lead to under-reporting. Our observed rate of transfusion reactions for fresh components is 2% from audits of over 1000 transfusion episodes, whereas our reported rate via the haemovigilance program is 0.3%. Transfusion reactions associated with GTX are, however, less likely to have been unreported compared with those associated with other fresh components as provision of therapeutic granulocyte products demands close communication among the treating clinicians, the transfusion medicine specialists and the NZBS staff during the treatment course.

There are potential ethical and safety concerns pertaining to exposing healthy volunteer donors to medications, such as G-CSF and dexamethasone, for mobilization of granulocytes. Quillen et al. followed 83 out of 92 apheresis granulocyte donors who received three or more doses of G-CSF at 5 mcg/kg and 8 mg of oral dexamethasone between 1994 and 2002 for a median follow-up period of 10 years. They compared the health outcomes of these granulocyte donors with matched control platelet donors and found there was no difference in the incidence of malignancies, coronary artery disease and thrombosis [13]. To our knowledge, there is no definite evidence in the current literature that a short-term use of G-CSF and dexamethasone leads to any significant long-term morbidity or mortality. These medications, however, still have potential side effects, such as bone pain, tenderness at the injection site, transient hyperglycaemia and so forth. Therefore, it is important to counsel the potential donors carefully before obtaining their informed consent.

Improvements in diagnostic strategies, antimicrobial therapy and supportive care in modern medicine have reduced the perceived need for GTX, and there is lack of strong evidence for GTX in treating neutropaenic patients with severe infection; a limited number of published randomized trials were underpowered to demonstrate potential benefit of GTX owing to small sample sizes and slow recruitment. As the latest 2015 Cochrane review has concluded, there is also not enough evidence to refute the benefit of GTX, and there are case series and anecdotal evidence that show potential benefit of GTX; hence, it seems premature and scientifically unjustified to dismiss therapeutic utility of GTX entirely [9, 14]. We believe GTX should be considered in critically unwell patients with severe neutropaenia or neutrophil dysfunction who fail to respond to conventional antimicrobial or antifungal therapy and surgical measures to control an infective source but otherwise have good long-term prognosis from their underlying diagnoses. Without firm evidence directing the clinicians and patients in one way or another, full discussion should be held with regards to the potential benefits and risks of GTX, and the controversial nature of the treatment before a consensus decision is reached for each patient. It is essential that such a decision is made promptly so that the search for suitable donors can begin without delay and GTX can be delivered in a timely manner at an appropriate dose, before the patient is in extremis.

Our study has a number of limitations. It is a retrospective data analysis, and the data quality was dependent on the availability and completeness of historic documentation. The number of subjects included in this study is small although we were able to capture all patients who received GTX during the study period by virtue of the presence of a single national electronic platform for patient blood management. There is no comparison group because we anticipated it would be difficult to define a matched historic control group as the GTX recipients included in this analysis were a heterogeneous group with varying underlying diagnoses, different types of infections and a wide range of baseline neutrophil counts.

In order to prove or refute the clinical benefit of GTX in septic patients with severe neutropaenia or neutrophil dysfunction, a randomized controlled trial with an adequate power is needed. Such efforts to date have been hindered by failure to enrol enough participants to achieve an adequate power. The next best approach would be to set up an international prospective registry of therapeutic GTX, so the outcome data can be collected in a much larger scale. The Biomedical Excellence for Safer Transfusion (BEST) Collective has set up a web-based international collaborative registry of GTX [15]. NZBS has been working with this international registry to establish participation since 2018. It is anticipated that as the Registry data matures over time, more information will become available to shed more light on real-life experience in therapeutic use of GTX and trend in clinical outcome.

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CONFLICT OF INTEREST

There is no conflict of interest to declare. This reserach received no specific grant from any funding agency in the public, commercial, or not-for-profic sectors.

ORCID

Shanee Chung https://orcid.org/0000-0002-0375-4858
Richard Charlewood https://orcid.org/0000-0002-1798-1189

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ORIGINAL ARTICLE

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Early packed red blood cell transfusion in major trauma patients: Evaluation and comparison of different prediction scores for massive transfusion

Arnaud Cassignol[1] | Pauline Bertein[1] | Paul Botti[2] | Aurélien Renard[3] | Jean Cotte[4] | Julien Bordes[4]

[1]SMUR Department, Sainte-Musse Public Hospital, Toulon, France

[2]Radiology Department, University Hospital of Geneva, Genève, Switzerland

[3]Emergency Department, Sainte-Anne Military Hospital, Toulon, France

[4]Anesthesia and Intensive Care Department, Sainte-Anne Military Hospital, Toulon, France

Correspondence
Arnaud Cassignol, 80 rue du Corail, Résidence Eden Park, Bâtiment Kalmia, 83220, Le Pradet, France.
Email: acassignol@yahoo.fr

Abstract

Background and Objectives: Our study sought to evaluate and compare different prediction scores for massive transfusion in-hospital packed red blood cell (PRBC) transfusions.

Materials and Methods: Between January 2013 and December 2018, 1843 trauma patients were enrolled in the registry of a level-1 trauma centre. All prehospital and in-hospital variables needed to calculate the Shock Index and RED FLAG, Assessment of Blood Consumption (ABC) and Trauma Associated Severe Hemorrhage (TASH) scores were prospectively collected in the registry. The primary endpoint was the initiation of transfusion within the first hour of the patient's arrival at the hospital.

Results: A total of 1767 patients were included for analysis with a mean age of 43 years (±19) and a mean Injury Severity Score of 15 (±14). The in-hospital TASH score had the highest predictive performance overall (area under the curve [AUC] = 0.925, 95% confidence interval [CI] [0.904–0.946]), while the RED FLAG score (AUC = 0.881, 95% CI [0.854–0.908]) had the greatest prehospital predictive performance compared to the ABC score (AUC = 0.798, 95% CI [0.759–0.837]) and Shock Index (AUC = 0.795, 95% CI [0.752–0.837]). Using their standard thresholds, the RED FLAG score was the most efficient in predicting early transfusion (sensitivity: 87%, specificity: 76%, positive predictive value: 25%, negative predictive value: 99%, Youden index: 0.63).

Conclusion: The RED FLAG score appears to outperform both the ABC score and the Shock Index in predicting early in-hospital transfusion in trauma patients managed by pre-hospital teams. If adopted, this score could be used to give advance warning to trauma centres or even to initiate early transfusion during pre-hospital care.

KEYWORDS
early transfusion, major trauma, massive transfusion, pre-hospital score, RED FLAG

INTRODUCTION

Trauma is the primary cause of death among people under 45 years of age worldwide [1], and 66% of trauma deaths occur during pre-hospital care, while 27% occur within the first 48 h in hospital [2]. Between 30% and 40% of these deaths are attributable to massive haemorrhage [3]. In recent years, various scores have been developed to quickly predict the need for massive transfusion. There are

14 prediction scores and algorithms for massive transfusion in major trauma patients [4]. Of these, the Assessment of Blood Consumption (ABC) score and the Shock Index are most frequently used in pre-hospital care [5, 6], while the Trauma Associated Severe Hemorrhage (TASH) score remains the gold standard in hospitals [7]. The objective of these scores is to identify major trauma patients requiring transfusion at an early stage in order to anticipate the amount of labile blood products needing to be ordered by the receiving trauma centre [8]. Although the capability for pre-hospital blood transfusion is being implemented in some areas, it is not yet widely accepted as a standard practice [9]. Effective pre-hospital management and triage of these major trauma patients to dedicated trauma centres reduce their mortality by 25% [10]. In 2018, a French trauma team developed the RED FLAG prediction score for massive transfusion, which is applicable from pre-hospital care [11]. The main objective of our study was to evaluate the RED FLAG score in predicting early packed red blood cell (PRBC) transfusion, defined as the initiation of blood transfusion within the first hour of hospital arrival of major trauma patients in a level-1 trauma centre, as well as comparing this score to other transfusion scores currently in use in pre-hospital (ABC score and Shock Index) and in-hospital (TASH score) care.

MATERIALS AND METHODS

Study type

We conducted a monocentric retrospective study in the Sainte Anne Military Hospital of Toulon (Southeast of France), a level-1 trauma centre with every necessary medical and surgical resources for the treatment of any type of traumatic injury. It receives an average of 300 trauma patients per year. A registry of the hospital's trauma patients was started in 2013 to collect data prospectively in accordance with the Utstein-style guidelines [12]. Raw data were collected prospectively on paper by physicians and then entered into an electronic database. A clinical research assistant regularly verifies the integrity and completeness of the data and collects patient outcomes upon discharge. All patients in the registry from January 2013 to December 2018 were included in the study so long as major trauma was suspected in pre-hospital care. The calculation of prediction scores for transfusion in each trauma patient (RED FLAG, ABC score, Shock Index and TASH score) was performed retrospectively by a single operating physician using the registry data. Both the Establishment Ethics Committee and the Data Protection Committee have agreed to the study (DR-2016-234, request for authorization no. 911461v2).

Scores included in the study

Pre-hospital scores

The RED FLAG score is calculated using five variables: Shock Index ≥1, mean arterial blood pressure ≤ 70 mmHg, capillary haemoglobin ≤13 g/dl, pre-hospital orotracheal intubation and unstable pelvic fracture upon clinical examination. Each variable counts as 1 point. A RED FLAG score ≥ 2 is considered positive.

The ABC score (pre-hospital gold standard) is calculated using four variables: heart rate ≥ 120 bpm, systolic blood pressure ≤ 90 mmHg, the penetrating effect of the trauma and presence of free intraperitoneal fluid (e.g., by Focused Assessment with Sonography for Trauma [FAST]). Each variable counts as 1 point. An ABC score ≥ 2 is considered positive.

The Shock Index is calculated using two variables: maximum heart rate and minimum systolic arterial blood pressure. A Shock Index ≥1 is considered positive.

In-hospital score

The TASH score (in-hospital gold standard) is calculated using eight variables (Table 1): patient gender, haemoglobin level, base excess, systolic blood pressure, heart rate, presence of free intraperitoneal fluid (e.g., by FAST), unstable pelvic fracture and open femur fracture. A simplified point score can be directly correlated to the probability of massive transfusion.

Epidemiological analysis

Data collected during pre-hospital care include age, gender, type of trauma, mechanism of trauma, Glasgow Coma Scale (GCS), heart rate, systolic and diastolic blood pressure, respiratory rate, pulse oximetry, pre-hospital resuscitation (orotracheal intubation, catecholamines and fluids administered), capillary haemoglobin and FAST ultrasound results. These variables were used to calculate the Shock Index and RED FLAG and ABC scores. In-hospital data included clinical examination (GCS, heart rate, systolic and diastolic blood pressure, respiratory rate, pulse oximetry), biological analysis (standard blood, coagulation and arterial blood gas tests) and medical imaging (chest and pelvis x-rays, FAST ultrasound and whole-body scan). The primary endpoint was early transfusion, defined as the transfusion of PRBCs within the first hour after the trauma patient's arrival at the hospital.

Statistical analysis

We first performed a descriptive analysis of our study population. Descriptive statistics included frequencies and percentages for categorical variables and mean values (standard deviation [SD]) for continuous variables. We then compared early transfused patients with non-transfused patients. The Mann–Whitney *U*-test was used to compare quantitative variables, while the chi-squared test was used for qualitative variables. Comparison of each score's diagnostic performance to predict early in-hospital PRBC transfusion was evaluated via analysis of the receiver operating characteristic curve (ROC curve) using the same method as DeLong et al. [13]. Sensitivity, specificity,

TABLE 1 Trauma associated severe hemorrhage (TASH) score and probability of massive transfusion (MT)

Variables	Values	Points	TASH score	Probability of MT
Haemoglobin (g/dl)	<7	8	1–8	<5%
	<9	6	9	6%
	<10	4	10	8%
	<11	3	11	11%
	<12	2	12	14%
Base excess (mEq/L)	<−10	4	13	18%
	<−6	3	14	23%
	<−2	1	15	29%
Systolic blood pressure (mmHg)	<100	4	16	35%
	<120	1	17	43%
Heart rate (bpm)	>120	2	18	50%
Free intraperitoneal fluid (via FAST)		3	19	57%
Clinically unstable pelvic fracture		6	20	65%
Open or displaced femur fracture		3	21	71%
Male sex		1	22	77%
Total points = TASH score			23	82%
			24+	>85%

Abbreviation: FAST, focused assessment with sonography for trauma.

positive predictive values and negative predictive values were calculated with their standard thresholds in order to examine each score's predictive capability regarding early PRBC transfusion (RED FLAG score ≥ 2, ABC score ≥ 2, Shock Index ≥1, TASH score ≥ 16).

The statistical software XLSTAT pro v.22.1.3 was used for data acquisition and analysis, as well as for the comparison of ROC curves. Statistical significance was set at $p < 0.05$.

RESULTS

Population

Of the 1843 trauma patients included in the registry during the study period, 1767 patients (96%) were included in the analysis. A study population flowchart and reasons for exclusion can be seen in Figure 1. Study population characteristics are reported in Table 2.

Aims

After admission to the level-1 trauma centre, 151 patients were transfused within the first hour. Table 3 presents the clinical, paraclinical and severity differences between trauma patients who received early PRBC transfusion and those who did not. The early transfused population was more often in critical condition than the non-transfused population. The ROC curves and AUC differences for each score studied are included in Figure 2 and Table 4. The in-hospital TASH score was better than all pre-hospital scores studied ($p < 0.05$), while the RED FLAG score was the best performing pre-hospital score ($p < 0.05$). Table 5 presents the predictive performance of each score with their standard thresholds, with the RED FLAG score demonstrating the best predictive performance for early PRBC transfusion. However, the TASH score's predictive performance improved (sensitivity [Se] = 87%, specificity [Sp] = 86%, positive predictive value [PPV] = 37%, negative predictive value [NPV] = 99%, Youden index = 0.73) after the positivity threshold was lowered (TASH ≥9).

DISCUSSION

Our study aimed to evaluate and compare prediction scores for massive transfusion, especifically, in the case of early PRBC transfusion defined as the initiation of transfusion within the first hour of arrival at our level-1 trauma centre. The RED FLAG score appeared to be the highest performing pre-hospital scores studied in predicting the need for early PRBC transfusion with AUC = 0.88, 95% CI [0.85–0.91].

The RED FLAG score was recently developed by a French trauma team and includes the Shock Index and additional clinical variables that denote a patient's severity of trauma [11]. As such, it can be considered as a slightly more adapted score than the Shock Index in the early detection of trauma-induced haemorrhage. To our knowledge, it has never been compared to other in-hospital and pre-hospital scores that predict the need for massive transfusion. Our study is also original in introducing a notion of temporality with the concept of early transfusion in the comparison of these scores. Although we were unable to verify whether patients who received an early PRBC

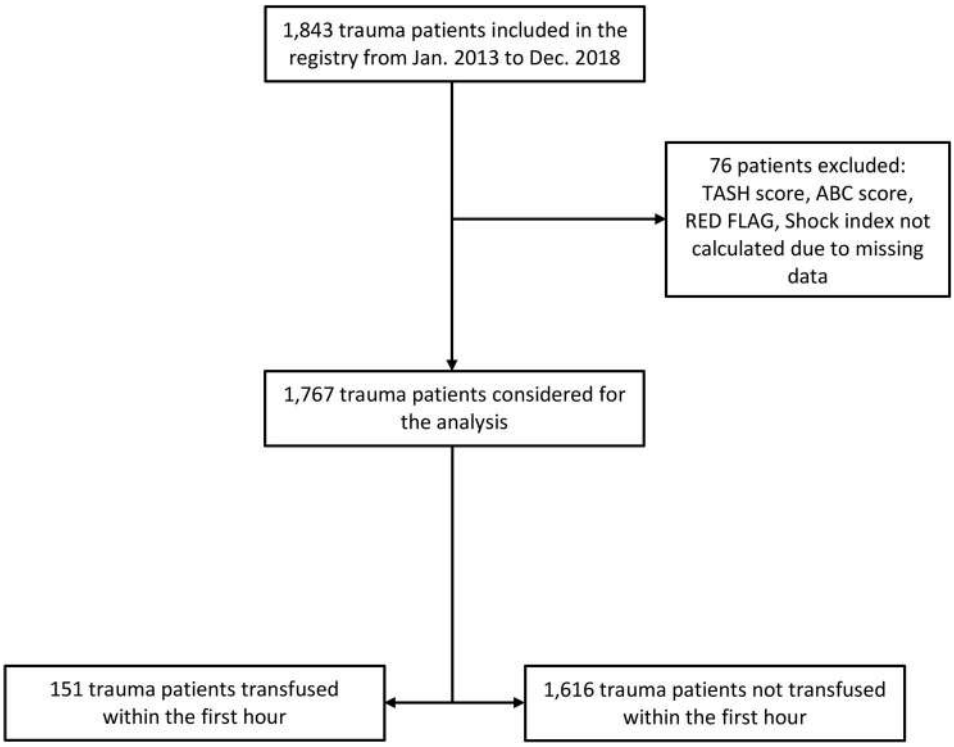

FIGURE 1 Study population flowchart. ABC, assessment of blood consumption; TASH, trauma associated severe hemorrhage

transfusion required a massive transfusion thereafter, the results of our study suggest an interest in the RED FLAG score as it seemed to perform better than both the ABC score and the Shock Index in predicting the need for early PRBC transfusion.

The ABC score is the standard reference in pre-hospital care [5]. In the hospital, it has demonstrated greater diagnostic performance than the TASH score in predicting the need for transfusion in trauma patients [14]. In our study, the ABC score appeared to perform less well than the TASH score in predicting early PRBC transfusion. This could be explained by the use of pre-hospital variables in its calculation versus in-hospital variables for the TASH score. It also appeared to be less effective than the RED FLAG score and comparable to the Shock Index. In addition to systolic blood pressure and heart rate (also Shock Index components), the ABC score considers free intraperitoneal fluid via FAST and penetrating trauma as well. These last two variables did not prove to be significant in predicting transfusion in a recent study [15], which may explain the comparability of the two scores.

The Shock Index is the oldest score included in our study. It was first used as an indicator of shock severity [16], then, as a prediction score for trauma mortality [17] and, finally, as a predictive test for massive transfusion [6]. The Shock Index has been demonstrated to be an early indicator of hypovolaemic shock and blood transfusion upon hospital arrival [18]. It is a measure of the severity of hypovolaemia, particularly, in patients in the compensatory stage of shock who have a heart rate and/or systolic blood pressure within the tolerance limits, despite significant blood loss [19]. By selecting the threshold of SI ≥1, its calculation can be simplified for pre-hospital care [20]. The Shock Index has also been relevant in geriatric and paediatric populations [21, 22].

In the hospital, the TASH score is the gold standard to predict transfusion needs [23], which acts as a simplified scoring system where increasing points can be directly correlated to an increasing probability of massive transfusion without having a clear positivity threshold. We used the threshold ≥16 in our study as it has been commonly used in the literature [24], though different thresholds for the TASH score have been suggested [25, 26], and a threshold of ≥8.5 was found to have greater predictive performance (Se = 84.4% and Sp = 78.4%) [27]. In our study, by decreasing the positivity threshold to ≤9, the predictive performance of the score was increased regarding early PRBC transfusion. However, having to wait for pathological and/or imaging results at the hospital may delay both calculating the score and starting transfusion. The relevance of the RED FLAG score in initiating early in-hospital PRBC transfusion is interesting but should be the subject of a study specifically dedicated to this objective.

In the majority of studies comparing massive transfusion prediction scores, the TASH score appears to perform the best [24, 26, 28]. However, there are limitations to using the TASH score in pre-hospital care, such as the large number of variables required for its calculation (eight variables) and the absence of a real threshold for initiating transfusion. The Shock Index (two variables), the ABC score (four variables) and the RED FLAG score (five variables) with their fixed thresholds (≥1, ≥2 and ≥2, respectively) are more appropriate in pre-hospital emergency care for starting PRBC transfusion on scene and/or to alert the trauma centre.

TABLE 2 Study population characteristics (n = 1767 patients)

Characteristics	Value
Sex: Male, *n* (%)	1359 (77)
Age, mean (SD)	42.8 (±19.2)
Mechanism of injury, *n* (%)	
• Blunt	1616 (91.5)
• Penetrating	151 (8.5)
Type of trauma, *n* (%)	
• Motorcycle	709 (40)
• Car crash	397 (22.5)
• Fall	322 (18.4)
• Pedestrian	107 (6)
• Gunshot	82 (4.6)
• Stab wound	62 (3.5)
• Other	88 (5)
Transport to hospital, *n* (%)	
• EMS	1079 (61)
• HEMS	622 (35)
• Private transportation	66 (4)
Severity scores	
MGAP score, *n* (%)	
• 3–17[a]	196 (11.1)
• 18–22[b]	311 (17.6)
• 23–29 [c]	1260 (71.3)
Injury severity score, mean (SD)	15 (±14)
Prediction scores for MT, *n* (%)	
• TASH score ≥ 16	82 (4.6)
• ABC score ≥ 2	149 (8.4)
• Shock Index ≥1	310 (18)
• RED FLAG ≥2	527 (30)
In-hospital evolution	
• Blood transfusion within the first hour, *n* (%)	151 (8.5)
• Length of stay, day, mean (SD)	11 (±16)
• Mortality, *n* (%)	129 (7.3)

Abbreviations: ABC, assessment of blood consumption; EMS, emergency medical service; HEMS, helicopter emergency medical service; MGAP score, mechanism, glasgow coma scale, age, systolic blood pressure; MT, massive transfusion; TASH, trauma associated severe hemorrhage.
[a]High risk of mortality.
[b]Intermediate risk of mortality.
[c]Low risk of mortality.

Many of the existing scores that attempt to predict massive transfusion are not derived from pre-hospital variables [4]. Our study was based solely on pre-hospital variables used in the design of the RED FLAG score, ABC score and Shock Index. Only the TASH score was designed using in-hospital variables. As Hamada et al. suggest, the RED FLAG score has the advantages of simplicity and pragmatism due to its use of variables corresponding to criteria that are directly accessible to the pre-hospital care team. It permits the rapid identification of trauma patients who require the mobilization of significant human and material resources to control bleeding.

It is important to use the appropriate algorithms and standard procedures in pre-hospital care in order to anticipate the transfusion requirements of major trauma patients and to alert the trauma centre. Without advance warning from pre-hospital teams, trauma leaders are often restricted to awaiting the patient's arrival at the hospital to activate a massive transfusion protocol. In this context, the scores are of less interest as patients are already in the hospital, leading to a loss of preparedness for ordering labile blood products. Thus, advance warning of early PRBC transfusion by pre-hospital teams is necessary for increasing preparedness by anticipating the orders of labile blood products in the hospital and readying operating rooms before the arrival of the major trauma patient.

Our study has several limitations. Although the data collected were prospective and mostly complete, the study design was retrospective, monocentric and observational. Scores were calculated retrospectively, and it was impossible to determine whether their use during pre-hospital care would have affected in-hospital care. The retrospective nature also prevented us from analysing the temporal component of score use, a fundamental element of all pre-hospital care. It was not possible for us to confirm whether patients who had received an early PRBC transfusion received a massive transfusion afterward, as our study focussed on early PRBC transfusion upon arrival in hospital. Neither can we confirm whether patients who were not transfused in the first hour did not later require a blood transfusion. RED FLAG had been developed primarily in a blunt trauma population and reflects distinct local procedures (medicalization of pre-hospital emergency care), such as orotracheal intubation in the field or measuring haemoglobin levels by capillary test. Consequently, RED FLAG might not be applicable when either pre-hospital haemoglobin assessment is unavailable or pre-hospital intubation is not commonly performed, or in the context of penetrating trauma, particularly concerning military combat where high-energy penetrating trauma is more frequent. Penetrating trauma accounted for only 8.5% of our cohort, and no subgroup analysis was performed. Therefore, we cannot determine the relevance of the scores evaluated in this population. A multicentric prospective study is necessary to both validate our results and allow us to standardize our pre-hospital care protocols accordingly.

In summary, it remains difficult to detect active bleeding and anticipate the need for transfusion in major trauma patients. The RED FLAG score appears to outperform both the ABC score and the Shock Index in predicting the necessity of early PRBC transfusion in major trauma patients during pre-hospital care. Its use in current practice could provide two significant advantages: (i) providing advance warning to receiving trauma centres of the need to initiate an early transfusion and (ii) beginning blood transfusion during pre-hospital care for teams equipped with labile blood products in their mobile emergency units (EMS and/or HEMS).

TABLE 3 Comparison of patient transfused or not within first hour in hospital

	Blood transfusion within first hour		
	YES	NO	
Characteristics	***n* = 151**	***n* = 1616**	***p*-value**
Sex male, *n* (%)	105 (70)	1254 (78)	0.025
Age, mean (SD)	46 (±21)	43 (±19)	0.099
Prehospital physiological variables, mean (SD)			
• GCS	9 (±5)	13 (±4)	<0.001
• Heart rate (bpm)	103 (±31)	89 (±21)	<0.001
• Systolic blood pressure (mmHg)	94 (±37)	123 (±24)	<0.001
• Diastolic blood pressure (mmHg)	53 (±28)	72 (±18)	<0.001
• Mean blood pressure (mmHg)	66 (±31)	88 (±19)	<0.001
• Peripheral oxygen saturation (%)	89 (±18)	97 (±6)	<0.001
• Respiratory rate (cpm)	20 (±7)	19 (±5)	0.082
• Capillary haemoglobin (g/dl)	11 (±3)	13 (±2)	<0.001
Prehospital resuscitation			
• Mechanical ventilation, *n* (%)	88 (58)	245 (15)	<0.001
• Catecholamine administration, *n* (%)	90 (60)	103 (6)	<0.001
• Tranexamic acid administration, *n* (%)	102 (68)	240 (15)	<0.001
• Osmotherapy, *n* (%)	18 (12)	56 (4)	<0.001
• Fluid administration, ml, mean (SD)	944 (±786)	378 (±399)	<0.001
In-hospital physiological variables, mean (SD)			
• GCS	8 (±6)	12 (±5)	<0.001
• Heart rate (bpm)	102 (±34)	87 (±21)	<0.001
• Systolic blood pressure (mmHg)	86 (±34)	122 (±24)	<0.001
• Diastolic blood pressure (mmHg)	46 (±24)	72 (±17)	<0.001
• Mean blood pressure (mmHg)	58 (±27)	88 (±18)	<0.001
• Peripheral oxygen saturation (%)	91 (±17)	99 (±23)	<0.001
• Respiratory rate (cpm)	19 (±6)	19 (±5)	0.99
Biological parameters, mean (SD)			
• Haemoglobin (g/dl)	9.9 (±2.4)	13.6 (±4.3)	<0.001
• Blood platelets (G/L)	185 (±76)	229 (±66)	<0.001
• Prothrombin	50 (±20)	83 (±15)	<0.001
• Fibrinogen	1.6 (±1)	2.7 (±1)	<0.001
Blood gas parameters, mean (SD)			
• pH	7.18 (±0.18)	7.37 (±0.19)	<0.001
• PO_2	206 (±128)	169 (±104)	0.002
• PCO_2	46 (±17)	39 (±10)	<0.001
• HCO_3^-	16.7 (±4.6)	22.3 (±3.3)	<0.001
• Base excess	−10.6 (±6.4)	−2.6 (±3.7)	<0.001
• Lactate	6.1 (±4.8)	2 (±1.5)	<0.001
Medical imagery, *n* (%)			
• Pneumothorax	17 (11)	57 (3.5)	<0.001
• Haemothorax	37 (25)	41 (2.5)	<0.001
• Unstable pelvic fracture	38 (25)	99 (6.1)	<0.001
• Free intraperitoneal fluid (via FAST)	40 (26)	81 (5)	<0.001

(Continues)

TABLE 3 (Continued)

Characteristics	Blood transfusion within first hour		
	YES	NO	
	n = 151	*n* = 1616	*p*-value
Severity, *n* (%)			
• Emergency haemostatic surgery	51 (34)	52 (3.2)	<0.001
• ISS > 15	132 (87)	622 (39)	<0.001
• In-hospital death	55 (36)	74 (4.6)	<0.001

Abbreviations: FAST, focused assessment with sonography for trauma; GCS, glasgow coma scale; ISS, injury severity score.

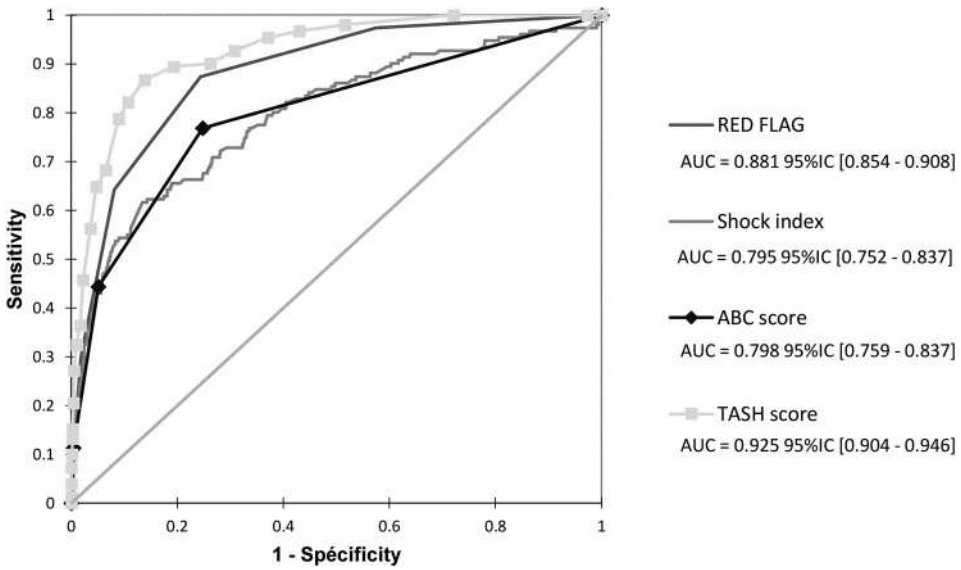

FIGURE 2 Receiving operating curves of four scores (Shock Index and RED FLAG, ABC and TASH scores) for early packed red blood cell transfusion. ABC, assessment of blood consumption; TASH, trauma associated severe hemorrhage

TABLE 4 Comparison of the predictive performance of the Shock Index and TASH, RED FLAG and ABC scores in the cohort (n = 1767 patients)

Scores	AUC difference	95% CI	*p*-value
RED FLAG versus Shock Index	0.086	0.08–0.10	<0.001
RED FLAG versus ABC score	0.083	0.07–0.09	<0.001
ABC score versus Shock Index	0.003	−0.01–0.10	0.539
TASH score versus RED FLAG	0.044	0.04–0.05	<0.001
TASH score versus ABC score	0.127	0.12–0.14	<0.001
TASH score versus Shock Index	0.130	0.12–0.14	<0.001

Abbreviations: ABC, assessment of blood consumption; AUC, area under the curve; TASH, trauma associated severe hemorrhage.

TABLE 5 Diagnostic properties of each score (Shock Index and TASH, RED FLAG and ABC scores) with standard thresholds

Scores	Thresholds	Sensitivity	Specificity	PPV	NPV	Youden index
RED FLAG	≥2	87%	76%	25%	99%	0.63
Shock Index	≥1	62%	87%	30%	96%	0.48
ABC score	≥2	44%	95%	45%	95%	0.39
TASH score	≥16	36%	98%	67%	94%	0.35

Abbreviations: ABC, assessment of blood consumption; NPV, negative predictive value; PPV, positive predictive value; TASH, trauma associated severe hemorrhage.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

ORCID

Arnaud Cassignol https://orcid.org/0000-0002-8403-0076

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

The impact of COVID-19 outbreak on the Transfusion Medicine Unit of a Northern Italy Hospital and Cancer Centre

Davide Schiroli[1] | Lucia Merolle[1] | Giuseppe Molinari[1] | Erminia Di Bartolomeo[1] | Daniela Seligardi[1] | Laura Canovi[1] | Thelma A. Pertinhez[1,2] | Pamela Mancuso[3] | Paolo Giorgi Rossi[3] | Roberto Baricchi[1] | Chiara Marraccini[1]

[1]Transfusion Medicine Unit, AUSL-IRCCS di Reggio Emilia, Reggio Emilia, Italy

[2]Department of Medicine and Surgery, Università di Parma, Parma, Italy

[3]Epidemiology Unit, AUSL-IRCCS di Reggio Emilia, Reggio Emilia, Italy

Correspondence
Lucia Merolle, Transfusion Medicine Unit, AUSL-IRCCS di Reggio Emilia, Reggio Emilia, Italy.
Email: lucia.merolle@ausl.re.it

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Abstract

Background and Objectives: The first wave of coronavirus disease-2019 (COVID-19) dramatically affected the Transfusion Medicine Unit of the Azienda Unità Sanitari Locale - Istituto di Ricovero e Cura a Carattere Scientifico (AUSL-IRCCS) di Reggio Emilia, which faced a total rearrangement of the procedures for donors and patients. This study aims to assess the major implications of COVID-19 on our department, focusing on the blood transfusion chain and therapies, in order to support transfusion specialists in seeking efficient ways to face similar future emergencies.

Materials and Methods: This retrospective study compares our Transfusion Medicine Unit data collected between February and May 2020 with the same period in 2017–2019. Data on red blood cells and platelets donations, transfusions and clinical procedures were collected as aggregates from our internal electronic database.

Results: During the lockdown, donor centres were re-organized to reduce the risk of contagion and avoid unnecessary blood collection. Blood donations were re-scheduled to meet the decrease in elective surgery; consequently, plateletapheresis was implemented to supply the reduction of buffycoat-derived platelets. Transfusions significantly decreased together with orthopaedic and vascular surgery, while they were only marginally diminished for both cancer and onco-haematological patients. Reduced procedures for inpatients and outpatients were matched by remote medicine, addressing the need of a constant healthcare support for patients with chronic diseases.

Conclusions: The described measures were adopted to avoid excessive blood collection and expiration, guarantee the safety of our ward (for both patients and staff) and supply the necessary transfusion therapies. These measures may support the development of appropriate risk management plans and safety procedures for other hospitals and transfusion services that have to face similar events.

KEYWORDS
blood collection, donor recruitment, platelet transfusion, transfusion medicine (in general), transfusion therapy

INTRODUCTION

The first wave of coronavirus disease-2019 (COVID-19) significantly impacted Northern Italy, where hospitals faced a rapid and profound re-organization of the healthcare services supply [1, 2]. The Italian Government declared a lockdown period that, for the Reggio Emilia province, started on 9 March 2020, with a gradual loosening from 3 May 2020 [3]. During this period, all the wards of the AUSL-IRCCS of Reggio Emilia rearranged their routine procedures to meet the requirements imposed by the COVID-19 outbreak.

Reggio Emilia hospital is located in Emilia Romagna (Northern Italy) and experienced thousands of COVID-19 cases, with different grades of severity during the lockdown period [4]. The impact of the pandemic on the healthcare system negatively affected a large number of patients with different pathologies [5–9]. Emilia Romagna Local Governance listed the healthcare services that could not be postponed during the lockdown, as well as those that could be delayed or developed through remote medicine (i.e., telemedicine, electronic prescriptions, phone consultations), in order to limit the access to hospitals and avoid overcrowding [10].

Despite not being involved in first-line treatment of COVID-19 patients, even our Transfusion Medicine Unit faced many changes in its routine clinical practice, due to the close connection with the other hospital departments [11]. To ensure high standards of blood donation and blood safety during the emergency, the Italian National Blood Centre (Centro Nazionale Sangue, CNS), in compliance with the Ministry of Health and the COVID-19 Technical Scientific Committee (CTS), issued a series of measures that our ward promptly adopted [12, 13].

In the present study, we investigate the effects of the COVID-19 pandemic and lockdown-related restrictions on the activity of our Transfusion Medicine Unit, providing a description of the adopted measures and of their effectiveness on blood collection, blood transfusions, transfusion medicine and immune-haematology clinical practice.

Our aim is to point out, through an evidence-based analysis, the practices that may help transfusion specialists worldwide to deal with similar emergencies in the future.

MATERIALS AND METHODS

Reggio Emilia is one of the nine provinces located in the Emilia-Romagna region, accounting for about 532,000 inhabitants. The Emilia-Romagna region coordinates blood availability and distribution between the provinces via the Regional Blood Centre (Centro Regionale Sangue [CRS]). Reggio Emilia province harbours five hospitals. Nevertheless, there is only a Transfusion Medicine Unit that centralizes the necessity for transfusions, therapies and medical consultations.

Study design

The study was carried out at the Transfusion Medicine Unit of the Azienda USL-IRCCS di Reggio Emilia. This is a retrospective study comparing data from February to May 2020 with the same period of the previous triennium (2017–2019).

Since one-way analysis of variance (ANOVA) analysis did not reveal significant difference ($p > 0.05$) in the mean transfusions among the tested groups (2017–2019), total red blood cells (RBCs) transfusions performed during the 2020 lockdown were compared with the mean transfusions performed during the same period of the previous triennium.

PLTs transfusions' comparison, instead, was performed on 2020 versus 2019. In our OECI Clinical Cancer Centre, between 65% and 90% of PLTs usage is generally destined to haematological and onco-haematological patients. The number of these patients increased since July 2017, when our hospital established the Cancer and Haematology Centre (CORE). This pavilion became fully functional by the end of 2018 and now centralizes all the onco-haematological patients of the Reggio Emilia province.

No ethical approval was necessary for collecting the aggregated, anonymous data presented herein.

Transfusion Medicine Unit procedures and organization

Our Transfusion Medicine Unit accounts for 24 collection points (donor centres) covering the whole Reggio Emilia province. The management is equally distributed between the hospital and the volunteer associations (Associazione Volontari Italiani del Sangue, AVIS). Blood processing and distribution are exclusively managed by the Transfusion Medicine Unit.

Our procedures involve therapies and consultations for patients coming from other wards, for inpatients temporarily hospitalized within our ward and for outpatients. Medical offices that compose our Transfusion Medicine Unit are dedicated to patient blood management, bloodlettings/phlebotomies, transfusions, apheresis and sideropaenic anaemia treatment.

Data management and statistical analysis

Data concerning hospitalizations and COVID-19 patients were collected as aggregates from our hospital discharge database. Data concerning the Transfusion Medicine Unit procedures were collected as aggregates using our internal database interface (Eliot, Engineering Ingegneria Informatica S.p.A., Italy) and downloaded as Excel spreadsheets. Subsequent data analysis was performed on Excel (Microsoft Office 2010, Microsoft Corp., Redmond, WA), Python (3.8) and GraphPad Prism 7.02 (GraphPad Software, San Diego, CA). Data were expressed as number of patients or blood/platelets (PLTs) units and, when possible, as a mean of three different years (2017, 2018 and 2019) with its maximum–minimum range. Statistical significance was assessed using a t-test when comparing the same time lapse of two different years, while one-way ANOVA was used to determine statistical significance of the same time lapse in the last 3 years (from 2017 to 2019) either including 2020 or not within the comparison. The significance was set at $^{*}p \leq 0.05$, $^{**}p \leq 0.001$ and $^{***}p \leq 0.0001$.

RESULTS

Management of the donor centres

During the national lockdown period, Reggio Emilia donor centres were re-organized to:

1. Lower the risk of contagion.
2. Avoid unnecessary blood collection.

To lower the risk of contagion, two small donor centres were temporarily closed and converted into COVID clinics. In the remaining operating centres, some of the personnel were appointed to check the temperature and health status of all donors immediately before their access. Social distancing measures were adopted to avoid overcrowding, and, after each donation, rooms and seats were sanitized. Donations were suspended for students and first donors; when not necessary, eligibility and control exams were halted.

Blood collection

Blood, PLTs and plasma donations are routinely planned with the donors by SMS alert or phone call and scheduled in order to adequately manage the blood bank. During the 2020 lockdown, the schedule was deeply modified to meet the hospital requirements.

According to the Italian Government instructions [3], our hospital re-scheduled a large majority of surgical procedures. When possible, non-urgent orthopaedics, cardiovascular and cancer surgeries were postponed or moved to non-COVID hospitals. This had an impact on the total number of surgical interventions, especially during the most critical lockdown phase (i.e., March and April 2020, data not shown). As a consequence, the Transfusion Medicine Unit faced a decrease in blood components' requests, and blood donation schedules were all re-considered in order to avoid excessive stored blood units.

Figure 1(a) shows that the number of red blood cells (RBCs) units prepared from whole blood donations between February and May 2020 decreased, particularly between March and April. Consequently, the available buffycoat-derived PLTs units also diminished. Plateletapheresis was, thus, increased to allow a constant PLTs supply to our blood bank (Figure 1(b)).

Whole blood donations dedicated to group 0 negative (0−) (Supplementary Figure S1) and rare phenotypes reserved for specific cohorts of patients (i.e., thalassaemics and myelodysplastics) were preserved. Rare group donors were scheduled by direct phone call, while all the other appointments were cancelled by SMS.

Since plateletapheresis is a complex and invasive procedure, PLTs donors are generally fewer than blood donors. The collection of a sufficient amount of plateletapheresis-derived units, therefore, represented a challenge for our recruiting personnel. To overcome this problem, our Transfusion Medicine Unit established a bank of frozen PLTs, which can be stored for up to 2 years. Between August and October 2020, our personnel collected and stored over 50 frozen PLTs units, according to Italian standards [14].

Furthermore, the CRS coordinated blood availability and distribution between the provinces according to the epidemic diffusion. In order to determine how this internal and local re-organization of blood supply and transfusions affected the coordination with the other transfusion centres of our region, we also analysed the influx/efflux of blood units. Results are reported in Figure S2 of the Supplementary information.

Finally, since plasma can be stored frozen for several months, its collection was not interrupted. The donor centres extended the opening time, while appointments for plasma donations were re-scheduled to avoid overcrowding and to allow the necessary sanitization (data not shown).

Blood transfusion—RBCs and PLTs

To understand how the pandemic affected the transfusion therapies, we compared the percentages of hospitalized patients transfused between February and May 2020 with those of 2017–2019 (Table 1). Taking into account the number of non-COVID patients, the 2020 RBCs transfusion rate increased from 18.5% to 22.5%, while only a small number of COVID-19 patients underwent a transfusion (161 over 1720, 9.4%). These data are in agreement with the hospitalizations that, during the lockdown period, dropped from a 15,771

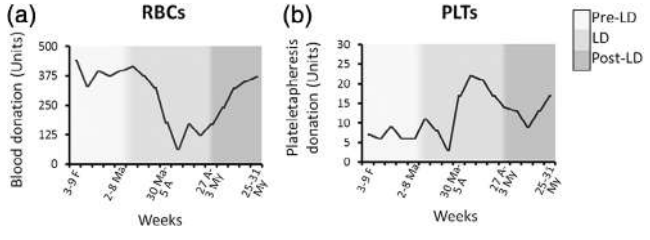

FIGURE 1 (a) Whole blood–derived RBCs, weekly donations between February and May 2020, and (b) platelets apheresis donations collected in the same period. LD, lockdown; RBCs, red blood cells; PLTs, platelets

TABLE 1 Patients transfused and total patients hospitalized by the AUSL-IRCCS di Reggio Emilia between February and May 2020 (absolute number), compared to the same period of 2017–2019 (mean)

February–May	Transfused patients	Hospitalized patients	% Transf./ Hosp.
2017–2019 (mean value)	2920	15,771	18.5
2020 COVID negative	2373	10,520	22.5
2020 COVID positive	161	1720	9.4

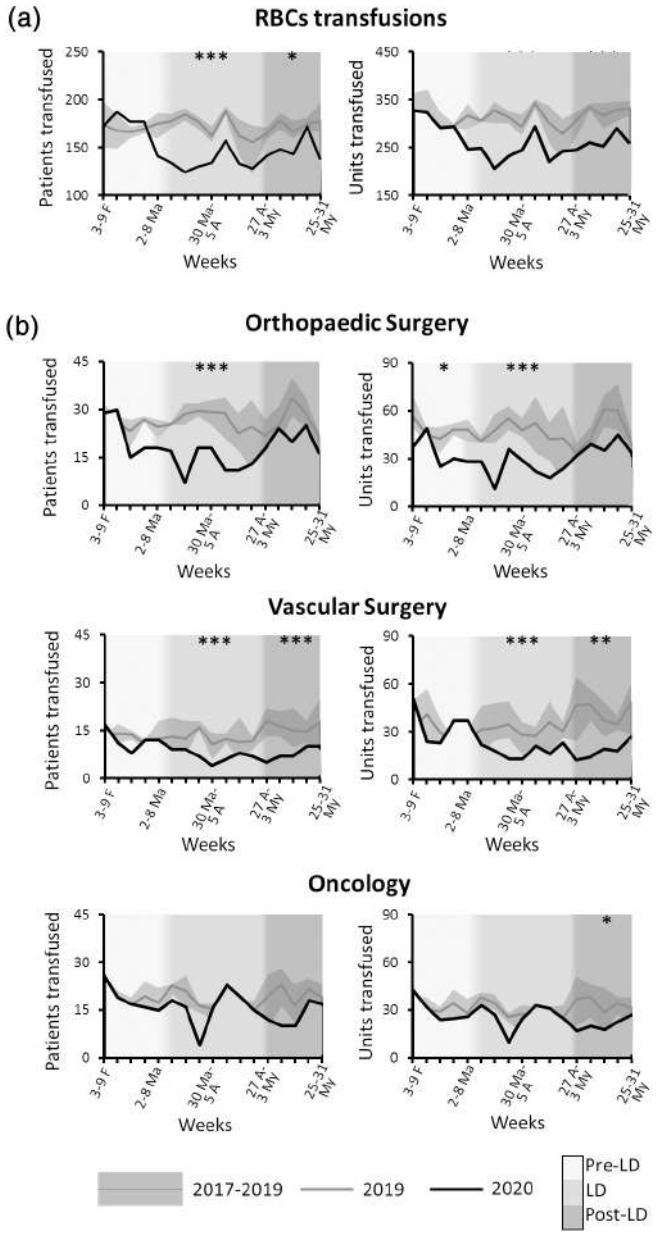

FIGURE 2 RBCs transfusions in 2020 (black lines), compared with the same period of the previous triennium. (a) Patients transfused with RBCs (left panel) and RBCs units transfused (right panel); (b) patients and units transfused for orthopaedics surgery; (c) patients and units transfused for cardiovascular surgery and (d) patients and units transfused for cancer other than haematological. *$p \leq 0.05$, **$p \leq 0.001$ and ***$p \leq 0.0001$. RBCs, red blood cells

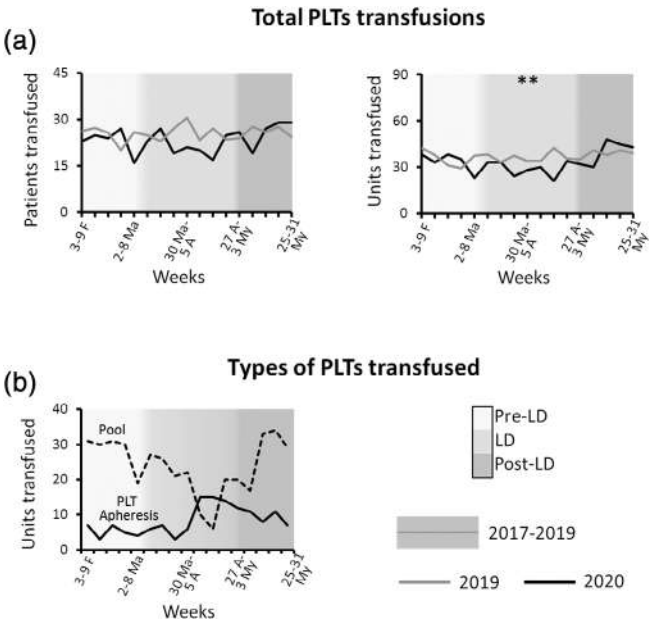

FIGURE 3 (a) Patients transfused with PLTs (left panel) and PLTs units transfused in 2020, compared to the same period of 2019 and (b) buffycoat-derived (pool) and apheresis-derived (PLT apheresis) PLTs units transfused between February and May 2020. *$p \leq 0.05$, **$p \leq 0.001$ and ***$p \leq 0.0001$. LD, lockdown; PLT, platelet

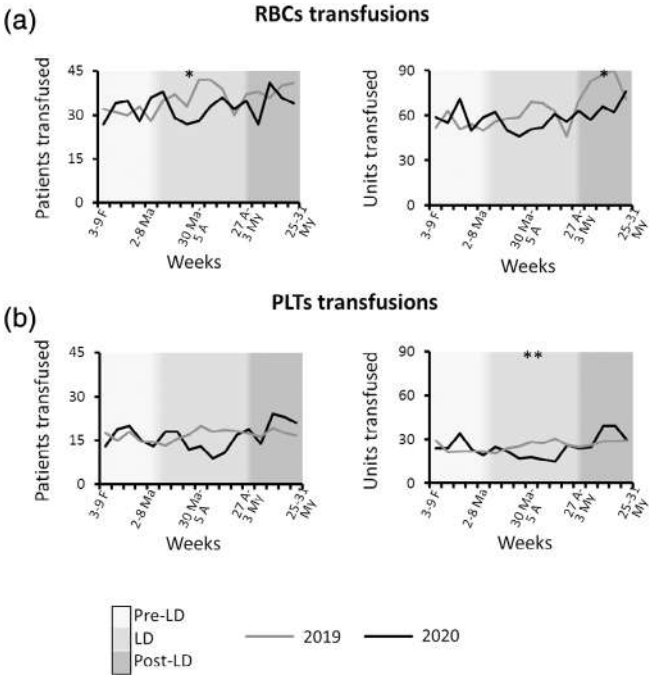

FIGURE 4 Patients and units transfused for onco-haematological diseases: (a) RBCs and (b) PLTs. *$p \leq 0.05$, **$p \leq 0.001$. LD, lockdown; PLTs, platelets; RBCs, red blood cells

mean in 2017–2019 to 10,520 in 2020 (that becomes 12,240 if we include COVID-19 patients, Table SI).

Following the suspension of elective and non-urgent surgical interventions, RBCs transfusions significantly decreased when compared to the previous triennium, both in terms of patients and total units transfused (Figure 2(a)). Starting from this evidence, we further dissected the RBCs transfusions according to the applicant ward. Our data revealed that the most significant decrease of RBCs transfusions was observed for orthopaedic patients and, to a lesser extent, for cardiovascular surgery (Figure 2(b),(c), respectively). Interestingly, the decrease of RBCs usage in the orthopaedic interventions' accounts for about 35%

of the total reduction and started around 15 days before the national lockdown. Conversely, there was no significant decrease in RBCs transfusion of cancer patients during the lockdown period and was only a slight decrease of the transfused units in the post-lockdown period (Figure 2(d)).

In parallel, we investigated PLTs transfusions, we observed a slight decrease in patients and units transfused during the lockdown (Figure 3(a)), although only these last data are significant ($p < 0.01$). PLTs transfusions started decreasing 3 weeks after the beginning of lockdown and came back to the 2019 level within 1 month. The largest amount of PLTs units transfused during the second month of lockdown (i.e., April 2020) came from plateletapheresis, as a consequence of the reduction of buffycoat-derived pools following the decrease of whole blood donations (Figure 3(b)).

Blood transfusion—RBCs and PLTs in onco-haematological patients

As mentioned earlier, the establishment of the CORE pavilion implied an increase of hospitalized onco-haematological patients between 2017 and 2019. Therefore, the effects of the lockdown on transfusion therapies for this sub-group of patients are described in comparison with 2019 only.

In our hospital, onco-haematological patients transfused with RBCs account for about 20% of the total, and their caregiving was preserved as much as possible during the first wave of the pandemic. Nevertheless, during the lockdown, we also observed a slight decrease of RBCs transfusions for these patients (Figure 4(a)). Although these data were not highly significant, it is supported by a similar observation on PLTs transfusions (Figure 4(b)).

Transfusion medicine and immune-haematology clinical practice

Following the official resolution of the Emilia-Romagna region [14], our Transfusion Medicine Unit totally re-organized its services to:

- Limit the access of patients to emergencies and unavoidable therapies;
- Guarantee transfusions and other therapies to patients coming from other wards;

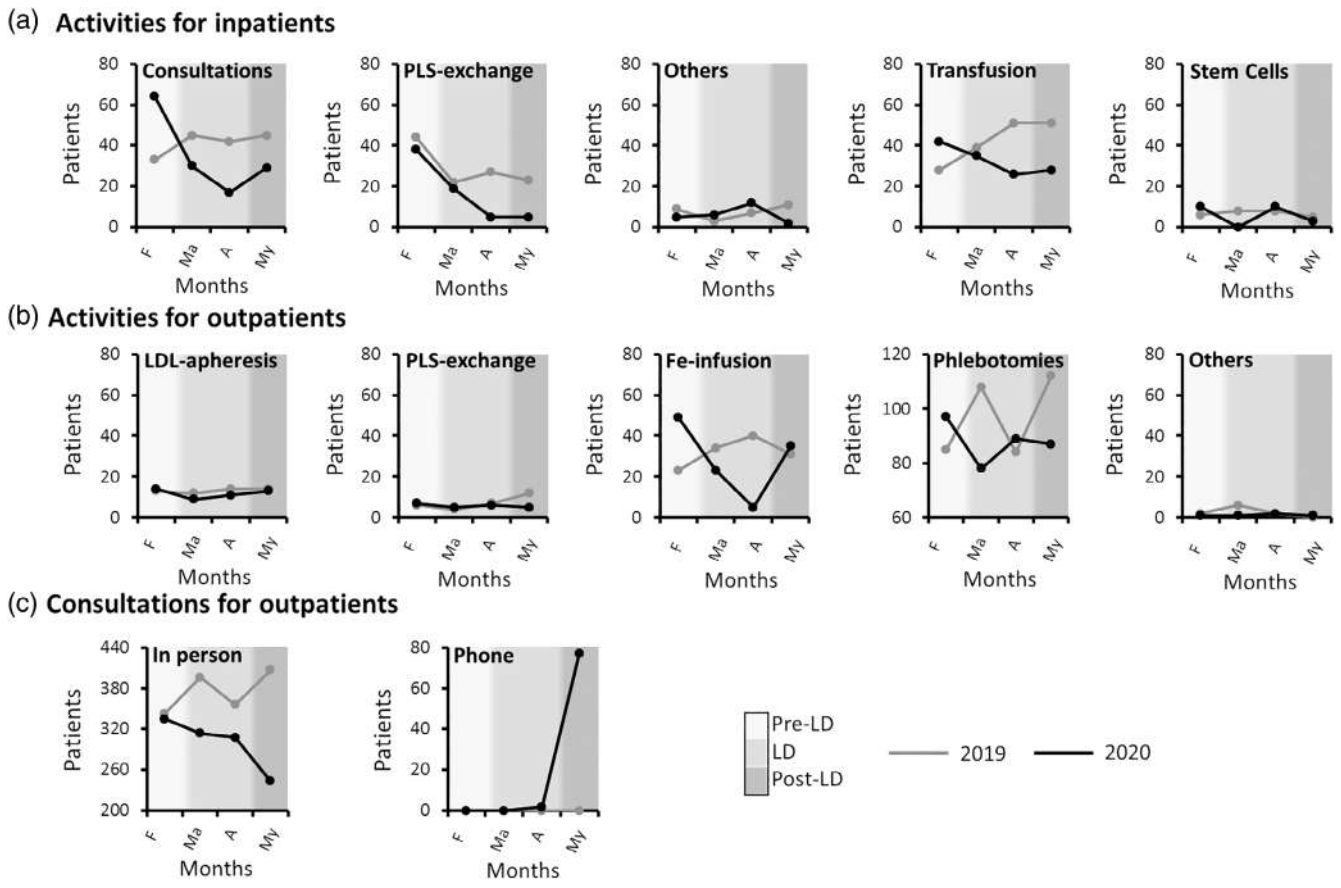

FIGURE 5 Transfusion medicine and immune-haematology clinical practice procedures performed for: (a) inpatients coming from other wards; (b) outpatients and (c) medical consultations for outpatients. Black line indicates 2020 procedures, while grey line indicates 2019 procedures. LD, lockdown; LDL, low density lipoprotein; PLS, plasma

- Extend the opening times of medical offices to ensure an adequate number of procedures and, at the same time, allow the necessary room sanitization after each procedure;
- Encourage medical phone consultations to guarantee therapeutic continuity for non-urgent and chronic patients.

These measures affected all the inpatients' and outpatients' rooms that compose our Transfusion Medicine Unit: patient blood management; bloodlettings/phlebotomy; transfusion; apheresis and sideropaenic anaemia.

Figure 5 resumes the main procedures, procedures and medical consultations performed between February and May 2020, compared with the same period of 2019. The large majority of our procedures decreased during the lockdown. A significant decrease was observed in particular for medical consultations and plasma -exchange procedures required for inpatients coming from other hospital wards, as a consequence of the decreased hospitalizations. Despite Reggio Emilia province trying to preserve the healthcare services provided to oncological patients, the number of hospitalized cancer patients decreased, especially during the first weeks of lockdown. Moreover, no autologous or allogeneic stem cell collection for bone marrow transplantation was performed in March 2020 (Figure 5(a)).

Procedures on outpatients decreased as well, particularly iron infusions on anaemic pre-operative patients (Figure 5(b)). Between April and May, our physicians started implementing medical phone consultations for the outpatients (Figure 5(c)). The Patient Blood Management medical office, which routinely performs medical consultations to pre-operative patients, temporarily closed due to the lack of elective surgeries imposed by the lockdown.

The treatment of chronically ill patients (thalassaemic, polycythaemic, familial hypercholesterolaemia patients among all) was preserved as much as possible, albeit the patients' appointments were spread over a longer range of time (from 8 AM–12 noon to 8 AM–6 PM).

All patients were only allowed to stay for the exact time of the hospital procedure. Visitors were not allowed, except when accompanying minors or fragile patients. When possible, on-site appointments were substituted by remote consultations. Although not shown in this study, medical assistance by phone was implemented during the whole summer in order to be prepared for a second pandemic wave.

DISCUSSION

Between March and May 2020, the Italian Government declared a national lockdown, accompanied by further regional indications on the management of healthcare services that significantly changed the routine and emergency procedures all over the country [3, 10, 15]. The COVID-19 outbreak also severely affected the procedures of the AUSL-IRCCS di Reggio Emilia: the number of non-COVID patients accessing the hospital between February and May 2020 was only 10,520, which is over 33% less than the same period of the previous triennium (15,771).

In accordance with what was previously reported, in our hospital, only a few hospitalized COVID-19 patients might have received a blood transfusion as a consequence of the pathology (161 over 1720, 9.4%) [16].

However, the temporary suspension of elective surgeries, alongside the need to keep unaltered the clinical routine in some other departments (in particular, oncology and haematology), drastically impacted on our Unit activity. If we consider the non-COVID patients hospitalized between February and May 2020 (Table 1), the RBCs transfusion rate rose from 18.5% to 22.5%. This evidence suggests that, in a scenario where the hospitalization of non-serious patients was postponed or even diminished, it was chosen to prioritize the emergencies and the most severe cases that often require blood transfusions.

In terms of organization, the main changes for our Transfusion Medicine Unit involved blood donations, which were significantly reduced and re-scheduled to meet the decrease need of blood components, as similarly described in other blood centres [17–19]. To avoid a surplus of RBCs, with the risk of discarding a large amount of expired units, all blood donations were temporarily reduced, except for those of rare groups. Adopting this strategy, our Transfusion Medicine Unit avoided RBCs units expiration for almost the whole lockdown. Moreover, the regional blood supply-chain system, set up by CRS, buffered the distribution of RBCs units through the Emilia-Romagna provinces from the least affected to the most affected by the pandemic (Figure S2 panels B and C). Reggio Emilia, for instance, received blood units from the CRS in April, after having faced the peak of COVID-19 diffusion in the previous month.

After the initial reduction of the routine procedures, the healthcare supply for cancer and onco-haematological patients was gradually restored following the national and regional indications [10, 15, 20]. Accordingly, a decrease in blood transfusions for the onco-haematological patients was mainly concentrated in the most critical weeks (between the 20th of March and the 5th of April, Figure 4), while PLTs transfusion (mainly administered to prevent or treat bleeding episodes of onco-haematological patients) remained constant for the whole lockdown duration.

To compensate for the decrease in buffycoat-derived PLTs units, we increased the number of PLTs donations (Figure 2(c)). The latter are not easily collected in an adequate quantity to satisfy the request, and we thus adopted a procedure to store frozen PLTs [21]. PLTs cryopreservation is an alternative to the conventional storage temperature (25°C) that allows the product shelf-life to increase from 5 days to up to 2 years.

Following the decrease of inpatients hospitalized by other wards, our physicians faced a significant decrease of medical consultations (Figure 5(a)) and plasma-exchange procedures.

In summary, the COVID-19 pandemic deeply impacted the healthcare systems at all levels, with important implications also for blood donation and transfusion [12, 22]. The mechanisms and procedures adopted to face this serious emergency were necessary to meet the safety requirements for donors, patients and staff. Our experience has, actually, many points in common with other Transfusion Medicine Units in Italy and other parts of the world [11, 12, 23, 24]. Among the strategies developed to meet this health emergency, we

believe that, alongside the consolidated practices such as sanitizing procedures, re-modulation of access and the use of personal protective equipment [25], a provision of frozen blood components that can be stored for several months could be helpful during emergency periods and should be promptly planned. Moreover, remote appointments were revealed as effective, especially for chronically ill patients, and it has been implemented after the lockdown.

Although our data are in agreement with recent literature about transfusion in COVID-19 patients, [16] we must point out that they come from our internal database, which is not directly connected to clinical records and other hospital databases. Therefore, it is plausible that some data are underestimated, especially those relating to cancer patients (other than onco-haematological). The latter can be hospitalized by several wards in our hospital, depending on tumour localization, and this implies higher tracing complexity; furthermore, when a physician prescribes a blood component, the diagnosis can sometimes not be well specified, thus generating missing data in our internal database. Nevertheless, our electronic system blocks blood component requirements in case of missing diagnosis, and we are confident that, despite a slight underestimation, the data presented herein are close to reality.

Finally, between February and May 2020, a 'COVID-19 diagnosis' was not present in our database. Therefore, we cannot discriminate between RBCs and PLTs units transfused for COVID-19 or for medical/surgical reasons. Nevertheless, we traced the requests for transfusions coming from the wards that hosted COVID-19 patients, and we did not find any significant variation (data not shown).

In conclusion, during the first wave of the pandemic, our Transfusion Medicine Unit both avoided unnecessary blood collection and excessive stored blood units, preserving donor and staff safety. This result was reached by coordinating with the internal (hospital wards and donor centres) and external (i.e, CRS) stakeholders. However, these measures come from an emergency phase, and, despite their effectiveness, they should be implemented with other strategies (such as prioritizing blood use for hospitalized patients during blood shortage events) and integrated in appropriate risk management plans and safety procedures. At the time of writing, the spread of COVID-19 is still beyond control in many countries worldwide; the knowledge acquired during the emergency should not be dispersed, but exploited, in order to define a more efficient healthcare management.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Lucia Merolle https://orcid.org/0000-0001-5712-4272
Chiara Marraccini https://orcid.org/0000-0002-8053-498X

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Immediate intravenous iron administration improves anaemia recovery following total knee arthroplasty: A propensity-matched analysis

Hee-Sun Park[1] | Seong-Il Bin[2] | Ha-Jung Kim[1] | Jinsun Kim[1] | Hyungtae Kim[1] | Youngjin Ro[1] | Won Uk Koh[1]

[1]Department of Anesthesiology and Pain Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea

[2]Department of Orthopaedic Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea

Correspondence
Won Uk Koh, Department of Anesthesiology and Pain Medicine, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic Ro 43-gil, Songpa-gu, Seoul 05505, South Korea.
Email: koh9726@naver.com

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Abstract

Background and Objectives: Patients who undergo total knee arthroplasty (TKA) have a risk of postoperative anaemia. This observational study evaluated whether single-dose intravenous ferric carboxymaltose (FCM) administered immediately after TKA facilitates the correction of anaemia.

Materials and Methods: We retrospectively analysed 722 patients who underwent primary TKA. The FCM group receiving 1000 mg intravenous FCM within one postoperative hour was compared with the non-FCM group that did not receive the medication. A propensity score matching with multiple logistic regression analysis was used to minimize intergroup differences in the baseline characteristics and postoperative blood loss. The rate and severity of postoperative anaemia were compared between the groups, along with haemoglobin (Hb) value, transfusion rate and complications.

Results: After propensity score matching, 231 patients were included in each group. In the FCM group, the rate of anaemia at postoperative day (POD) 7 ($p = 0.021$) and postoperative week (POW) 5 ($p < 0.001$) and the transfusion rate were significantly lower ($p = 0.008$). The rate of moderate to severe anaemia at POW-5 was also significantly lower in the FCM group ($p < 0.001$). In patients without preoperative anaemia ($n = 322$), the transfusion rate and rate and severity of anaemia at POD-7 and POW-5 were significantly lower in the FCM group than in the non-FCM group.

Conclusion: Postoperative intravenous FCM administration facilitated recovery of surgery-related anaemia by improving Hb and may reduce the need for transfusion in TKA patients. Preoperative non-anaemic patients could also benefit from accelerated recovery by intravenous iron treatment.

KEYWORDS
anaemia, ferric carboxymaltose, intravenous iron, iron deficiency, patient blood management, total knee arthroplasty

INTRODUCTION

Postoperative anaemia is common after total knee arthroplasty (TKA), due to considerable postoperative blood loss of >500 ml [1, 2]. Anaemia is associated with increased cardiovascular risk as well as increased morbidity and mortality after non-cardiac surgery and among elderly patients [3–5]. Allogeneic blood transfusion is among the methods used for rapidly correcting anaemia, but it can be associated with adverse outcomes, such as surgical site infection (2.88%) [6], transfusion-related acute lung injury (0.08%–15%), and circulatory overload (1%–11%) [7].

To correct preoperative anaemia, iron preparations with or without erythropoiesis-stimulating agents (ESAs) are recommended for patients who have iron deficiency with preoperative haemoglobin (Hb) less than 13.0 g/dl [8]. This process should be worked up and initiated 4–6 weeks before surgery. Multiple publications have reported on the impact of preoperative iron treatment in the context of elective surgery [9–12].

Many patients who have undergone major surgery are at risk of being discharged with moderate to severe anaemia. The presence of moderate or severe anaemia in elderly patients who have multiple comorbidities is known to negatively affect clinical and functional outcomes [13]. As part of the Patient Blood Management (PBM) [8] for managing individualized care for each patient, iron replacement can be used to improve Hb levels and may reduce the need for allogeneic blood transfusion. However, few studies have focused on the efficacy of postoperative intravenous (IV) iron therapy to improve postoperative anaemia in patients who undergo elective surgery [14–19].

We retrospectively evaluated whether immediate postoperative single high-dose IV iron treatment could facilitate recovery of postoperative anaemia, mitigate the severity of postoperative anaemia and decrease transfusion rate among patients who have undergone unilateral TKA.

METHODS

This retrospective observational study was approved by the institutional review board of the study centre (2019-0223). Data of patients who underwent elective primary unilateral TKA between January 2016 and December 2018 at a single tertiary teaching hospital were collected. The requirement for informed consent was waived.

Study population

Among 1195 identified patients, the following were excluded: (1) patients who underwent bilateral staggered TKA (the two arthroplasty are performed on different days, within 7 days after the first procedure) [20], (2) patients with haematological and renal disorders (serum creatinine level > 1.5 mg/dl), (3) patients who received iron preparations or transfusion preoperatively, (4) patients with no Hb results from postoperative week (POW) 5, (5) patients with 48 h postoperative blood loss exceeding 1000 ml and (6) patients with popliteal artery injury during surgery. This study compared patients who received IV ferric carboxymaltose (FCM; Ferinject®, Vifor Int., St. Gallen, Switzerland) (the FCM group) with a non-FCM group, who did not receive this treatment.

Data collection

We reviewed the electronic medical records and collected data on age, sex, height, weight, body mass index, hypertension, diabetes, underlying disease, and American Society of Anaesthesiologists (ASA) physical status. Preoperative medication included the use of anticoagulants or non-vitamin K–antagonist oral anticoagulants.

Intraoperative variables included the use of tranexamic acid, type of anaesthesia (general or regional anaesthesia), duration of operation and tourniquet use. Postoperative data included the rate and volume of perioperative allogenic red blood cell transfusion, oral iron administration (200 mg iron acetyl transferrin or 256 mg dried ferrous sulphate), postoperative blood loss (the amount of postoperative drains during 48 h), postoperative clinical complications, readmission rate until 3 months after discharge, 1-year mortality and overall mortality. The length of hospital stay was calculated from the day of surgery until hospital discharge. We collected serious adverse events associated with FCM infusion [21].

Postoperative Hb and anaemia were evaluated at three designated time-points: PODs 1 and 7 and POW-5. For some patients (if they did not have Hb results from POD-7), Hb results from PODs 4 to 6 were considered in POD-7s results. The POW-5 Hb results included Hb results from between PODs 29 and 45.

Perioperative patient blood management and iron preparation administration strategy

Intraoperatively, a tourniquet was applied to all patients, and it was removed at the end of surgery after the application of the surgical wound dressing. Since the end of 2016, a bolus dose of tranexamic acid (TXA) (10 mg/kg) has been administered intraoperatively to patients at our institution. However, TXA was not used in patients allergic to TXA and in case of other contraindications, such as a past medical history of cerebral ischaemia or infarction, ischaemic heart disease, deep vein thrombosis and other thromboembolic conditions.

Patients in the FCM group received 1000 mg IV FCM for 30–60 min on the operation day 1 h after surgery. The patients were closely monitored and evaluated during and after FCM injection for any discomfort or possible side effects. If the severity of reaction was severe/life-threatening, it was recorded as a serious adverse event [21]. We calculated the estimated mean iron requirement according to the adopted Ganzoni formula, with a target Hb of 13.0 g/dl [22].

Surgeons and the attending anaesthesiologists were encouraged to follow the restrictive transfusion triggers of Hb <8.0 g/dl throughout the entire perioperative period. If patients presented with

symptoms of significant hemodynamic instability despite adequate fluid administration and the use of vasopressor was essential, allogeneic transfusion of packed red blood cells was permitted even for Hb ≥8.0 g/dl. Every patient received postoperative oral iron during the hospital stay unless they could not tolerate it.

Outcome variables

The primary outcomes included the difference in Hb and rate of anaemia at each time-point. The secondary outcome of the study was the difference in transfusion rate between two groups. Anaemia was defined as an Hb concentration < 12.0 g/dl in women and < 13.0 g/dl in men. Anaemia was further categorized into mild (women: Hb 11.0–11.9 g/dl; men: Hb 11.0–12.9 g/dl), moderate (Hb 8.0–10.9 g/dl for women and men) and severe (Hb < 8.0 g/dl for women and men) according to World Health Organization (WHO) guidelines [23]. We also performed the analysis of postoperative Hb, perioperative transfusion and rate of postoperative anaemia in preoperatively non-anaemic patients between both study groups.

Statistical analysis

Continuous variables are reported as mean with standard deviation or median with interquartile range (IQR), as appropriate. Student's *t*-test or Mann–Whitney test was used to compare continuous variables. Categorical variables are expressed as frequencies and percentages and were analysed using the chi-square test or Fisher's exact test, as appropriate.

To reduce the effect of potentially confounding factors, we used propensity score (PS) matched analysis to modify intergroup differences between patients with and without FCM administration. PS was calculated through multiple logistic regression, including the baseline characteristics shown in Table 1 and anaesthetic method as covariates. To minimize the effect of surgery-associated blood loss, postoperative blood loss—whether above 500 ml or not—was also included as a covariate. There were no missing values in the baseline characteristics. Model discrimination was assessed with a *c*-statistic of 0.732, and model calibration was assessed with Hosmer–Lemeshow statistics (chi-square = 4.017, degrees of freedom = 8, $p = 0.856$). We subsequently used the derived PS to create PS-matched pairs at a ratio of 1:1 using greedy matching with a calliper width of 0.1 without

TABLE 1 Baseline characteristics of the entire cohort

	Total set				After PS matching			
	Non-FCM group	FCM group			Non-FCM group	FCM group		
	N = 386	*N* = 336	*p*-Value	Stdiff	*N* = 231	*N* = 231	*p*-Value	Stdiff
Age (y)	70.1 ± 6.11	70.0 ± 6.40	0.786	0.020	70.2 ± 5.96	70.1 ± 6.68	0.848	0.018
Female	333 (86.3)	309 (92.0)	0.021	0.184	213 (92.2)	211 (91.3)	0.866	0.032
Height (cm)	153.8 ± 7.00	153.4 ± 6.37	0.514		153.1 ± 6.72	153.6 ± 6.46	0.323	
Weight (kg)	66.5 ± 14.66	64.5 ± 14.52	0.073		64.7 ± 13.69	65.4 ± 16.28	0.654	
Body mass index (kg/m^2)	28.1 ± 5.62	27.3 ± 5.64	0.090	0.127	27.6 ± 5.19	27.6 ± 6.41	0.939	0.007
Diabetes	106 (27.5)	78 (23.2)	0.222	0.099	55 (23.8)	54 (23.4)	1.000	0.010
Hypertension	260 (67.4)	211 (62.8)	0.228	0.096	145 (62.8)	152 (65.8)	0.560	0.063
Underlying disease	85 (22.0)	94 (28.0)	0.078	0.138	61 (26.4)	48 (20.8)	0.189	0.133
Ischaemic heart disease	35 (9.1)	35 (10.4)	0.628		24 (10.4)	18 (7.8)	0.418	
Cerebrovascular disease	33 (8.55)	32 (9.5)	0.744		26 (11.3)	18 (7.8)	0.267	
Pulmonary disease	11 (2.85)	21 (6.2)	0.042		8 (3.5)	9 (3.9)	1.000	
Nephrotic disease	16 (4.15)	19 (5.7)	0.442		11 (4.8)	18 (7.8)	0.418	
ASA			0.056	0.154			1.000	<0.001
1 or 2	344 (89.1)	314 (93.5)			216 (93.5)	216 (93.5)		
3	42 (10.9)	22 (6.5)			15 (6.5)	15 (6.5)		
Anticoagulants/NOACs	126 (32.6)	100 (29.8)	0.452	0.062	72 (31.2)	66 (28.6)	0.611	0.057
Preoperative Hb (g/dl)	12.7 ± 1.27	12.6 ± 1.16	0.212	0.093	12.5 ± 1.21	12.6 ± 1.15	0.419	0.075
Postoperative blood loss[a]			<0.001	0.325			0.626	0.054
≤500 ml	200 (51.8)	227 (67.6)			155 (66.2)	149 (63.7)		
>500 ml	186 (48.2)	109 (32.4)			78 (33.8)	84 (36.4)		

Note: Data are expressed as number of patients (%), mean ± standard deviation or median [interquartile range].
Abbreviations: ASA, American Society of Anesthesiologists physical status; FCM, ferric carboxymaltose; NOACs, non-vitamin K-antagonist oral anticoagulants; PS, propensity score; Stdiff, standardized difference.
[a]48 h postoperative drainage amount.

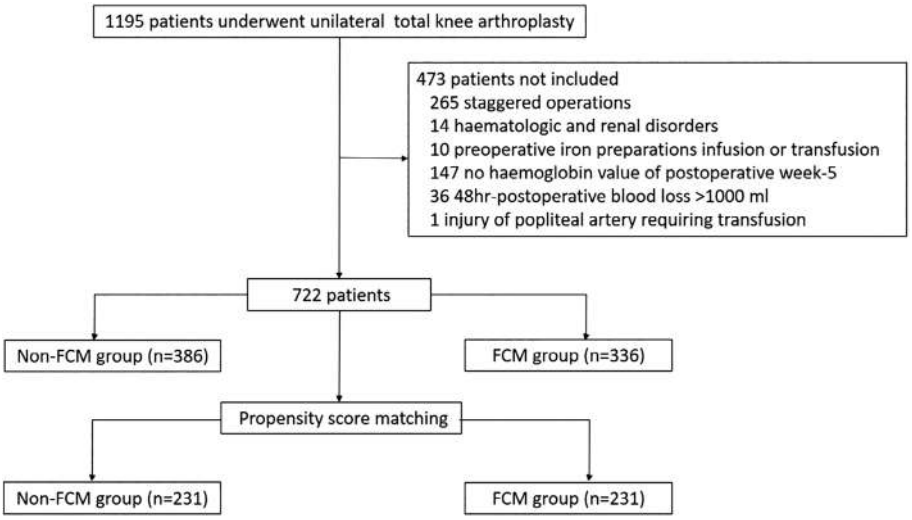

FIGURE 1 Flowchart of the study

TABLE 2 Intraoperative and postoperative variables and outcomes after propensity score matching

	Total set			After PS matching			
	Non-FCM group	FCM group		Non-FCM group	FCM group		
	N = 386	*N* = 336	*p*-Value	*N* = 231	*N* = 231	*p*-Value	Stdiff
Intraoperative variables							
Tranexamic acid use	235 (60.9)	231 (68.8)	0.033	152 (65.8)	167 (72.3)	0.159	0.141
General anaesthesia	304 (78.8)	154 (45.8)	<0.001	152 (65.8)	145 (62.8)	0.56	0.063
Operation time (min)	106.2 ± 18.9	124.8 ± 14.1	<0.001	105.8 ± 19.3	124.8 ± 13.4	<0.001	1.137
Tourniquet time (min)	105.1 ± 20.2	118.5 ± 18.4	<0.001	105.0 ± 19.4	118.7 ± 19.1	<0.001	0.713
Postoperative variables							
Transfusion	46 (11.9)	20 (6.0)	0.008	31 (13.4)	12 (5.2)	0.008	0.286
Red blood cell (units)	0.26 ± 0.77	0.09 ± 0.46	0.001	0.28 ± 0.75	0.07 ± 0.32	<0.001	0.360
Postoperative blood loss[a] (ml)	496.3 ± 285.7	402.1 ± 245.3	<0.001	423.4 ± 279.7	418.5 ± 244.2	0.842	0.019
Postoperative oral iron[b]	365 (94.6)	322 (95.9)	0.534	214 (92.6)	225 (97.4)	0.032	0.220
Length of stay (days)[c]	8.4 ± 2.9	7.9 ± 2.2	0.003	8.2 ± 2.9	7.6 ± 1.4	0.004	0.220
Readmission rates	4 (1.0)	4 (1.2)	1.000	0	2 (0.9)	0.479	0.132
1-Year mortality	0	0	1.000	0	0	1.000	<0.001
Overall mortality	2 (0.5)	2 (0.6)	1.000	2 (0.8)	1 (0.4)	1.000	0.054
Complication	83 (21.5)	67 (19.9)	0.671	51 (22.1)	45 (19.5)	0.566	0.064
Nephrotic	13 (3.4)	18 (5.4)	0.276	8 (3.5)	13 (5.6)	0.834	
Cerebrovascular	20 (5.2)	19 (5.7)	0.946	10 (4.3)	13 (5.6)	0.834	
Pulmonary	14 (3.6)	7 (2.1)	0.297	10(4.3)	6 (2.6)	0.786	
Vascular	12 (3.1)	0	0.010	8 (3.5)	0	0.013	
Psychiatric	12 (3.1)	15 (4.5)	0.471	8 (3.5)	11 (4.7)	0.323	
Neurologic	16 (4.2)	9 (2.7)	0.364	11(4.8)	5 (2.2)	0.574	
Infection	8 (2.1)	9 (2.7)	0.796	5 (2.2)	7 (3.0)	0.770	
Urologic	12 (3.1)	0	0.010	8 (3.5)	0	0.013	

Note: Data are expressed as number of patients (%), mean ± standard deviation or median [interquartile range].
Abbreviations: FCM, ferric carboxymaltose; PS, propensity score; Stdiff, standardized difference.
[a]48 h postoperative drainage amount.
[b]Oral iron administration from postoperative day 1.
[c]Length of hospital stay after surgery.

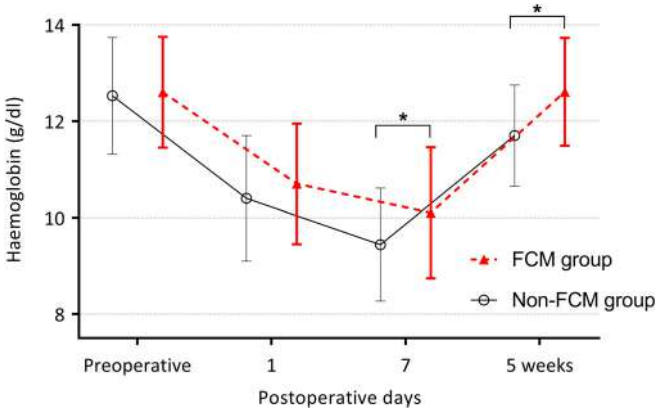

FIGURE 2 Haemoglobin alteration after unilateral total knee arthroplasty. *$p < 0.05$ [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 The rate of postoperative anaemia and its severity after propensity scored matching

	Non-FCM group	FCM group	
	N = 231	*N* = 231	*p*-Value
Overall anaemia			
POD-1	207 (89.6)	197 (85.3)	0.206
POD-7	225 (97.4)	213 (92.2)	0.021
POW-5	140 (60.6)	75 (32.5)	<0.001
Moderate to severe anaemia			
POD-1	180 (77.9)	161 (69.7)	0.057
POD-7	197 (85.3)	162 (70.1)	<0.001
POW-5	54 (23.4)	19 (8.2)	<0.001

Note: Data are expressed as number of patients (%). Anaemia grade according to WHO guidelines: mild 11.0–11.9 g/dl for women and 11.0–12.9 g/dl for men, moderate 8.0–10.9 g/dl and severe <8.0 g/dl.
Abbreviations: FCM, ferric carboxymaltose; POD, postoperative day; POW, postoperative week.

replacement. After all PS matches were completed, we assessed the balance of baseline covariates between PS-matched pairs using paired *t*-tests or McNemar's tests for continuous and categorical variables, as appropriate. *p*-Values < 0.05 were considered statistically significant. Statistical analyses were performed using R software version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The data of 722 patients who underwent primary unilateral TKA were analysed in this study (Figure 1). The baseline characteristics included in the PS matching are shown in Table 1. The calculated mean estimated iron requirement was 847.7 ± 219.8 mg and 899.2 ± 192.5 mg at POD-1 in the FCM and non-FCM groups ($p = 0.63$), respectively.

TABLE 4 Comparison of variables among preoperative non-anaemic patients after propensity score matching

	Non-FCM group	FCM group	
	N = 161	*N* = 161	*p*-Value
Preoperative Hb (g/dl)	13.1 ± 0.83	13.2 ± 0.79	0.438
Postoperative Hb (g/dl)			
POD-1	10.8 ± 1.12	11.2 ± 1.07	0.001
POD-7	9.7 ± 1.16	10.5 ± 1.31	<0.001
POW-5	12.1 ± 0.90	12.9 ± 1.01	<0.001
Transfusion	11 (6.8)	2 (1.2)	0.024
Red blood cells (units)	0.12 ± 0.47	0.01 ± 0.11	0.010
Postoperative anaemia			
POD-1	137 (85.1)	127 (78.9)	0.192
POD-7	155 (96.3)	143 (88.8)	0.020
POW-5	74 (46.0)	30 (18.6)	<0.001
Ratio moderate to severe anaemia			
POD-1	112 (69.6)	96 (59.6)	0.080
POD-7	132 (82.0)	98 (60.9)	<0.001
POW-5	19 (11.8)	4 (2.5)	0.002
Length of stay days[a]	8.1 ± 2.4	7.7 ± 1.3	0.107
Postoperative blood loss[b] (ml)	442.5 ± 279.9	437.6 ± 255.1	0.870

Note: Data are expressed as number of patients (%), mean ± standard deviation or median [interquartile range].
Abbreviations: FCM, ferric carboxymaltose; POD, postoperative day; POW, postoperative week.
[a]Length of hospital stay after surgery.
[b]48 h postoperative drainage amount.

Intraoperative and postoperative outcome variables are presented in Table 2. The non-FCM group had a significantly higher rate of transfusion in both the crude (11.9% and 6.0%, $p = 0.008$) and PS-matched set (13.4% and 5.2%, $p = 0.008$). The duration of hospital stay was significantly shorter in the FCM group than in the non-FCM group ($p = 0.004$). Readmission within 3 months after discharge and mortality did not differ significantly.

The changes in Hb at each time-point are shown in Figure 2. At each time-point in the postoperative period, the Hb level was significantly higher in the FCM group (10.4 ± 1.3 vs. 10.7 ± 1.25, 9.4 ± 1.17 vs. 10.1 ± 1.36 and 11.7 ± 1.05 vs. 12.6 ± 1.12, $p = 0.008$, <0.001 and <0.001), respectively for POD-1, POD-7 and POW-5 (Figure 2).

The overall rate and the severity of postoperative anaemia are summarized in Table 3. The rate of anaemia was significantly lower in the FCM group at POD-7 (97.4% vs. 92.2%, $p = 0.021$) and POW-5 (60.6% vs. 32.5%, $p < 0.001$). In addition, the FCM group showed significantly lower moderate to severe anaemia at POD-7 (85.3% vs. 70.1%, $p < 0.001$) and POW-5 (23.4% vs. 8.2%, $p < 0.001$). Among preoperative non-anaemic patients in the matched set (Table 4), the rate of anaemia and its severity were significantly lower in the FCM

group. No serious adverse events associated with IV FCM administration were noted in the electronic medical records.

DISCUSSION

This study evaluated the effect of immediate postoperative 1000 mg IV iron supplementation on the recovery of anaemia after unilateral TKA. Iron supplementation was associated with a lower rate of anaemia due to increase in Hb. The rate of moderate to severe anaemia at POD-7 and POW-5 was significantly lower in the FCM group. Furthermore, postoperative IV iron therapy was associated with a reduced requirement for perioperative transfusion.

Even patients without preoperative anaemia will frequently develop anaemia following major surgery due to blood loss. In addition, non-anaemic patients may have undetected iron deficiency [2]. Surgery-associated inflammation also hinders normal erythropoiesis in patients. Inflammation increases the synthesis of hepcidin, which inhibits the mobilization of iron from macrophages and enterocytes into the systemic circulation [24]. Therefore, despite an adequate iron store, it cannot be used effectively for erythropoiesis; this is known as iron sequestration, which delays anaemia recovery even in patients without pre-existing anaemia or iron deficiency.

Iron replacement following surgery has been studied for the management of postoperative anaemia and iron deficiency. Postoperative oral iron administration has been reported to be ineffective for treating functional iron deficiency or iron sequestration [25]. In contrast, parenteral iron administration bypasses hepcidine-mediated pathways. Thus, IV iron therapy is recommended to increase Hb levels in patients with functional iron deficiency [26]. Published studies [14–16, 18, 27, 28] and a systematic review and meta-analysis [29] reported that postoperative IV iron treatment can improve Hb levels, particularly at 2–4 weeks after surgery. Our results are consistent with those of previous studies. For this reason, the international consensus statement [26] recommends the early use of postoperative IV iron for patients with iron deficiency anaemia and functional iron deficiency.

The impact of iron replacement alone, particularly administered within 7 days before or after surgery, on reducing the requirement for transfusion is unclear [16, 18, 19, 27, 29]. Although IV iron therapy can rapidly replenish body iron stores, it still requires a sufficient duration of treatment before surgery [30]. In addition, protocols and guidelines often call for restrictive transfusion thresholds based on evidence regarding the risks associated with transfusion [31]. These factors may have contributed to the lack of difference in transfusion rates. According to the systematic review and meta-analysis in 2018, postoperative IV iron alone did not significantly decrease transfusion requirement (relative risk, 0.75; 95% confidence interval [CI]: 0.53–1.07) [29]. In our previous randomized controlled trial (RCT), IV iron therapy alone showed no difference in transfusion rate among patients who underwent TKA and total hip arthroplasty (THA) [32]. However, in other prospective RCT, IV iron combined with ESAs on the day before cardiac surgery was effective in decreasing the transfusion rate [28]. This combination method has recently been recommended at a PBM conference for major orthopaedic surgeries with preoperative Hb less than 13 g/dl [8].

The mortality and readmission rate in this study were not different, but we did not present other clinical outcomes such as anaemia-associated morbidity/mortality and quality of life (QoL)/functional outcomes. The effect of postoperative iron therapy on QoL/functioning has been assessed in several previous studies; however, the result was inconclusive for improving these patient-centred outcomes [14, 16, 19, 33]. For anaemia correction, postoperative IV iron administration increased the mean Hb level only by 0.34 g/dl (95% CI: 0.118–0.562) [29], which is a small increase and is not likely to have much clinical meaning. Moreover, the number and quality of studies investigating the effects of postoperative anaemia treatment on patient-centred outcomes are inadequate, as they were relatively small-sized studies and the timing of evaluation was different. Further studies are necessary to evaluate recovery from anaemia and its related outcomes following postoperative IV iron treatment.

The mean estimated iron requirement was 847.7 $\pm$ 219.8 mg and 899.2 $\pm$ 192.5 for the FCM and non-FCM groups at POD-1, respectively. Thus, if low-dose iron was administered (e.g., 200 mg of iron sucrose), patients would require repeated doses to replenish body iron stores. Study findings that did not support postoperative iron therapy for correcting anaemia after surgery [17, 34] may be attributed to relative low doses (100–600 mg) of iron. Patients who received high dose (≒1000 mg) IV iron have yielded favourable results of improved postoperative anaemia [19, 27]. This suggests that a sufficient dose of iron is required to facilitate recovery from anaemia after major surgery. Therefore, a single high dose (up to 1000 mg) of IV FCM is appropriate in the postoperative setting.

This study had several limitations. First, this was a retrospective observational study, and patients were given IV iron without pre-assessing iron metabolism test. This test reflects the cause of anaemia, which can be an important covariate with regards to the outcome of postoperative IV iron treatment [26]. The PBM recommendations include evaluating iron metabolism preceding surgery. If there are no preoperative tests, testing is recommended within 24 h after surgery when ferritin has not yet been elevated in response to inflammation [26]. Second, ESAs can be useful in iron sequestration and functional iron deficiency, particularly in the context of surgery-associated anaemia. Although IV iron with short-acting ESAs has been recommended for major orthopaedic surgery only [8], ESA use in these patients is not yet licenced in some country. Furthermore, the possible side effects and associated costs should be considered. Lastly, we used an anaemia criteria <12.0 g/dl for women, a lower cut-off than <13.0 g/dl for men. Some studies have questioned this definition and shown that mildly low and even low-normal Hb have been adversely associated with mobility function in elderly women [35]. We agree on the necessity for re-evaluating this criteria; however so far, the WHO guideline for anaemia has been used in most studies, even studies with older participants. Despite these limitations, our data demonstrated some benefit of single high-dose iron replacement given immediately postoperatively, without serious adverse reactions

associated with iron administration. Furthermore, this study used a relatively homogeneous study sample consisting of only TKA patients and minimized the potential effects of confounding factors through PS matching.

In conclusion, the administration of high-dose IV iron immediately after primary unilateral TKA in patients was associated with improved Hb recovery and reduced the severity of postoperative anaemia. Transfusion requirement was also reduced in the iron therapy group. This finding was also observed in patients without preoperative anaemia, suggesting the possible expansion of the clinical application of IV iron therapy to this group of patients.

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H.-S.P. analysed data and wrote the first draft and final draft of the manuscript. S.-I.B. designed study and wrote the manuscript. H.-J. K. and J.K. collected data and analysed of data. H.K. and Y.R. revised the manuscript. W.U.K. supervised the conduct of the study. All authors read and revised the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest associated with this study.

ORCID

Won Uk Koh https://orcid.org/0000-0003-4881-1884

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ORIGINAL ARTICLE

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Impacts of COVID-19 and elective surgery cancellations on platelet supply and utilization in the Canadian Province of British Columbia

Kevin E. Shopsowitz[1] | Christina Lim[2] | Andrew W. Shih[1,3,4] | Nick Fishbane[2] | Brian R. Berry[5] | Mark Bigham[6] | Tanya Petraszko[6,7] | Jacqueline Trudeau[8] | Maureen Wyatt[9] | Matthew T. S. Yan[1,6,10] | Douglas Morrison[1,2,11]

[1]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada

[2]BC Provincial Blood Coordinating Office, Vancouver, British Columbia, Canada

[3]Department of Pathology and Laboratory Medicine, Vancouver Coastal Health Authority, Vancouver, British Columbia, Canada

[4]Centre for Blood Research, University of British Columbia, Vancouver, British Columbia, Canada

[5]Department of Pathology, Vancouver Island Health Authority, Victoria, British Columbia, Canada

[6]Canadian Blood Services, Vancouver, British Columbia, Canada

[7]Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada

[8]Department of Anesthesia, Pharmacology, and Therapeutics, University of British Columbia, Vancouver, British Columbia, Canada

[9]Department of Pathology and Laboratory Medicine, Interior Health Authority, Kelowna, British Columbia, Canada

[10]Department of Pathology and Laboratory Medicine, Fraser Health Authority, New Westminster, British Columbia, Canada

[11]Department of Pathology and Laboratory Medicine, Children's and Women's Health Centre of BC, Vancouver, British Columbia, Canada

Correspondence
Douglas Morrison, BC Children's Hospital, 4500 Oak St., Vancouver, BC V6H 3N1, Canada.
Email: douglas.morrison@cw.bc.ca

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Abstract

Background and Objectives: The coronavirus disease 2019 (COVID-19) pandemic raised concerns about the vulnerability of platelet supply and the uncertain impact of the resumption of elective surgery on utilization. We report the impact of COVID-19 on platelet supply and utilization across a large, integrated healthcare system in the Canadian province of British Columbia (BC).

Materials and Methods: Historical platelet use in BC by indication was compiled for fiscal year 2010/2011–2019/2020. Platelet collections, initial daily inventory and disposition data were assessed pre-COVID-19 (1 April 2018–15 March 2020) and for two COVID-19 time periods in BC: a shutdown phase with elective surgeries halted (16 March–17 May, 2020) and a renewal phase when elective surgeries resumed (18 May–27 September 2020); comparisons were made provincially and for individual health authorities.

Results: Historically, elective surgeries accounted for 10% of platelets transfused in BC. Initial daily supplier inventory increased from baseline during both COVID-19 periods (93/90 units vs. 75 units pre-COVID-19). During the shutdown phase, platelet utilization decreased 10.4% (41 units/week; $p < 0.0001$), and remained significantly decreased during the ensuing renewal period. Decreased platelet utilization was attributed to fewer transfusions during the shutdown phase followed by a decreased discard/expiry rate during the renewal phase compared to pre-COVID-19 (15.2% vs. 18.9% pre-COVID-19; $p < 0.0001$). Differences in COVID-19 platelet utilization patterns were noted between health authorities.

Conclusion: Decreased platelet utilization was observed in BC compared to pre-COVID-19, likely due to a transient reduction in elective surgery as well as practice and policy changes triggered by pandemic concerns.

KEYWORDS
platelet transfusion, transfusion medicine, transfusion strategy

INTRODUCTION

Blood products rely on a complex infrastructure of donors, distribution networks and hospital transfusion services to meet clinical demand. Platelets, with a relatively short shelf-life of 5–7 days, are particularly vulnerable to perturbations to the supply–demand equilibrium. Concerns over potential platelet shortages are not new, for example, related to ageing donor and recipient populations [1]; however, the COVID-19 pandemic had the potential to exacerbate these vulnerabilities in ways not previously experienced by healthcare systems. Potential problems included the disruption of donor networks and blood collection, production and logistics networks (reduced supply) and/or by increasing platelet use through large numbers of critically ill COVID-19 patients (increased demand). On the other hand, decreases in non-COVID-19-related healthcare visits due to both behavioural changes and policies like halting elective surgeries [2, 3], or policies to extend platelet shelf life, for example, through cold storage [4], could counter-balance these stressors by reducing demand.

Understanding the multifaceted influence of COVID-19 on platelet supply and demand is crucial for ongoing resource management and future planning. Data are emerging on the effects of the pandemic on platelet utilization [5]. Studies from diverse geographical regions have suggested that although COVID-19 infection is associated with thrombocytopaenia [6], significant bleeding is uncommon, and hospitalized patients have relatively low platelet transfusion requirements, with some centres reporting decreased overall platelet utilization during the first wave of the pandemic [7–10]. In one study from a tertiary care centre in India, a diminished inventory caused by decreased donations was offset by a lower demand for platelets during the early phase of the pandemic [11]. Additional data from large health systems over longer time periods are useful to further understand the manifold impacts of COVID-19 on platelet supply and utilization.

The Canadian province of British Columbia (BC) maintains a centralized transfusion database for six health authorities (HAs) that service a population of roughly 5 million residents. Here, we report on BC's baseline platelet use patterns and experience with platelet supply and utilization during the initial 6 months of the COVID-19 pandemic. This time period encompasses an initial response phase when all elective surgeries were suspended in the province and, subsequently, when elective surgeries were resumed. To our knowledge, this study represents the largest analysis to-date of system-wide platelet use during the COVID-19 pandemic.

MATERIALS AND METHODS

Platelet supply in BC

BC receives blood components from Canadian Blood Services (CBS), which operates the national blood system outside the province of Quebec. While the majority of platelets issued to hospitals in BC are collected in the province, some are imported from other provinces. Platelets are produced from both whole blood and apheresis collections. Buffy coat platelet units, each manufactured from four whole blood units, comprise ~70% of platelet production in the province. Pre-hospital bacterial testing by CBS allows for an extended 7-day platelet shelf life. CBS's BC inventory fluctuates during the day as hospital orders are filled and donor collections from previous days become available for release to hospitals. At the start of each day, initial daily CBS inventory is communicated to hospitals.

Data sources

The BC Provincial Blood Coordinating Office Central Transfusion Registry (CTR) is a comprehensive database of all transfusions that have occurred in the province of BC since 1999. We performed a retrospective analysis of BC platelet utilization for pre- and COVID-19 time periods. Historical data on platelet use, by indication, were analysed for fiscal years 2010/2011–2019/2020 (FY: 1 April–31 March). Platelet transfusions were linked to surgical procedures via the provincial Surgical Patient Registry and considered related to the surgical event when a patient received platelets from 1 day prior to surgery to 30 days after the surgery. Surgeries were further differentiated between elective and urgent/emergency procedures. All other platelet transfusions were considered to be non-surgical. Surveillance data on provincial COVID-19 cases and hospitalizations were obtained from the publicly available COVID-19 Tracker Canada [12]. Total hospital admission, intensive care unit (ICU) admissions and surgical volumes in BC from March to June 2020 with year-over-year comparisons to 2019 were available from the Canadian Institute for Health Information [13]. Total population and age data for BC and its HAs were obtained from publicly available government data [14]. Median ages were estimated from population data capped at age 90 years (i.e., individuals aged ≥90 were binned together as 90+).

Data related to blood donations and the number of platelet units supplied to hospitals within BC were obtained from CBS for the period from 1 December 2019 to 27 November 2020. Initial daily supplier platelet availability within BC (CBS BC inventory at midnight) was obtained for the period from 1 April 2018 to 27 September 2020 (last available data point at the time of writing). Platelet disposition is reported as transfused units and expired/discarded units. The total number of units is the sum of transfused and expired/discarded units. Daily platelet disposition data were obtained from CTR for 1 April 2018 to 27 September 2020. Three time periods are considered throughout the study: pre-COVID-19 baseline (until 15 March 2020); COVID-19 shutdown period, when elective surgeries in BC were halted (16 March 2020 to 17 May 2020), and COVID-19 renewal phase, when elective surgeries resumed in BC (18 May 2020 to 27 September 2020).

Statistical analysis

The mean of total weekly platelet use in BC was calculated for the three time periods noted above (pre-COVID-19, shutdown, renewal),

and statistical testing was performed by analysis of variance with a post-hoc Tukey test for pairwise comparisons. Linear regression was used to assess trend over time for historical annual platelet usage. Initial daily platelet inventory and platelet usage are reported as 7-day rolling average to remove the periodicity of daily counts related to different transfusion practices on weekend versus weekdays. Statistical values are all reported as mean with 95% confidence intervals (CIs) unless otherwise indicated. Weekly mean with 95% CI was also calculated for each of the three largest HAs within the province and for the sub-categories of weekly transfused and expired platelet units (formal statistical testing not carried out for these sub-categories). Province-wide and HA-specific discard rates were calculated as expired/discarded units divided by total units for the three time periods. For the total provincial data, these rates were statistically compared by chi-squared test for both overall trend and pairwise comparisons with Bonferroni correction. *p*-values <0.05 were considered to be statistically significant for all comparisons.

RESULTS

Historical platelet use in BC

Over the 10-year period beginning with FY 2010/2011, an average of 15,642 platelet units per year were transfused in BC, with 17,120 units transfused over the last complete fiscal year with available data (2019/2020; Figure 1a). Over the past 10 years, non-surgical indications have accounted for 67.0% of platelet transfusions (yearly range = 63.4%–72.5%; Figure 1b). The remaining third of platelet units were associated with surgical procedures, of which 23.2% of the provincial total were transfused for emergency procedures (range = 19.4%–27.2%) and 9.8% for elective procedures (range = 7.4%–12.6%). Eighty percent of platelets transfused during the peri-operative period occur within 5 days of surgery (Figure S1). Two surgical specialties – general surgery (which includes trauma) and cardiac surgery – were historically the greatest platelet users by a large margin, each accounting for approximately 33% of surgical platelet usage. We found there was a significant increase in platelet transfusions over time from FY 2010/2011 to 2019/2020 (+248 units/year; $p = 0.001$), but we did not detect a significant trend when correcting for population growth ($p = 0.7$) nor in the proportion of platelets transfused for surgical events ($p = 0.4$) over this time period.

Effect of COVID-19 on provincial platelet supply and demand

At the outset of the COVID-19 pandemic, several precautionary measures were put into place in BC, including the suspension of elective surgeries between 16 March 2020 and 17 May 2020, which led to a large decrease in planned surgeries in the province from March to June 2020 compared to the previous year (Figure S2A). In March 2020, day surgeries decreased by 35% in BC compared to the previous year (although BC-specific data were not available for the months leading up to the shutdown, national surgical volumes had been stable through the end of February 2020) [13]. Surgical volume reached a nadir during the middle of the shutdown phase in April, when day surgeries, planned inpatient surgeries and total surgeries had decreased by 81%, 59% and 70%, respectively, compared to April 2019. Of note, in addition to the expected decrease in planned surgeries, April also saw a 27% decrease in unplanned surgeries. During this initial shutdown period, BC experienced its first wave of COVID-19, reaching a peak of 149 hospitalized COVID-19 patients during the first week of April (including a peak of 72 patients in critical care; Figure S3). Concurrently, overall inpatient occupancy and ICU occupancy were down by 33% and 20%, year over year, respectively (Figure S2B). Elective surgeries were resumed on 18 May 2020, and continued unabated throughout the second wave of COVID-19 in BC (renewal phase), with surgeries beginning to approach baseline levels by June (Figure S2A). In late August, COVID-19 hospitalizations again rose

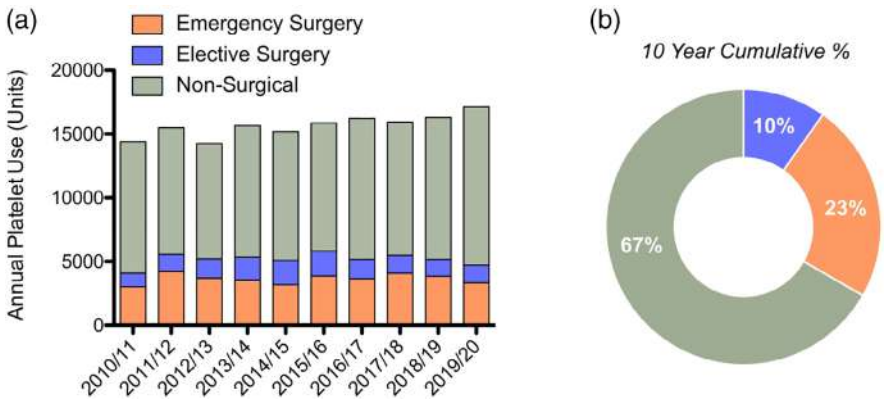

FIGURE 1 Platelets transfused by indication in BC from fiscal year 2010/2011–2019/2020. (a) Annual transfused platelet units in BC by indication. (b) Cumulative percentage of transfused platelets by indication over the 10-year time period. BC, British Columbia [Colour figure can be viewed at wileyonlinelibrary.com]

from a minimum of <10 to 69 by the end of September with 19 in critical care (Figure S3).

The trends in whole blood donations (a proxy for buffy coat–derived platelets) and apheresis platelet donations for baseline and COVID-19 time periods, both nationally and specifically for BC, are shown in Figure 2. There was a decrease in CBS national whole blood donations from late March to early June, only partly compensated for by an increase in apheresis platelet donations (Figure 2a). Towards the end of May 2020, there was a steep increase in whole blood donations (similar to pre-COVID-19 levels) that was sustained until the end

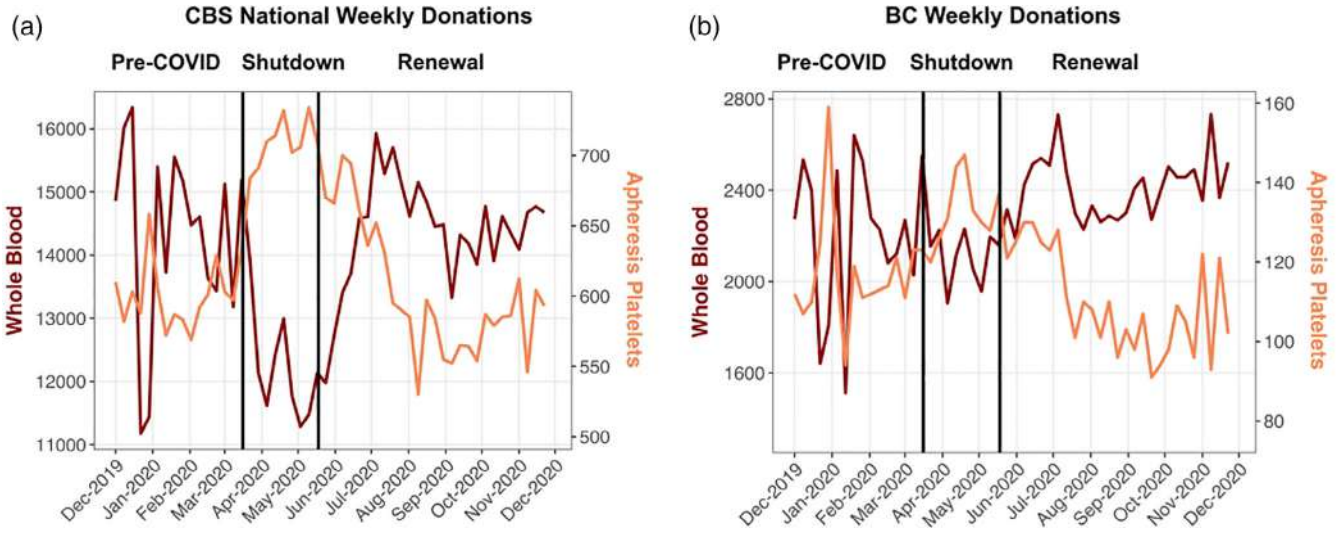

FIGURE 2 Weekly platelet donations for (a) all of Canada (excluding Quebec) and (b) British Columbia, before and after the onset of the coronavirus disease 2019 (COVID-19) pandemic. Whole blood donations are used as a proxy for buffy coat-derived platelets [Colour figure can be viewed at wileyonlinelibrary.com]

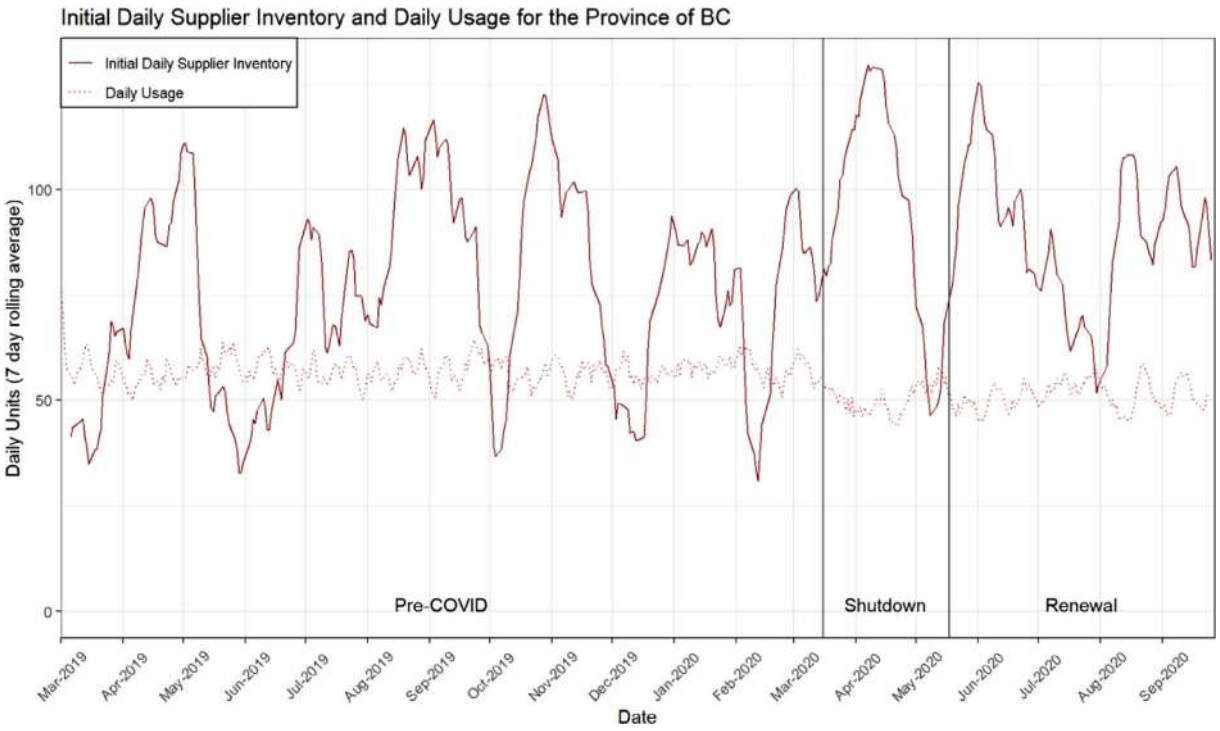

FIGURE 3 Initial daily CBS platelet inventory (solid) compared to daily usage (dashed). Both curves represent 7-day rolling averages. The average initial daily supplier inventory for different time periods are 75.1 units (pre-COVID-19), 92.8 units (shutdown) and 90.1 units (renewal). The average daily platelet usages (transfused + expired/discarded) for the same time periods are 56.4 units (pre-COVID-19), 50.5 units (shutdown) and 51.1 units (renewal), respectively. Note that for calculation of averages, a longer pre-COVID-19 period was used than what is shown on the graph (April 2018–March 2020) CBS, Canadian Blood Services; COVID, coronavirus disease 2019 [Colour figure can be viewed at wileyonlinelibrary.com]

of 2020, whereas apheresis donations decreased towards baseline. In BC specifically, donations remained comparatively stable during the initial shutdown phase and showed similar trends of increasing whole blood and decreasing apheresis donations from June onwards (Figure 2b). Overall, the number of platelets issued to BC by CBS decreased during both the shutdown and renewal phases compared to the preceding months (Figure S4).

Initial CBS daily inventory and daily usage for the year leading up to COVID-19, the initial shutdown period and the renewal period are shown in Figure 3. Initial daily CBS platelet inventory for BC was considerably more variable than daily platelet use, with large oscillations between ~25 and 150 units at baseline. The initial daily CBS inventory increased from a pre-COVID-19 baseline average of 75.1 units to 92.8 units for the shutdown phase and remained elevated at 90.1 units during the renewal phase. Overall, the platelet supply in BC was more secure from the onset of COVID-19 with initial daily CBS inventory minima remaining higher than pre-COVID-19, both in absolute terms and relative to daily usage. Daily platelet usage decreased from a baseline average of 56.4 units to 50.5 and 51.1 units during the shutdown and renewal periods, respectively (Figure 3). Comparing weekly platelet utilization to remove periodicity of variable weekday and weekend practices, this decrease in platelet usage was found to be statistically significant (Table 1 and Figure 4a,e). Weekly platelet usage decreased from a pre-COVID-19 mean of 394.8 units/week (95% CI, 391.2–398.5) to 353.7 units/week (95% CI, 337.2–370.1) during the shutdown period ($p < 0.0001$) and did not significantly change from this level during the renewal period (357.7 units/week [95% CI, 346.5–368.9; $p = 0.9$]).

Effect of COVID-19 on platelet disposition provincially and in separate health regions

To further understand the effects of the COVID-19 pandemic on platelet disposition in BC, we broke down overall platelet use into units transfused and units expired/discarded for the pre-COVID-19, shutdown and renewal time periods (Table 1 and Figure 4). Figure 4a shows that the decrease in platelet use throughout the pandemic was

TABLE 1 Pairwise statistical comparisons between time periods for platelet use in British Columbia (BC)

Comparison	Mean of total weekly units used	Expired/discarded rate (chi-squared)
Pre-COVID-19 versus shutdown	395 versus 354 ($p < 0.0001$)	18.9% versus 19.8% ($p = 0.6$)
Pre-COVID-19 versus renewal	395 versus 358 ($p < 0.0001$)	18.9% versus 15.2% ($p < 0.0001$)
Shutdown versus renewal	354 versus 358 ($p = 0.9$)	19.8% versus 15.2% ($p < 0.0001$)

Abbreviation: COVID, coronavirus disease 2019.

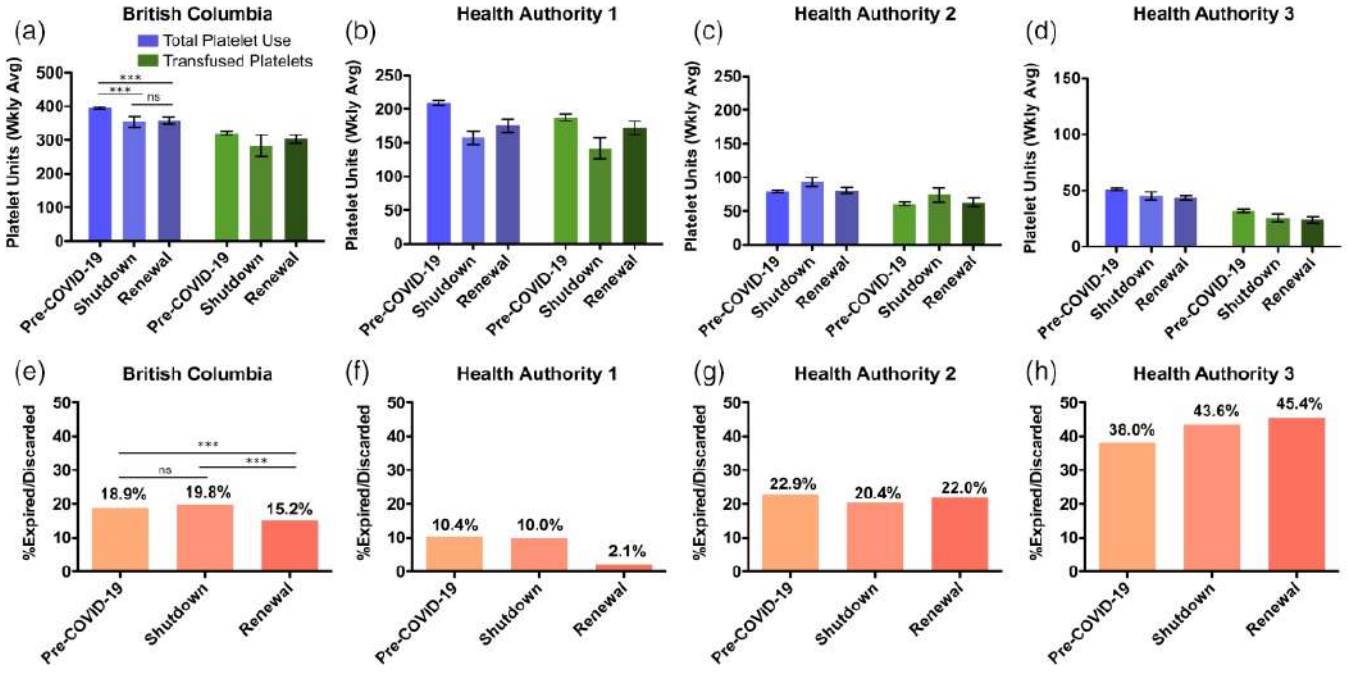

FIGURE 4 Platelet usage in BC and its three largest health authorities before and after COVID-19. (a–d) Total platelet use and transfused platelets for the pre-COVID-19, shutdown and renewal periods (weekly mean ± 95% CI). (e–h) Expired/discarded rate for the same three time periods. For statistical comparisons: *** indicates $p < 0.0001$, ns indicates $p \geq 0.05$. BC, British Columbia; CI, confidence interval; COVID, coronavirus disease 2019 [Colour figure can be viewed at wileyonlinelibrary.com]

due to a combination of fewer platelet transfusions and fewer expired units; however, the timing of these influences has been different. The decrease in transfused units reached a nadir during the shutdown phase when elective surgeries were halted (11% decrease from baseline), whereas a decrease in expired units was observed only during the renewal phase (15.2% discard/expiry rate vs. 18.9% at baseline; $p < 0.0001$).

Key features of the three HAs in BC that utilize the most platelets (86% of total) are summarized in Table 2. These HAs vary by population, geographical breadth and clinical services. Over the past 10 years, the three HAs had a similar proportion of platelet transfusions related to elective surgeries (range 8.3%–10.1%), with more variability for platelet use by emergency surgeries and non-surgical indications (see Figure S5).

Although HA-1 encompasses only ~20% of the population, it includes two cardiovascular surgery centres and contains the province's major quaternary referral centre, which provides level-1 trauma care and a bone marrow transplant (BMT) program. At baseline, HA-1 accounted for 53% of platelet use in the province. During the shutdown phase, HA-1 experienced a mean decrease of 52.1 platelet units used per week, which was greater than the province-wide decrease of 41.1 units per week (Figure 4b). This was primarily driven by a mean decrease in transfused platelets in HA-1 of 45.8 units per week during the shutdown phase (24% decrease from baseline). During the renewal phase, platelet transfusions increased in HA-1 from the shutdown nadir but still remained below baseline levels by 8%. The rate of expired/discarded units in HA-1 decreased from a baseline of 10.4% to 2.1% during the renewal phase, compared to virtually no change from baseline during the initial shutdown (Figure 4f).

HA-2 comprises ~37% of the provincial population and contains a level-1 trauma centre and a cardiovascular surgery service. HA-2 accounted for 20% of provincial platelet use at baseline. In contrast to HA-1, HA-2 saw an increase in total platelet use (18% increase from baseline) and transfused platelet units (22% increase from baseline) during the initial shutdown but returned to baseline levels during the renewal phase, while expired/discarded units remained largely unchanged throughout both periods in HA-2 (Figure 4c,g).

HA-3 comprises ~15% of the provincial population and includes a regional hospital with a cardiovascular surgery service and other geographically dispersed hospital services with significant resupply logistics challenges. HA-3 was responsible for 13% of provincial baseline platelet use. Similar to HA-1, HA-3 experienced a decrease in total platelet use starting with the shutdown (12% decrease from baseline) that was sustained during the renewal (Figure 4d). However, in HA-3, this was driven by decreased platelet transfusions during both periods, whereas the expired/discarded platelet rate in HA-3 increased relative to baseline during both the shutdown and renewal phases (Figure 4d,h).

TABLE 2 Description of the three health authorities in British Columbia (BC) with the highest platelet demand

Health authority (baseline platelet use)	Population[a]	Major services
HA-1 (53%)	• Predominantly urban • Pop. 1,193,977 • Median age: 41 years	• Level-1 trauma centre • Quaternary referral centre • Two cardiovascular surgery centres • Bone marrow transplantation (BMT) service • Solid organ transplant service
HA-2 (20%)	• Mixed urban/rural • Pop. 1,906,933 • Median age: 39 years	• Level-1 trauma centre • Cardiovascular surgery • No BMT service
HA-3 (13%)	• Dispersed with mixture of rural and small urban centres • Significant resupply logistics challenges • Pop. 827,314 • Median age: 47 years	• Cardiovascular surgery • No level-1 trauma or BMT service

[a]Population data from 2019[14].

DISCUSSION

The COVID-19 pandemic has placed an incredible strain on healthcare systems around the world, including sustaining a stable inventory of platelets and other blood products. Initial studies have noted reduced platelet demands for critically ill COVID-19 patients compared to other ICU patients [8], and several tertiary care centres in New York [9], Washington [10] and India [11] reported decreased platelet transfusions during the first 1–2 months of the pandemic, possibly due to a combination of low platelet requirements for COVID-19 patients and policies that decreased healthcare utilization (e.g., halting elective surgeries). In our healthcare jurisdictions, we found similar findings that platelet transfusions decreased concurrently with decreased surgeries and hospital admissions. We also found that platelet use remained decreased even when elective surgeries resumed, largely driven by decreased expired/discarded units. Our comprehensive study of system-wide platelet use during the COVID-19 pandemic also demonstrates differential effects on platelet use by health region, potentially related to their different demographics and characteristics.

The overall balance of blood product supply and demand depends on many interacting factors: donors, collection services, distribution networks, hospitals and patients requiring transfusions. All of these are potentially impacted directly and indirectly by COVID-19 and related policies. The Canadian province of BC, with a large integrated healthcare system and transfusion database, provides a unique opportunity to examine these various factors. We found that platelet use significantly decreased compared to baseline during the initial shutdown phase of BC's response and remained decreased during the

subsequent renewal phase when elective surgeries were resumed. On the other hand, platelet supply declined during the first 2 months of the pandemic but subsequently rebounded to levels at or slightly above the pre-COVID-19 baseline. The initial decrease in supply was driven by fewer whole blood collections and reduced capacity to process blood, related to cancellations of mobile clinics along with decreased capacity at donation and manufacturing sites due to public health orders. Apheresis donations were less impacted by these factors because they largely occur at fixed sites and could be increased during the shutdown phase to partially compensate for the decrease in buffy coat platelets. Possible explanatory factors for the subsequent increase in whole blood collections during the renewal phase include greater donor awareness of the need for blood and the safety of donor collection sites, operational changes by CBS such as increased clinic hours/staffing and comfort with efficient donor turnaround time in the new, physically distanced environment. Importantly, by this time point, efficient COVID-19 safety procedures were in place at manufacturing sites, allowing production capacity to be safely increased. As a result, at no point during the first 6 months of COVID-19 emerging in BC, did the province observe a platelet shortage; if anything, there was a greater average platelet reserve in the province compared to the year prior to the pandemic.

The decreased platelet utilization in BC during COVID-19 was driven by different factors during the distinct periods of the province's response to the pandemic. During the initial shutdown phase, when elective surgeries were halted and overall healthcare utilization was dramatically decreased from baseline, reduced platelet utilization was mostly due to fewer platelet transfusions. Based on provincial historical data, halting elective surgeries was expected to decrease platelet transfusions by ~10% – close to the observed provincial decrease of 11%. However, the considerable differences seen between HAs, which have similar baseline proportions of platelet utilization from elective surgeries, suggest that additional factors also played a role. In BC, HA-1 saw a considerably larger decrease in platelet transfusions during the shutdown compared to the provincial average. This difference might be explained by decreased utilization of non-surgical services with high platelet requirements, only offered in HA-1, such as a BMT/dedicated haematology–oncology service. On the other hand, HA-2 saw an increase in platelet transfusions during the shutdown phase. Given the retrospective nature of our study and our inability to account for a multitude of complex confounders including clinician practice patterns and other contingency plans, which may have affected platelet usage, we are unable to determine the exact reasons why HAs were differentially affected during the pandemic.

In addition to shutting down surgeries, various provincial efforts were made across different HAs to preserve blood products. Led primarily by HA-1 physicians, these initiatives included clinical advisories and education to encourage appropriate blood utilization, screening platelet orders for appropriateness and improving inventory management to reduce wastage [15]. Of note, platelet utilization remained significantly less in BC during the renewal phase, when elective surgeries resumed. While platelet transfusions remained slightly less compared to baseline during the renewal phase, possibly related to persistent decreases in healthcare utilization, the main driver of decreased platelet utilization after the shutdown ended was a significant decrease in the platelet expiration/discard rate. This was mainly driven by one HA (HA-1) that also implemented specific policies including a shared platelet inventory among different hospitals and prospective isoagglutinin titres of all group O platelets to facilitate their transfusion into non-group O patients [15]. The degree to which the significant decrease in platelet expiration/discard rate observed is explained by these policy changes warrants further study.

Strengths of this study include the availability of historical platelet transfusion data as well as the availability of platelet disposition data across a large population/geographical region throughout distinct phases of the COVID-19 pandemic. An important limitation of this study is that the indications for platelet transfusions (i.e., surgical vs. non-surgical) were only available for the pre-COVID-19 period. In addition, data related to healthcare utilization during the pandemic were only available until the end of June 2020. Given these limitations and the inherent confounding factors in a retrospective observational study, we cannot definitively ascribe changes to platelet demand to specific causes or factors. Another limitation is that our findings may not be generalizable to other regions that had different strains on their healthcare system due to COVID-19 or with different patient demographics or resources/organizational structures with regards to blood product distribution.

In conclusion, to our knowledge, this is the largest analysis to-date of platelet supply and utilization during the COVID-19 pandemic. We found that the platelet supply in BC remained stable during the first 6 months of COVID-19, even with an initial decrease in blood donations. Platelet utilization was significantly decreased in BC during the first 6 months of the pandemic compared to baseline: this appears to have been due to province-wide policies that led to decreased healthcare utilization and platelet transfusions during the initial phase of the pandemic, followed by local practice changes that may have contributed to fewer wasted platelet units. The relatively modest contribution of elective surgery cancellations to decreased platelet utilization is noteworthy while other clinical and laboratory practice changes also likely contributed to significant reductions in platelet utilization in the HA with the highest baseline utilization. In particular, laboratory practice changes implemented in response to the threat of shortages appear to have contributed to a sustained reduction in utilization, extending for at least a 4-month period following the resumption of elective surgery and BMT activity. These findings may assist others in determining suitable practices for securing platelet supply throughout the ongoing pandemic and in other challenging scenarios.

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conception of this work and provided comments and revisions that were incorporated in the final manuscript.

CONFLICT OF INTEREST

No conflicts of interest were identified in the preparation of this manuscript.

ORCID

Kevin E. Shopsowitz https://orcid.org/0000-0003-3988-0837

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SUPPORTING INFORMATION

Additional supporting information may 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

Improvement of transfusion practice and reduction in red blood cell utilization in Belgian hospitals: Results of a national survey and benchmarking

Jana Vanden Broeck[1,2] | Katrien Beeckman[3,4,5] | Evelyne Van Gastel[6] | Luc De Keersmaecker[7] | Timothy Devos[8] | Christiane Gérard[9] | Lucien Noens[10] | Dominique Putzeys[11] | Karin Van Poucke[12] | Margareta Haelterman[2] | Véronique Deneys[13] | Rik Schots[1]

[1]Department of Hematology, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel (VUB), Brussels, Belgium

[2]Federal Public Service Health, Food Chain Safety and Environment, Brussels, Belgium

[3]Nursing and Midwifery Research Unit, University Hospital Brussels, Brussels, Belgium

[4]Nursing and Midwifery Research Unit, Faculty of Medicine and Pharmacy, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel (VUB), Brussels, Belgium

[5]Centre for Research and Innovation in Care, Midwifery Research Education and Policymaking (MIDREP), Verpleeg- en vroedkunde, Universiteit Antwerpen, Antwerp, Belgium

[6]Federal Agency for Medicines and Health Products, Brussels, Belgium

[7]Clinical Laboratory, Jan Yperman Hospital, Ypres, Belgium

[8]Department of Hematology, Universitair Ziekenhuis Gasthuisberg, Katholieke Universiteit Leuven, Leuven, Belgium

[9]Blood Bank, Centre Hospitalier Universitaire Liège, Liège, Belgium

[10]Blood Bank, Universitair Ziekenhuis Gent, Ghent, Belgium

[11]Department of Nursing, Centre Hospitalier Régional de la Citadelle Liège, Liège, Belgium

[12]Clinical Laboratory, Algemeen Ziekenhuis Nikolaas, Sint-Niklaas, Belgium

[13]Blood Bank, Département des Laboratoires Cliniques, Cliniques universitaires Saint-Luc, Université Catholique de Louvain Brussels, Brussels, Belgium

Correspondence
Jana Vanden Broeck, Secretariaat Hematologie, UZ Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium.
Email: jana.vandenbroeck@health.fgov.be

Abstract

Background and Objectives: Belgian health authorities launched a national platform in 2011 to improve the quality of transfusion practices and blood use in Belgian hospitals. No data were available about the quality of hospital transfusion practice at the national level.

Materials and Methods: Three consecutive national surveys (2012, 2014 and 2016) were performed in all 111 Belgian hospitals to assess the degree of implementation of standards in four process domains related to red blood cell (RBC) transfusion: general quality aspects, ordering of RBC, electronic traceability and reporting of adverse events. The surveys were part of a methodology based on informing, feedback and benchmarking. Responses to questions were analysed semi-quantitatively, and hospitals could score 10 points on each of the domains.

Results: The proportion of hospitals scoring below 5 per domain decreased from 16%, 70%, 14% and 11% (2012) to 2%, 17%, 1% and 1% (2016), respectively. Similarly, scores above 7.5 increased from 25%, 1%, 23% and 36% (2012) to 64%, 30%,

68% and 81% (2016), respectively. In 2016, overall quality of transfusion practices, including the four pre-specified domains, improved continuously with an average total score (max = 40) increasing from 24.2 to 30.5 ($p = 0.0005$). In addition, there was a decrease in the number of distributed and transfused RBC per 1000 population between 2011 and 2019 from 47.0 to 36.5 and 43.5 to 36.1, respectively.

Conclusion: These data show that the applied methodology was a powerful tool to improve quality of transfusion practices and to optimize utilization of RBC at the national level.

KEYWORDS
benchmark, national survey, red blood cell utilization, transfusion practice

INTRODUCTION

Transfusion of red blood cells (RBCs) is a frequent medical intervention in many fields of medicine. In a typical European context, more than 50% of RBC are administered to patients older than 70 years and around 25%–30% to patients older than 80 years. Major indications for RBC transfusion are haemato-oncology and cancer followed by traumatology-orthopaedics, gastrointestinal–liver diseases and cardiovascular disorders [1–3]. According to a national survey performed in England and North Wales in 2014, 67.4% of RBC transfusions were for medical indications including 20% for haematological and 10% for non-haematological cancers [3]. Taken into account the ageing of the Western population and a higher incidence of cancer in the elderly, it might be expected that there will be a growing gap between supply and demand of RBC. On the other hand, the overall improvement in the quality of transfusion practice, the implementation of more restrictive transfusion strategies and the gradual introduction of patient blood management (PBM) programmes have led to a decrease in RBC transfusions over the past decade [4]. However, there is substantial heterogeneity, ranging between 27 and 55, in the number of RBC transfused per 1000 population in developed countries suggesting that the degree of implementation of these measures at the national level is highly variable [5]. In several countries, national surveys have been conducted to assess the quality of transfusion practice processes [6–9]. These surveys used variable quality indicators and showed incomplete participation rate among hospitals, whereas the impact on nation-wide RBC utilization rate was not studied. Some nation- or community-wide studies could show that the implementation of transfusion guidelines [10] or PBM measures [11, 12] had a positive impact on RBC utilization. In Belgium, the RBC utilization rate in 2011 was 47.0 per 1000 population, and there were no data on the quality of hospital transfusion practice at the national level. BeQuinT stands for Belgian Quality in Transfusion and is an initiative of the Federal Public Service (Ministry) of Public Health, Food Chain Safety and Environment since 2011. Its mission is to support the transfusion and haemovigilance policy and to optimize the utilization of blood components.

The primary aims of this study were to measure and improve the quality of the transfusion practices in Belgian hospitals in a period of 6 years with a 2-year interval. The secondary aim was to examine whether the measured quality had an impact on national transfusion rates of RBC.

MATERIALS AND METHODS

General aspects

BeQuinT includes a central steering committee and several working parties with the participation of experts and stakeholders in transfusion. The methodology used was based on repetitive national surveys in combination with feedback and information sessions and benchmarking as depicted in Figure 1. The surveys were sent to the chairs of the transfusion committees of all 111 Belgian acute hospitals, including university hospitals. The chairs of the transfusion committees received a link and an individual code to complete the survey online in a period of 3 months with a deadline at the end of September 2012, 2014 and 2016, respectively. The Red Cross of Flanders (Dutch speaking part) and the Red Cross of Belgium (French speaking part) were trusted third parties and used the coding system to ensure an anonymous data analysis. Participation in the survey was mandatory and promoted by a Royal Decree, a government decision, which twice a year allocates a budget to hospitals to establish and maintain a quality system relating to transfusion. The results of the benchmarking were sent to all chairs of the transfusion committees, individually, in a closed envelope by the trusted third parties. The overall results of each survey were disseminated through a report published on the website of BeQuinT and presented during a yearly information session organized by BeQuinT. The information sessions were combined with a scientific symposium and interactive workshops. Based on the overall results, a number of general recommendations related to the most frequently observed deficiencies were included in the report, whereas the benchmarking allowed hospitals to prioritize on areas of deficiencies at the local level.

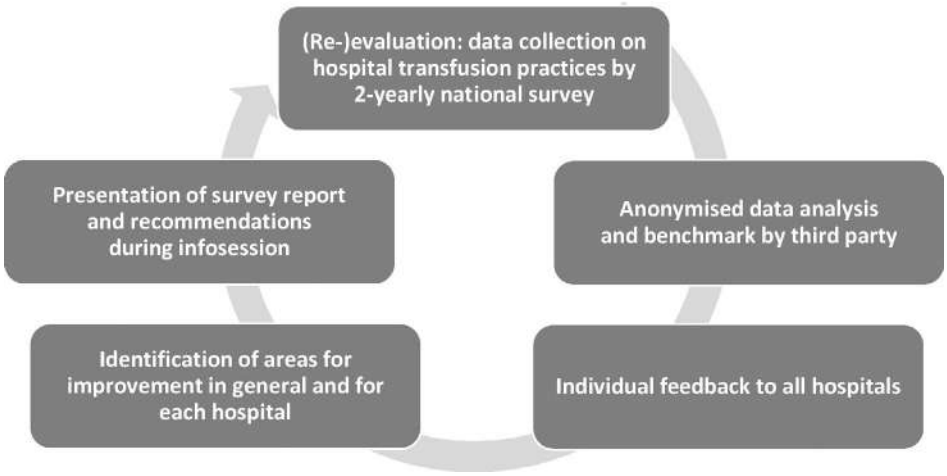

FIGURE 1 Summary of methodology based on national surveys

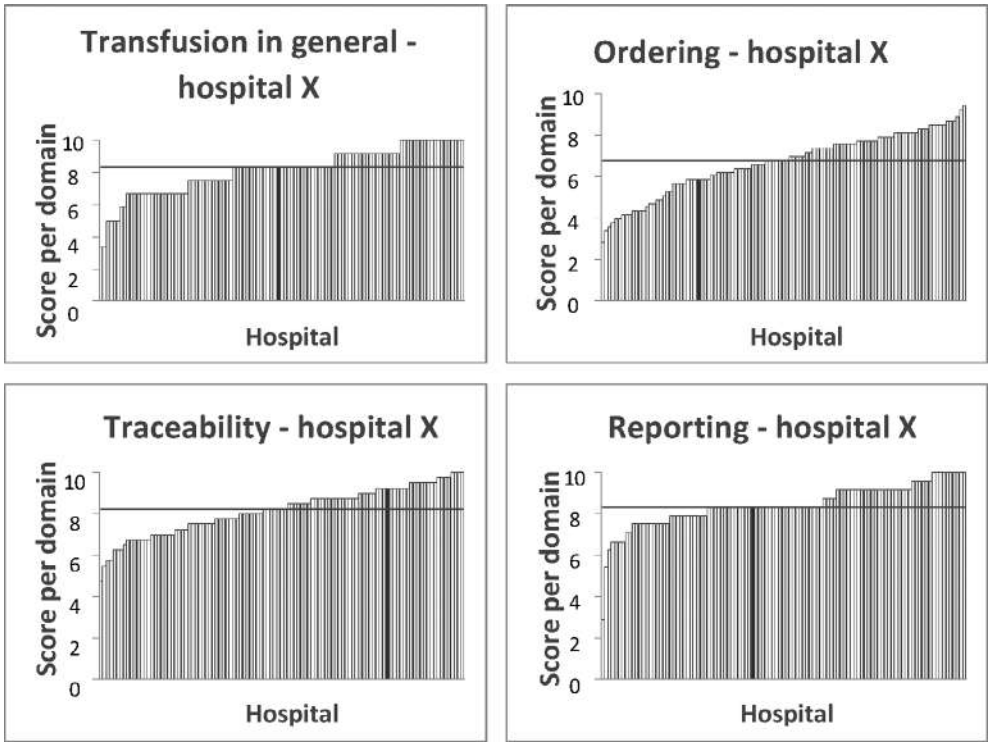

FIGURE 2 Benchmarking report: example of a report as sent to each hospital individually. Results are shown for hospital X of which scores are represented by the vertical red bar. Other vertical bars represent the scores of all hospitals participating to the survey. The median scores for each process domain are indicated by the horizontal bars

Survey content

Legal requirements, guidelines, expertise and consensus were used by the steering committee of BeQuinT to identify structural and process measures associated with best practices ('standards') in transfusion. Several issues of transfusion practices were grouped into four process domains, and for each domain, standards were defined (the complete survey is available in the Supplementary Appendix in the online version of the manuscript). The domain 'Transfusion general' relates to the organizational structure of the transfusion practice including the number of meetings of the transfusion committee, the appointment of a transfusion practitioner and/or a multidisciplinary transfusion team. In addition, training of physicians and nurses in transfusion practice, the availability of transfusion procedures and the availability of data on the number of transfused and invoiced blood components were included into this domain. Standards in the domain 'Ordering' related to the availability of procedures regarding blood sampling for pre-transfusion testing, the use

TABLE 1 Reported frequencies (%) for (a) 'transfusion general' and 'ordering' and (b) 'traceability' and 'reporting'

(a) 'Transfusion general' and 'ordering'			
	2012 (*n* = 111)	**2014 (*n* = 111)**	**2016 (*n* = 107)**
Transfusion general			
Meeting transfusion committee >2 times/year	89.2	95.5	98.1
Multidisciplinary transfusion team appointed	[a]	67.6	82.2
Transfusion practitioner appointed	82.8	80.2	92.5
Digital transfusion handbook with procedures available	86.5	96.4	96.2
Educational programme physicians (min 1×/year)	13.5	26.1	32.7
Transfusion history available in patient record	33.3	50.5	57.0
Ordering			
Electronic order implemented	6.3	7.2	15.0
Electronic order in development	12.6	28.8	39.3
Ordering always by Medical Doctor	29.7	29.7	43.0
Blood samples identified with barcode	34.2	52.2	59.8
Procedure pre-transfusion testing available	87.4	94.6	97.2
Procedure urgent transfusion available (hospital wide)	56.8	77.5	79.4
Procedure urgent transfusion available (some units)	14.4	8.1	11.2
Indication for transfusion systematically mentioned in order (hospital wide)	[a]	[a]	42.1
Systematic analysis prescriptive behaviour (hospital wide)	[a]	11.7	13.1
Systematic analysis prescriptive behaviour (some units)	[a]	27.9	32.7
(b) 'Traceability' and 'reporting'			
	2012 (*n* = 111)	**2014 (*n* = 111)**	**2016 (*n* = 107)**
Traceability			
Emergency stock of RBC in separate refrigerator with T° monitoring, data storage and alarm in hospital care unit(s)	66.1[b]	61.2[b]	60.8[b]
24 h/24 h intervention in case of alarm for stored RBC in remote refrigerator in hospital care unit(s)	53.6[b]	71.4[b]	86.3
Person responsible for follow-up emergency stock in hospital care unit(s)	69.6[b]	83.6[b]	82.4[b]
Identification with wristband upon admission	92.8	98.2	99.1
Partial electronic blood tracking system	53.1	35.1	41.1
Complete electronic blood tracking system	9.9	18.9	25.2
Scanning identification wristband before transfusion	15.3	22.5	32.7
Responsible medical doctor for transfusion identifiable in patient record in >90% of cases	58.6	69.4	78.5
Administrator blood component identifiable in file in >90% of cases	55.9	67.6	86.0
Procedure for transport and storage of blood components	57.6	85.6	95.3
Reporting			
Procedure administration blood components available	91.0	95.5	98.2

(Continues)

TABLE 1 (Continued)

(b) 'Traceability' and 'reporting'			
	2012 ($n = 111$)	**2014 ($n = 111$)**	**2016 ($n = 107$)**
Procedure patient follow-up 24 h after transfusion available	27.9	36.9	47.7
Procedure patient long-term follow-up available	14.4	19.8	23.3
Registration vital signs in patient record in >90% of cases	55.0	70.3	84.1
Registration serious transfusion reactions in patient record in >90% of cases	71.2	81.1	90.7
Reporting of serious transfusion reactions to staff member responsible for notification to the national haemovigilance office	81.1	94.6	94.4
Reporting of serious transfusion incidents to staff member responsible for notification to the national haemovigilance office	81.1	93.7	92.5
Serious transfusion reactions and incidents discussed by transfusion committees at least once a year	91.9	99.1	99.1
Methodology for analysis of serious adverse events	37.8	70.3	85.0

Abbreviations: FAMHP, Federal Agency for Medicines and Health Products; RBC, red blood cell.
[a]Not questioned in the first survey.
[b]Only applicable in 56 (in 2012), 49 (in 2014) and 51 (in 2016) hospitals, respectively.

TABLE 2 Reported frequencies (%) of hospitals performing internal audits to check the implementation of some important transfusion-related procedures at least once a year[a]

	2012 ($n = 111$)	**2014 ($n = 111$)**	**2016 ($n = 107$)**
Audit of procedure on blood sampling for pre-transfusion testing (e.g., patient identification and labelling of blood samples)	20.2 (18/89)	45.5 (46/101)	64.1 (66/103)
Audit of procedure on pre-transfusion testing (type of analyses)	32.0 (31/97)	34.3 (36/105)	56.0 (56/100)
Audit of procedure on patient identification upon admission	46.6 (48/103)	67.0 (73/109)	90.6 (96/106)
Audit of procedure on transport and storage blood components from delivery by the blood establishment to the patient	29.3 (22/75)	44.2 (42/95)	53.9 (55/102)
Audit of procedure on patient identification before starting the transfusion	18.9 (21/111)	50.5 (56/111)	74.8 (80/107)

[a]Not all hospitals responded to the questions, and, therefore, absolute numbers are mentioned below the %.

of an electronic method to prescribe blood components, the evaluation of the prescription behaviour and adherence to transfusion guidelines and the use of O-negative RBC. In addition, the legal requirements regarding the ordering of RBC are questioned such as mentioning of the indication for transfusion on the order and adherence to the exclusive restriction of the act of ordering to physicians. In the domain 'Traceability' patient identification, the implementation of pre-transfusion identity checks, the availability of an electronic blood tracking system, the storage of RBC in the hospital blood bank and care units and the number of wasted RBC are assessed. Finally, the availability of a procedure about the administration of blood components and the patient monitoring post transfusion, the registration of vital signs during transfusion and transfusion reactions in the patient record and the notification and analysis of transfusion reactions and incidents are grouped in the domain 'Reporting'. On several occasions throughout the survey, the implementation of internal auditing procedures is assessed. The survey was repeated twice with an interval of 2 years in 2012, 2014

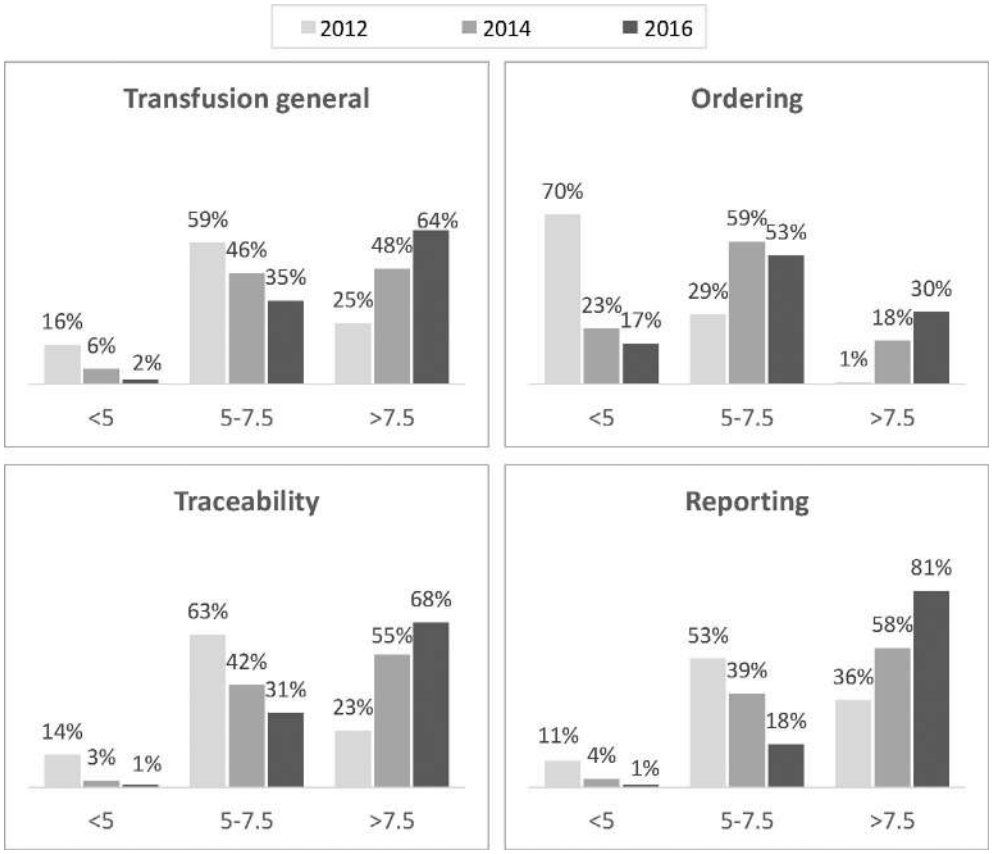

FIGURE 3 Percentage of hospitals scoring low (<5), average (5–7.5) or high (>7.5) for the four transfusion process domains with comparison of scores between the three surveys

and 2016. Consecutive survey contents were identical although some terms were clarified in the questions and answer categories in the second and third survey by means of an explanatory glossary.

Scoring and data analysis

For each hospital, scores were attributed to several answer categories and converted to a score out of 10 for each of the four domains and a total score on 40 for the entire survey, being the sum of the four scores for each domain. Scoring could be 2, 1 or 0 depending on whether or not a standard was implemented or, for example, a written procedure was available hospital-wide (2 points), in some departments (1 point) or not (0 points), respectively. These scores provided information on the degree of implementation of standards per domain and enabled to analyse trends over time and to perform benchmarking of the hospitals on a national scale. The results of the anonymous benchmarking of the scores for the four domains for all of the hospitals for each survey were sent individually to all the chairs of the transfusion committees. An example of such reporting per hospital is illustrated in Figure 2. For the scores on each domain, the frequencies of hospitals scoring low (below 5), intermediate (5–7.5) or high (above 7.5) were calculated. As for the total score, average, median and percentiles were calculated, and differences between the first (2012) and last survey (2016) were analysed with the paired samples *t*-test using SPSS Statistics.

Utilization of red blood cells

The data on the total national RBC distribution by the transfusion establishments as well as the total number of RBC transfused in Belgian hospitals are provided in an official yearly report by the Federal Agency for Medicines and Health Products. Data on Belgian population are obtained at the Belgian be.STAT official website. The distribution and transfusion rates per 1000 capita are calculated by the ratio of total number of transfused RBC during a particular year divided by the total population (×1/1000) as reported in January of the next year.

RESULTS

Characteristics of the hospitals

All the Belgian hospitals represented by 111 transfusion committees participated in the first two surveys. Due to three fusions of hospitals,

there were 107 responders to the third survey. In 2016, 25.2% of the hospitals had less than 250 acute beds, 44.1% between 250 and 500 beds and 33.6% more than 500 beds; seven hospitals were academic hospitals. In terms of activities with higher RBC transfusion needs, 23.4% had a clinical haematology department with transplantation, 9.3% performed solid organ transplantation, 29.0% had cardiac surgery and 20.6% had neonatal intensive care.

Survey data analysis

For each of the surveys, the degree of implementation of the most important standards per domain is shown in Table 1. Major and continuous progress could be noticed between 2012 and 2016, for instance an increase in: the existence of multidisciplinary transfusion teams (only measured since 2014) (67.6% ⇨ 82.2%), a readily available documented patient transfusion history (33.3% ⇨ 57.0%); the development of an electronic ordering system (12.6% ⇨ 39.3%), identification of blood samples with barcode (34.2% ⇨ 59.8%), the availability of a hospital-wide procedure for urgent transfusion (56.8% ⇨ 79.4%); the availability of a complete electronic tracking system (9.9% ⇨ 25.2%), procedures for transport and storage of blood components (28.8% ⇨ 54.2%); the implementation of a systematic methodology to analyse serious adverse events in the transfusion chain (37.8% ⇨ 85%). Also, an increase in the number of hospitals performing internal audits related to several aspects of the transfusion chain was observed (Table 2). The frequency of scores per process domain was calculated for each survey (Figure 3). In 2016, except for the domain 'Ordering', less than 3% of hospitals had scores below 5 and 64%, 30%, 68% and 81% instead of 25%, 1%, 23% and 36% had high (>7.5) scores on each of the domains, respectively. The average scores per domain were significantly better for 'Transfusion general', 'Ordering', 'Traceability' and 'Reporting' (Table 3) and the average total scores were significantly better, increasing from 24.2 in 2012 to 30.5 in 2016 ($p = 0.0005$) (Table 4).

TABLE 3 Average scores (max 10) (SD) per domain (first and third survey)

Process domain	2012 ($n = 111$)	2016 ($n = 107$)[a]	*p*-Value
Transfusion in general	6.3 (2.1)	8.1 (1.5)	0.001
Ordering	4.4 (1.3)	6.7 (1.5)	0.397
Traceability	6.4 (1.4)	8.2 (1.1)	0.003
Reporting	7.0 (1.6)	8.4 (1.1)	0.001

Abbreviation: SD, standard deviation.
[a]Four hospitals less due to fusion.

TABLE 4 Total scores (max 40) (first, second and third survey)

	2012 ($n = 111$)	2014 ($n = 111$)	2016 ($n = 107$)[a]
Average (SD)	24.2 (4.1)	28.1 (4.4)	30.5 (3.7)[b]
Median	24.4	28.4	30.6
Range	14.5–36.5	13.8–36.5	14.1–38.1
P25	20.8	25.5	28.5
P75	27.2	31.4	32.8

Abbreviation: SD, standard deviation.
[a]Four hospitals less because of fusion.
[b]$p = 0.0005$.

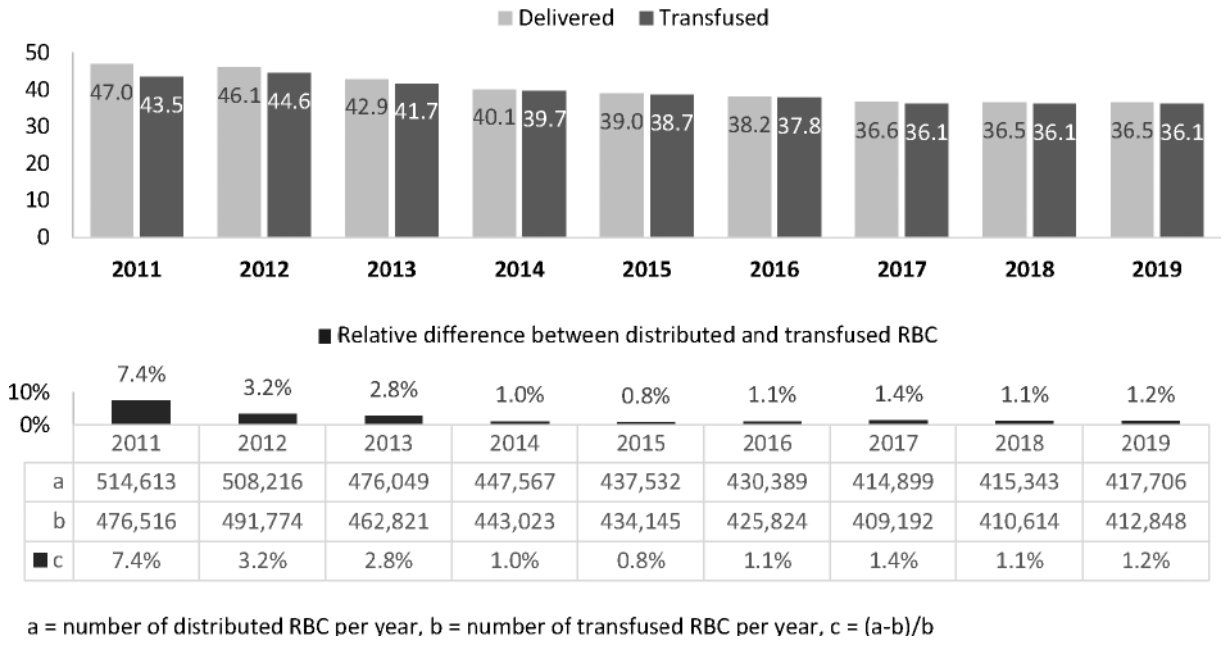

FIGURE 4 Utilization of red blood cells (RBC) in Belgian hospitals (expressed as units per 1000 population) and indicator for wastage of RBC

Utilization of red blood cells

Figure 4 shows that from 2011 (the year before the launch of BeQuinT) to 2017 (the year after the last survey was performed), the number of distributed and transfused RBC per 1000 population decreased from 47.0 to 36.6 and from 43.5 to 36.1, respectively. This decrease was sustained but did not further improve in 2018 and 2019. In addition, the difference between distributed and transfused RBC decreased from 7.4% in 2011 to 1.4% in 2017 and further down to 1.2% in 2019.

DISCUSSION

BeQuinT conducted three consecutive national surveys to study the quality of transfusion practices in Belgian hospitals by measuring the degree of implementation of numerous standards related to the major processes involved in RBC transfusion: the functioning of the transfusion committees, the presence or not of dedicated personnel involved in transfusion, the processes of ordering, transport, storage and tracing of blood components and the management of transfusion-related adverse events and reactions. The surveys were part of a methodology based on providing information through yearly information sessions, individualized feedback to the hospitals and anonymized benchmarking (Figure 1). This methodology was very successful to increase awareness and involvement of transfusion committees and hospital managements leading to numerous structural measures at the local level to improve transfusion policy. The result of this approach was that the quality of transfusion practice in Belgian hospitals increased significantly and continuously with an average score increasing from 24.2 (2014) to 30.5 (2016). Also, the maximum scores increased overtime, whereas the minimum scores remained the same, indicating that some hospitals at the bottom of the range had difficulties in improving their transfusion processes. A major strength was the 100% participation rate of the Belgian hospitals, so that the results of the surveys generate a complete set of data on national transfusion practices over a 6-years' period. The endorsement by national health authorities, the possibility to complete the survey online and the financial support to the individual hospitals were factors contributing to the success. In 2016, the majority of hospitals had high scores for the implementation of general transfusion measures, traceability and reporting of adverse events and reactions. Although markedly improved, only 30% of the hospitals had high scores on the process of ordering of RBC (Figure 3). BeQuinT is currently facilitating the implementation of a computerized physician order entry (CPOE) system in Belgian hospitals. A CPOE should include immediate access to key medical information related to the indication for transfusion, incorporate a clinical decision support system and facilitate data collection to document compliance with evidence-based transfusion guidelines. Clinical decision support systems have been shown to reduce transfusions and lead to substantial cost savings in clinical practice [13].

A major limitation of our methodology is that the surveys were not based on existing electronic sources but through web-based self-reporting. Hence, the data were not verified by external audits, and this is mainly due to the practical problems to perform such audits in more than 100 hospital sites. In addition, the impact of the surveys on the number of transfusion errors or near misses in Belgian hospitals was not examined, although this can be considered as an important measure of transfusion quality. Data of Federal Agency for Medicines and Health Products showed that in the period 2012–2016, 19 hospitals did not report any adverse event to the national haemovigilance system, and in 2016, 45 hospitals did not report any adverse event, introducing a bias for the reliability of this indicator. More action is needed to encourage hospitals to report serious errors and near misses.

An albeit indirect but objective evidence of improved transfusion practices in Belgium is the RBC distribution and transfusion rate, which decreased significantly from 2011 to 2017 (Figure 4). Most of the decrease occurred from 2013 to 2017, concurrently with the BeQuinT surveys. We showed that a substantial part of decreased RBC utilization is related to a marked reduction in RBC wastage since the difference between distributed and transfused RBC decreased from 7.4% (38,097 units) in 2011 to 1.2% (4858 units) in 2019. In addition to less wastage, we hypothesized that our survey-based methodology and associated increased awareness also led to a more rational transfusion policy and associated reduction in RBC transfusion. The introduction of PBM has reduced RBC utilization in some areas of clinical practice over the past decade in several countries. This was particularly the case in cardiovascular and orthopaedic surgery. However and based on data (2016) provided by the Data and Strategic Analysis, Directorate-General for Health Care and Federal Public Service of Public Health, RBC transfusion in surgical areas only accounted for 30% of the total. Even a 50% reduction of RBC transfusion in surgical areas would have accounted for much less than the reduction of the total utilization rate as was observed between 2011 and 2017. We, therefore, concluded that the BeQuinT survey-based methodology has significantly contributed to less wastage of RBC and a more rational transfusion policy introduced in a broad field of medical and surgical disciplines. Data from 2017 to 2019 showed no further decrease but stabilization, suggesting that the impact of improved transfusion practices on RBC utilization is durable but levelling off and that other measures are needed for further optimization. This should be possible since in many European countries lower than 35 per 1000 population utilization rates were noted [5, 14]. Further improvement will require a more direct impact on clinical-oriented as opposed to process-oriented transfusion practices. PBM is an evolving multidisciplinary concept shifting from a 'product-centred' towards a 'patient-centred' evidence-based approach to minimize unnecessary exposure of patients to blood components. Recently, PBM recommendations have been published including diagnosing and medical treatment of pre-operative anaemia, minimizing perioperative blood loss and using restrictive triggers to transfuse in most clinical situations [15, 16]. A survey in some European university hospitals showed that the degree of implementation of PBM was highly variable [17]. The implementation of PBM in clinical practice has a significant effect on RBC utilization. The combination of measuring, auditing and providing feedback resulted in a marked

decrease RBC utilization in intraoperative RBC usage in a cardiac surgery setting [18]. Although some PBM elements were questioned, the BeQuinT surveys were not designed to assess the degree of PBM implementation in the hospitals. Based on the success of the methodology used, BeQuinT has launched a national PBM survey in all Belgian hospitals in 2020 with the aim of further improving transfusion practices and optimizing RBC utilization.

We concluded that this nation-wide survey–based benchmarking initiative combining information, data collection and individualized feedback has contributed to a measurable and durable improvement of the quality of transfusion practices in Belgian hospitals. We also demonstrated that improving transfusion quality is associated with a marked and structural decrease in RBC utilization rate.

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All authors have actively contributed to the conception and design of the survey. K.B. realized the statistical analysis. J.V.B., K.B., R.S., C.G. and V.D. were involved in the interpretation of the data. J.V.B., K.B. and R.S. drafted the manuscript. All authors did a critical revision of the manuscript and gave final approval of the version to be published.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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None.

ORCID

Jana Vanden Broeck https://orcid.org/0000-0002-8514-9765

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SUPPORTING INFORMATION

Additional supporting information may 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

A comparative study on perinatal outcomes of red blood cell–alloimmunized pregnancies with anti-RhD in combination and anti-RhD alone in China

Muxue Yu | **Tonghui Tang** | **Rujiang Zheng** | **Miaoqiong Situ** | **Jiali Feng**

Department of Pediatrics, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

Correspondence
Muxue Yu, Department of Pediatrics, The First Affiliated Hospital, Sun Yat-sen University, 58 Zhongshan Road 2, Guangzhou 510080, China.
Email: yumuxue@mail.sysu.edu.cn

Abstract

Background and Objectives: The advent of intrauterine transfusion (IUT) has improved the survival of severe foetal anaemia. The aim of this study was to compare the perinatal outcomes of red blood cell (RBC)–alloimmunized pregnancies with anti-RhD in combination and anti-RhD alone in China.

Materials and Methods: A retrospective study was conducted involving RBC–alloimmunized pregnancies with anti-RhD in combination and anti-RhD alone admitted to The First Affiliated Hospital, Sun Yat-sen University, between January 2007 and December 2019. Obstetric data and neonatal outcomes were compared.

Results: A total of 165 alloimmunized pregnancies were identified, with 32 pregnancies in the anti-RhD-in-combination group (25 pregnancies with anti-RhD + anti-RhC and 7 pregnancies with anti-RhD + anti-RhE) and 133 pregnancies in the anti-RhD-alone group. The anti-RhD-in-combination group had significantly higher frequency of IUTs than the anti-RhD-alone group (59.4% [19/32] vs. 30.1% [40/133]; $p < 0.01$). The postnatal frequency of top-up transfusions was significantly higher in the anti-RhD in combination group than the anti-RhD-alone group (90.6% [29/32] vs. 70.7% [94/133]; $p = 0.02$). There was no significant difference in the frequency of exchange transfusions (ETs) between the two groups (15.6% [5/32] vs. 17.3% [23/133]; $p = 0.82$).

Conclusions: Compared to alloimmunized pregnancies with anti-RhD alone, pregnancies with anti-RhD in combination with anti-RhC or anti-RhE have an increased requirement for antenatal IUTs and postnatal top-up transfusions but do not have an increased need for ETs.

KEYWORDS
alloimmunization, anti-RhD alone, anti-RhD in combination, pregnancy, red blood cell antibodies

INTRODUCTION

Rh red blood cell (RBC)-alloimmunized pregnancies are associated with foetal or newborn haemolysis due to incompatibility of the Rh RBC antigen. The Rh antigens are highly immunogenic. RhD-negative individuals will produce anti-RhD if they encounter the D antigen. Anti-RhD is the most common antibody implicated in foetal or newborn haemolysis [1, 2]. Antibodies to other Rh antigens, such as C, c, E and e, also contribute to the haemolytic effect [3, 4].

Studies [5–7] from the United States and Europe have shown that pregnancies with anti-RhD combined with other RBC antibody specificities are likely to develop more aggressive immune responses than

pregnancies with anti-RhD alone. The ethnicities of the pregnant women were mainly Native Americans, Caucasians and blacks. The frequency of Rh antigens and phenotypes varies across ethnicities throughout the world [1]. No published studies have compared Rh RBC alloimmunization perinatal outcomes caused by anti-RhD in combination with anti-RhD alone among Asians.

In mainland China, anti-RhD immunoglobulin is not available because of import restrictions. The prevalence of RhD-negative women varies widely around the globe, and it is only approximately 0.5% in China. Nevertheless, maternal RhD alloimmunization occurs frequently, given the high number of births and the very high likelihood that the father is RhD-positive. In addition, the implementation of the 'more than one child' policy is expected to increase the occurrence of Rh disease because the response to Rh incompatibility increases in severity with subsequent pregnancies [8]. The advent of intrauterine transfusion (IUT) has improved the perinatal outcomes and survival of severe foetal anaemia [9]. IUT is carried out to treat foetal anaemia due to maternal alloimmunization in our hospital [10–12]; as a result, the healthcare team at our hospital manages most patients with RBC alloimmunization in pregnancy throughout China.

The aim of this study was to compare perinatal outcomes of RBC-alloimmunized pregnancies that received routine antenatal and neonatal management caused by anti-RhD in combination with anti-RhD alone in China.

MATERIALS AND METHODS

Study population

This retrospective single-centre study was conducted at The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China. Following approval of the Institutional Review Board, the medical records were searched to identify pregnant patients diagnosed with Rh RBC alloimmunization and their infants who received routine antenatal and neonatal management in The First Affiliated Hospital, Sun Yat-sen University, between January 2007 and December 2019.

The derivation of the included pregnancies and the reasons for exclusion are shown in Figure 1. Of the 181 potentially eligible pregnancies with Rh RBC alloimmunization, five pregnancies with non-anti-RhD (two pregnancies with anti-RhE alone, one pregnancy with anti-RhC + anti-RhE, one pregnancy with anti-RhE + anti-RhC + anti-Jk^b and one twin gestation with anti-RhC + anti-RhE) were excluded. There were 35 pregnancies with anti-RhD in combination. Two pregnancies with anti-RhD + non-anti-Rh (one pregnancy with anti-RhD + anti-M and one pregnancy with anti-RhD + anti-A) were excluded. One pregnancy with anti-RhD in combination with anti-RhC was excluded because an emergency caesarean section was performed for foetal distress 8 days after an IUT. We also excluded three pregnancies with twins, one pregnancy with an incompetent cervix, one pregnancy requiring emergency delivery (due to foetal distress 2 days after an IUT), one pregnancy with rupture of membranes (occurred 4 days after an IUT that resulted in a preterm delivery) and two pregnancies with severe neonatal complications (congenital cytomegalovirus infection and intestinal perforation complicated with peritonitis, respectively) among 141 pregnancies with anti-RhD alone. In summary, twin gestations, pregnancies with other RBC antibodies (except for anti-RhC and anti-RhE) in combination with anti-RhD and pregnancies requiring emergency delivery for foetal distress during antenatal management were excluded, as well as pregnancies with severe neonatal complications. Altogether, 32 pregnancies with anti-RhD in combination (anti-RhD + anti-RhC [$n = 25$] or anti-RhD + anti-RhE [$n = 7$]) and 133 pregnancies with anti-RhD alone were eligible to enrol our study.

Obstetric data collection

Obstetric data were recorded on the following: maternal age, number of pregnancies, maternal indirect anti-globulin tests (IATs), maximum antibody titre, major obstetric complications, past obstetric history, gestational age at the first IUT, foetal haemoglobin (Hb) concentration and haematocrit (Hct) levels at the first IUT, number of IUTs, foetal direct agglutination test (DAT) and elution analysis and foetal RBC antigen type.

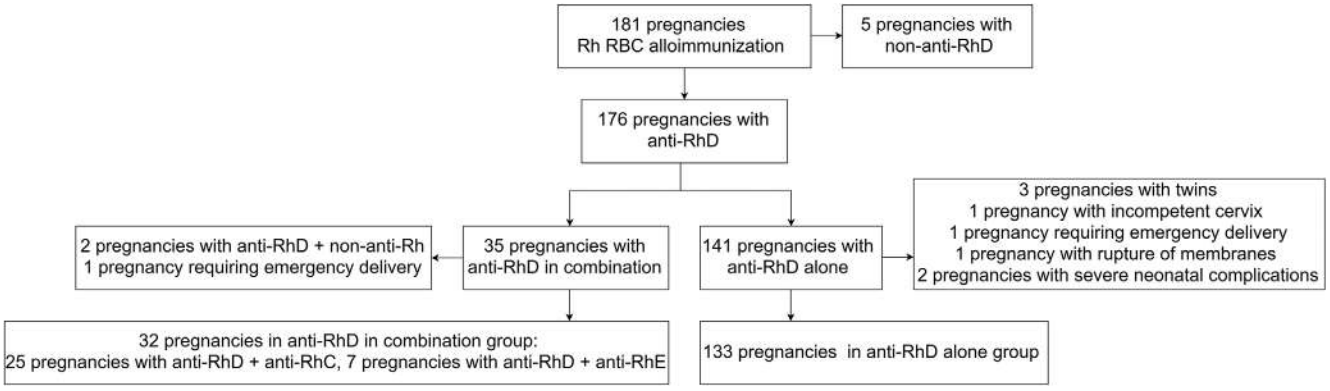

FIGURE 1 Diagram of inclusion of pregnancies for anti-RhD-in-combination versus anti-RhD-alone study

The alloimmunized pregnant women had routine antibody screening at the first antenatal visit. The Rh phenotypes of the pregnant women were available. They were RhD-negative and were shown not to be D variants by IATs with a monoclonal human IgM + IgG blend. The Rh phenotypes of the husbands were also available. Repeat anti-RhD, anti-RhC and anti-RhE titres were performed each month until 24–28 weeks' gestation and every 2 weeks thereafter. For pregnancies that had reached 16–20 weeks' gestation, the middle cerebral artery peak systolic velocity (MCA-PSV), as measured by ultrasound Doppler interrogation, was used to detect foetal anaemia. Measurements of the MCA-PSV were repeated every 1–2 weeks and were performed more frequently in pregnancies with higher multiples of the mean (MoM) levels. Cordocentesis was performed to determine the foetal Hct when the MCA-PSV value was ≥1.5 MoM. An IUT was indicated if the foetal Hct was ≤30%. With the exception of two pregnancies, gravidas presenting at >34 weeks' gestation were typically offered delivery. The two gravidas (one in the anti-RhD-in-combination group and one in the anti-RhD-alone group) who had undergone seven or six IUTs, respectively, were delivered at <33 weeks' gestation because the risk of continued monitoring and IUT was balanced.

Neonatal data collection

Neonatal data were recorded on the following: gender, gestational age at birth, Apgar score at 1 and 5 min, birth weight, Hb concentration, Hct, bilirubin levels at birth, blood DAT and elution analysis, RBC antigen status, maximum bilirubin level during hospitalization, duration of phototherapy, number of exchange transfusions (ETs) required and number of top-up transfusions received during the first 3 months of life.

After delivery, the newborns were transferred to the Neonatal Unit and received neonatal intensive care. Phototherapy was initiated and intravenous immunoglobulin (IVIG) was administered to the newborns at the time of admission to the Neonatal Unit. Top-up RBC transfusions were performed when Hb levels were < 8.0 g/dl or at higher levels if clinical symptoms of anaemia (lethargy, feeding problems or failure to thrive) were present. Data on the number of top-up transfusions during the 3 months of life were collected. The indication for ET was based on the consensus from the Society of Pediatrics, Chinese Medical Association [13, 14].

Statistical analysis

Data are presented as mean ± standard deviation (SD), median (interquartile range [IQR]) or numbers and percentage as appropriate. For comparisons, the *t*-test was used if the values were normally distributed, otherwise the Mann–Whitney U test was applied. The chi-square test or Fisher's exact test were used for distribution-based comparison. A *p*-value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS (version 20; IBM Corp., Armonk, NY).

RESULTS

Altogether 165 alloimmunized pregnant women and their infants were included in the study. Anti-RhD with anti-RhC or anti-RhD with anti-RhE antibodies were identified in pregnant women in the anti-RhD-in-combination group, and only anti-RhD antibodies were identified in pregnant women in the anti-RhD-alone group.

TABLE 1 Maternal characteristics of the anti-RhD-in-combination and anti-RhD-alone groups

	Anti-RhD in combination (*n* = 32)	Anti-RhD alone (*n* = 133)	*p*-value
Maternal age (years)[a]	31.1 ± 4.3	31.1 ± 4.0	0.92
Median number of pregnancies[b]	4 (3.8–5)	3 (2–4)	<0.01
Maximum anti-RhD titre – *n* (%)			
4-1024	26 (81.3)	121 (91.0)	0.20
≥2048	6 (18.7)	12 (9.0)	
Gestational diabetes – *n* (%)	4 (12.5)	18 (13.5)	1.00
Pregnancy-induced hypertension – *n* (%)	1 (3.1)	0 (0)	0.19
Placental abruption – *n* (%)	0 (0)	2 (1.5)	1.00
Intrauterine growth restriction – *n* (%)	1 (3.1)	1 (0.8)	0.35
Caesarean section – *n* (%)	24 (75.0)	99 (74.4)	0.95
History of IUT – *n* (%)	1 (3.1)	3 (2.3)	0.58
History of IUFD – *n* (%)	14 (43.8)	17 (12.8)	<0.01
History of neonatal death – *n* (%)	12 (37.5)	22 (16.5)	<0.01

Abbreviations: IUFD, intrauterine foetal death; IUT, intrauterine transfusion.
[a]Value given as mean ± SD.
[b]Value given as median (IQR).

TABLE 2 IUT characteristics of the anti-RhD-in-combination and anti-RhD-alone groups

	Anti-RhD in combination ($n = 32$)	Anti-RhD alone ($n = 133$)	p-value
IUT required – n (%)	19 (59.4)	40 (30.1)	<0.01
Median number of IUTs[a]	1.5 (0–5)	0 (0–1)	<0.01
Median number of IUTs in subgroup requiring IUT[a,b]	4 (2–6)	3 (1–5)	0.05
Gestational age at first IUT (weeks)[b,c]	26.1 ± 4.8	29.2 ± 4.0	0.01
Foetal Hb level at first IUT (g/dl)[b,c]	6.6 ± 2.5	7.4 ± 2.1	0.22
Foetal Hct level at first IUT (%)[b,c]	20.9 ± 7.2	22.5 ± 6.0	0.36

Abbreviations: Hb, haemoglobin; Hct, haematocrit; IUT, intrauterine transfusion.
[a]Value given as median (IQR).
[b]Assessed in pregnancies requiring IUT.
[c]Value given as mean ± SD.

TABLE 3 Neonatal outcomes and management of the anti-RhD-in-combination and anti-RhD-alone groups

	Anti-RhD in combination ($n = 32$)	Anti-RhD alone ($n = 133$)	p-value
Male – n (%)	17 (53.1)	69 (51.9)	0.90
Gestational age at birth (weeks)[a]	36.0 ± 1.7	36.6 ± 1.4	0.04
Birth weight (g)[a]	2672 ± 480	2804 ± 361	0.08
Apgar score < 7 at 1 min – n (%)	2 (6.3)	6 (4.5)	1.00
Apgar score < 7 at 5 min – n (%)	1 (3.1)	0 (0)	0.19
RDS – n (%)	4 (12.5)	6 (4.5)	0.20
Hypoglycaemia – n (%)	1 (3.1)	2 (1.5)	0.48
Hb level at birth (g/dl)[a]	11.8 ± 2.4	13.1 ± 2.5	<0.01
Hct level at birth (%)[a]	34.7 ± 6.9	38.9 ± 6.8	<0.01
Bilirubin level at birth (μmol/L)[a]	99.0 ± 32.8	84.2 ± 33.8	0.03
Maximum bilirubin level (μmol/L)[a]	203.3 ± 62.2	207.1 ± 67.8	0.78
Duration of phototherapy (days)[a]	5 (4–6)	5 (4–7)	0.88
Top-up transfusion required – n (%)	29 (90.6)	94 (70.7)	0.02
Median number of top-up transfusions[b]	2 (1–3)	1 (0–2)	0.02
ET required – n (%)	5 (15.6)	23 (17.3)	0.82
Median number of ETs[b]	1 (0–0)	0 (0–0)	0.87
Median number of ETs in subgroup requiring ET[b,c]	1 (1–2)	1 (1–2)	0.81

Abbreviations: ET, exchange transfusion; Hb, haemoglobin; Hct, haematocrit; RDS, respiratory distress syndrome.
[a]Value given as mean ± SD.
[b]Value given as median (IQR).
[c]Assessed in newborns requiring ET.

Maternal and foetal data

The maternal characteristics of the anti-RhD-in-combination and anti-RhD-alone groups are depicted in Table 1. The anti-RhD-in-combination and anti-RhD-alone groups were comparable with respect to maternal age, distribution of maximum anti-RhD titre, frequencies of major obstetric complications and caesarean section rate. The median number of pregnancies was higher in the anti-RhD-in-combination group than the anti-RhD-alone group (4 [IQR 3.8–5, range 2–9] vs. 3 [IQR 2–4, range 1–7]; $p < 0.01$). All pregnancies in the anti-RhD-in-combination group were subsequent pregnancies, and 2 of the 32 gravidas had histories of RBC transfusions. Two pregnancies in the anti-RhD-alone group were the first pregnancy; the two gravidas had histories of RBC transfusions. The other 131 pregnancies in the anti-RhD-alone group were subsequent pregnancies, and 9 of the 131 gravidas had histories of RBC transfusions. No significant difference was demonstrated in the history of IUT frequency between the two groups. The frequencies of intrauterine foetal death (IUFD) and neonatal death histories were significantly higher in the anti-RhD-in-combination group than the anti-RhD-alone group (43.8% [14/32] vs. 12.8% [17/133]; $p < 0.01$ and 37.5% [12/32] vs. 16.5% [22/133]; $p < 0.01$).

The IUT characteristics are presented in Table 2. The anti-RhD-in-combination group had significantly higher frequency of IUTs

during pregnancy than the anti-RhD-alone group (59.4% [19/32] vs. 30.1% [40/133]; $p < 0.01$). The anti-RhD-in-combination group also had significantly higher median number of IUTs during pregnancy than the anti-RhD-alone group (1.5 [IQR 0–5, range 0–8] vs. 0 [IQR 0–1, range 0–7]; $p < 0.01$). In the subgroup analysis of pregnancies requiring IUT, the median number of IUTs tended to be higher in the anti-RhD-in-combination group than the anti-RhD-alone group (4 [IQR 2–6, range 1–8] vs. 3 [IQR 1–5, range 1–7]; $p = 0.05$). The gestational age at the first IUT was significantly lower in the anti-RhD-in-combination group compared to the anti-RhD-alone group (26.1 weeks vs. 29.2 weeks; $p = 0.01$). No significant differences were demonstrated in foetal mean Hb concentration and Hct at the first IUT between the anti-RhD-in-combination and anti-RhD-alone group.

Neonatal outcomes and management

Table 3 shows the neonatal outcomes and management of the anti-RhD-in-combination and anti-RhD-alone groups. The neonatal gender and frequencies of Apgar score < 7 at 1 min, Apgar score < 7 at 5 min, respiratory distress syndrome (RDS) and hypoglycaemia were not significantly different between the two groups. A lower gestational age at birth existed in the anti-RhD-in-combination group compared to the anti-RhD-alone group (36.6 weeks vs. 36.0 weeks; $p = 0.04$). The birth weight was not significantly different between the anti-RhD-in-combination group and the anti-RhD-alone group, but a trend was noted (2672 g vs. 2804 g; $p = 0.08$).

The Hb concentration and Hct at birth were significantly lower in the anti-RhD-in-combination group than the anti-RhD-alone group (11.8 g/dl vs. 13.1 g/dl; $p < 0.01$ and 34.7% vs. 38.9%; $p < 0.01$). The bilirubin levels at birth were significantly higher in the anti-RhD-in-combination group than the anti-RhD-alone group (99.0 μmol/L vs. 84.2 μmol/L; $p = 0.03$).

The anti-RhD-in-combination group had significantly higher frequency of top-up transfusions than the anti-RhD-alone group (90.6% [29/32] vs. 70.7% [94/133]; $p = 0.02$). The median number of top-up transfusions per infant was also higher in the anti-RhD-in-combination group compared to the anti-RhD-alone group (2 [IQR 1–3, range 0–5] vs. 1 [IQR 0–2, range 0–8]; $p = 0.02$). There were no significant differences in the mean maximum bilirubin level, median duration of phototherapy, frequency of ETs and median number of ETs between the two groups. In the subgroup analysis of newborns requiring ET, no significant difference in the median number of ETs between the two groups was found.

DISCUSSION

This study showed that compared to alloimmunized pregnancies with anti-RhD alone, alloimmunized pregnancies with anti-RhD-in-combination with anti-RhC or anti-RhE increased the need for antenatal IUTs and postnatal top-up transfusions. The need for ETs was similar in both groups.

There were alloimmunized pregnancies with anti-RhD plus anti-RhC or anti-RhE, while there were no pregnancies with anti-RhD plus two other Rh antibody specificities in our study. Other authors have reported alloimmunized pregnancies by anti-RhD plus anti-RhC plus anti-RhE [6, 7, 15, 16]. This is due to the low occurrence of the RhD-negative ccee phenotype in Asians. Anti-RhD plus anti-RhC plus anti-RhE could be produced through pregnancy with the RhD-negative ccee phenotype. The frequency of the RhD-negative ccee phenotype in Caucasians and blacks is 15.1% and 6.8%, respectively. Nevertheless, the frequency of the RhD-negative ccee phenotype in Asians is only 0.1% [17].

In our study, alloimmunized pregnancies with anti-RhD in combination with one Rh antibody specificity were more likely to have foetal and neonatal anaemia compared with pregnancies with anti-RhD alone. As a result, there was an increased requirement for IUTs and postnatal top-up transfusions. These findings could account for anti-RhD in combination with anti-RhC or anti-RhE enhancing the immune response. IUT appears to increase and prolong the risk of anaemia, probably by reducing erythropoiesis [2, 18]. These findings are in accordance with the results of previous studies [5–7]. In the study conducted by Markham et al. [5], alloimmunized pregnancies with anti-RhD alone versus alloimmunized pregnancies with anti-RhD plus one or more additional RBC antibodies were compared. In the study of Nordvall et al. [6], alloimmunized pregnancies with anti-RhD alone versus alloimmunized pregnancies with anti-RhD plus one other RBC antibody were compared. In Spong et al.'s study [7], alloimmunized pregnancies with anti-RhD alone versus alloimmunized pregnancies with two to four RBC antibodies were compared.

Phung et al. [15] and Walsh et al. [16] focussed on those pregnancies requiring IUT. Phung et al. [15] showed that in pregnancies requiring IUT, the daily decrease in Hb between the first and second IUT did not detect a significant difference between alloimmunized pregnancies with anti-RhD alone and anti-RhD plus one other RBC antibody while the daily decrease in Hb were significantly lower in alloimmunized pregnancies with anti-RhD alone compared with anti-RhD plus two RBC antibodies. Walsh et al. [16] reported that there were no differences in the number of IUTs per affected alloimmunized pregnancy when comparing anti-RhD alone with anti-RhD plus one other RBC antibody.

There was a lack of data on postnatal ETs in the studies of Markham et al. [5] and Sponges et al. [7]. The Nordvall et al.'s study [6] showed that alloimmunized pregnancies with anti-RhD in combination required more postnatal ETs compared with anti-RhD alone. Indeed, we detected no difference in the postnatal maximum bilirubin level, duration of phototherapy, frequency of ETs and median number of ETs in both groups. In our department, phototherapy was initiated, and IVIG was administered when the newborn was admitted to the Neonatal Unit because Rh alloimmunization is a risk factor for unfavourable neonatal outcomes. The American Academy of Pediatrics suggests that intensive phototherapy and IVIG administration may reduce the need for ETs in infants with Rh haemolytic disease [19]. Studies of infants with Rh incompatibility supported a reduction

in the use of ET with IVIG treatment [20]. Intensive phototherapy for neonatal hyperbilirubinaemia has resulted in a worldwide decrease in the need for ETs during the last two to three decades [21–23]. In addition, replacement of foetal RBCs by antigen-negative donor RBCs during the IUT could result in less active postnatal neonatal alloimmune haemolysis [18]. Therefore, although alloimmunized pregnancies with anti-RhD in combination compared to alloimmunized pregnancies with anti-RhD alone had more severe foetal haemolysis and an increased need for antenatal IUTs, an increased need for postnatal ETs was not required. Currently, most foetal therapy centres opt for postnatal top-up transfusions rather than postnatal ETs [9, 24]. Theoretically, more bilirubin is produced after birth because of more severe haemolysis in alloimmunized pregnancies with anti-RhD in combination [25]. However, we did not demonstrate a difference in the postnatal maximum bilirubin level and duration of phototherapy between the two groups. This finding could be explained that the severe haemolysis in pregnancies underwent antenatal IUTs and neonates in both groups received aggressive phototherapy. Previous studies did not show the neonatal bilirubin level and phototherapy data [5–7].

Although alloimmunized pregnancies with anti-RhD in combination increased the need for antenatal IUTs and postnatal top-up transfusions, there were no differences in type of delivery and major obstetric and neonatal complications in both groups. RDS is associated with pre-term delivery [26]. Gestational age at birth was lower in the anti-RhD-in-combination group than the anti-RhD alone group in our study; however, the mean gestational age at birth in both groups was >36 weeks. RDS is more common in neonates born at a gestational age < 35 weeks when compared to a gestational age > 35 weeks [27]. Therefore, the risk of RDS decreased in the anti-RhD-in-combination and anti-RhD-alone groups. A fetus with immune haemolysis destined to become hydropic after 32–34 weeks' gestation might survive after early delivery and prompt ET, before the development of hydrops [28]. Currently, the therapeutic options for pregnancies with RBC alloimmunization consist of controlled early delivery and IUT. Pregnancies with a foetus at significant risk for foetal anaemia are delivered at 37–38 weeks' gestation unless indications develop prior to this time [29]. The last foetal transfusion is given at approximately 35 weeks with delivery at 37–38 weeks' gestation in preference to early delivery and ET because the foetus tolerates larger transfusion volumes than the neonate due to the large capacity of the placenta [30].

There are several limitations to this study. First, these data were limited to alloimmunized pregnancies with anti-RhD alone and anti-RhD plus one other anti-Rh antibody specificity. The number of pregnancies with anti-RhD plus non-anti-Rh or with multiple RBC antibodies was less, thus these pregnancies were not included in our study. We collected data after the introduction of MCA-PSV in our hospital, which could cause a limited number of pregnancies with multiple RBC antibodies. Additionally, we did not compare the use of erythropoietin (EPO) in both groups. Small case series reported the use of EPO to prevent late anaemia in neonates with Rh haemolytic disease [2, 31]. EPO administration to some of the infants with anaemia in both groups were performed in our study; however, the initial infant's age at the time of EPO administration, duration and dosages of EPO varied, and a number of infants were treated by EPO after discharge. We did not collect completed information regarding the use of EPO in both groups. Finally, our study was retrospective and was limited to obstetric and neonatal outcomes. There was also a lack of data about long-term growth development of the infants.

In conclusion, alloimmunized pregnancies with anti-RhD in combination with anti-RhC or anti-RhE need more perinatal treatment than alloimmunized pregnancies with anti-RhD alone, including IUTs and top-up transfusions. More severe haemolysis in pregnancies with anti-RhD in combination with anti-RhC or anti-RhE resulting in foetal anaemia leads to an increased need for IUTs, and pregnancies with anti-RhD in combination with anti-RhC or anti-RhE require more postnatal transfusions for neonatal anaemia. Pregnancies with anti-RhD in combination with anti-RhC or anti-RhE, however, do not have an increased need for ETs.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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ORCID

Muxue Yu https://orcid.org/0000-0002-1122-3918

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

A first WHO reference reagent for the detection of anti-human platelet antigen-15b

Giles Sharp[1] | Anthony Poles[2] | Lucy Studholme[1]

[1]Biotherapeutics Division, National Institute for Biological Standards and Control (NIBSC), Potters Bar, UK

[2]Histocompatibility and Immunogenetics, NHSBT, Filton, UK

Correspondence
Lucy Studholme, Biotherapeutics Division, National Institute for Biological Standards and Control (NIBSC), Potters Bar, Herts, EN6 3QG, UK.
Email: lucy.studholme@nibsc.org

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Abstract

Background and Objectives: Alloantibodies to human platelet antigen-15b (anti-HPA-15b) have been detected in mothers with foetal–neonatal alloimmune thrombocytopenia and in multiply transfused patients. Assays used to detect this antibody, which aids in disease diagnosis, can be unreliable and vary in sensitivity. The objective was to generate a stable, lyophilized anti-HPA-15b preparation and evaluate its suitability as a World Health Organization (WHO) reference reagent for use in the quality control of platelet alloantibody detection assays. Results from an international collaborative study to evaluate the preparation were used to assign a minimum potency at which laboratories can be expected to detect the antibody.

Materials and Methods: Recalcified plasma containing anti-HPA-15b was aliquotted, lyophilized and coded 18/220. Twenty-five laboratories in 16 countries tested doubling dilutions of the reconstituted material in glycoprotein-specific assays such as the monoclonal antibody–specific immobilization of platelet antigen assay and reported the last positive (or endpoint) dilution.

Results: Twenty-four laboratories (96%) detected antibodies with HPA-15b specificity in preparation 18/220. Reported endpoint dilutions were normally distributed with a modal dilution of 1 in 16 and ranged from 1 in 2 to 1 in 128. Only two laboratories (8%) failed to detect anti-HPA-15b at 1 in 8 dilution.

Conclusions: When diluted 1 in 8, most laboratories detected anti-HPA-15b in preparation 18/220 using HPA-15bb platelets but not with HPA-15aa platelets. The participants agreed this to be an appropriate dilution for assignment as the minimum potency. In October 2020, the WHO Expert Committee on Biological Standardization approved 18/220 as an International Reference Reagent.

KEYWORDS
CD109, HPA-15b, MAIPA, platelet alloantibody detection, reference reagent

INTRODUCTION

To date, a total of 41 platelet alloantigens have been defined serologically, of which 12 are grouped in six bi-allelic systems (human platelet antigens; HPA -1, -2, -3, -4, -5, -15) [1, 2]. The molecular basis of the 41 antigens has been resolved, and in all but one (HPA-14b), the difference between self and non-self is defined by a single nucleotide polymorphism in the gene encoding the relevant membrane

glycoprotein [2]. Alloantibodies against HPAs are involved in foetal–neonatal alloimmune thrombocytopenia (FNAIT), a disease in which foetal/neonatal platelets are destroyed by IgG alloantibodies from the incompatible, HPA-sensitized mother. HPA antibodies are also involved in platelet transfusion refractoriness (PTR), the repeated failure to achieve the desired level of blood platelets following transfusion and in post-transfusion purpura (PTP), the delayed reaction to a transfusion caused by recipient antibodies to platelet antigens. Identification of the HPA antibody specificity is essential to the diagnosis and treatment of the patient. In severe cases of thrombocytopenia requiring treatment with a platelet transfusion, it is important that the transfused platelets are negative for the target alloantibody specificity [3–6].

A variety of techniques are in use across the world to detect HPA antibodies. The monoclonal antibody–specific immobilization of platelet antigen (MAIPA) assay is considered the gold standard method for the determination of platelet (IgG) alloantibody specificity. The method involves using specific HPA-typed platelets to capture alloantibodies in human serum/plasma. Mouse monoclonal antibodies specific for glycoproteins on which the platelet antigens of interest are located are used to capture the platelet antigen—alloantibody complexes after solubilization of the platelets. This design ensures the accurate identification of alloantibody specificity and avoids false-positives resulting from, for example, HLA antibodies. The MAIPA does not distinguish between IgG alloantibody subclasses in patient samples. To date, there are three World Health Organization (WHO) reference reagents established (anti-HPA-5b, anti-HPA-3a and anti-HPA-1a) [7–9], which are used for assay quality control. These reagents are used to validate the minimum sensitivity of tests for the respective HPA antibodies.

In addition to HPA-1a, -3a and -5b, HPA-15 is of clinical relevance. Studies have shown this antigen to be as immunogenic as HPA-5, and alloantibodies against HPA-15b can be detected in patients receiving multiple transfusions and mothers with FNAIT [10–12]. The platelet-based methods used for the detection of anti-HPA-15 antibodies can be unreliable and vary in their sensitivity because CD109, the glycoprotein on which HPA-15b is located, is expressed in low numbers by platelets and is labile [13, 14]. An anti-HPA-15b minimum potency reference reagent would allow clinical laboratories to validate their methods. The need for this important reference material was emphasized in the proficiency testing scheme organized by National Institute for Biological Standards and Control (NIBSC) in 2017 showing that anti-HPA-15b detection in the samples distributed was generally poor. Furthermore, it was concluded in a report by the Platelet Immunobiology Working Party of the International Society of Blood Transfusion (ISBT) in 2018 [15] that a more standardized approach to the CD109 MAIPA is required. The purpose of this study was to produce and evaluate a candidate WHO anti-HPA-15b reference reagent (minimum potency) for use as a quality control reagent in glycoprotein-specific assays used to detect/identify alloantibody specificity. The authors describe the evaluation of an anti-HPA-15b lyophilized preparation coded 18/220 in an international collaborative study, the results of which are used to assign a minimum potency to this preparation. Clinical laboratories testing the established reference reagent at the assigned minimum potency should expect a positive result if the sensitivity of their method is acceptable.

MATERIALS AND METHODS

Candidate material

The material was provided to NIBSC by the National Blood Service, Oxford, United Kingdom. All donations of recalcified plasma came from just one consenting donor, and each was screened using the MAIPA to identify the anti-HPA-15b titre. Those with the highest titres were pooled to make a bulk (coded 18/220) prior to filling 0.5 ml/ampoule into 1896 glass ampoules (size 2.5 ml). Filled material was freeze-dried under conditions established *in house* for the routine lyophilization of WHO serum standards/reference reagents; a summary of the product information is shown in Table 1. The individual donations from which the candidate reference material was prepared and the pooled bulk were found to be negative for HBsAg, anti-HIV1 + 2 and anti-hepatitis C virus (HCV). HCV PCR testing of the pooled bulk was also negative, and no microbial contaminants were detected.

A trial-sized plasma pool containing the same proportions of plasma donations as for the definitive bulk (18/220) and selected single donations used to prepare 18/220 were evaluated by two independent clinical laboratories; both laboratories were able to detect anti-HPA-15b antibodies but could not detect antibodies with any other HPA specificity. Both laboratories also confirmed the presence of anti-HLA class 1 antibodies, and the following specificities were detected: A2, A68, A69, C5, C8 and C15. Furthermore, constituent

TABLE 1 Product summary

Code number	18/220
Presentation	Heat sealed, 2.5-ml glass ampoules
Number available	1830
Date filled	1 March 2019
Mean fill mass ($n = 96$)	0.52 g
Fill mass CV ($n = 96$)	0.65%
Residual moisture by coulometric Karl Fischer titration ($n = 6$)	0.20%
Residual moisture CV ($n = 6$)	24.8%
Mean dry weight ($n = 6$)	0.04 g
Dry weight CV ($n = 6$)	0.44%
Mean oxygen in head space by lighthouse FMS670 ($n = 6$)	0.20%
Oxygen in head space CV ($n = 6$)	44.69%
Storage conditions	−20°C
Address of processing facility and custodian	NIBSC, Potters Bar, UK

Abbreviation: CV, Coefficient of Variation.

TABLE 2 List of participating laboratories

Institute	City, Country
Australian Red Cross Blood Service, Victoria	Melbourne, Australia
Australian Red Cross Blood Service, Queensland	Brisbane, Australia
Hospital Sirio Libanês	São Paulo, Brazil
Canadian Blood Services	Winnipeg, Canada
Institute of Blood Transfusion, Zhejiang Blood Centre	Hangzhou, China
Institute of Blood Transfusion, Guangzhou Blood Centre	Guangzhou, China
French Blood Establishment (EFS), HFNO	Lille, France
French Blood Establishment (EFS), Brittany	Rennes, France
French Blood Establishment (EFS), Aura	Lyon, France
Centre Hospitalier Universitaire de Nantes	Nantes, France
Institut National de la Transfusion Sanguine	Paris, France
Red Cross Blood Transfusion Services, NSTOB	Dessau, Germany
Zentrum für Transfusionsmedizin und Zelltherapie	Berlin, Germany
Institute of Clinical Immunology and Transfusion Medicine	Giessen, Germany
National Blood Centre	Kuala Lumpur, Malaysia
Sanquin Diagnostic Services	Amsterdam, Netherlands
University Hospital of North Norway	Tromsø, Norway
Institute of Haematology and Transfusion Medicine	Warsaw, Poland
Banc de Sang i Teixits	Barcelona, Spain
Karolinska University Hospital	Stockholm, Sweden
University Hospital Geneva	Geneva, Switzerland
The Thai Red Cross Society	Bangkok, Thailand
National Health Service Blood and Transplant	Filton, UK
Welsh Blood Service	Pontyclun, UK
National Institute for Biological Standards and Control	Potters Bar, UK
Versiti	Milwaukee, USA
Bloodworks	Seattle, USA

donations of the definitive pool have been evaluated in previous proficiency testing schemes organized by NIBSC; no other antibody specificities were reported other than anti-HPA-15b and anti-HLA.

Participants

The invitation was distributed to participants of the ISBT Platelet Immunology Workshop and to participants of the HPA antibody detection quality assessment scheme organized by the National External Quality Assessment Site UK (NEQAS). In total, 27 clinical laboratories located across the globe accepted the invitation to participate (see Table 2).

Study design

Four ampoules of the definitive freeze-dried material (coded 18/220) were sent to each laboratory. Participants were asked to reconstitute the material immediately before testing and to titrate at doubling dilutions in one or more assay method(s) routinely used in clinical practice. Participants were required to use a CD109 glycoprotein–specific method such as the MAIPA because the material contains anti-HLA antibodies, which could otherwise cause false-positive results. Clinical laboratories would normally be required to use such a method since patient samples can also contain HLA antibodies. Two ampoules were to be tested, each on a different day, with HPA-15bb and HPA-15aa platelets or similar on both days. Ideally, different platelet donors with the same HPA-15 type were to be used over 2 days. Laboratories were asked to report their interpretation of the results for each test by recording 'positive' or 'negative' for each dilution tested. The endpoint dilution (antibody titre) for 18/220 in each test was assigned as the largest (maximum) dilution, which the laboratory reported to be 'positive.' Laboratories reporting results as 'weak positive' or results that were defined as borderline in 'grey zones' of defined optical density ranges were also deemed negative.

Stability studies

To predict loss in stability over time, accelerated thermal degradation studies were performed at NIBSC using ampoules of lyophilized 18/220 stored at −70, −20, +4, +20, +37 and +45°C for 13 months. Reconstituted material from two ampoules at each temperature was tested in duplicate at a range of twofold serial dilutions in the MAIPA assay with HPA-15bb platelets. Absorbance readings were used to calculate the relative potencies of the accelerated thermal degradation samples by parallel-line analysis using the −70 sample as the reference. Relative potency calculations were performed using the European Directorate for the Quality of Medicines software CombiStats, version 6.0, using a sigmoid curve model and logit transformation of responses. The Arrhenius equation, relating degradation rate to absolute temperature assuming first-order decay [16], was used to predict the degradation rates for each storage temperature.

RESULTS

A total of 27 clinical laboratories accepted the invitation to participate in the study, however, only 25 laboratories returned results. Each laboratory was assigned a code number, which does not reflect the order of listing shown in Table 2. The methods used by participants are summarized in Table 3. All 25 laboratories performed glycoprotein-specific assays, all of which were a version of

TABLE 3 Laboratory method summary

Method	Laboratory code	Number of entries
Rapid MAIPA	3, 4[a], 4a[a,b], 5, 7, 7a[c], 9, 11, 17, 17a[d], 18, 21, 23	13
2-Day MAIPA	1, 10[b], 14, 16	4
In-house MAIPA	2, 6, 12, 13, 15, 19, 20, 22	8
Other	8[b,e], 18a[e]	2

Note: Labs providing supplementary datasets from a second method have an 'a' suffix.
Abbreviation: MAIPA, monoclonal antibody–specific immobilization of platelet antigen.
[a]Frozen platelets.
[b]CD109 antibody other than mAb TEA 2/16.
[c]K562 Recombinant cells used.
[d]Lyophilized platelets.
[e]Modified MAIPA method.

the MAIPA using a monoclonal antibody specific for CD109. Some laboratories provided supplementary datasets from additional platelet donors or from a second method. A full summary of the results from each laboratory and details of their assay protocols are shown in Appendix S1.

Reported endpoint dilutions against HPA-15bb antigen

The majority of laboratories tested two ampoules of the material on two separate occasions, using different donors of HPA-15bb platelets, as described in the study protocol. Laboratory 20 reported results from four different HPA-15bb donors using three of the ampoules provided and laboratory 1 reported results using three different HPA-15bb donors from three ampoules provided. Out of

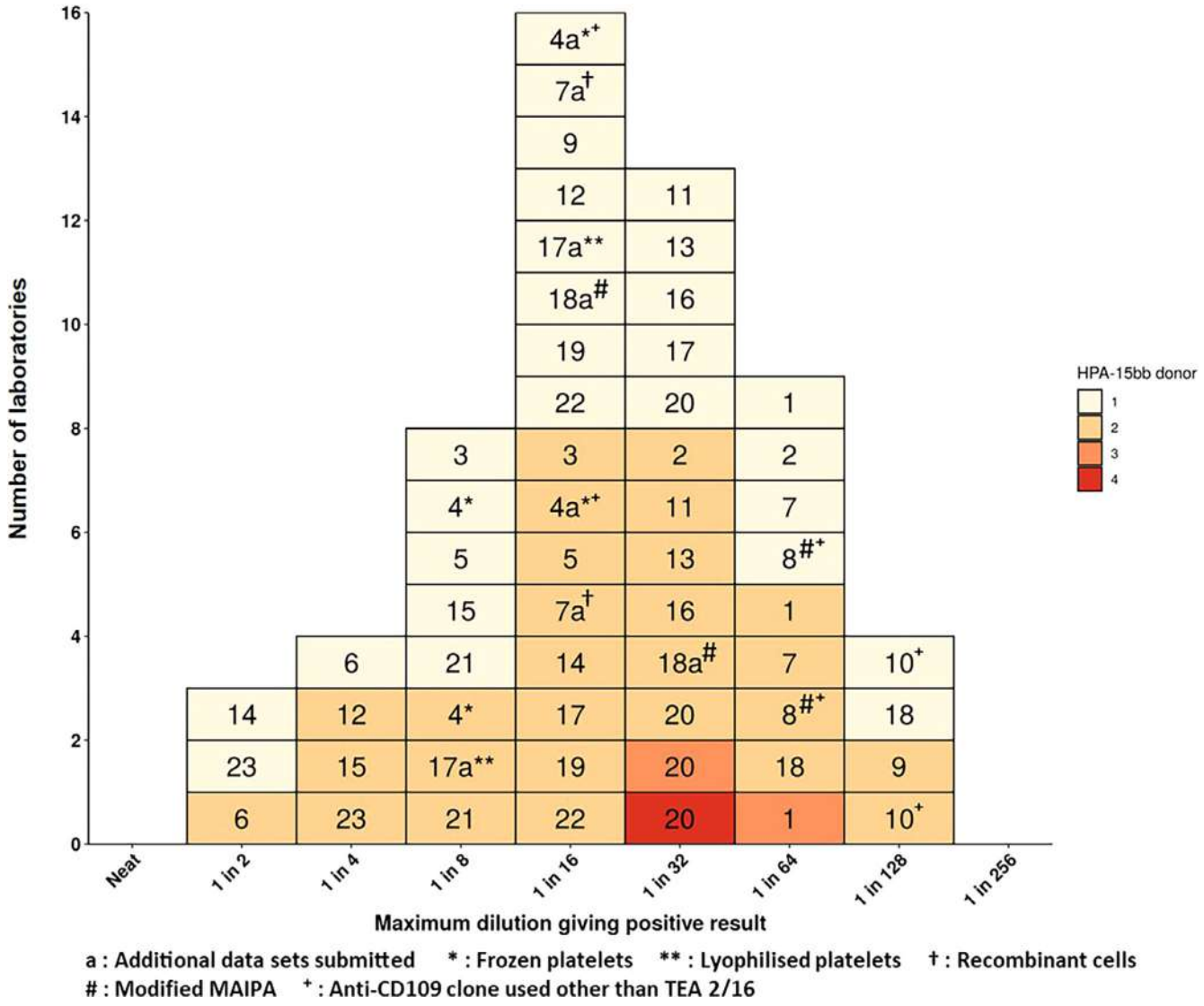

FIGURE 1 Maximum (endpoint) dilutions of anti-HPA-15b reference reagent (18/220) giving a positive result, as reported by international collaborative study participants for HPA-15bb platelets/cells in CD109-specific assays for the detection of anti-HPA antibodies. Laboratory codes are shown in boxes; box colours refer to assay repetitions with different platelet donors except for 7 where the same donor for each repetition was used and 7a where recombinant cells were used

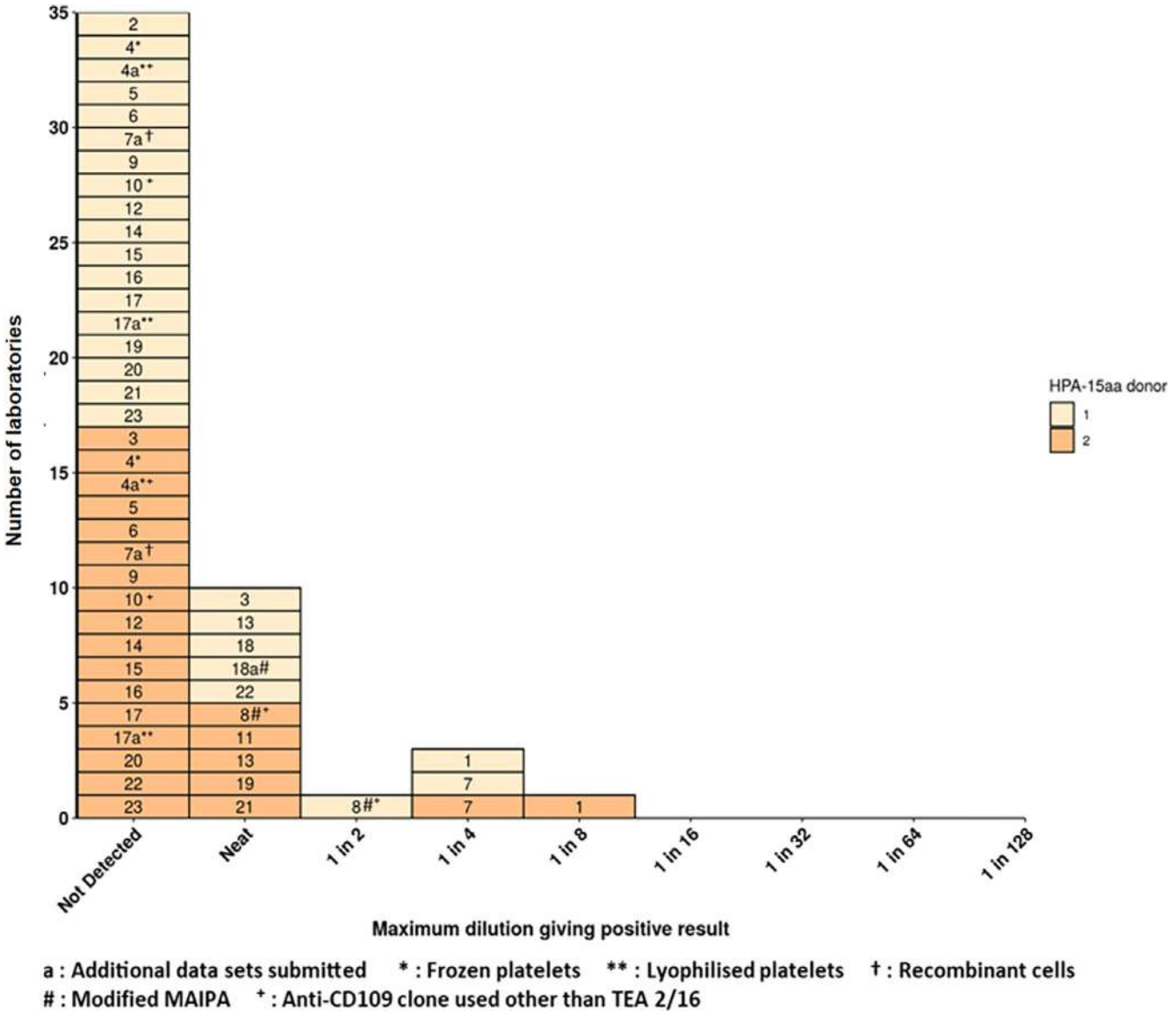

FIGURE 2 Maximum (endpoint) dilutions of anti-HPA-15b reference reagent (18/220) giving a positive result, as reported by international collaborative study participants for HPA-15aa platelets/cells in CD109-specific assays for the detection of anti-HPA antibodies. Laboratory codes are shown in boxes; box colours refer to assay repetitions with different platelet donors except for 4, 4a and 7 where the same donor for each repetition was used and for 7a where recombinant cells were used

25 laboratories, 24 were indeed able to detect antibodies with HPA-15b specificity in preparation 18/220 with all HPA-15bb platelet donors/cells used and in all methods performed. The remaining one laboratory reported a positive result for undiluted material using both HPA-15aa and HPA-15bb platelets but failed to titrate the material, and so endpoint dilutions could not be recorded. Another laboratory which did detect antibodies with HPA-15b specificity failed to titrate the material enough; consequently, an endpoint dilution could not be recorded for this laboratory either. Figure 1 shows the endpoint dilutions reported by 23 laboratories for each method and for each donor of HPA-15bb platelets/cells. The reported endpoint dilutions were normally distributed with a modal dilution of 1 in 16 and a range from 1 in 2 to 1 in 128 as shown in Figure 1. The intra-laboratory variation in reported endpoint dilutions was significant in this study. Several laboratories (13 methods in total) reported different endpoint dilutions for 18/220 in each independent assay using the same method but where different HPA-15bb platelet donors were used. For example, three of the five laboratories that reported poor endpoint dilutions (i.e., 1 in 2 or 1 in 4), only did so for one of two donors, with endpoint dilutions for a second donor of either 1 in 8 or 1 in 16. This difference could be attributed to the levels of CD109 expression on the platelets [13, 14]. The remaining two of the five laboratories reported 1 in 2 or 1 in 4 for both donors used.

The majority of laboratories used CD109-specific, mouse monoclonal antibody TEA 2/16 from BD Pharmingen. There was no clear trend in reported endpoint dilutions for 18/220 relating to the different CD109-specific monoclonal antibodies used as shown in Figure 1.

TABLE 4 Accelerated degradation studies

Storage temperature (°C)	Relative potency (−70°C reference, $n = 4$)	95% lower confidence limit	95% upper confidence limit
−20	1.00	0.92	1.09
+4	1.28	1.18	1.40
+20	1.13	1.04	1.23
+37	0.98	0.90	1.07
+45	0.72	0.66	0.79

Note: Ampoules stored for 13 months at each temperature.

As expected, most laboratories (22/23) used fresh HPA-15bb platelets. Laboratory 4 used only frozen platelets characterized as having high CD109 expression levels prior to freezing and achieved an endpoint dilution in a MAIPA of 1 in 8 for 18/220 using the anti-CD109 clone TEA 2/16 and an endpoint dilution of 1 in 16 using the anti-CD109 clone HU17. Laboratory 17 compared lyophilized and fresh platelets in the MAIPA, with comparable endpoint dilutions being reported for both types (1 in 8 and 1 in 16 for two donors of lyophilized platelets and 1 in 16 and 1 in 32 for two donors of fresh platelets). Laboratory 7a used recombinant K562 cells with a reported endpoint dilution of 1 in 16.

Participants were asked to provide technical information on their protocols; this is summarized in Appendix S1. Indeed, there was considerable variation among the MAIPA methods performed, the most notable differences (further to those described earlier) being the number of platelets and sample volume used. Platelet numbers ranged from 0.4×10^6 to 300×10^6 per well and sample volumes ranged from 20 to 120 μl per well.

Reported endpoint dilutions against HPA-15aa antigen

To confirm the specificity of the HPA-15 antibodies within the candidate material, laboratories were also asked to test 18/220 with HPA-15aa platelets from two different donors where possible at doubling dilutions and to report the endpoint dilution; these results are shown in Figure 2. There were only two laboratories that reported results from just one HPA-15aa donor. In summary, as expected, nearly all laboratories reported that for all HPA-15aa platelet donors (or alike) used with each method performed; they were unable to detect HPA-15a antibodies or reported a positive result only for the undiluted (neat) material. However, three labs did report endpoint dilutions of 1 in 2, 1 in 4 or 1 in 8 with one or more donors. This could be explained by the presence of anti-HLA antibodies at high serum concentrations and incomplete solubilization of the platelet membrane during the MAIPA procedure. Indeed, of both laboratories which reported endpoint dilutions of 1 in 4 or 1 in 8, each did so for different HPA-15aa platelet donors suggesting that it is not a rare donor-specific phenomenon but rather a procedural effect. One laboratory reported an endpoint dilution of 1 in 32 (result not shown in Figure 2); this appears to be anomalous in comparison with the results from all other laboratories. The anomaly could have arisen from the incorrect typing of the alleged HPA-15aa platelets or may be attributed to the fact that these platelets were 9 days old. Indeed, the same laboratory reported 'neat' as the endpoint for a second donor of HPA-15aa platelets which were only 6 days old. While some laboratories have reported a positive result with HPA-15aa platelets at high concentrations of 18/220, there still remains a clear distinction between reported endpoint dilutions with HPA-15bb and HPA-15aa types.

Stability

Estimates of the potency of 18/220 stored at elevated temperatures for a period of 13 months relative to 18/220 stored at −70°C for 13 months are summarized in Table 4. Tests for non-parallelism and non-linearity in the parallel-line analysis to estimate relative potencies were not statistically significant ($p = 0.14$ and 0.59, respectively). There was insufficient degradation at the elevated temperatures, even after 13 months of storage, to fit the Arrhenius model, with only a clear relative potency loss at +45°C. This indicates that 18/220 will be stable for long-term storage at −20°C and sufficiently stable to allow for shipment of ampoules at ambient temperature.

DISCUSSION

The aim of the study was to prepare and evaluate a stable, reference reagent for anti-HPA-15b detection that clinical laboratories can use to assess and validate the sensitivity of their routine assays. The collaborative study has shown that candidate preparation 18/220 contains anti-HPA-15b antibody that could be detected at a dilution of 1 in 8 by 21 of 23 laboratories with at least one donor. Setting the minimum potency at 1 in 8 dilution removes ambiguity resulting from positivity with HPA-15aa platelets or alike at lower dilutions. The results of the study in general show good consistency across most laboratories but also indicate that some laboratories should consider further improving the sensitivity of their assay method. Since the material contains an anti-HLA component (as may any patient clinical sample), it should only be used in techniques that are glycoprotein-specific (i.e., for CD109) such as the 'gold standard' MAIPA which all laboratories used in this study or where it can be ensured that the anti-HLA antibodies will not cause a false-positive reaction (i.e., through chloroquine treatment of platelets to remove HLA-class 1 epitopes).

A report of the 19th ISBT Platelet Immunology Workshop gave a comprehensive summary of the variations in the MAIPA procedure used by workshop participants and concluded that the MAIPA is far from harmonized which may contribute to variations in results [15]. While our study also showed a lack of inter-laboratory harmonization of the CD109 MAIPA procedure, we were also able to demonstrate

notable intra-assay variability in reported endpoint dilutions that are likely due to the differential levels in CD109 expression by platelets from different donors, as previously reported [14]. Therefore, the harmonization of test methods using optimized assay conditions may well improve the sensitivity of testing across clinical laboratories to some degree but cannot overcome test variability as a result of donor–donor differences. The use of the anti-HPA-15b reference reagent will at least provide some guarantee as to the sensitivity of the platelets used.

All participants of the international collaborative study were invited to comment on the final study report, and their approval for use of 18/220 as a reference reagent for human IgG antibodies against HPA-15b with a minimum potency of 1 in 8 was sought. No objections were received from the participants, and, in addition, this proposal was endorsed by the ISBT Working Party on Platelet Immunology. In October 2020, all data were reviewed by the WHO Expert Committee on Biological Standardization, and 18/220 was approved for use as a first WHO anti-HPA-15b reference reagent. This material should be used at a dilution of 1 in 8 for assay validation (i.e., the minimum potency which should test positive) and may be used to qualify 'in-house' controls. It is hoped that wide use of this reference reagent will improve the sensitivity of test methods giving more confidence to the results generated.

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G.S. and L.S. were responsible for the generation of the reference material, internal evaluation of the material, running the collaborative study and report and manuscript preparation; A.P. contributed significantly to pre-collaborative study experimental work.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ORCID

Lucy Studholme https://orcid.org/0000-0002-2935-2048

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SUPPORTING INFORMATION

Additional supporting information may 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

Estimation of Lewis-negative alleles by high-resolution melting analysis of three tag SNPs of *FUT3*

Mikiko Soejima | **Yoshiro Koda**

Department of Forensic Medicine, Kurume University School of Medicine, Kurume, Japan

Correspondence
Yoshiro Koda, Department of Forensic Medicine, Kurume University School of Medicine, Kurume 830-0011, Japan.
Email: ykoda@med.kurume-u.ac.jp

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Abstract

Background and Objectives: The expression of type 1 chain Lewis blood group antigens is regulated by secretor-type α(1,2)fucosyltransferase, encoded by *FUT2*, and Lewis α(1,3/1,4)fucosyltransferase, encoded by *FUT3*. Accumulating evidence has linked Lewis phenotypes or genotypes to various clinical conditions. Thus, in addition to *FUT2*, large-scale *FUT3* genotyping is important. Because *FUT3* has two paralogous genes (*FUT5* and *FUT6*) with high DNA sequence similarity, we should select the polymerase chain reaction (PCR) primers carefully for *FUT3* genotyping. Previously, we suggested that 13G>A (rs28362458), 59T>G (rs28362459) and 202T>C (rs812936) could be selected as tag single nucleotide polymorphisms (SNPs) for detection of Lewis-negative alleles (*le*).

Materials and Methods: In this study, three high-resolution melting (HRM) analyses for genotyping these SNPs were developed and applied for 140 Japanese, eight Ghanaians and four Sinhalese subjects.

Results: Each of three genotypes of 13G>A (G/G, G/A, A/A), 59T>G (T/T, T/G, G/G) and 202T>C (T/T, T/C, C/C) was discriminated clearly. Although we need to be careful in interpretation of results due to SNPs other than the 59T>G in the amplicon, the results of 59T>G genotyping were in full agreement with the results by a previous PCR-restriction fragment length polymorphism analysis in 140 Japanese. In addition, three heterozygotes of 202C substitution were identified, and no one having a 13A substitution was found in 140 Japanese.

Conclusion: The present HRM analyses are useful and reliable methods for large-scale estimation of *le* alleles.

KEYWORDS
FUT3, high-resolution melting, Lewis-negative allele, rs28362458, rs28362459, rs812936

INTRODUCTION

Type 1 chain Lewis blood group antigens are composed of Lewis a (Le^a) and Lewis b (Le^b) antigens and belong to the ABH blood group–related antigens. The expression of these antigens is regulated by secretor-type α(1,2)fucosyltransferase (Se enzyme), encoded by *FUT2*, and Lewis α(1,3/1,4)fucosyltransferase (Lewis enzyme), encoded by *FUT3* [1]. Functional *FUT2* and *FUT3* alleles (*Se* and *Le*) are dominant over non-functional alleles (*se* and *le*). Accordingly, individuals who lack the Lewis enzyme (*le/le*) have Le (a-b-) red cells irrespective of secretor status, while among individuals who have the active Lewis enzyme (*Le/Le* or *Le/le*), red cells of secretors (*Se/Se* or *Se/se*) are Le(a-b+), those of non-secretors (*se/se*) are Le(a+b-) and those of weak secretors are Le(a+b+) [2, 3]. However, since the determination

of secretor status by traditional serological Lewis phenotyping is difficult [3], a reliable method of genotyping of *FUT2* and *FUT3* is important for estimation of the Lewis phenotype as an alternative to phenotyping. Previous studies suggested that the expression of Lewis blood group antigens is associated with *Helicobacter pylori* infection, ischemic heart disease, ulcerative colitis, ankylosing spondylitis and obesity [4–8].

According to previous reports, a 1000-genome browser (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/) and Erythrogene v0.8 (27 November 2017) (http://www.erythrogene.com/) [9, 10], four single nucleotide polymorphisms (SNPs), 202T>C (rs812936), 484G>A (Trp68Arg, rs28362463), 508G>A (Gly170Ser, rs3745635) and 1067T>A (Ile356Lys, rs3894326) are SNPs that frequently inactivate Lewis enzyme. They often accompany other SNPs respectively, and many *le* alleles have been reported [11–14]. 484G>A is in almost absolute linkage disequilibrium (a complete dependence of allele distribution, e.g., there are G–G and A–A combinations but not G–A and A–G combinations at the positions of 13 and 484) with 13G>A (Gly5Ser, rs28362458) and in complete linkage disequilibrium (there are three combinations of two SNPs, e.g., there are G–G, G–A and A–A combinations but not A–G combination at the positions of 13 or 484 and 667) with 667G>A (Gly223Arg, rs144569478), while 508G>A and 1067T>A are in almost complete linkage disequilibrium with 59T>G (Leu20Arg, rs28362459), and the 59G allele without 508A or 1067A is quite rare (Table 1). In addition, the 508A or 1067A allele without 59G is also rare. Accordingly, we suggested that three tag SNPs, 13G>A, 59T>G and 202T>C, are useful for estimation of *le* alleles in many populations in a previous study [14]. On the other hand, *FUT3* has two paralogous genes, *FUT5* and *FUT6*, with high sequence similarity [15]. Thus, we need to design polymerase chain reaction (PCR) primers to amplify *FUT3* specifically.

High-resolution melting (HRM) analysis is a simple and relatively high-throughput method for the detection of variations such as SNPs, small insertion/deletion in PCR amplicons. This method does not require post-PCR processing and is based on detecting subtle differences in the melting curve and melting temperature of short PCR amplicons, using saturated fluorescent dyes [16]. In the present study, three HRM analyses for detection, each of 13G>A, 59T>G and 202T>C, were developed.

MATERIALS AND METHODS

DNA samples

The study protocol was approved by the Ethical Committee of Kurume University (Bioethics approval No. 342).

We used genomic DNAs from 140 Japanese whose genotypes of three SNPs (59T>G, 508G>A and 1067T>A of *FUT3*) had been already determined by PCR-restriction fragment length polymorphism (PCR-RFLP) [17]. We also used eight Ghanaians (in Ghana) and fours Sinhalese (in Sri Lanka) whose *FUT3* haplotypes had been already determined by DNA sequencing of PCR amplicons [14, 18]. The 202T>C polymorphism of selected Japanese samples was determined by Sanger sequencing methods as described previously [14].

PCR and HRM conditions

Primers for amplification of only *FUT3*, but not paralogous genes *FUT5* and *FUT6*, were designed by Primer3 software (https://bioinfo.ut.ee/primer3-0.4.0/, [19]). Primer sequences for detection of 13G>A, 59T>G and 202T>C and amplicon length are indicated in Table 2. Real-time PCR and HRM analyses were performed in a single run on a LightCycler 480 instrument II and gene scanning software (Roche Diagnostics, Tokyo, Japan) as described previously with a slight modification [20]. The reaction was performed in a mixture containing 2–20 ng of genomic DNA, 5 μl of Premix Ex Taq (Probe qPCR) (Takara, Tokyo, Japan), 125 nM of each primer and 0.1 μl of LightCycler 480 ResoLight Dye (Roche Diagnostics, Tokyo, Japan) with PCR-grade water adjusted to a final volume of 10 μl. The temperature conditions of all three analyses are identical and as follows: initial denaturation and enzyme activation at 95°C for 30 s, followed by 45 cycles of denaturation at 95°C for 5 s and annealing/extension

TABLE 1 Major non-functional alleles of *FUT3* and their tag single nucleotide polymorphism (SNP) and distributions

		Frequency (%)				
Allele	Tag SNP	Africa	America	E. Asia	Europe	S. Asia
$le^{202,314}$	202	7.7	12.0	3.1	16.2	11.2
$le^{47,202,314}$	202	1.1	1.4	0	1.3	1.1
$le^{13,484,667}$	13	8.7	0.9	0	0	0
$le^{13,484,667,807}$	13	3.8	0.4	0	0	0
$le^{59,1067}$	59	3.9	4.2	13.5	6.2	16.0
$le^{59,508}$	59	18.9	19.9	13.6	1.5	5.4
$le^{59,508,858}$	59	3.7	0.1	0.2	0	0.1
$le^{59,61,508}$	59	3.1	1.3	0	0	0

Note: Allele frequencies were obtained from Erythrogene. Allele frequency of 1% or more in global population was indicated.
Abbreviations: E. Asia, East Asia; S. Asia, South Asia.

TABLE 2 Primer for detection of single nucleotide polymorphisms (SNPs)

Primer sequences	Position of *FUT3*	Differences with *FUT5*	Differences with *FUT6*	Amplicon length
Detection of 13G>A				
Forward primer: 5′-CCTCTCTCCTCTCTTCCCAGA-3′	−32 to −12 bp	1	2	63 bp
Reverse primer: 5′-ATTGTGGCTTGGCTGCAC-3′	14 to 31 bp	3	4	
Detection of 59T>G				
Forward primer: 5′-GTGCAGCCAAGCCACAAT-3′	14 to 31 bp	3	4	68 bp
Reverse primer: 5′-AGCCACCAGCAGCTGAAATA −3′	62 to 81 bp	1	2	
Detection of 202T>C				
Forward primer: 5′-CCACCCTCCTGATCCTGCTA-3′	182 to 201 bp	2	3	50 bp
Reverse primer: 5′-AGCCACAGGGATGTGGAA-3′	214 to 231 bp	5	7	

Note: 'Differences with *FUT5* (or *FUT6*)' indicate the number of base differences in each of the primer with corresponding *FUT5* and *FUT6* sequences.

TABLE 3 Relationships of nucleotide positions, used DNA samples and belonging groups by high-resolution melting (HRM) analysis for 59T>G

	47	59	61	Group
Ghanaian 1	G/G	T/T	C/C	I
Ghanaian 2	G/G	T/G	C/C	II
Ghanaian 3	G/G	G/G	C/C	III
Ghanaian 4	G/G	T/G	C/T	IV
Ghanaian 5	G/G	G/G	C/T	II
Ghanaian 6	G/G	G/G	T/T	I
Sinhalese 1	C/G	T/T	C/C	V
DNA is not available	C/G	T/G	C/C	
DNA is not available	C/C	T/T	C/C	
DNA is not available	C/G	T/G	C/T	

at 60°C for 20 s. Before HRM analysis, the samples were heated to 95°C for 1 min and rapidly cooled to 60°C for 1 min, and fluorescence data for HRM analysis were collected over the range from 72 to 92°C, increasing at 0.02°C/s with 25 acquisitions/s. The raw melting curve data were normalized by manual adjustment of linear regions pre- and post-melt signals of all samples. Temperature shift correction for all analyses to clearly discriminate wild-type and mutated allele homozygotes was not performed.

RESULTS

59T>G genotyping

According to the 1000 Genome Project and Erythrogene databases [10], two other SNPs, 47G>C (Cys16Ser, rs145362171) and 61C>T (synonymous SNP, rs28362460), in addition to 59T>G, were present within the short amplicon sequence (68 bp, Table 2) with a frequency of more than 1%. The SNP 47G>C seems to be present in many

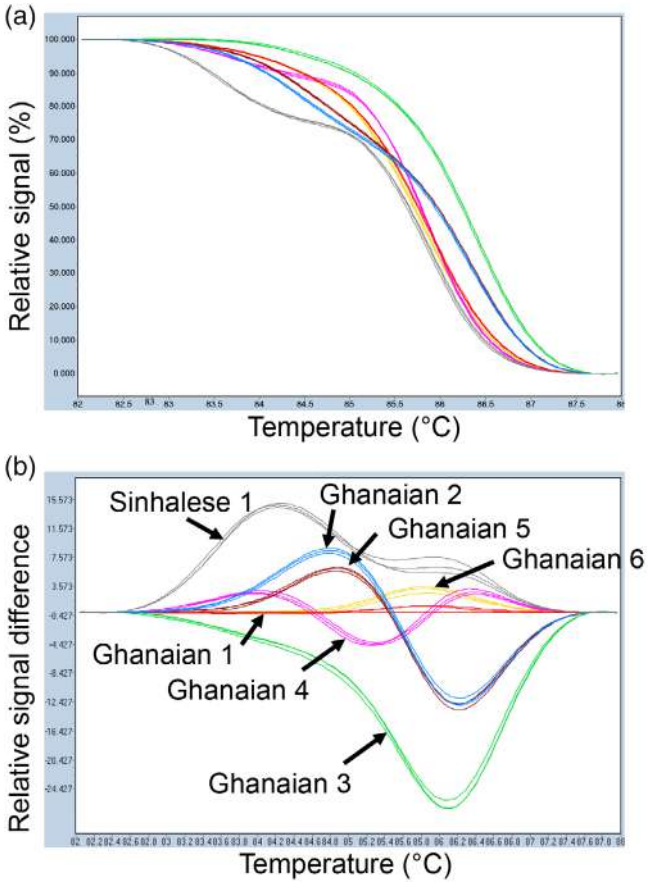

FIGURE 1 Normalized melting curves (a) and difference plot curves (b) for genotyping of 59T>G obtained from six selected Ghanaians and a Sinhalese (Ghanaian 1: red, Ghanaian 2: blue, Ghanaian 3: green, Ghanaian 4: pink, Ghanaian 5: brown and Ghanaian 6: yellow, Sinhalese 1: grey, see Table 2). High-resolution melting (HRM) analysis separated seven individuals into five groups. Individuals having 47G/G, 59T/T, 61C/C (Ghanaian 1) and 47G/G, 59G/G, 61T/T (Ghanaian 6) and individuals having 47G/G, 59T/G, 61C/C (Ghanaian 2) and 47G/G, 59G/G, 61C/T (Ghanaian 5) were not separated [Colour figure can be viewed at wileyonlinelibrary.com]

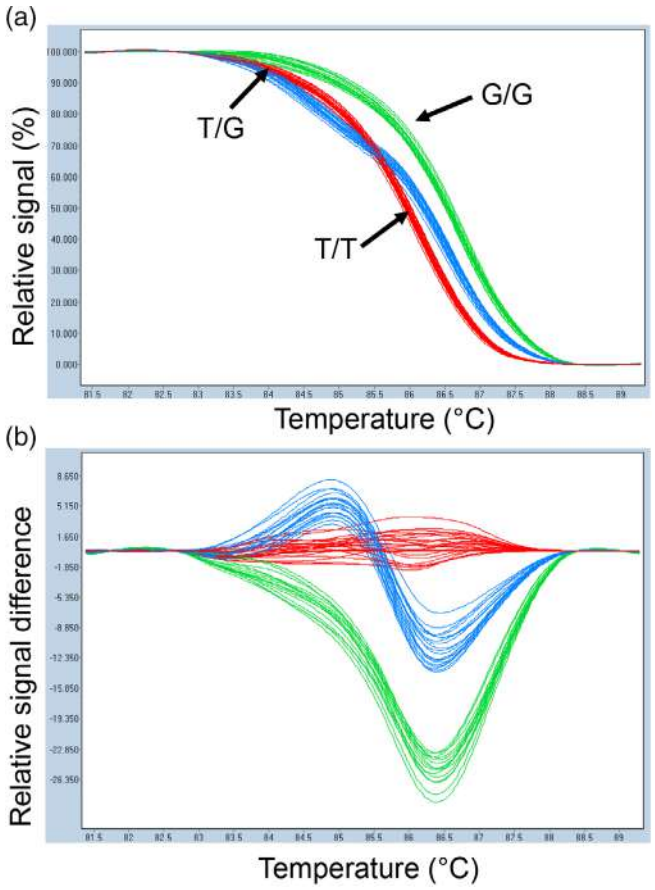

FIGURE 2 Normalized melting curves (a) and difference plot curves (b) for genotyping of 59T>G obtained from 60 to 140 randomly selected Japanese. The individuals having genotypes of T/T (red), T/G (blue) and G/G (green) at 59T>G were completely separated [Colour figure can be viewed at wileyonlinelibrary.com]

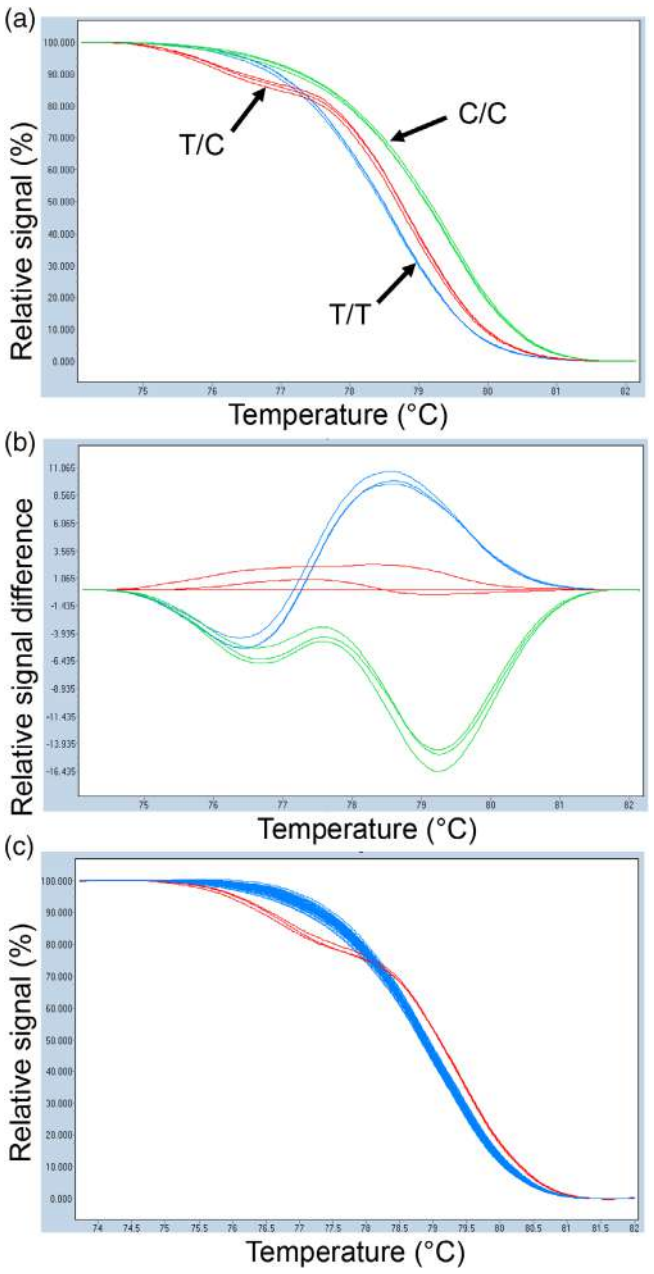

FIGURE 3 Normalized melting curves (a) and difference plot curves (b) for genotyping of 202T>C obtained from three selected Sinhalese. Normalized melting curves (c) for genotyping of 202T>C obtained from 60 to 140 randomly selected Japanese. The individuals having genotypes of T/T(blue), T/C (red) and C/C (green) at 202T>C were completely separated [Colour figure can be viewed at wileyonlinelibrary.com]

populations but not East Asians, while 61C>T seems to be specific to Africans and Americans (probably African origin). The 47C allele is in complete linkage disequilibrium with the 59T allele, while the 61C allele is in complete linkage disequilibrium with the 59G allele. Therefore, 10 genotypes by combinations of three sites (haplotypes) are expected. However, we did not have DNA samples with 3 of 10 haplotypes (see Table 3); HRM analysis was able to be performed only in seven samples. As shown in Figure 1(a, b), HRM analysis separated seven individuals into five groups. Group I included Ghanaian 1 and Ghanaian 6, group II included Ghanaian 2 and Ghanaian 5, group III included Ghanaian 3, group IV included Ghanaian 4 and group V included Sinhalese 1.

We then analysed 140 Japanese whose genotypes of 59T>G, 508G>A and 1067T>A of *FUT3* were previously determined by PCR-RFLP [17]. Figure 2(a, b) show results of normalized melting curves and difference plots of 60 of 140 Japanese subjects. Three genotypes of 59T>G could be separated clearly (T/T = 55, T/G = 63, G/G = 22). The present results were completely in agreement with previous PCR-RFLP results [17]. Thus, the HRM analysis for 59T>G seems to be valid and reliable, especially in a population without 47G>C and 61C>T such as East Asians. We confirmed the repeatability of the method by two independent HRM analyses.

202T>C genotyping

We next performed the HRM analysis for 202T>C using a 50-bp amplicon (Table 2) on three Sinhalese having genotypes of T/T

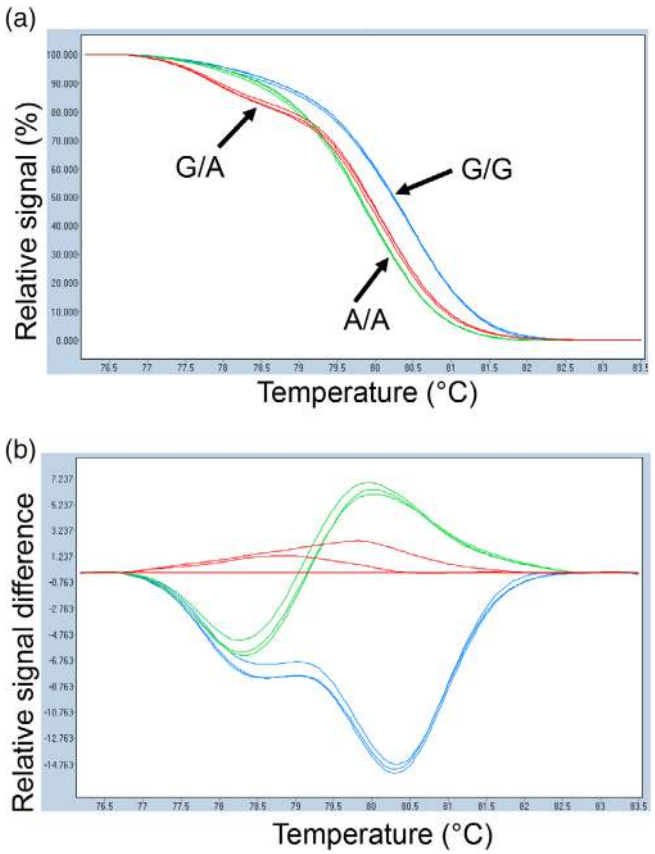

FIGURE 4 Normalized melting curves (a) and difference plot curves (b) for genotyping of 13G>A obtained from three selected Ghanaians. The individuals having genotypes of G/G (blue), G/A (red) and A/A (green) at 13G>A were completely separated [Colour figure can be viewed at wileyonlinelibrary.com]

(wild type), T/C and C/C at 202T>C. No SNP with a frequency of more than 1% seems to be present within this amplicon according to the database. As shown in Figure 3(a, b), we clearly distinguished the three genotypes from each other.

Although we have never examined this polymorphism in 140 Japanese subjects, the 1000 Genome Project and Erythrogene databases indicated that this SNP is present in East Asian populations including Japanese with a frequency about 1%–6% [9, 10]. Therefore, we then analysed 140 Japanese and found three heterozygotes of 202T>C (Figure 3(c)) by two independent HRM analyses. In addition, we demonstrated this SNP in these three individuals by Sanger sequencing of the PCR products of coding region of *FUT3*. Accordingly, the frequency of 202C is estimated to be 1.07% in our Japanese samples.

13G>A genotyping

Finally, we performed the HRM analysis for 13G>A using a 63-bp amplicon (Table 2) on three Ghanaians having genotypes of G/G (wild type), G/A and A/A at 13G>A. As well as the 202T>C amplicon, no SNP with a frequency more than 1% seems to be present within this amplicon. As shown in Figure 4(a, b), we clearly distinguished three genotypes from each other by two independent assays. We then analysed 140 Japanese and did not find any individuals containing the 13G>A polymorphism, as expected from the databases (data not shown).

DISCUSSION

Accumulating evidence suggested that *FUT3* polymorphisms (Lewis blood group status) seemed to be associated with various disease [4–8]. Large-scale replication studies are desirable to confirm these associations, and thus, a reliable method of genotyping *FUT3* is required. The findings of this study achieved with the application of the HRM method to validate FUT3 SNPs are in agreement with those of other previous studies such as PCR-RFLP, PCR using sequence-specific primers (PCR-SSP), an allele-specific oligonucleotide hybridization method, hydrolysis probe-based real-time PCR (TaqMan) assay, nucleotide sequencing of PCR products and a multiplex SNaPshot assay [6, 14, 21–23]. Compared with other methods, the HRM analysis does not require post-PCR processing as a TaqMan assay does. In addition, this method is simple, cost-effective, accurate, high-throughput and faster than the conventional PCR.

As mentioned earlier, PCR primers for the detection of SNPs of *FUT3* should be selected to avoid amplification of two paralogous genes, *FUT5* and *FUT6*. Because all three HRM analyses could distinguish three genotypes of *FUT3*, primers using the present three HRM analyses seemed to amplify *FUT3* sequence specifically.

The presence of SNPs other than the target SNP in the amplified region is likely to affect the melting curve profiles of amplicons obtained by HRM analysis. Thus, we investigated whether the three amplicons contain SNPs with a high frequency or not by searching the 1000 Genome Browser and found two SNPs (47G>C and 61C>T) with the frequency of more than 1% in the amplicon only for 59T>G detection. The SNP 47G>C was found in many populations (1.2%–1.5% and 1.02% in global populations) except East Asians, and 61C>T was found in African and Americans (4.24% in Africans, 1.30% in Americans and 1.30% in global populations). 47C was in complete linkage disequilibrium with 202C and in almost complete linkage disequilibrium with 314T, while a synonymous SNP, 61T, was in complete linkage disequilibrium with both 59G and 508A. However, there is no East Asian having these two SNPs in the 1000 Genomes Project database.

Although these SNPs, particularly 61C>T, have an impact on the melting curve profiles in the HRM analysis for detection of 59T>G, the influence of these SNPs seems to be limited even in large-scale association studies in an East Asian population considering their allele frequencies. In addition, a reliable estimate of *le* could be made by combining HRM analyses for 202T>C and 59T>G in European subjects because 47C was in complete linkage disequilibrium with 202C. Thus, for large-scale association studies of *FUT3*, examination of 59T>G and 202T>C might be sufficient for East Asian and European populations. On the other hand, in addition to 59T>G and 202T>C,

we should consider 13G>A, which contains the second highest *le* allele in African populations, with a frequency of 18.4% (and in American populations with a frequency of 1.7%).

In conclusion, the present HRM analyses for 59T>G and 202T>C are valid and feasible for association studies of *FUT3*, particularly in East Asian populations. Addition of HRM analysis for 13G>A to those for 59T>G and 202T>C is desirable for a more correct estimation of the *le* alleles in African and American populations, although the presence of the 61T>C polymorphism slightly complicates the results of HRM analysis for 59T>G genotyping.

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M.S. investigated and wrote the original draft; Y.K. supervised the methodology, validated the results and reviewed and edited the manuscript.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

ORCID

Mikiko Soejima https://orcid.org/0000-0001-9721-5881
Yoshiro Koda https://orcid.org/0000-0003-1368-0116

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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

Using blood services platforms to facilitate COVID-19 vaccination programs

Implementing an effective coronavirus disease-19 (COVID-19) vaccination program presented several challenges, including approaches to convince people to get vaccinated, setup of convenient vaccination locations, building and operating the logistics involved in the cold-chain needed standards and the optimal use of thawed vaccines to prevent wastage.

Most vaccination plans prioritized first responders and other medical teams, followed by the at-risk elderly people. Currently, a significant number of younger working individuals, initially not eligible for vaccination, show hesitancy to get the vaccine, expressing doubts regarding its safety and efficacy, fear of serious side effects and reluctance to invest time and efforts, even when the vaccine is offered 'for free.' While the trade-off of their health is important, the public has already adjusted to live with the pandemic. An 'out-of-the-box' approach can be useful to overcome such issues.

Blood services worldwide use existing platforms for blood drives operation in easily accessible locations, including working places, educational institutions and social or religious gathering places (churches, synagogues or mosques). The blood services' personnel involved in blood drives organization is familiar with the leading figures in the communities, who are known to positively influence the public to donate blood and can be easily recruited to support the vaccination programs. Chief executive officers hosting a vaccination event 'at the expense of the employer' where employees can get vaccinated at work may be viewed as a tribute to the employees, both by them, by the workers' union organizations and by the general population, as participating in an important life-saving project.

Additional numerous factors that affect the success of a blood drive, such as accessibility, location and time, approval of family members, friends, managers and colleagues, may be used for the vaccination program.

Organizing 'vaccination drives' in these convenient, familiar locations may provide a possible solution. Moreover, blood drives sites can be easily adapted as suitable vaccination locations, since the setup for both operations is similar, including proper sites for assessment of individuals' eligibility before vaccination, the actual procedure and the post-vaccination rest period needed.

Since March 2020, Magen David Adom (MDA) is Israel's National Blood Bank and Blood Services organization in addition to being the national Red Cross organization and Emergency Medical Services organization. MDA participated in the national effort to mitigate the COVID-19 pandemic in the 9.33 million population, through more than 4.5 million swabs collection for polymerase chain reaction testing and by providing over 1 million vaccines, carefully matching expected number of individuals and vaccines needed, to prevent wastage of precious resources. This includes successful vaccination events in workplaces, cooperating with the Manufacturers Association and Unions. A small survey showed that employees desired to be vaccinated at work (scale 6.2/7). MDA Blood Services' experience in operating mobile blood drives was utilized to conduct an efficacious program nationwide.

CONFLICT OF INTEREST

There are no conflicts of interests relevant to this letter for all authors.

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Eli Jaffe PhD[1]
Itamar Abramovich MA[1]
Roman Sonkin MD[1]
Eilat Shinar MD[2]

[1]*Community Division, Magen David Adom, Or-Yehuda, Israel*
[2]*Blood Services, Magen David Adom, Or-Yehuda, Israel*

Correspondence
Roman Sonkin, Community Division, Magen David Adom, Ha-Plada 5, Or-Yehuda, 6021805 Israel.
Email: romans@mda.org.il

ORCID

Eli Jaffe https://orcid.org/0000-0002-8380-0219
Roman Sonkin https://orcid.org/0000-0001-8662-3273
Eilat Shinar https://orcid.org/0000-0002-5658-248X

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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

The association of B blood group with SARS-CoV-2-induced death in Babol, north of Iran

Relationship between blood types and incidence and fatality of coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2, has not been consistent in the studies performed around the world [1–5]. Here, we investigate the association between ABO and RhD blood types with incidence and fatality of COVID-19 in the ethnically homogenous population of Babol, north of Iran. The ABO and RhD distributions of 955 RT-PCR-confirmed COVID-19 patients, hospitalized between 20 March and 21 September of the year 2020 at the affiliated hospitals of Babol university of medical sciences, and 639 local COVID-19 convalescent plasma (CCP) donors were compared with 618 randomly selected pre-COVID-19 era hospitalized patients and 19,668 pre-COVID-19 era local healthy blood donors. Blood groups' frequencies were compared using IBM SPSS Statistics, Version 23.0 (Armonk, NY) via chi-square and logistic regression tests. In our study region, blood type B individuals are more frequent than group A (Table 1), in contrast to the most other parts of the world [2–5]. Blood type B was associated with highest odds of testing positive for COVID-19 compared to the local healthy blood donors (OR = 1.142, CI = 0.99–1.32, $p = 0.06$). Further, B type was significantly higher in the deceased COVID-19 patients compared to the both non-COVID-19 patients and local healthy blood donors ($p = 0.048$ and $p = 0.028$, respectively). In agreement, B-type individuals were significantly lower in the CCP donors compared to the local healthy blood donors ($p = 0.03$). This reduction of blood type B in the CCP donors could also be attributed to more men than women in the deceased group and male sex of all CCP donors. No significant changes in the prevalence of RhD were found between the different groups analysed.

In conclusion, these results propose that blood type B may possibly be connected to SARS-CoV-2-induced fatality in homogeneous population of Babol, north of Iran.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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Saeed Hassani[1,2]
Narges Kalantari[3]
Seyedeh Farzaneh Jalali[1]
Mahmood Shams[1]
Zahra Ahmadnia[4]
Meysam Aghajani Daroonkola[1]
Masomeh Bayani[5]
Housein Ghorbani[4]

[1]*Department of Medical Laboratory Sciences, Faculty of Paramedical Sciences, Babol University of Medical Sciences, Babol, Iran*
[2]*Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Arak University of Medical Sciences, Arak, Iran*

TABLE 1 ABO and RhD distributions among COVID-19 patients, COVID-19 convalescent plasma (CCP) donors and controls in the north of Iran, Babol

	Number	Male	Female	O (%)	A (%)	B (%)	AB (%)	RhD^+ (%)
COVID-19 patients	955	471 (49.3)	484 (50.7)	371 (38.8)	227 (23.8)	295 (30.9)[a]	62 (6.5)	877 (91.8)
Deceased COVID-19 patients	124	70 (56.5)	54 (43.5)	45 (36.3)	25 (20.2)	46 (37.1)[b]	8 (6.5)	109 (87.9)
CCP donors	639	639 (100)	0 (0)	254 (39.7)	176 (27.5)	154 (24.1)[c]	55 (8.6)	594 (93.0)
Non-COVID-19 patients	618	247 (40)	371 (60)	242 (39.2)	164 (26.5)	174 (28.2)	38 (6.1)	565 (91.4)
Healthy blood donors	19,668	18,681 (95)	987 (5)	7773 (39.5)	5028 (25.6)	5531 (28.1)	1336 (6.8)	17,845 (90.7)

[a]COVID-19 patients versus healthy blood donors: OR = 1.142, CI = 0.99–1.32, $p = 0.06$.
[b]Deceased COVID-19 patients versus non-COVID-19 patients and healthy blood donors: OR = 1.51, CI = 1.004–2.255, $p = 0.048$ and OR = 1.507, CI = 1.046–2.173, $p = 0.028$, respectively.
[c]CCP donors versus healthy blood donors: OR = 1.23, CI = 1.025–1.481, $p = 0.03$.

[3]*Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran*
[4]*Pathology Department, Babol University of Medical Sciences, Babol, Iran*
[5]*Infectious Diseases and Tropical Medicine Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran*

Correspondence
Saeed Hassani, Department of Laboratory Sciences, Faculty of Paramedical Sciences, Babol University of Medical Sciences, Ganj-Afroz Ave. Babol, Mazandaran, Iran.
Email: saeedhassaniz@yahoo.com; s.hassani@mubabol.ac.ir

ORCID

Saeed Hassani https://orcid.org/0000-0001-5444-9987

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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

Risk of future haemolytic disease of the fetus and newborn following the transfusion of Rh(D)-positive blood products to Rh(D)-negative children

Recently, the risks of an Rh(D)-negative female of child-bearing potential (FCP) developing haemolytic disease of the newborn (HDFN) following receipt of Rh(D)-positive red blood cells (RBCs) or low-titre group O whole blood during her trauma resuscitation was modelled using her age at the time of transfusion and several other important societal factors that impact the development of HDFN [1]. In that study, the FCP age range was 18–49 years. Since its publication, questions have arisen about the future HDFN potential following the transfusion of Rh(D)-positive units to injured Rh(D)-negative children. The previously published model was adapted for, and applied to, patients between 0 and 17 years [1]. For this new model, the Rh(D) alloimmunization risk had to be derived for a paediatric population as it has not been published and might be difficult to obtain as it is uncommon to provide Rh(D)-positive RBCs to Rh(D)-negative/type unknown children in trauma. Age-specific paediatric alloimmunization rates to all RBC antigens were obtained from a study of paediatric patients in Japan; these age-specific rates might be different in populations with different antigen frequencies [2]. These age-specific rates were divided by the overall alloimmunization rate to all RBC antigens in adults who received a median of four RBC units (2.2%) [3], then separately multiplied by 7.8% (low estimate) or 42.7% (high estimate) as these rates reflect the ends of the spectrum of Rh(D) alloimmunization that have been reported in trauma populations where the patients' ages reflected the child-bearing age range [4, 5]. These mathematical manipulations attempted to correct the paediatric-specific overall RBC alloimmunization risk for the adult Rh(D)-alloimmunization risk, although this created the potential for slightly underestimating the paediatric Rh(D)-alloimmunization rate. The age-specific Rh(D)-alloimmunization rates are shown in the inset table in the Figure 1. For the current model, 7.8% and 42.7% were also used for the adult (18–49 years) Rh(D)-alloimmunization rates instead of 21.2% from the previous model [1]. The 28-day mortality rate used for injured patients, 0–17 years, was 36% as recently described [6]. Although it is known that injured adult females have lower mortality than males, it is not currently known if this finding also applies to injured girls. All other assumptions were unchanged from the previous model. The model, implemented in Microsoft R Open version 3.5.3 (Microsoft, Redmond, WA), simulated 1 million Rh(D)-negative females from 0 to 49 years of age who received an Rh(D)-positive-unit during trauma resuscitation and estimated her risk of developing HDFN of any severity. The rate of future HDFN increased as the child's age increased, largely due to the increasing rates of alloimmunization as the child's age increased, reaching a plateau between approximately 18–20 years (Figure 1); at this age, the alloimmunization rate is at its maximum, and the length of time for future pregnancies is approximately 30 years.

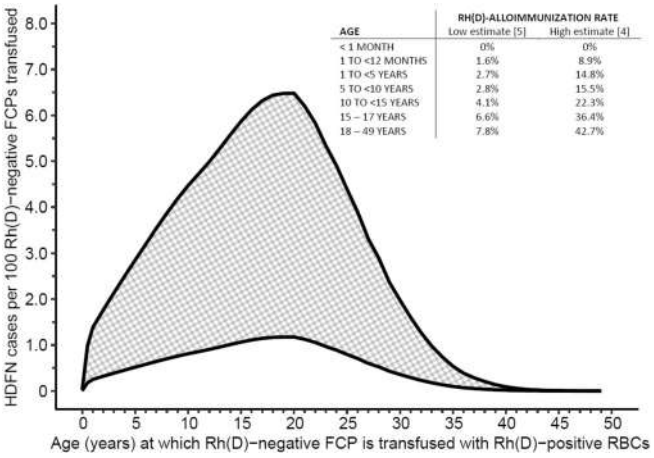

FIGURE 1 Anti-Rh(D)-mediated HDFN risk estimate based on the patient's age and age-specific RhD alloimmunization rates. The HDFN risk was modelled using Rh(D)-negative alloimmunization rates of 7.8% (low estimate, lower solid line) and 42.7% (high estimate, upper solid line), with an age correction factor for patients between 0 and 17 years of age as described in the text. The hatched area between these lines indicates the range of risk for HDFN as defined by these two Rh(D)-alloimmunization rates. Locally estimated scatterplot smoothing (LOESS) was used for HDFN rates between ages 0 and 17 years

These data indicate that if Rh(D)-negative children, especially very young children, are resuscitated with Rh(D)-positive units, the overall risk of developing HDFN is relatively small compared to the mortality reduction of receiving pre- and early in-hospital transfusions. However, Rh(D)-positive transfusions should only be administered when the Rh(D)-negative patient's survival would be compromised by waiting for Rh(D)-negative units.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

Mark H. Yazer[1,2]
Philip C. Spinella[3]
Jansen N. Seheult[1,2]

[1]*Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA*
[2]*Vitalant, Pittsburgh, Pennsylvania, USA*
[3]*Department of Pediatrics, Division of Critical Care Medicine, Washington University in St Louis, St Louis, Missouri, USA*

Correspondence
Mark H. Yazer, Department of Pathology, University of Pittsburgh School of Medicine, 3636 Blvd of the Allies, Pittsburgh, PA 15213, USA.
Email: myazer@itxm.org

ORCID

Philip C. Spinella https://orcid.org/0000-0003-1721-0541

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CORRIGENDUM

In the article by Bell et al. 2020, there were typographical errors in Table 1. The overall data in the 'Weight at time of donation' (kg) row, under the 'Yes' and 'No Skin Marker' columns, should be (84.8 ± 19.4) and (85.2 ± 19.0) instead of (48.6 ± 66.9) and (46.5 ± 65.9).

The authors apologize for this error.

Reference:

Bell B, O'Donovan J, Wright ST, Gemelli CN, Knight E, Hirani R. Evaluation of a sterile surgical skin marker to indicate the optimal vein for venepuncture in the blood donation setting. Vox Sang. 2020;115:377-387.

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DIARY OF EVENTS

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

