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TRANSFUSION MEDICINE

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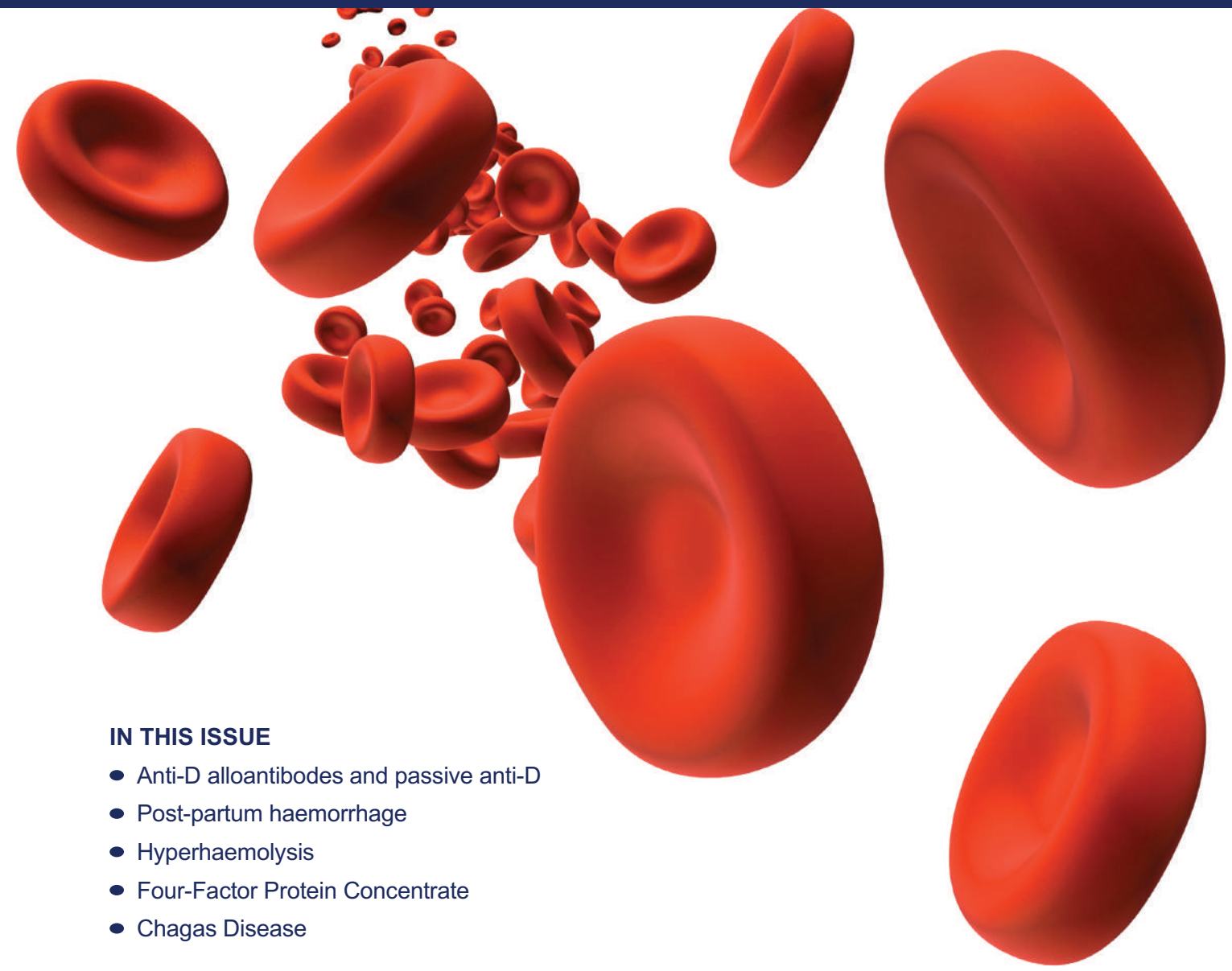

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Transfusion Medicine

An international journal published for the British Blood Transfusion Society

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EDITORIAL

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Time to be proactive about passive D: Distinguishing anti-D alloimmunisation from RhIG

Although Rh immune globulin (RhIG) has been in use clinically for over 50 years, pregnant patients continue to become alloimmunised to the Rh D antigen due to inadequate perinatal care or RhIG failure.[1-3] In order to identify patients at risk for Haemolytic Disease of the Fetus and Newborn (HDFN), blood banks generally screen for anti-D using a qualitative assay. Once identified, further tests are used to quantify anti-D, with levels used to estimate the risk for HDFN. Although most blood banks throughout the world use the saline indirect antiglobulin test (SIAT; also known as tube test) to determine anti-D titre levels, the British Society of Haematology (BSH) revised guidelines recommend using continuous flow analysis (CFA), which yields a concentration of anti-D measured in international units/mL (IU/mL).

The level of anti-D is critical as it is used to guide patient care. Patients with levels above an accepted threshold are considered to be at high risk for HDFN and must be closely monitored by the obstetric service. Conversely, very low concentrations of anti-D can be categorised as low risk for HDFN, although correlation with clinical history is needed to distinguish between alloimmunisation, which carries the potential for HDFN, and passive antibodies from RhIG treatment. As a result, the test used to quantify antibody levels should be easy to use, allow for reasonable turnaround time and be reproducible.

Based on these requirements, the CFA and tube test both have critical drawbacks. The CFA test requires expensive equipment and specially trained technicians. As a result, the majority of labs in the United Kingdom must send their samples to a reference lab, creating potential delays in critical antenatal care. Also, significant inter-laboratory variability has been reported for the CFA.[4] Conversely, titre levels obtained by tube testing are inexpensive but are time intensive and prone to variability. A third alternative uses automated platforms to run column agglutination technology (CAT) or solid phase technology (SPT). CAT and SPT are affordable test options with decreased variability in methodology and interpretation, as recently demonstrated in evaluation of isohaemagglutinins.[5,6]

Automated platforms are commonplace within modern hospital laboratories, enabling improved workflow for blood typing and antibody screening. Automated platforms may also improve consistency of results, though this has not been extensively demonstrated. However, assessment of antibody levels by automated titration has lagged behind tube testing, as clinically actionable anti-D levels were previously defined by manual tube methods.[7] As studies have shown increased sensitivity in CAT and solid phase when compared to tube, there is concern that these modalities may result in relatively higher titres, which may lead to unwarranted testing as well as undue stress for the patient.

Prior evaluation by Mikesell et al showed that gel testing for RhIG with CAT was more sensitive than SIAT but less sensitive than when using SPT.[3] This group also showed that passive D reactivity can persist for up to 3.5 to 4.5 months after administration with expected variation among different commercially available formulations.[3] As most half-lives range between 20 and 30 days, with more sensitive testing, persistence of antibodies can become problematic as 5 to 6 half-lives are required for drug clearance.[8-11] As RhIG may be detected for long periods of time after prenatal administration, it is critical to delineate the true nature of an antibody and categorise it as passive and benign vs immunogenic with a concomitant risk of HDFN.

As such, it is with great interest that we read Evans and colleagues' work evaluating antibody titre scores by automated CAT vs CFA in the assessment of immune and passive anti-D antibodies. Herein, they describe their experience using the ORTHO VISION automated CAT platform to evaluate nearly 200 anti-D samples in five separate UK hospital transfusion laboratories. This study builds on the work of Bruce et al that initially compared anti-c and anti-D titre scores by manual CAT vs CFA, showing increasing manual CAT titre scores with higher concentrations by CFA.[4] A titre score is a value assigned to assess an antibody's level and avidity. It is calculated using the strength of reactivity at each titration with levels of reactivity assigned with scores (4+ 12, 3+ 10, 2+ 8, 1+ 5, ± 3, 0 0).[4] Evans et al expand on this work with a larger cohort and application of the titre score in conjunction with clinical history of RhIG administration.

The group shows automated CAT testing can effectively distinguish between high and low antibody levels. These low levels defined by titre scores align with currently in use definitions of high and low by CFA (low likely passive<0.4 IU/mL < high likely immune). This would make automated CAT testing an appropriate screening test for the identification of true immune anti-D antibodies vs passive antibodies not requiring CFA. Likewise, they suggest a testing algorithm in which patients could be screened out using titre score. Based on this schema, in which indeterminate results would be reflexed to CFA, all patients would have received appropriate testing in their study.

As current UK standards dictate quantification of anti-D to rule out alloimmunisation, availability of testing is a key factor.[12] This pilot study shows promising results and may represent a solution to the problem of anti-D level assessment. Moreover, this important work helps establish a correlation between automated CAT titre scores and absolute levels by CFA. Though no linear correlation was

demonstrated, understanding this correlation is key for the management of HDFN and ensuring appropriate perinatal care.

Anti-D antibody titre scores are a reasonable starting point for assessment of automated CAT as an antenatal testing modality, as Rh-alloimmunisation represents the prototypic cause of HDFN. Evaluation of maternal antibodies to other blood group systems is a key area of future investigation and is necessary for generalisation of results. This is a pilot study and the group intend to continue their work adding additional clinical correlation and interlaboratory comparison in further studies. Whether labs will internationally adopt this testing is unclear; however, the results point to automated CAT testing as an attractive possibility. Currently, automated CAT titre scores represent a practical screening test for passive anti-D antibody identification.

CONFLICT OF INTEREST

The authors declare no competing interests.

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TRANSFUSION PRACTICE

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A survey of nurses to assess transfusion practice at the bedside using an electronic identification system: Experience at a university hospital

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Abstract

Objectives: The objective of this study was to assess the performance and recognition of transfusion practice at the bedside by nurses in our hospital, where a barcode-based electronic identification system (EIS) has been used since 2002.

Background: More than half of the steps in the transfusion chain are dependent on nurses' awareness and skills.

Methods: Our transfusion policy at the bedside includes two-person checking of the patient and two-person signing of the label at the time of collecting blood samples for pre-transfusion testing and two-person blood administration, which generally involved a doctor–nurse pair but sometimes involved two nurses. Anonymous, paper-based questionnaires were sent in January 2018 to 1051 nurses who were working in Juntendo University Hospital, Tokyo, Japan. The questionnaire consisted of three parts: (a) background of respondents, (b) performance of collection of blood samples for pre-transfusion testing and (c) performance of pre-transfusion check procedures at the bedside using an EIS based on a total of 20 questions.

Results: There was a good response rate of individual nurses (1006/1051, 96%). Most nurses (>90%) performed two-person checking of the patient and two-person signing of the label at the time of collecting blood samples. Most nurses (>90%) performed two-person blood administration involving a doctor–nurse pair and electronic pre-transfusion check using an EIS before blood administration.

Conclusions: The survey revealed that most nurses complied with our transfusion policy at the bedside, but some nurses did not. Further education/training and continuous support by the transfusion service may be needed for all nurses.

KEYWORDS

bedside, electronic identification system, nurse, pre-transfusion check, questionnaire survey

1 | INTRODUCTION

Blood transfusion is a complex, multi-step process, and there is potential for error at each stage.[1] Physicians play a pivotal role in blood transfusion, including clinical decision-making, obtaining consent from the patient and prescribing blood components, whereas more than half of the steps in the transfusion chain are dependent on nurses' awareness and skills.[2] However, insufficient knowledge regarding transfusion medicine among

nurses has been reported.[3-6] The Serious Hazards of Transfusion (SHOT) scheme in the United Kingdom showed that approximately 70% of incorrect blood component transfused (IBCT) errors take place in clinical areas, with the most frequent error being failure to perform the final patient identification check at the bedside.[7] Two steps that are critical in the transfusion chain of events leading to IBCT errors are the collection of blood samples for pre-transfusion testing and checking the patient's identification at the bedside before blood administration,[1,8] being mainly attributed to nurses. Therefore, it is crucial to improve the knowledge and recognition of individual nurses who are involved in transfusion practice.

In this study, we assessed the performance and recognition of Japanese nurses, who were working in a hospital where a barcode-based electronic identification system (EIS) had been used since 2002, on transfusion practice at the bedside, especially collecting blood samples for pre-transfusion testing and pre-transfusion check procedures at the bedside before blood administration. Our observations suggest that most nurses (>90%) complied with our transfusion policy at the bedside, but some nurses did not.

2 | MATERIALS AND METHODS

2.1 | Study design

The questionnaire survey was conducted by the transfusion service in collaboration with the Department of Nursing, Juntendo University Hospital, Tokyo, Japan, a 1032-bed tertiary-care teaching hospital, including 37 clinical departments with approximately 350 000 admissions and over 1 200 000 outpatient visits annually. Anonymous, paper-based questionnaires were sent in January 2018 to 1051 nurses who were working in operating rooms and the outpatient clinic and on inpatient wards. The questionnaires were collected by the office of the Department of Nursing in February 2018 and finally analysed by both the transfusion service and Department of Nursing.

The pre-survey transfusion activity during 2017 included a total of 57 168 blood components that were transfused without a single mistransfusion, defined as the wrong blood being transfused to the wrong patient, that is, ABO-incompatible transfusion. Of these, 11 transfusions (0.02%) were performed without the "second" electronic pre-transfusion check, resulting in an overall compliance rate of the electronic pre-transfusion check at the bedside of 99.9%. There were no near-miss events during the study period. A total of 29 786 pre-transfusion tests were performed in 2017; of these, 410 blood samples (1.4%) were presented to the transfusion service without two-person signing of the blood sample label (not complying with our transfusion policy, as described below), being finally rejected. The number of cases of wrong blood in tube (WBIT), in which the blood group result differed from the result on file from prior testing, was one in 2016 and zero in 2017.

2.2 | Policy of collecting blood samples for pre-transfusion testing

The policy of collecting blood samples for pre-transfusion testing includes two-person checking of the patient at the bedside. First, the checker asks the patient to say both his or her name and date of birth and checks his or her wristband showing the label on the blood collection tube, and then, the checker puts his or her own signature on the label if the answer is correct. Second, the phlebotomist asks the patient to confirm that his or her identity matches the "signed" blood sample label, and then, the phlebotomist takes the patient's blood. Finally, the phlebotomist puts his or her own signature on the signed label, resulting in two signatures on the label, and the patient's blood sample is sent to the transfusion service.

2.3 | Policy of performing pre-transfusion check procedures at the bedside

A barcode-based EIS (Nursing Pass, Bio-Rad Laboratories, Tokyo, Japan), which links the transfusion management system to the hospital information system via a network, was implemented in Juntendo University Hospital in July 9, 2002.[9] The transfusion policy at the bedside in our hospital includes a standard two-person (generally a doctor–nurse pair and sometimes a two-nurse pair) visual and verbal double check, followed by an electronic pre-transfusion check using a hand-held device of the EIS just prior to the initiation of blood administration. The details of electronic pre-transfusion check procedures at the bedside were described previously.[9] After completing the electronic pre-transfusion check at the bedside, the nurse immediately initiates blood administration.

2.4 | Questionnaire survey

The questionnaire consisted of three parts: 1) background of respondents, 2) performance and recognition of collecting blood samples at the bedside and 3) performance and recognition of pre-transfusion check procedures at the bedside based on a total of 20 questions. The questionnaire was validated by both the Division of Nursing Education and laboratory technologists certified by the Japan Society of Transfusion Medicine and Cell Therapy (JSTMCT). The questionnaire is shown in Table S1.

2.5 | Statistical analysis

The significance of differences in the correct response rates of answers between the groups (e.g., clinical experience as a nurse, experience of transfusion practice) was determined using the chi-square test. Significance was defined as $P < .05$.

3 | RESULTS

3.1 | Background of respondents

There was a good response from individual nurses who returned completed questionnaires in the hospital (1006/1051, 96%). Details of the background of respondents are shown in Table 1.

TABLE 1 The number and rate of answers regarding backgrounds of respondents

Question[a]	Contents of questions	Answer
Q1 (n = 1006)	Clinical experience as a nurse	0-1 yr: 155 (15%)
		2-10 yr: 645 (64%)
		More than 11 yr: 206 (21%)
Q2 (n = 1006)	Experience of blood transfusion	0 times: 57 (6%)
		1-5 times: 133 (13%)
		6-10 times: 115 (11%)
		More than 11 times: 701 (70%)[b]
Q3 (n = 1006)	Frequency of blood transfusion in the department	0 times: 36 (4%)
		1-5 times/d: 125 (12%)
		1-5 times/wk: 292 (29%)
		1-5 times/mo: 395 (39%)
		1-5 times/yr: 158 (16%)

[a]The number of questions corresponded to that of the original questionnaire (Table S1).
[b]More than 11 times or too many times to remember.

3.2 | Performance and recognition of collecting blood samples for pre-transfusion testing at the bedside

Most nurses (>90%) performed two-person checking of the patient at the time of collecting blood samples for pre-transfusion testing (Table 2), whereas the rate of asking another person to accompany them at the bedside was significantly lower for nurses with clinical experience of ≤1 year and no experience of transfusion practice compared to other groups (Table 3). Although most nurses (>90%) recognised the necessity of two-person signing of the blood sample label, the rate of its performance was significantly lower for nurses with clinical experience of ≤1 year and no experience of transfusion practice compared to other groups.

3.3 | Performance and recognition of pre-transfusion procedures at the bedside

Most nurses (>90%) performed two-person blood administration involving a doctor–nurse pair, but its rate was significantly lower for nurses with clinical experience of ≤1 year compared to other groups (Table 4). Most nurses (>90%) performed an electronic pre-transfusion check using an EIS before blood administration, but its rate was significantly lower with clinical experience as a nurse of ≤1 year than other groups. Furthermore, most nurses (>90%) recognised the necessity of electronic pre-transfusion check before blood administration and were aware of the safety of blood transfusion after completing the electronic pre-transfusion check using an EIS. The reasons for non-compliance among the nursing staff were reported as follows: emergent situations, no attendance of a doctor at the bedside (generally a doctor–nurse pair perform pre-transfusion checking) and no attendance of the second checker (nurse) when collecting blood samples.

4 | DISCUSSION

In this study, we assessed the performance and recognition of transfusion practice at the bedside by nurses. For the group with clinical experience as a nurse of ≤1 year and no experience of transfusion practice, the correct response rate of recognition and performance of two-person signing of the blood sample label and performance of asking another person to accompany them at the bedside was significantly lower compared to other groups. As for the group with clinical experience as a nurse of ≥11 years and experience of transfusion practice of ≥11 times, the correct response rate of experience of asking another person who did not check the patient to sign the label or of being asked to sign the label by another person despite not having checked the patient was significantly lower than other groups. These findings suggest that nurses with a short career or fewer experiences may not fully understand our transfusion policy. Although transfusion education and training are performed when all nurses first start working as registered nurses, they may be insufficient for performing transfusion practice at the bedside. Furthermore, they have studied transfusion practice in clinical areas by learning with more experienced nurses and using a commercially available e-learning system (Nursing Skills Japan, Elsevier Japan, Tokyo, Japan). Further studies are needed to evaluate the effectiveness of e-learning in transfusion practice. Marked deficits in nurses' education and training and a low transfusion frequency had a strong negative impact on the incidence of transfusion errors.[10]

Before the questionnaire survey was conducted, the transfusion service held a series of educational courses for all healthcare professionals on transfusion practice every year. According to the results of the questionnaire survey, the hospital transfusion committee recommended more strict compliance with the transfusion policy by all healthcare professionals. Thus, the transfusion service held additional educational courses for all nurses involved in transfusion practice, especially regarding collecting blood samples and performing pre-transfusion check procedures at the bedside. We compared the number of blood samples without two-person signing of the blood sample label before (Period 1, from March to May 2018) and after (Period 2, from October to December 2018) the additional educational courses by the transfusion service (from June to September 2018). The number of non-compliant blood samples in Period 1 was 54, whereas that in Period 2 was 49, without a significant difference (P = 0.665, Student's t-test). These findings suggest that transfusion education and training for all nurses may be insufficient for complying with the transfusion policy and that nurses certified by the JSTMCT (Transfusion Practitioner/Nurse) may be expected to lead transfusion education and training for small groups or individual nurses. Furthermore, it may be necessary to positively utilise e-learning, although combining e-learning with face-to-face teaching was suggested to support skill development in nurses.[11,12]

TABLE 2 The overall correct response rates for each answer

Question[a]	Contents of questions	Correct answer	Rate (%)
Q4 (n = 901)	Performance of two-person checking of the patient at the time of collecting blood samples	Two-person	92
Q5 (n = 954)	Recognition of necessity of personally signing of the blood sample label	Know	95
Q6 (n = 942)	Recognition of necessity of two-person signing of the blood sample label: the checker and phlebotomist	Know	99
Q7 (n = 857)	Performance of signing of the blood sample label	Performed every time	88
Q8 (n = 816)	Performance of asking another person to accompany them at the time of collecting blood samples	Performed every time	83
Q9 (n = 709)	Experience of asking another person who did not check the patient to sign the blood sample label	No experience	74
Q10 (n = 739)	Experience of being asked to sign the blood sample label by another person despite not having checked the patient	No experience	74
Q11 (n = 985)	Recognition of one-nurse performance of blood administration at the bedside	Not allowed	99
Q12 (n = 953)	Experience of one-nurse blood administration at the bedside	No experience	96
Q13 (n = 923)	Experience of two-person blood administration at the bedside involving a doctor/nurse pair	Performed every time	93
Q14 (n = 843)	Recognition of two-nurse (not complying with our transfusion policy) blood administration at the bedside	A pair of doctor/nurse	84
Q15 (n = 953)	Performance of electronic pre-transfusion check at the bedside before blood administration	Performed every time	96
Q16 (n = 997)	Recognition of necessity of electronic pre-transfusion check at the bedside before blood administration	Necessary	100

[a]The number of questions corresponded to that of the original questionnaire (Table S1).

TABLE 3 The correct response rates of answers regarding collection of blood samples for pre-transfusion testing at the bedside

	Clinical experience as a nurse (yr)				Experience of transfusion practice (times)				
Question[a]	≤1	2-10	≥11	*P*-value	0	1-5	6-10	≥11	*P*-value
Q4	92	91	94	NS	85	93	93	91	NS
Q5	85	96	100	$P < .0001$	77	91	96	98	$P < .0001$
Q6	99	98	100	NS	96	98	98	99	NS
Q7	71	89	95	$P < .0001$	39	80	92	92	$P < .0001$
Q8	70	84	90	$P < .0001$	37	81	88	87	$P < .0001$
Q9	83	72	74	$P < .05$	91	81	74	72	$P < .01$
Q10	93	73	65	$P < .0001$	94	89	85	68	$P < .0001$

Abbreviations: NS, not significant.
[a]The number of questions corresponded to that of the original questionnaire (Table S1).

When an EIS is implemented in a hospital, pre-transfusion check procedures at the bedside may involve one or two healthcare professionals. When one individual carries out the pre-transfusion check procedure using an EIS, it may be considered possible. However, if electronic pre-transfusion check at the bedside fails due to human error,[13] one-person checking without an EIS may present a higher risk of mistransfusion than a standard two-person double check, although the number of people required to check the identity of the patient and blood unit at the bedside is controversial.[14] The British Committee for Standards in Haematology (BCSH) Guidelines state that "the use of a bedside blood tracking system does not replace the role of the well trained and competency assessed clinician who administers blood components."[15] We have recommended that the electronic pre-transfusion check at the bedside should be basically carried out by two persons, one of whom should be the nurse or doctor and the other should be the second checker.[16]

WBIT errors may be caused by mislabelling of a blood sample tube or miscollection with an otherwise appropriately labelled tube, leading to catastrophic harm to patients.[17] The rate of WBIT errors is 1 in every 1986 samples,[18] and one of the causes of WBIT errors is non-compliance with existing guidelines.[19] The most effective interventions are probably the implementation of end-to-end electronic systems.[20] The use of electronic patient identification at the time of pre-transfusion sample collection has been associated with fewer WBIT errors than that of manual patient identification.[21] Although our EIS has not been used for collecting blood samples for pre-transfusion testing, our transfusion policy prescribes (a) a two-sample rule before the first crossmatch is undertaken and (b) two-person

TABLE 4 The correct response rates of answers regarding performance of pre-transfusion check at the bedside

	Clinical experience as a nurse (yr)				Experience of transfusion practice (times)				
Question[a]	≤1	2-10	≥11	*P*-value	0	1-5	6-10	≥11	*P*-value
Q11	99	99	99	NS	DE	99	98	99	NS
Q12	97	96	94	NS	DE	99	97	95	NS
Q13	79	95	97	$P < .0001$	DE	96	99	96	NS
Q14	83	87	77	$P < .0001$	DE	87	94	84	$P < .05$
Q15[b]	82	99	99	$P < .0001$	DE	99	99	100	NS
Q16[b]	100	99	100	NS	DE	100	99	99	NS
Q17[b]	94	92	88	NS	DE	93	97	90	$P < .05$
Q18[b]	80	71	60	$P < .001$	DE	79	73	67	$P < .05$
Q19[b]	81	83	82	NS	DE	85	82	83	NS
Q20	95	92	83	$P < .0001$	DE	93	91	90	NS

Note: DE, Data were excluded from analyses because these questions were regarding transfusion practice at the bedside.
Abbreviations: NS, not significant.
[a]The number of questions corresponded to that of the original questionnaire (Table S1).
[b]Questions were about using an electronic identification system (EIS).

checking of the patient at the bedside. We found that a minority of respondents did not perform two-person checking of the patient at the time of collecting blood samples. It has been reported that, concerning the question of the number of nurses involved in the patient identification process, 22% and 11% of respondents answered "always one nurse" and "often one nurse," respectively.[10] These findings suggest that some nurses routinely perform one-person checking of the patient at the bedside despite the existence of transfusion guidelines specifying two-person checking. Further studies are needed to assess whether one-person checking of the patient at the bedside is safely performed.

The limitations of the present study were as follows: (a) It was conducted in a single university hospital. The background of nurses who were involved in transfusion practice may be different among institutions. We performed a survey of nurses who were working in a hospital where a barcode-based EIS had been used. To our knowledge, this is the first report of a survey of nurses to assess the performance of pre-transfusion check procedures using an EIS. (b) Although 1006 nurses were included in this survey, some respondents did not completely answer some questions. We analysed the data regarding the group rates using the sum of answers.

In conclusion, the survey revealed that most nurses complied with our transfusion policy at the bedside, but some nurses did not. Further education/training and continuous support by the transfusion service may be needed for all nurses.

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CONFLICT OF INTEREST

The authors have no competing interests.

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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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TRANSFUSION PRACTICE

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Practical approach to transfusion management of post-partum haemorrhage

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Abstract

Objectives: To describe transfusion management during post-partum haemorrhage (PPH) and the usefulness of standard or point-of-care (POC) laboratory tests for guiding haemostatic management.

Background: PPH is the leading cause of maternal mortality and severe maternal morbidity worldwide. Despite the efforts made in recent years, PPH is often burdened by preventable death. Recent data from the active Italian Obstetric Surveillance System (ItOSS) highlighted the following main critical issues: inadequate communication between healthcare professionals, inability to correctly and promptly assess the severity of haemorrhage, delays in diagnosis and treatment, failure to request blood promptly and inappropriate monitoring post-partum.

Materials and Methods: Data in the literature have been compared with the rotational thromboelastometry (ROTEM)- and the thromboelastography (TEG)-guided algorithms applied in the authors' departments.

Results: PPH transfusion therapy may have an empirical approach based on the standard use of blood products or a targeted approach based on coagulation monitoring by laboratory or POC tests. Here, the authors describe how they manage PPH in their departments, according to the Italian guidelines, along with the addition of a ROTEM- and a TEG-guided algorithms developed by themselves.

Conclusion: Although the proposed algorithms have not been validated by trials or observational studies conducted in our departments, we believe that these indications could be useful for supporting clinical practice. Furthermore, we deem it appropriate to emphasise the importance of a multidisciplinary approach and the need for standardised and shared protocols to support the decisions of healthcare professionals.

KEYWORDS

blind transfusion therapy, coagulation, postpartum haemorrhage, pregnancy, rotational thromboelastometry-guided algorithm, thromboelastography-guided algorithm

1 | INTRODUCTION

Post-partum haemorrhage (PPH) is the world's leading cause of maternal mortality and severe maternal morbidity; it is responsible for approximately 25% of deaths that occur during pregnancy, childbirth and puerperium.[1] A systematic review reported that the rate of PPH with blood loss ≥500 ml is 10.8% (95% confidence interval [CI], 9.6–12.1), with wide regional variability.[2,3] However, the prevalence

of major PPH with blood loss ≥1000 ml is significantly lower, with an overall estimated rate of 2.8% (95% CI, 2.4–3.2).[2,3]

Although the number of maternal deaths is higher in low-income countries, the incidence of PPH is increasing, even in high-income countries.[4] Mortality related to PPH is usually due to a suboptimal clinical response because of limited or absent resources in low-income countries, whereas in high-income countries, it is related to poor resuscitation management.[1,5] Deaths attributable to PPH usually occur in the context of coagulopathy, which severe PPH patients may develop as a result of the administration of crystalloids or as acute consumption coagulopathy and disseminated intravascular coagulation (DIC).[5]

Obstetric bleeding and surgical bleeding are the most common causes of massive PPH.[5] In Italy, PPH has been confirmed as the most common cause of maternal death by a record linkage analysis[6] and by the active Italian Obstetric Surveillance System (ItOSS).[7]

Confidential investigations conducted as part of the ItOSS project highlighted the following main critical issues: inadequate communication between healthcare professionals, inability to correctly and promptly assess the severity of haemorrhage, delays in diagnosis and treatment, failure to request blood promptly, inappropriate indications for caesarean delivery and inappropriate monitoring post-partum.[7]

As attested by a recent work by McNamara,[8] in our experience, we have been able to verify, in practice, that "effective treatment of PPH relies on teamwork," striving to a dual objective: obstetric and anaesthetic teams should cooperate in stopping the bleeding and managing fluid resuscitation and blood transfusion, respectively.[8] Teamwork should also include a transfusion medicine specialist, who should work in close cooperation with other healthcare professionals.

Although PPH treatment can be performed even if no point-of-care (POC) coagulation laboratory tests have been conducted, the ability to perform POC laboratory tests could improve the therapeutic approach for these patients. The objectives of this work were to describe transfusion management during PPH and to highlight the usefulness of standard or POC laboratory tests for guiding haemostatic management during massive PPH. Data in the literature have been compared with those of a rotational thromboelastometry (ROTEM)-guided algorithm and a thromboelastography (TEG)-guided algorithm developed by the authors and applied in their departments.[9]

2 | COAGULATION PROFILE OF WOMEN WITH PPH

The peculiar coagulation profile correlated with pregnancy may contribute to worsening the bleeding caused by obstetric complications.[10] For effective management of PPH, it is essential to be aware that the reference values for the coagulation system are extremely different from those of non-pregnant patients. There are many haemostatic changes in normal pregnancy, including an increase in procoagulant factors (V, VII, VIII, IX, X, XII and von Willebrand factors), a decrease in some of natural anticoagulants (reduced protein S activity and acquired activated protein C resistance) and a decrease in fibrinolytic activity. These changes occur to support placental function but may lead to a state of hypercoagulability, resulting in an increased risk of thrombosis and placental vascular complications.[11]

Both plasma volume (+40%) and red blood cell volume (+25%) increase, leading to a state of haemodilution; as a result, approximately one-third of pregnant women are anaemic at term.[11] Due to dilution and consumption by the uteroplacental unit, platelet count decreases during pregnancy from the first trimester, with the greatest reduction occurring during the third trimester[8]; however, it increases immediately after delivery, especially in cases of caesarean delivery.[11]

For non-pregnant women, normal fibrinogen values are 2–4 g/L, but they increase incrementally to 4–6 g/L by the third trimester.[8] Fibrinogen is the first coagulation factor to decrease below critical levels during haemorrhage and haemodilution.[8] Furthermore, it has been shown that decreased fibrinogen is an early predictor of the severity of PPH.[12]

3 | HAEMATOLOGIC MANAGEMENT OF MASSIVE PPH AND HOW WE TREAT IT

Transfusions may be an essential part of PPH treatment and can determine maternal outcomes, especially in the most severe cases. As blood loss is often underestimated, the clinical picture is the main element used to assess the need for transfusion.[3] The most recent Italian guidelines define PPH as minor when blood loss is between 500 and 1000 ml and as major when blood loss is >1000 ml.[3] Major PPH can be defined as controlled or persistent if bleeding persists or if there are signs of clinical shock.[3]

As reported in the literature, the appropriate transfusion therapy for massive PPH should include the rapid administration of crystalloids to ensure adequate circulating blood volume, the transfusion of red blood cells to guarantee tissue oxygenation and the use of appropriate blood and plasma components (i.e., platelets, fresh-frozen plasma [FFP] to provide clotting factors and cryoprecipitate or fibrinogen concentrates as a source of fibrinogen) in order to reverse or prevent coagulopathy.[13] To ensure the optimal function of the transfused factors, hypothermia, acidosis and hypokalaemia must be prevented or, if necessary, treated promptly.[13]

Several algorithms and massive transfusion protocols have been implemented to integrate clinical, laboratory and logistic responses to PPH into structured pathways. When these protocols have been applied in clinical practice, a reduction in haemorrhage-related morbidity and in transfusion requirements has been demonstrated.[8]

During the past few years, the Italian Istituto Superiore di Sanità (ISS), which coordinates the ItOSS surveillance system, and the Italian Ministry of Health have developed important public health actions to improve the clinical and multidisciplinary approach to PPH, such as the drafting and updating of guidelines for the prevention and treatment of PPH.[3,14] Key goals for coagulation during massive PPH as set by the Italian guidelines[3] are as follows:

- Haemoglobin concentration >8 g/dL
- Platelet count >50 × 10^9/L
- Prothrombin time (PT) and activated partial thromboplastin time (aPTT) ratio <1.5 times the normal
- Fibrinogenaemia >2 g/L

Recently, an approach based on patient blood management (PBM) has been introduced as a way to optimise anaemia in obstetrics on the basis of the positive results obtained in other settings, such as cardiac surgery and traumatology.[15] There are three pillars of PBM: identification and treatment of preoperative/pre-partum anaemia, reduction of peri-operative/peri-partum blood loss and optimisation of the treatment of postoperative/post-partum anaemia.[15]

Although the reference values of haemoglobin in pregnancy are still debated, it is important to evaluate the trend in the different trimesters in order to prevent the onset of anaemia: According to the World Health Organization, anaemia during pregnancy is diagnosed with haemoglobin values <11 g/dL, and between the first and second trimester, the values are reduced by 0.5 g/dL.[15]

Transfusion of blood products is a critical component of resuscitation in response to PPH. Available products include red blood cells, FFP, platelets, cryoprecipitate, fibrinogen concentrate (FIB) and tranexamic acid.[8] PPH transfusion therapy may have an empirical approach based on the standard use of blood products or a targeted approach based on coagulation monitoring by laboratory or POC tests.[8]

Coagulation laboratory testing may be useful for rapid assessment and guiding haemostatic management during massive PPH.[13,16] PT, aPTT and plasma FIB are usually measured during standard coagulation tests. They indicate clot initiation but are not informative about the quality and strength of the clot and require long response times. Moreover, aPTT and PT have limited value in PPH because they persist within normal values even in the presence of massive bleeding.[8]

POC tests such as ROTEM and TEG are being increasingly considered alternative or complementary tools for guiding haemostatic management during massive PPH. The European Society of Anesthesiologists and the American Society of Anesthesiologists Task Force on Perioperative Blood Management recommend the use of POC monitoring to guide transfusion during PPH.[17,18]

According to the Italian guidelines[3,14] for women with major PPH, transfusions with red blood cells and other blood components (FFP, FIB, platelets) should be performed. Based on the results of the WOMAN study, an international, double-blind, multicentre, randomised clinical trial,[19] the Italian guidelines strongly recommend the early use of tranexamic acid.[14]

Here, we present the algorithms used by our departments, based on Italian guidelines,[3,14] along with the addition of a differentiated pathway, depending on whether POC monitoring is available.[9]

Before initiating transfusion therapy, whether blindly or ROTEM/TEG-guided, early administration of tranexamic acid at a dose of 1 g intravenously is recommended in addition to the standard treatment with uterotonics. If bleeding persists beyond 30 min, or if it resumes within 24 h of the first administration, a second dose of tranexamic acid is recommended.[3,14]

If POC tests are not available, then we perform blind transfusion therapy (Figure 1).[9] According to the Italian guidelines, transfusion of packed red blood cells must be performed as soon as possible, without waiting for laboratory results.[3,9] Therefore, we transfuse either four bags of packed red blood cells to four units of plasma from a single donor or of an industrial type or four bags of packed red blood cells to two units of apheresis plasma when waiting for laboratory test results.[9] For platelet concentrates, the use of one unit by apheresis or buffy coat for every eight bags of packed red blood cells is suggested.[9] When the results of coagulation tests are available, if the aPTT or international normalised ratio (INR) is >1.5, then we transfuse plasma and packed red blood cells at an initial dose of 20 ml/kg and up to a dose of 30 ml/kg in case of persistent or worsening

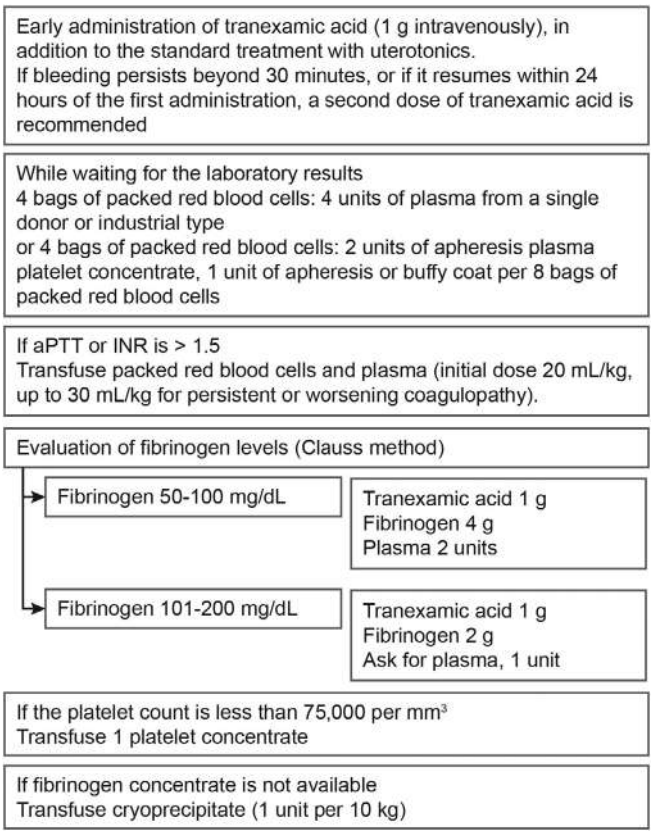

FIGURE 1 Blind transfusion therapy.[9] aPTT, activated partial thromboplastin time; INR, international normalised ratio

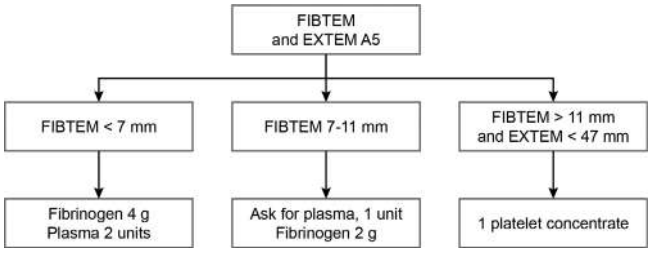

FIGURE 2 ROTEM-guided transfusion therapy

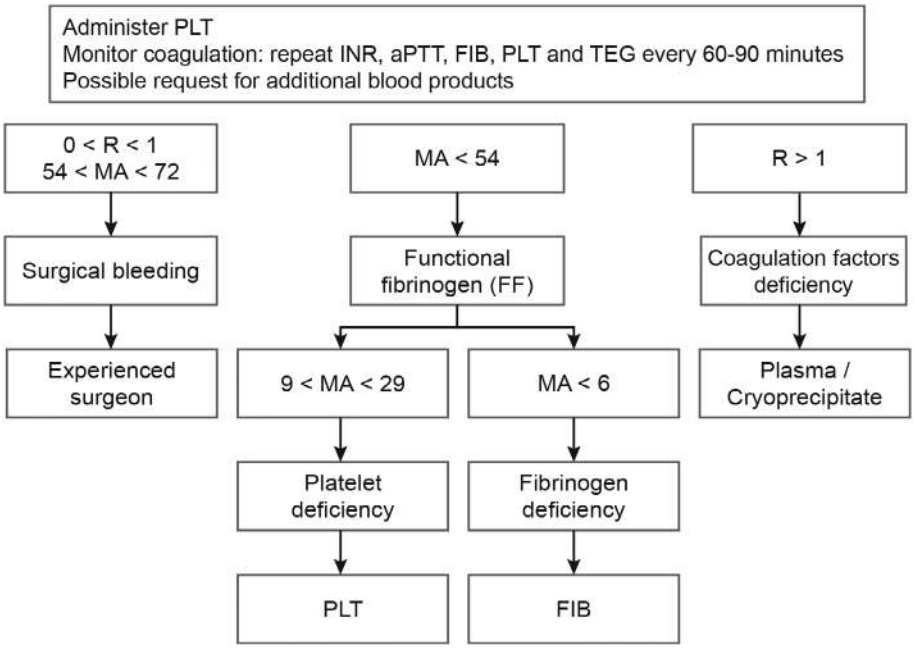

FIGURE 3 TEG-guided transfusion therapy.[9] aPTT, activated partial thromboplastin time; FIB, fibrinogen; INR, international normalised ratio; PLT, platelets

coagulopathy.[9] Fibrinogen levels are evaluated using the Clauss method, and transfusion of tranexamic acid, fibrinogen and plasma is performed (Figure 1). If the fibrinogen concentrate is not available, then using cryoprecipitate is suggested. If the patient has thrombocytopaenia with platelet counts <75 000 mm^3, then we suggest transfusing one platelet concentrate.

If POC tests are available, then coagulation monitoring can be performed using ROTEM or TEG viscoelastic tests. Therefore, we have presented both ROTEM- (Figure 2) and TEG-guided therapies (Figure 3).[9]

Several studies have shown that the use of ROTEM-based algorithms to guide transfusion for PPH leads to a reduction in overall blood component transfusions, consumption of FFP, incidence of transfusion-associated circulatory overload and cost of care.[20-23] A recent retrospective analysis of the implementation of an individualised ROTEM-guided transfusion management approach has confirmed the utility of routine bedside thromboelastometry to promptly and precisely identify the coagulation status of patients with PPH.[23] The correlation between specific ROTEM/TEG parameters and fibrinogen concentration has also been confirmed.[20-22] The FIBTEM and EXTEM A5 are useful ROTEM parameters for rapid clotting assessments. In literature, their correlation with maximum clot firmness (MCF) and their rapidity of the response are confirmed (6–7 min from the beginning of the test).[8] Several studies have shown the excellent correlation between FIBTEM A5 and Clauss fibrinogen in women with PPH, thus indicating FIBTEM A5 to be a useful early biomarker.[8,21,22] Fibrinogen replacement is required when plasma levels are <2 g/L or when FIBTEM A5 is <12 mm.[10]

Recently, a prospective study showed for the first time that there are no significant differences in ROTEM reference ranges of pregnant women of different nationalities/ethnicities.[24]

Some studies have reported that even the TEG-based functional fibrinogen (FF) assay correlates well with the standard von Clauss fibrinogen assay[9,25] and that TEG provides rapid and reliable detection of hypofibrinogenaemia ≤2 g/L and/or thrombocytopaenia ≤80 000/mm.[26]

Although the proposed algorithms have not been validated by trials or observational studies conducted in our departments, we believe that these indications could be useful for supporting clinical practice and could be applied based on the clinical situation of the patient and on the availability of blood components in specific departments. We hope to collect data in the future that will allow us to evaluate the protocol in practice with the aim of comparing the clinical results in the management of PPH before and after the application of the algorithms in our hospitals.

In conclusion, we deem it appropriate to emphasise the importance of a multidisciplinary approach to the management of PPH and the need for structure with standardised protocols that have been proven effective to support the decisions of healthcare professionals and improve clinical outcomes of a condition that is often burdened by preventable death.

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CONFLICT OF INTEREST

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ORIGINAL ARTICLE

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Prospective audit of blood transfusion request forms and continuing medical education to optimise compliance of clinicians in a hospital setting

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Abstract

Objectives: The aim of this study was to analyse blood requisition forms sent by clinicians in a tertiary care hospital to the transfusion service to ascertain their completeness and correctness. A secondary objective was to study the effect of continuing medical education (CME) in a hospital setting on clinician's behaviour regarding the importance of details that ought to be mentioned on blood requisition forms.

Background: Transfusion audits are useful tools in the evaluation and education of those requesting blood components.

Methods/Materials: This was a prospective, observational study conducted in the department of Transfusion Medicine at a tertiary-level healthcare centre from June 2019 to December 2019. The study was divided into two phases: pre-CME (P1) and post-CME (P2). In both phases, an audit for assessing completeness and correctness of blood requisition forms, which were divided into four sections, was performed. A scoring system was devised to compare both phases.

Results: In the P1 phase, 45.77% of the blood requisition form entries were complete and correct; 23.45% of incomplete entries were generated by emergency and trauma. In the P2 phase, 76.75% of the blood requisition form entries were complete and correct; 35.09% of the incomplete entries were generated by obstetrics and gynaecology. Complete and correct entries increased from 45.7% (P1) to 76.75% (P2). Scores of P1 were found to be lower than scores of P2 for all four sections. Cumulative mean score for P1 (20687) was found to be significantly lower than the mean score for P2 (30870).

Conclusion: Audit and CME regarding different aspects of transfusion medicine practices play a major role in the improvement of transfusion practices in hospitals.

KEYWORDS

audit, CME, training, transfusion medicine

1 | INTRODUCTION

An audit is a written series of simple, direct questions that, when answered and reviewed, indicate whether the procedures, activities and policies are correct. Audits prove to be valuable in reviewing crucial systems within a laboratory.[1] It is a system to investigate, evaluate and measure and also a means of continuous assessment and improvement, which is based on a set of guidelines and consists of

determining the difference between directions given and what has actually been done. Transfusion services are expected to audit practices appropriate to their needs.[2]

Transfusion medicine, as a medical specialty, is expected to ensure that every transfusion procedure must be indicated correctly. Transfusion audits are useful tools in the evaluation of a procedure and education of those requesting blood components. Blood requisition forms provide information regarding patient demographics, diagnosis, haematological reports, details of blood component requested and relevant previous transfusion history, along with a history of sensitising events. More often than not, clinicians ignore details on laboratory requisition forms and the completeness of these forms in general, which might result in medical errors.[3-8] Performing audits ensures the identification of missing information that has the potential to cause delays in treatment process, can lead to chances of errors and can cause a transfusion reaction, as has been shown by various studies.[9,10] Callum et al analysed their near-miss events and reported that, over a period of 14 months, a median frequency of three component request errors occurred per month (range, 0-5), including orders for blood for the incorrect patient.[9] Similarly, Linden et al reported that a total of 5% (22 of 462) of transfusion-associated errors were due to incorrect orders on the blood requisition forms.[10]

The periodic appraisal of transfusion practices highlights the shortcomings that can be addressed by education in the form of continuing medical education (CME). CME is defined as any attempt to persuade physicians to modify their practice performance by communicating clinical information.[11,12] The purpose is to help maintain and update the competency of healthcare-related individuals. CMEs have often been used to improve patient care.

In this study, we prospectively analysed blood requisition forms sent by the clinicians in a tertiary care hospital to the transfusion service to ascertain their completeness and correctness. A secondary objective was to study the effect of CME in a hospital setting on clinician's behaviour regarding the importance of details that ought to be mentioned on blood requisition forms.

2 | MATERIALS AND METHODS

2.1 | Settings and design

This was a prospective, observational study conducted in the department of Transfusion Medicine at a tertiary-level healthcare centre from June 2019 to December 2019.

2.2 | Audit of requisition forms

Transfusion medicine (TM) residents in the department were given 10 requisition forms daily. This number of forms was chosen so that the study could be conducted without disturbing the routine functioning of the department. The department receives 9000 to 11 000 component requests annually. The residents would check the completeness and correctness of the form and identify missing and/or incomplete information on each form. The residents did not assess the appropriateness of the entries on the blood requisition forms. For example, they did not assess whether the patient required the particular component requested or if the requested dose was adequate or not. Each requisition form was divided into four sections for the purpose of evaluation. For each section, the information could either be complete or incomplete. Complete information could be correct or incorrect. Correctness of information was confirmed with the help of patient records in the hospital information system (HIS) and the patient file. For example, the unique hospital identification number (UHID) is alphanumeric and 12 characters in length. If the UHID mentioned consisted of fewer than 12 characters, it would be incomplete. However, if there were 12 characters, the UHID could be correct or incorrect depending upon whether it was the same as that mentioned in the HIS and the patient file. Similarly, if the recipient blood group was A Positive, forms that mentioned only A were considered incomplete, and forms with complete group mentioned were checked for correctness in the HIS and patient investigation file. For each parameter, the information provided could be complete and correct (CC) or complete and incorrect (CI), or it could be incomplete (I). Assessment of incorrect information was important because it had the potential to cause harm to the recipient. However, transfusion-associated errors were not studied because the aim of this study was only to assess the completeness and correctness of blood requisition forms received. The scoring system used has been given in Table 1. For complete and correct information, a score of 2 was given; for complete and incorrect information, a score of 1 was given, whereas for incomplete information, a score of 0 was given. An Excel sheet (version 16.0, 2016, Microsoft Corporation, Redmond, Washington) was maintained to record the results of the audit.

2.2.1 | Section I

Patient demographic data included name, father's name, hospital identification number, location, age, gender, country of origin, height and weight.

2.2.2 | Section II

Clinical and laboratory details included patient diagnosis; previous history of transfusion; history of transplant or pregnancy/abortion;

TABLE 1 Scoring criteria for entries used in the study

Assessment of entries in the form	Abbreviation used	Score awarded
Complete and correct entry	CC	2
Complete and incorrect entry	CI	1
Incomplete entry	I	0

Abbreviations: CC, complete and correct; CI, Complete and incorrect; I, incomplete.

indication for transfusion; and haematological parameters including haemoglobin, coagulation profile (PT/aPTT/INR), platelet count and history of transfusion reaction.

2.2.3 | Section III

Component and transfusion details included number and type of component required, expected date of transfusion, recipient blood group, donor blood group (ABO-incompatible transplant), history of irregular antibodies, request for phenotype-matched red cell units and special processing (irradiation/washing).

2.2.4 | Section IV

Miscellaneous details included urgent/lifesaving/routine request, new/repeat request, doctor's name and signature, department and request generated by (name of nursing).

2.3 | Continuing medical education

A CME was planned, for all clinicians working in the hospital, to emphasise the importance of each entry on the blood requisition form and the implications of incomplete or incorrect entries. This CME was planned to be conducted after an audit of 600 requisition forms. The CME was organised once a week for eight consecutive weeks, and all clinicians were invited. Attendance in any one was mandatory for consultants and senior and junior residents, and a clinician could attend more than once if desired.

2.4 | Inclusion and exclusion criteria

All consecutive blood requisition forms that requested a single type of component from the department of TM were included in the study. Blood requisition forms received from outside and those requesting multiple components were excluded from the study. Requisitions with multiple components were excluded for ease of scoring and interpretation by the TM residents because multiple components could require special processing or a different blood group for either of the components. Multiple requisition forms for a particular patient were considered individual entries in the Excel sheet (version 16.0, 2016, Microsoft Corporation).

2.5 | Algorithm of the study

The first 10 blood requisition forms received in the red cell serology laboratory in a day were given to a resident for audit. The same forms were given to an independent resident the next day. The results reported by both residents were given to a consultant who would check for discrepancies and decide the final result. After audit of 600 forms, a CME was conducted. After the CME, an audit of 600 forms was conducted in a similar manner. Pre-CME (P1) results were compared with post-CME (P2) results. The pre-CME and post-CME phases were spread over 2 to 3 months each (P1-10 weeks, P2-11 weeks), and the CME was conducted weekly for 8 weeks.

2.6 | Ethical committee approval

Institutional review board and ethical committee approval was obtained for the purpose of this study and for conducting a CME.

2.7 | Statistical analysis

The outcome parameter was percentage of incomplete forms, with a 95% confidence level and 3.8% margin of error, and a sample size of 599 was estimated. Data was entered in an MS excel sheet (version 16.0, 2016, Microsoft Corporation). Statistical analysis was performed using SPSS software (Version 25.0.0.0, Chicago, Illinois). Independent student *t* test was applied to calculate the *P*-value for comparing mean scores in the two groups (P1 and P2). *P*-value was considered significant if it was <.05.

3 | RESULTS

The study was conducted over a period of 7 months, with 600 forms being audited each in the P1 and P2 phases. In P1, the number of requests for red cells was 278 (46%), for random-donor platelet concentrates was 311 (52%) and for single-donor platelet concentrates was 11 (2%), whereas in P2, the number of requests for red cells was 345 (57%), for random-donor platelet concentrates was 226 (38%) and for single-donor platelet concentrates was 29 (5%).

3.1 | Demographics

Department-wise distribution and component-wise distribution of blood requisition forms included in both phases has been illustrated in Figures 1 and 2, respectively. After evaluation of 600 P1 blood requisition forms, the target areas that needed to be emphasised were identified, and the CME was conducted. A total of 202 clinicians participated in the CME. After the CME, 600 forms were audited, and target areas were reviewed for improvement.

3.2 | P1 audit results

Almost half (45.77%) of the blood requisition form details were complete and correct, and 19.68% of these entries were complete but

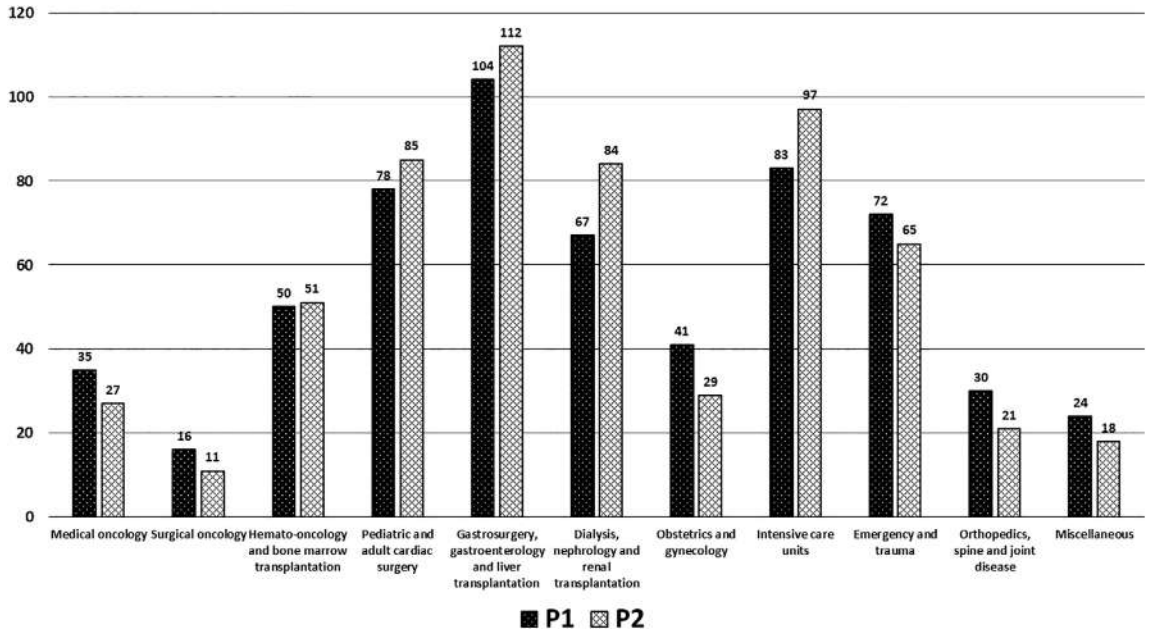


FIGURE 1 Department-wise distribution of blood requisition forms in P1 and P2

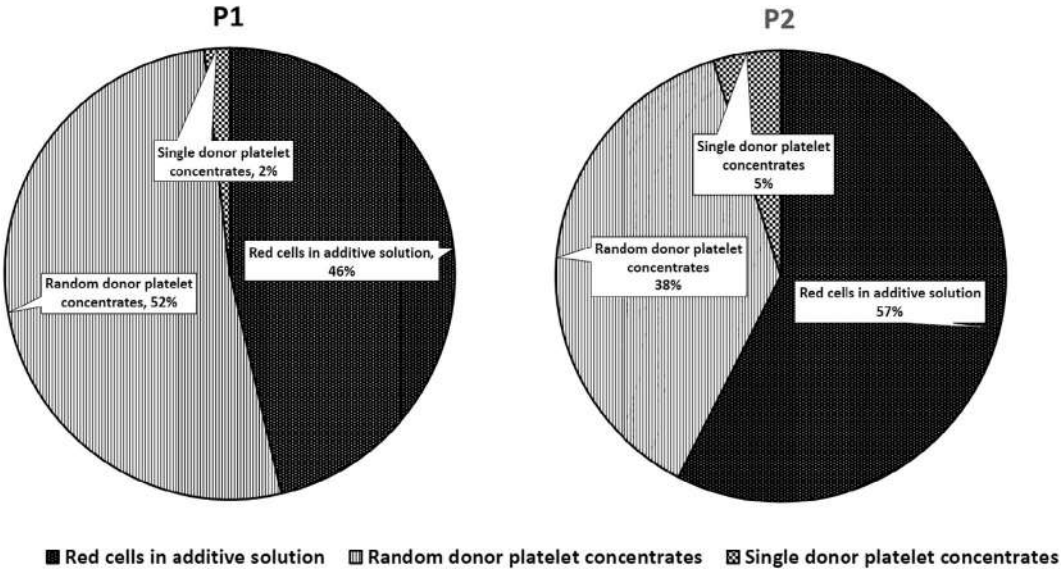


FIGURE 2 Component-wise distribution of blood requisition forms in P1 and P2

incorrect. However, almost one-third (34.55%) of the requisitions were incomplete. A distribution of CC, CI and I blood requisition form entries in the P1 phase has been illustrated in Figure 3. Of the 6426 incomplete entries, 1507 (23.45%) were generated by emergency and trauma. Department-wise distribution of incorrect entries in the P1 phase has been illustrated in Figure 4. Entries of patient name (84%), hospital identification number (87%), father's name (83%), age (55.83), history of pregnancy (62.67%), component type (65.17%), number of components (63%), patient blood group (71.33%), history of irregular antibodies (64.17%), requirement for special processing (56%) and requesting physician details (51.83%) were mostly complete and correct. Entries of expected date of transfusion (59.17%), urgent or routine request (73.5%) and new or repeat patient's request (62.33%) were mostly complete but incorrect. Entries of patient gender (61.17%), height (56.17%), weight (56.17%), country of origin (69%), patient diagnosis (79.5%), history of transfusion (41.33%) or transplant (52.17%), indication for transfusion (45%) and history of transfusion reaction (84%) were mostly incomplete. Details of the P1 audit results have been listed in Table 2.

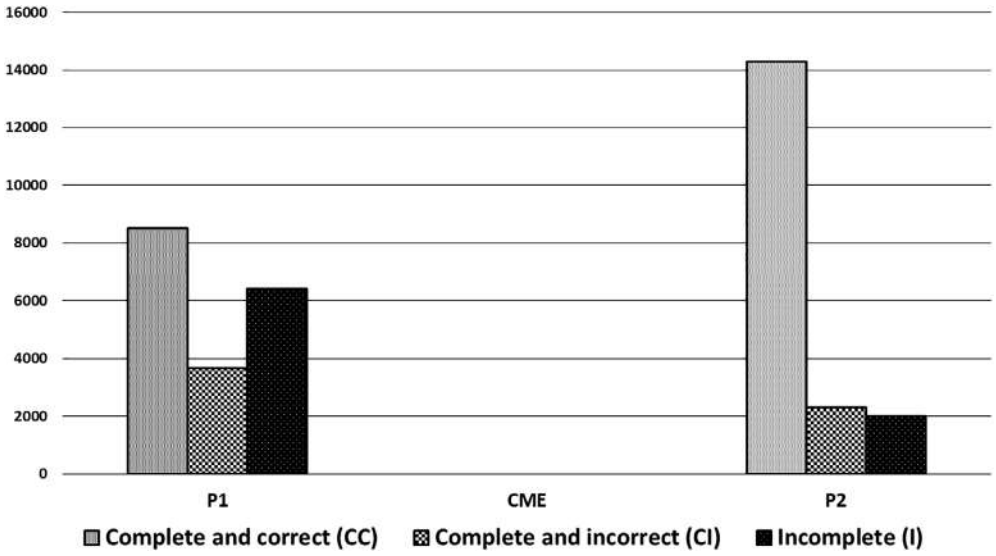
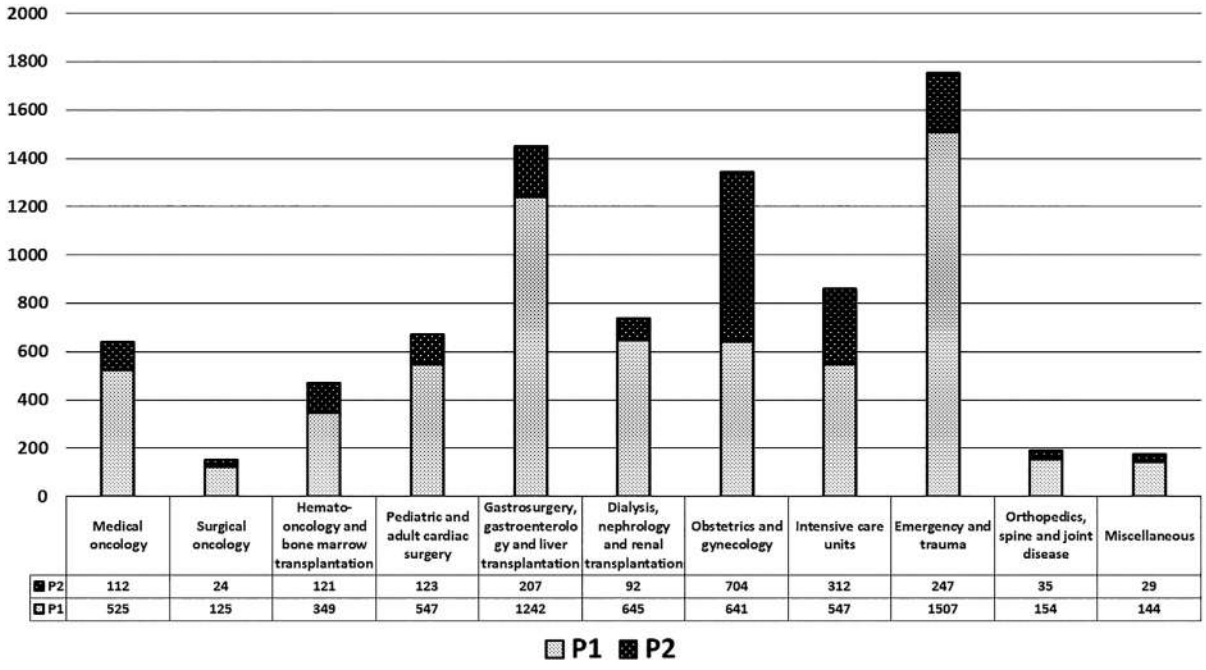


FIGURE 3 Distribution of CC, CI and I blood requisition form entries in P1 and P2 (Miscellaneous: paediatrics, neurosurgery, plastic surgery)



	Medical oncology	Surgical oncology	Hemato-oncology and bone marrow transplantation	Pediatric and adult cardiac surgery	Gastrosurgery, gastroenterology and liver transplantation	Dialysis, nephrology and renal transplantation	Obstetrics and gynecology	Intensive care units	Emergency and trauma	Orthopedics, spine and joint disease	Miscellaneous
P2	112	24	121	123	207	92	704	312	247	35	29
P1	525	125	349	547	1242	645	641	547	1507	154	144

FIGURE 4 Department-wise distribution of incorrect entries in P1 and P2

3.3 | P2 audit results

A majority (76.75%) of the blood requisition forms was complete and correct. One-tenth (10.78%) of the requisitions were incomplete. A distribution of CC, CI and I blood requisition form entries in P2 phase has been illustrated in Figure 3. Of the 2006 incomplete entries, 704 (35.09%) were generated from obstetrics and gynaecology. Department-wise distribution of incorrect entries in the P2 phase has been illustrated in Figure 4. All details were found to be mostly complete and correct. However, entries of history of previous transfusion (I = 26.67%), coagulation profile (I = 31.67%), request for phenotype matched units (CI = 28.33%), urgent or routine requests (CI = 36.83%) and new or repeat patient's requisition (CI = 48.17%) did not show the increase that was expected. Details of the P2 audit results have been listed in Table 2.

3.4 | Comparison of P1 and P2 results

Most of the requests in P1 (544 of 600, 90.67%) were marked as urgent requests. However, on checking patient records in the file, only 192 (32%) requests in P1 were actually urgent. This number went down to 115 (19.17%) requests in P2, which were marked as urgent requests. Only 17 of these 115 requests were urgent requests as per

TABLE 2 Results of P1 and P2

S. no.	Entry	P1 (n = 600)			P2 (n = 600)		
		CC, n (%)	CI, n (%)	I, n (%)	CC, n (%)	CI, n (%)	I, n (%)
	Section I	*2935*	*636*	*1829*	*4747*	*441*	*212*
1	Patient name	504 (84)	55 (9.17)	41 (6.83)	538 (89.67)	42 (7)	20 (3.33)
2	UHID	522 (87)	69 (11.5)	9 (1.5)	541 (90.17)	56 (9.33)	3 (0.5)
3	Father's name	498 (83)	71 (11.83)	31 (5.17)	526 (87.67)	51 (8.5)	23 (3.83)
4	Location	551 (91.83)	12 (2)	37 (6.17)	579 (96.5)	07 (1.17)	14 (2.33)
5	Age	335 (55.83)	09 (1.5)	256 (42.67)	547 (91.17)	02 (0.33)	51 (8.5)
6	Gender	228 (38)	05 (0.83)	367 (61.17)	555 (92.5)	03 (0.5)	42 (7)
7	Country of origin	57 (9.5)	129 (21.5)	414 (69)	458 (76.33)	101 (16.83)	41 (6.83)
8	Height	104 (17.33)	159 (26.5)	337 (56.17)	497 (82.83)	97 (16.67)	06 (1)
9	Weight	136 (22.67)	127 (21.17)	337 (56.17)	506 (84.33)	82 (13.67)	12 (2)
	Section II	*1945*	*799*	*2656*	*3939*	*568*	*893*
1	Patient diagnosis	24 (4)	99 (16.5)	477 (79.5)	486 (81)	62 (10.33)	52 (8.67)
2	Previous history of transfusion	131 (21.83)	221 (36.83)	248 (41.33)	377 (62.83)	83 (13.83)	140 (26.67)
3	History of transplant	285 (47.5)	02 (0.33)	313 (52.17)	394 (65.67)	101 (16.83)	105 (17.5)
4	History of pregnancy/abortion	376 (62.67)	45 (7.5)	179 (29.83)	511 (85.17)	29 (4.83)	60 (10)
5	Indication for transfusion	124 (20.67)	206 (34.33)	270 (45)	468 (78)	57 (9.5)	75 (12.5)
6	Haemoglobin	302 (50.33)	125 (20.83)	173 (28.83)	426 (71)	74 (12.33)	100 (16.67)
7	Coagulation profile (PT/aPTT/INR)	243 (40.5)	27 (4.5)	330 (55)	364 (60.67)	46 (7.67)	190 (31.67)
8	Platelet count	402 (67)	36 (6)	162 (27)	516 (86)	21 (3.5)	63 (10.5)
9	History of transfusion reaction	58 (9.67)	38 (6.33)	504 (84)	397 (66.17)	95 (15.83)	108 (18)
	Section III	*2511*	*834*	*1455*	*3543*	*518*	*739*
1	Type of component required	391 (65.17)	152 (25.33)	57 (9.5)	488 (81.33)	63 (10.5)	49 (8.17)
2	Number of components required	378 (63)	84 (14)	138 (23)	413 (68.33)	72 (12)	115 (19.17)
3	Expected date of transfusion	219 (36.5)	355 (59.17)	26 (4.3)	396 (66)	109 (18.17)	95 (15.83)
4	Recipient blood group	428 (71.33)	14 (2.33)	158 (26.33)	515 (85.83)	10 (1.67)	75 (12.5)
5	Donor blood group (ABO-incompatible transplant)	254 (42.33)	51 (8.5)	295 (49.17)	469 (78.17)	12 (2)	119 (19.83)
6	History of irregular antibodies	385 (64.17)	49 (8.17)	166 (27.67)	498 (83)	27 (4.5)	75 (12.5)
7	Special processing (irradiation/washing)	336 (56)	12 (2)	252 (42)	418 (69.67)	55 (9.17)	127 (21.17)
8	Phenotype-matched units	120 (20)	117 (19.5)	363 (60.5)	346 (57.67)	170 (28.33)	84 (14)
	Section IV	*1122*	*1392*	*486*	*2047*	*791*	*162*
1	Urgent/lifesaving/routine request	126 (21)	441 (73.5)	33 (5.5)	359 (59.83)	221 (36.83)	20 (3.33)
2	New/repeat request	100 (16.67)	374 (62.33)	126 (21)	255 (42.5)	289 (48.17)	56 (9.33)
3	Department	258 (43)	264 (44)	78 (13)	463 (77.17)	122 (20.33)	15 (2.5)
4	Doctor's name and signature	311 (51.83)	159 (26.5)	130 (21.67)	517 (86.17)	58 (9.67)	25 (4.17)
5	Request generated by (name of nursing)	327 (54.5)	154 (25.67)	119 (19.83)	453 (75.5)	101 (16.83)	46 (7.67)
Overall (n)		8513	3661	6426	14 276	2318	2006
Overall (%)		45.77	19.68	34.55	76.75	12.46	10.78

Abbreviations: CC, complete and correct; CI, Complete and incorrect; I, incomplete; P1, Pre-CME; P2, Post-CME.

clinician orders. CC entries increased from 45.7% (P1) to 76.75% (P2). CI entries decreased from 19.68% (P1) to 12.46% (P2), and I entries decreased from 34.55% (P1) to 10.78% (P2). Department-wise distribution of incorrect entries in P1 and P2 has been illustrated in Figure 4. There was 30.98% overall improvement in completeness of request forms. Complete scores of P1 were found to be lower than the scores of P2 for all four sections. Cumulative mean score for P1 was found to be significantly (P-value < .05) lower than the mean score for P2, as mentioned in Table 3, which indicates that CME helped improve the completeness and correctness of request forms.

TABLE 3 Scores of P1 and P2

Section	P1 score		P2 score		*P*-value
	Total	Mean	Total	Mean	
Section I	6506	722.89	9935	1103.89	
Section II	4689	521	8446	938.44	
Section III	5856	732	7604	950.5	
Section IV	3636	727.2	4885	977	
Overall	20 687	667.32	30 870	995.81	<.00001

Abbreviations: P1, Pre-CME; P2, Post-CME.

4 | DISCUSSION

Clinical audit is a management tool for appraisal and is also an important part of quality assurance, which provides necessary information for improving TM practices.[1,5] The blood request form is an important method of communication through which clinicians help TM specialists regarding the patient's clinical details. It also helps the transfusion service identify the potential for long-term transfusion therapy.[4] Transfusion services receive incomplete forms without adequate patient information.[13-15] A common myth among healthcare providers is that a blood sample or a blood group report is all that is required for requesting components without providing clinical details of the patient. A handful of studies has examined the frequency of incomplete blood requisition forms and highlighted the fact that there are deficits in completing requisition forms. Clinicians who counsel and advise patients about transfusion therapy are responsible for accurately describing the patient's details, as well as the component details.[4]

Jain et al found that 19.8% of blood requisition forms were incomplete, whereas Jegede et al found that 18.8% of forms had incomplete patient details.[16,17] In the present study, 54.23% of the blood requisition forms were incomplete in the P1 phase. Oyedeji et al found that only 1.3% of requisition forms reviewed were fully filled.[13] Patient's name, the referring physician's name and gender were the most completed information on the forms evaluated, with 99.0%, 99.0% and 90.3% completion, respectively. Patidar et al found that the highest number of incomplete fields observed (91.42%) varied from second identification (91.03%) to medical officer's name and signature (1.48%).[18] They also found that 51.44% of forms did not mention urgency of requirements, and pretransfusion haematological parameters were not provided in 53.67% of the request forms. Deb et al audited blood requisition forms and found that 56% of forms did not mention the blood group of patients and urgency of requirement.[19] They also found that 3.7% did not mention the indication of transfusion, 25.1% did not mention the history of previous transfusion, and 37.38% did not mention pregnancy history in the blood request forms. In the present study, entries of patient name (84%), hospital identification number (87%), father's name (83%), age (55.83), history of pregnancy (62.67%), component type (65.17%), number of components (63%), patient blood group (71.33%), history of irregular antibodies (64.17%), requirement for special processing (56%) and requesting physician details (51.83%) were mostly complete and correct.

CME is an established tool for increasing knowledge.[20] For improvement of filling of these incomplete request forms, we started educating clinicians and hospital staff through CMEs. Salem-Schatz et al suggested that educational strategies should target transfusing physicians rather than residents because knowledge varies among physicians depending on their age and working place.[21] Hence, all clinicians were invited to the CME, which focussed on the importance of filling each column in the blood requisition form. Solomon et al reported that auditing, introduction of a recurrent education programme for hospital staff and a request form requiring justification of component orders reduced blood component usage.[14] Gharehbaghian et al concluded that improvement in transfusion practices of physicians can be achieved by CME.[15] Osegbe et al educated clinicians about the completion of request forms, and they found significant improvement in the recording of patient age, hospital number, clinician's name, ward, clinical diagnosis and specimen type.[22] Patidar et al found that the most common incomplete column was second identification followed by previous transfusion history and adverse transfusion reactions due to previous transfusion.[18] After education of clinicians and their staff, there were significant improvements in the filling of blood request forms. Quantity of blood required, diagnosis of the patient and medical officer's name and signature were also major columns that were incomplete on initial assessment and improved after implementation of the CME. In the present study, CC entries increased from 45.7% (P1) to 76.75% (P2), CI entries decreased from 19.68% (P1) to 12.46% (P2), and I entries decreased from 34.55 (P1) to 10.78% (P2). There was 30.98% overall improvement in completeness of request form. Complete scores of P1 were found to be lower than scores of P2 for all four sections. Cumulative mean score for P1 was found to be significantly (P-value < .05) lower than the mean score for P2. Educating clinicians about the usefulness of completion of a requisition form was helpful. The results of the audit reflected that quite a number of forms with incomplete entries were received in the transfusion service and that incomplete requests had the potential to cause unnecessary delays in transfusion or transfusion reactions and increased the burden on the laboratory staff to obtain the correct details on the form. Audits, training and conducting regular CMEs are apt measures to improve transfusion practices.

5 | CONCLUSION

Audit and CME regarding different aspects of TM practices play a major role in the improvement of clinical practices in hospitals.

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CONFLICT OF INTEREST

The authors have no competing interests..

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ORIGINAL ARTICLE

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Clinical Implication of Immunohaematological Tests in ABO haemolytic disease of newborn: Revisiting an old disease

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Abstract

Objective: We aimed to assess the frequency distribution of of ABO haemolytic disease of newborn (ABO-HDN) and to know the predictive value of immunohaematological tests in identifying at risk neonates.

Background: ABO incompatibility, although a common cause of haemolytic disease of newborn, has several unaddressed issues related to it.

Material and methods: A prospective study over 20 months was carried out in a tertiary care centre in South India. Blood grouping, Direct Antiglobulin test (DAT) and elution studies were performed on neonatal samples, whereas blood grouping, antibody screening and antibody titration were performed on maternal samples. In suspected cases, ABO-HDN was diagnosed after excluding other possible causes for haemolysis. The laboratory results were correlated with the clinical details to assess the predictive value of the tests.

Results: Of the total 2856 pregnancies, 34% had ABO incompatibility. On testing with columnagglutination test (CAT), the overall DAT positivity and that among ABO-incompatible cases were 3.8% and 11.2%, respectively,) whereas by conventinal tube technique (CTT) it was 0.6% and 2.4% respectively. CAT was found to have higher sensitivity, and the predictive value was higher for CTT. Maternal IgG titre showed a positive linear relationship with the DAT strength and the rise in indirect bilirubin levels. The positive predictive value of combination of tests such as DAT, elution and titation was 94.12%, which was much higher than that of the individual tests.

Conclusion: DAT positivity is a predictor of early rise in serum bilirubin level, and a combination of tests has a better predictive value than individual tests towards development of clinically significant hyperbilirubinemia in ABO-HDN.

KEYWORDS

ABO HDN, fetal health, haemolytic disease of fetus and newborn, maternal health

1 | INTRODUCTION

ABO incompatibility between mother and fetus can lead to haemolytic disease of newborn (HDN), as first described by *Halbrecht* in 1944.[1] Following the advent of Rh immunoprophylaxis, ABO incompatibility is now the most common cause of HDN in the developed world.[2,3] Unlike the rhesus disease, it is usually a problem of the neonate rather than the fetus. About 15% to 25% of pregnancies can have ABO incompatibility, whereas only 10% develop HDN.[4,5] ABO HDN mostly has a benign course. However, with the recent practice

of early neonatal discharge from the hospital, it is a common cause of morbidity in the neonatal setup. ABO incompatibility leading to hyperbilirubinaemia is the one of the most common causes for neonatal readmission.[6] It is typically seen in group O mothers, who have IgG anti-A, anti-B and anti-AB, which crosses the placenta and reacts against the fetus red cell having A or B antigen.[7,8] Similarly, anti-H, -A and -B present in mothers with the Bombay phenotype (Oh) can also lead to HDFN.[9,10]

Although it is a common entity, there are many unresolved questions relating to the clinical course of affected neonates and the predictive factors for ABO HDN. Various tests are available for establishing the haemolysis and to estimate its severity; however, till date, there is no unified criteria or guidelines for the diagnosis and management of such cases. The significance of antenatal screening, the importance of maternal antibody titration and predictive value of immunohaematological laboratory tests are not addressed well in the literature. Identification of at-risk cases will help clinicians to reduce neonatal morbidity and will result in better patient management. Hence, we aimed to assess the frequency distribution of ABO HDN in our population and to assess the predictive value of immunohaematological tests to identify neonates at risk.

2 | MATERIAL AND METHODS

The study of the relevance of immunohaematological tests in neonates with ABO HDN was conducted prospectively over 20 months (October 2013 to May 2015) at a tertiary care referral centre in South India. All the patients who presented to the antenatal clinic and the samples that were sent for immunohaematological tests were included in the study. Ethical clearance was obtained from the Institutional Ethical Committee. Written informed consent was obtained from the study subjects. Medical history, obstetric history (including any stillbirths, abortions, medical termination of pregnancy [MTP] cases of HDN among siblings) and history of blood transfusions were recorded from the case file.

The newborns from ABO-incompatible pregnancies were closely monitored for signs of HDN. HDN was defined as severe anaemia (haematocrit <20%) with high reticulocyte count and symptomatic anaemia (tachycardia, tachypnoea, poor feeding) and appearance of clinical jaundice within 24 hours of life, with an increasing serum bilirubin of more than 5 mg/dL/day among these high-risk newborns.[11-13] Among neonates with early-onset hyperbilirubinaemia, ABO HDN was diagnosed after excluding other possible causes of haemolysis. The treatment received and the follow-up details were noted from the hospital records. Ethical clearance was obtained from the institutional ethics committee, No. IEC 353/2013.

2.1 | Laboratory investigations

Two samples of 2 mL each (with anticoagulant, Ethylenediaminetetraacetic acid and in a plain tube) were collected from study subjects during their visit to the antenatal clinic. The newborn blood grouping (cell grouping) along with a direct antiglobulin test (DAT) were performed on the neonatal cord blood or venous blood sample. The samples collected in a plain tube were stored at 37°C after collection prior to performing any immunohaematological tests. The blood grouping of the mother and newborn was performed using automated column agglutination technology (Ortho Auto-Vue). The presence of allo-antibodies was ruled out by performing an indirect antiglobulin test (IAT) on the mother's serum using inhouse prepared O-pooled cells.

Quantification of the maternal IgG antibody (anti-A and anti-B) level was carried out by a double dilution method using dithiothreitol (DTT) pre-treated maternal serum and conventional tube test (CTT) with appropriate controls as per the departmental Standard Operating Protocol (SOP) (prepared in accordance with AABB Methods).[14] The titre was defined as the reciprocal of the highest dilution that showed visible agglutination of 1+ reaction. The titre at which the neonates developed clinically significant hyperbilirubinemia was considered the critical level and was implemented following this study. The neonatal DAT was performed by both the CTT using Eryclone Polyspecific AHG, monoclonal anti-C3d reagent (Tulip Diagnostics, India) and column agglutination technology (CAT) (Bio-Rad LISS/Coombs Anti-IgG + C3d ID cards, Switzerland) along with auto-control to rule out spontaneous agglutination. Monospecific DAT was performed using DC-Screening I (Bio-Rad ID cards, Switzerland). All the neonatal samples with positive DAT results were further subjected to heat elution to characterise the antibody.[14]

2.2 | Statistical methods

Data management was performed using SPSS statistical software (version 14.0, USA). Descriptive analysis was used to calculate the frequencies and percentage. The association between the DAT status, jaundice and treatment was assessed using a chi-square test. The Mann-Whitney *U* test was used to compare differences between two independent groups when the dependent variable was either ordinal or continuous but not normally distributed. For analysing the correlation between two non-parametric variables, Spearman's ranked correlation coefficient was used. All the statistical analyses were carried out at 5% level of significance, and *P* value <.05 was considered significant.

3 | RESULTS

3.1 | Prevalence of ABO incompatibility

We reviewed the laboratory and clinical details of 2856 antenatal cases and their newborns during the study period. ABO incompatibility was seen in 962 (33.68%) cases. The O-A vs O-B incompatibility accounted for 13% and 12% of total cases studied (n = 2856), respectively. The distribution of the ABO blood group in the study group is

TABLE 1 Distribution of ABO blood group among the mothers and the newborns

	Mother					
Baby	A	B	AB	O	Oh	Total
A	229	*83 (8.6%)*[a]	30	*372 (38.6%)*[a]	*1 (0.1%)*[a]	715
B	*54 (5.6%)*[a]	303	39	*355 (36.9%)*[a]	—	751
AB	*43 (4.4%)*[a]	*53 (5.5%)*[a]	16	—	—	131
O	122	190	6	959	*1 (0.1%)*[a]	1259
Total	448	629	91	1686	2	2856

Note: ABO-incompatible pregnancies are highlighted in Italic.
[a]Percentage among ABO-incompatible group, total: 962.

TABLE 2 ABO incompatibility and DAT strength by CAT

	DAT strength					
Blood group combinations	4+	3+	2+	1+	WK+	No. of cases
O-A (n = 372)	2	13	37	14	12	78 (21%)
O-B (n = 355)	2	—	10	13	4	29 (8.2%)
Oh-A	—	—	—	1	—	1

TABLE 3 Relationship of DAT status with neonatal hyperbilirubinaemia

	DAT by CAT			DAT by CTT		
Clinically significant hyperbilirubinaemia	Negative N (%)	Positive N (%)	*P*-value	Negative N(%)	Positive N(%)	*P*-value
Present	30 (3.9%)	38 (35.2%)	<.001	60 (7%)	8 (47%)	<.001
Absent	747 (96.1%)	70 (64.8%)		810 (93%)	9 (53%)	

shown in Table 1, and the ABO-incompatible pregnancies are highlighted in italics.

3.2 | Direct antiglobulin test in ABO-incompatible HDN

Overall DAT positive results by CAT were seen in 3.8% of neonates and in 11.2% of neonates with ABO incompatibility. However, the frequency of positive DAT (n = 17) by CTT was 0.6% (of total cases) and 2.4% (among ABO-incompatible cases). As shown in Table 2, the strength of DAT by CAT is higher among neonates with O-A incompatibility than the neonates with O-B incompatibility.

The incidence of clinically significant hyperbilirubinaemia in a newborn with ABO incompatibility was compared with results of DAT. As shown in Table 3, it was significanlty higher in the group with positive DAT results by CAT ($P < .001$), and it was also higher among neonates with O-A incompatibility compared to the O-B group (65% Vs 45%).

The sensitivity, specificity and the positive predictive value of DAT by CAT and CTT are shown in Table 4. The tube technique showed higher specificity and predictive value for the occurrence of clinically significant hyperbilirubinaemia.

TABLE 4 Predictive value of direct antiglobulin test by CAT and CTT

	CAT (%)	CTT (%)	Elution test (%)
Sensitivity	51	11	17
Specificity	93	98	76
Positive predictive value	34	47	67
Negative predictive value	96	93	24

The clinical outcome of the newborn with respect to DAT status is given in Table 5. Based on the early onset of jaundice, a higher indirect bilirubin level (mean value) indicating rapid destruction of red cells was noted in the DAT positive group (Table 5). A significantly higher number of newborns required phototherapy in the DAT positive group compared to the DAT negative group (45.4% vs 24.4%).

3.3 | Eluate from DAT positive samples

Eluate was positive whenever the strength of the DAT was 3+ or above in CAT (17 cases). Antibody specificity was confirmed with the eluate, and the predictive value of the test is shown in Table 4.

TABLE 5 Clinical outcome vs the DAT result by CAT

Clinical outcome	DAT		*P* value
	Negative	Positive	
Onset of jaundice (h): median (range)	48 (18-72)	30 (12-36)	.005
Hyperbilirubinemia (number of cases)	3%	35%	<.001
Duration of phototherapy (h)	48(24-120)	72 (16-216)	.02

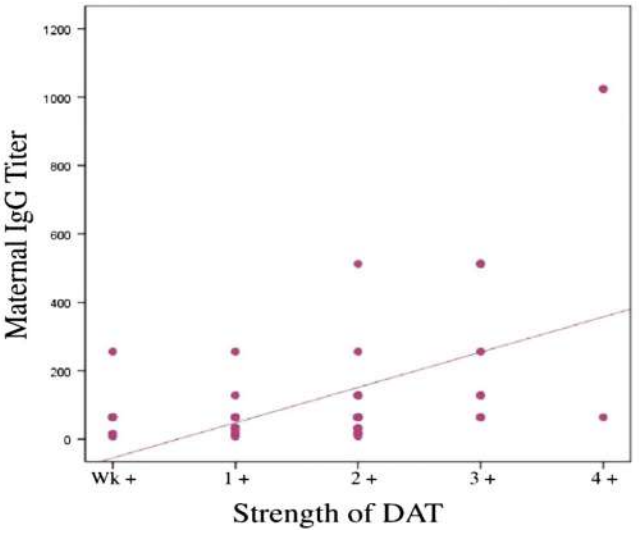

FIGURE 1 Linear relationship between maternal IgG titre and strength of DAT [Color figure can be viewed at wileyonlinelibrary.com]

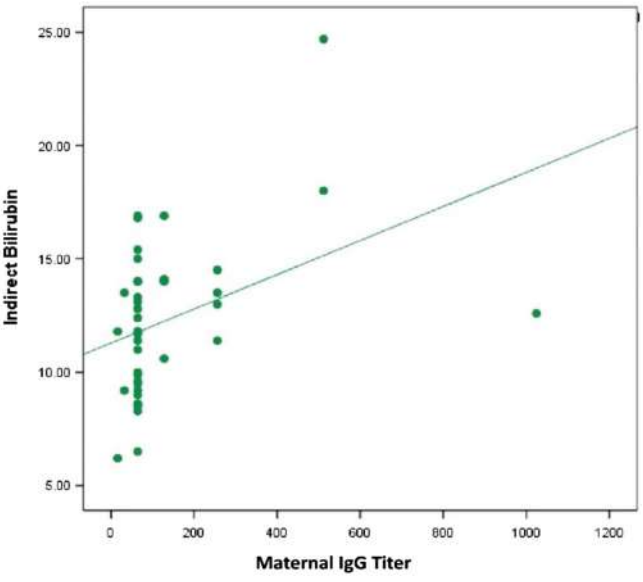

FIGURE 2 Maternal titre and rise in indirect bilirubin [Color figure can be viewed at wileyonlinelibrary.com]

3.4 | Maternal antibody titre

Maternal antibody titre (IgG) was performed in patients with ABO incompatibility. As shown in Figure 1, a positive relationship was observed between maternal IgG titre and the strength of the DAT (R = .408 and P-value = <.001). As shown in Figure 2, a positive association was also noted when we compared the indirect bilirubin level of the neonates with the maternal titre (R = .440; P = .004:Figure 2). Clinical intervention was required to manage hyperbilirubinaemia for all the neonates with O-A and O-B incompatibility at maternal titres of 16 and 64, respectively. This result was consistent with the already established critical titre levels of our laboratory. The number of cases with a critical or higher titre value was higher in the group with O-A incompatibility in comparison with the group with O-B incompatibility (8% vs 2.5%).

3.5 | Combination of tests and comparison of predictive value

The ability of a test to predict the occurrence of clinically significant hyperbilirubinaemia in a neonate with ABO incompatibility was estimated. The predictive values of the individual tests were as follows: positive DAT—40%, eluate—67% and maternal titres—41%. When immunohaematological tests such as DAT, elution test and maternal titres were combined, the positive predictive value increased to 94.12%.

4 | DISCUSSION

The ABO histo-blood system is the clinically most relevant blood group system in transfusion and transplantation medicine.[8] ABO HDN is gaining importance as the number of cases due to Rh incompatibility has reduced because of the routine immunoprophylaxis programme, and it is one of the most common reasons for neonatal readmission to the hospital.[12,13] In order to prevent bilirubin-induced encephalopathy, it is quite essential to detect the condition at the earliest and initiate appropriate treatment. Hence, establishing a predictive value of the laboratory tests will be of great clinical use in managing this condition. In the present study, we analysed the prevalence of ABO HDN in our population and assessed the clinical implication of the routinely performed immunohaematological tests.

The frequency of ABO blood groups in India varies from region to region.[15-17] In our study population, the blood group O (40.5%)was found to be the commonest one, followed by B (28.2%), A (26.6%), and AB (4.7%). Bombay Phenotype, a rare blood type was seen in 0.2% of the cases. In our study, the frequency of ABO incompatibility was seen in 33.68%, which is comparable with the study conducted by *Oseni* et al in a Nigerian population (38%).[18,19] The fetomaternal

ABO incompatibility was seen in 33.68% of the cases in the present study.

Toy et al showed that ethnic differences exert an influence on the DAT positive status of ABO-incompatible pregnancies but not in the clinical disease. Asians and Africans showed a higher prevalence of DAT positive status than the Caucasians in their study.[20] The incidence of DAT positivity in newborns with ABO incompatibility was observed to be 11.2% in the present study, which is nearly twice that of the observations made by *Toy* et al and *Dinesh D*.[20,21] Various studies have shown that DAT is not a very good predictor of clinical severity for ABO HDFN cases, and it ranges from 12% to 53% with a sensitivity of 15% to 64%.[16,19,20] The methodology used for testing influences the sensitivity of the test and hence the predictive value. We have assessed these parameters for both DAT by CAT and CTT. In our study, the positive predictive value of DAT was 34% by CAT and 47% by CTT, and the sensitivity by CAT was 51%, whereas with CTT, it was 11%, which is comparable to results of *Brouwers* et al (53%) and *Dittmar* et al.[1,22] The minimum number of IgG anti-A or anti-B molecules detectable on the red cells by the antiglobulin test is about 150 per cell for both cord and adult red cells by CTT.[23-25] The 'A' allele elicits a higher concentration of transferases activity than the B allele, which leads to conversion of all the H antigens on the Red Blood Cells (RBCs) to A antigen sites. Hence, in an adult RBCs, the number of antigen sites per red cell is estimated to be 8 to 12×10^5 for A1, 1 to 4×10^5 for A2 antigen and 6 to 8×10^5 for B antigen. However, when both A and B alleles are inherited, the B enzyme is more efficient for H substance than the A enzyme, leading to the expression of 6×10^5 A antigen sites on adult RBCs compared to 7.2×10^5 B antigen sites. As per literature, haemolysis due to anti-A is more common (1 in 150 births) than that due to anti-B, which was comparable in the present study.[26] The incidence of significant hyperbilirubinaemia, lab evidence of haemolysis and a positive DAT was higher among the O-A group (65% vs 45%) compared to the O-B group.

The poor development of ABO antigens on fetal RBCs, early destruction of antibody-coated red cells and presence of ABH substance in secretion leading to neutralisation of antibodies could be possible reasons for the weak reaction on DAT.[1,25]

The elution test is considered to a have high diagnostic value, and generally, the heat elution technique is performed in such cases. Eluate may be positive even in DAT negative cases; hence, elution may be considered to confirm ABO HDN wherever there is high clinical suspicion.[27] However, we succeeded in eluting the antibody only when the DAT strength was 3+ or more by CAT, and our finding is in concordance with results previously published by *Richa* et al.[28]

High titres of maternal antenatal IgG anti-A and anti-B have been associated with ABO HDFN.[29] Unlike in cases of Rh-negative pregnancy, maternal antibody titres are rarely performed to predict the ABO HDN. In our study, we noted a positive relationship between the levels of maternal IgG antibody titre and the strength of the DAT, which is inconsistent with the literature.[30,31] Early rise in serum bilirubin levels also exhibited a positive correlation with maternal antibody titres. This finding was similar to the previous study where a higher antibody titre was considered to be a risk factor for severe hyperbilirubinaemia.[30] *Bakkeheim* et al showed that IgG antibody titre below 512 in ABO-incompatible HDFN rarely requires aggressive treatment, which includes exchange transfusion and IvIg treatment.[32] ABO antibody titre of more than 512 had a sensitivity of 90% and a specificity of 72% for predicting immunoglobulin treatment. *Han* et al stated that patients with a maternal titre of 256 or above and a positive DAT result require treatment for hyperbilirubinaemia. In the present study, we have observed the features of ABO HDFN with a titre as low as 16 in the O-A group and 64 in the O-B group. The critical titres were set so low at our centre as the majority of newborns developed features of moderate HDFN, requiring conservative treatment such as phototherapy at this value.

Sarici et al proposed that reticulocyte count, a positive DAT and presence of a sibling with neonatal jaundice are good predictors for the development of significant hyperbilirubinaemia and severe HDN in full-term healthy newborns with ABO incompatibility.[6] A study conducted by *Procianoy* et al showed that the probability of clinically significant ABO HDN is approximately around 70% when we consider the combination of test results such as positive quantitative elution test, DAT and a bilirubin concentration of ≥4 mg/dL.[33] The negative predictive value of the combination of tests (at least two) was 93%. We estimated the positive predictive value of the combination of tests; it was 47.06% for the DAT and elution test and was 94% when we added the maternal antibody titre (value above the critical level) to the panel. However, we have not analysed the predictive value of the immunohaematological test in combination with other laboratory parameters, for example, reticulocyte count, bilirubin concentration.

Prediction of clinically significant hyperbilirubinaemia is challenging in neonates with ABO incompatibility. Although the specificity of the immunohaematological test is high, its ability to predict the severity of the disease individually is poor. It is advisable to combine the immunohaematological tests, clinical findings and haematological parameters to identify at-risk newborns.

CONFLICT OF INTEREST

None of the authors have any competing financial and/or non-financial interests in relation to the work described.

AUTHOR CONTRIBUTIONS

Soumya Das: Planned and performed the immunohaematology work-up in the study and writing the manuscript. Shamee Shastry: Planned, guided the study and corrected the manuscript. P Kalyana Chakravarthy: Data analysis, interpretation and correction of the manuscript. Poornima B Baliga: Corrected the manuscript.

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ORIGINAL ARTICLE

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Evaluating automated titre score as an alternative to continuous flow analysis for the prediction of passive anti-D in pregnancy

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Summary

Objectives: To evaluate the potential of the automated titre score (TS) as an alternative method to continuous flow analysis (CFA) for the prediction of the nature of anti-D in pregnancy.

Background: The 2016 revised British Society for Haematology (BSH) antenatal guidelines recommended a measurement of anti-D concentration by CFA to ensure the detection of potential immune anti-D. Due to high referral costs and resource pressures, uptake has been challenging for hospital laboratories. Serious Hazards of transfusion (SHOT) data have previously shown that this has contributed to missed antenatal follow ups for women with immune anti-D and neonates affected by haemolytic disease of the fetus/newborn.

Methods/Materials: In this multicentre comparative study, samples referred for CFA quantification were also tested by an ORTHO VISION automated anti-D indirect antiglobulin test (IAT) serial dilution and then converted to TS. CFA results and history of anti-D prophylaxis were used to categorise samples as passive or immune, with the aim of determining a potential TS cut-off for CFA referral of at risk patients.

Results: Five UK National Health Service (NHS) trusts generated a total of 196 anti-D TS results, of which 128 were classified as passive and 68 as immune. Diagnostic testing of CFA and TS values indicated a TS cut-off of 35 to assist in distinguishing the nature of anti-D. Using this cut-off, 175 (89%) results were correctly assigned into the passive or immune range, giving a specificity of 92.19% and a negative predictive value of 91.47%.

Conclusion: TS in conjunction with clinical and anti-D prophylaxis history can be used as a viable and cost-effective alternative to CFA in a hospital laboratory setting.

KEYWORDS

anti-D, automated titre, continuous flow analyser, HDFN, ortho vision, titre score

1 | INTRODUCTION

Close collaboration between the blood transfusion laboratory and obstetric teams is critical to identify the presence of maternal red cell antibodies (~1% of pregnancies)[1] and to monitor these levels throughout the antenatal period. Historically, anti-D was the most common cause of haemolytic disease of the fetus and newborn (HDFN), accounting for 18%–27% of all cases.[2] The advent of anti-D prophylaxis in the 1960s dramatically reduced the incidence of sensitisation and production of immune anti-D. Hence, the number of deaths from anti-D related HDFN cases reduced from 46/100000 to 1.6/100000, with a further reduction seen after the introduction of antenatal prophylaxis.[2] The anti-D prophylaxis programme continues to be hugely successful; however, sensitisation still occurs in ~500 pregnancies per year in the United Kingdom[3]; this would equate to ~78/100000 sensitisations to anti-D based upon current UK live birth data.[4]

Once anti-D has been detected in pregnancy, it needs to be quantified to categorise risk and to determine the need for further obstetric review. If the anti-D detected is prophylactic (passive) in nature, there is no risk; however, the nature of detected anti-D (passive or immune) cannot be determined by qualitative laboratory methods alone. The majority of transfusion laboratories in England currently refer samples to the National Health Service Blood and Transplant (NHSBT) for quantification by continuous flow analysis (CFA). The concentration of prophylactic anti-D rarely exceeds 0.4 IU/ml[5] by CFA, falling into the low risk of HDFN category. Difficulties in differentiating between immune and passive anti-D can lead to prophylaxis being omitted where it is required and women not receiving appropriate follow up during pregnancy. Six cases of newborns with HDFN were reported to Serious Hazards of transfusion (SHOT) in 2012 due to this incorrect assumption,[5,6] but the CFA results and clinical impact of such cases were not provided. To address these issues, the 2016 British Society for Haematology (BSH) antenatal guidelines recommended quantification of all samples containing anti-D by either CFA or a method that has been extensively validated against CFA.[5] However, the referral of these samples presents hospitals with many challenges, including high referral costs and resource pressures for both the hospital and the reference lab, as well as long turnaround times for obtaining results. Due to the issues discussed, many transfusion laboratories have been unable to implement the 2016 guidelines, meaning that patients are still potentially at risk.

In a climate of austerity, hospitals must develop strategies to provide the best care in a cost-effective manner. Some blood transfusion automated systems have the ability to perform automated titrations. Currently, reporting this method as an endpoint titre is considered to be semi-quantitative, does not accurately represent the clinical picture and correlates poorly with the severity prediction of HDFN.[7] The adoption of a titre score (TS), however, provides a more quantitative result that takes into account the strength of the reaction and the avidity of the antibody and is thought to better correlate with risk.[7-9] The aim of the first phase of this study was to assess if TS determined by automated ORTHO BioVue column agglutination technology (CAT) is a comparable alternative to the existing CFA for the categorisation of the nature of anti-D (prophylactic or immune).

2 | MATERIALS AND METHODS

Samples from pregnant women collected between April 2017 and December 2018, who were found to have a detectable anti-D (passive or immune), were included in the study.

A total of 196 samples were tested across five UK hospital transfusion laboratories using 10 ORTHO VISION platforms. All laboratories participating in the study referred whole-blood expaned to ethylenediamine tetraacetic acid samples from pregnant women with detectable anti-D to the NHSBT red cell immunohaematology (RCI) laboratory for anti-D quantification by CFA. Prior to referral, these samples were tested on the ORTHO VISION platform, an automated TS was determined and the results were compared with the CFA result.

Ethical approval was not obtained as only the CFA result was reported to the clinician; no additional samples were requested; and only samples with enough volume were tested in house prior to referral, thus not impacting patient care or management.

Samples with detectable anti-D were quantified at RCI laboratories using Astoria 2 flow analysers employing the reference method (White Horse Scientific Ltd. Pewsey, Wiltshire, United Kingdom).

The ORTHO VISION platform automated serial dilution functionality was used to make doubling dilutions of the patient's plasma in standard phosphate-buffered saline solution. Serial dilutions were prepared from neat to 1in 1024. The assay included a negative control to test for any antibody carryover from the neat plasma. These dilutions were tested against a pooled OR1r 0.8% reagent red cell (ref NHSBT PR045) using BioVue Anti-IgG cassettes (Ortho Clinical Diagnostics ref 707 450). The reaction grades were then read by the ORTHO VISION Cassette Imaging System (CIMS) and reported by the Image Processing System (IPS). Each positive reaction grade was manually converted into a score value (Table 1), and the sum of all scores gave the TS for each individual sample. See a calculated TS example in Figure 1.

The NIBSC anti-D standard (NIBSC Ref 73/515) was used as a quantitative positive control with a known concentration of 0.23 IU/ml.

To aid statistical analysis and result interpretation, the nature of the anti-D was assigned. Anti-D was classified as passive based on a combination of a CFA result of less than 0.4 IU/ml, patient clinical

TABLE 1 Automated titre reaction strengths are converted to titre score (TS) value

IAT reaction strength	4	3	2	1	0.5	0
Score value	12	10	8	5	3	0

	Plasma Dilution										Titre End point	Titre Score
	1	*2*	*4*	*8*	*16*	*32*	*64*	*128*	*256*	*512*		
Strength	*4*	*3*	*2*	*1*	*0.5*	*0*	*0*	*0*	*0*	*0*	***16***	***38***
Score	*12*	*10*	*8*	*5*	*3*	*0*	*0*	*0*	*0*	*0*		

FIGURE 1 A worked example of titre score (TS) application

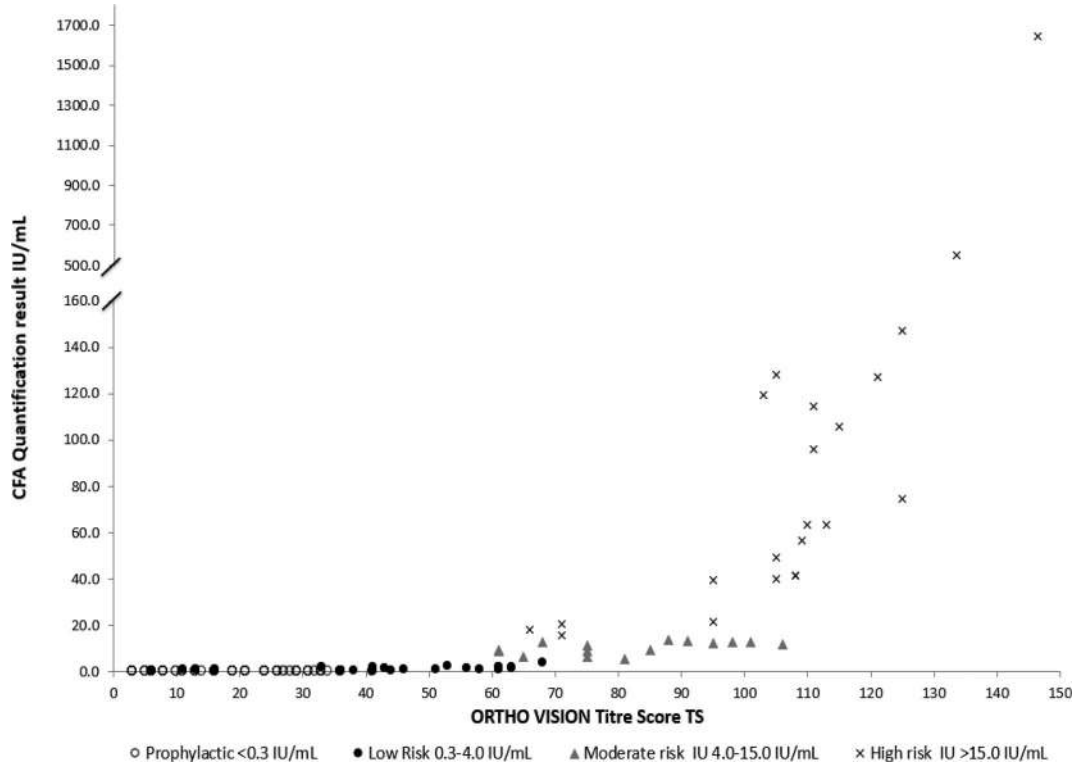

FIGURE 2 Titre score (TS) versus continuous flow analysis (CFA) quantification based upon haemolytic disease of the fetus/newborn (HDFN) risk categories

history and applicable evidence of anti-D prophylaxis and D status of the child where available. If no evidence to suggest a passive nature was identified, the antibody was assumed to be immune.

3 | DATA ANALYSIS

The CFA quantification results from NHSBT were directly compared to the TS. Diagnostic test (2 × 2) analysis was performed using MedCalc statistical software (© 2020 MedCalc Software bv). Sensitivity, specificity and positive/negative predictive values (NPVs) were determined at various TS cut-off points and used to propose the most appropriate score to differentiate between passive and immune anti-D in conjunction with patient clinical history.

4 | RESULTS

A total of 196 samples were tested across five UK hospital transfusion laboratories using 10 ORTHO VISION platforms. Of the anti-D detected in these samples, 128 were classified as passive and 68 as immune. The TS and CFA values were compared and presented based upon the HDFN clinical risk categorisation (prophylactic/low/moderate/high) (Figure 2).[5,10] The suggestive TS cut-off value range

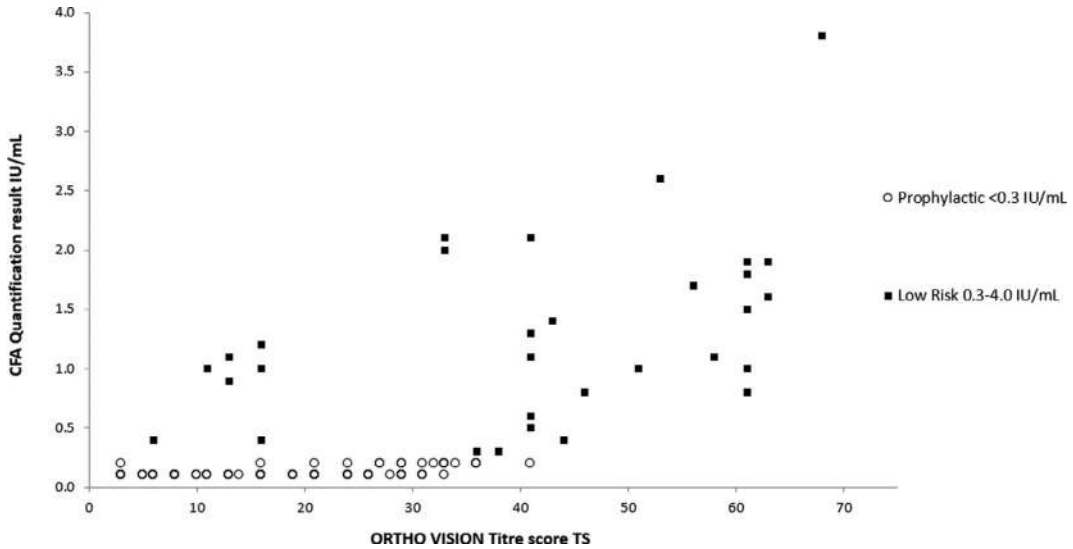

FIGURE 3 Passive and low-risk titre score (TS) versus continuous flow analysis (CFA) comparison

TABLE 2 Diagnostic testing of potential titre score (TS) cut-off values

Titre score	30	31	32	33	34	35
True positives (TP)	59	59	59	57	57	57
True negatives (TN)	106	110	111	117	118	118
False positives (FP)	22	18	17	11	10	10
False negatives (FN)	9	9	9	11	11	11
Sensitivity (%)	86.76	86.76	86.76	83.82	83.82	83.82
Specificity (%)	82.81	85.94	86.72	91.41	92.19	92.19
Positive predictive value (PPV) (%)	72.84	76.62	77.63	83.82	85.07	85.07
Negative predictive value (NPV) (%)	92.17	92.44	92.50	91.41	91.47	91.47

Note: TP All patient titre scores > 35 assumed immune, FP All patient titre scores > 35 assumed prophylactic, TN All patient titre scores ≤ 35 assumed prophylactic, FN All patient titre scores ≤ 35 assumed immune.

between prophylactic and the low-risk immune category results was informed by Figure 3. Diagnostic testing was then performed to define potential TS values (Table 2).

The mean quantification value was 22 IU/ml with a median of 0.2 IU/ml (range < 0.1–1643.6 IU/ml). The mean and the median TS values were 35 and 24, respectively (range 3–148).

Potential TS cut-off values were selected for analysis based on a passive and immune low-risk boundary as identified in Figures 2 and 3.

Using a TS cut-off value of 35, 175 (TP + TN)/196 (89%) were correctly classified as passive or immune. A TS cut-off value of 35 correctly categorised 118 (TN)/128 (92%) samples confirmed as prophylactic anti-D, signifying an NPV of 91.47% (95% CI of 86.17%–94.86%) and a negative likelihood ratio of 0.18.

For the detection of immune anti-D, a TS cut-off value of 35 correctly categorised 57 of 68 (83%) confirmed as immune anti-D by CFA, signifying a positive predictive value of 85.07% (95% CI of 75.70%–91.25%) and a positive likelihood ratio of 10.73.

5 | DISCUSSION

The recommendation in the 2016 BSH antenatal guidelines[5] for all anti-D detected in pregnancy to be quantified by CFA has a potential financial impact on hospital transfusion laboratories due to increased referral and turnaround times. The findings of this study demonstrate that an automated TS determined using ORTHO VISION can reliably predict the nature of anti-D detected in pregnancy as passive with a sensitivity of 83% and specificity of 92% when using a TS of 35.

CFA is recognised in the United Kingdom as the preferred technique for anti-D quantification[2] with cut off values embedded in clinical follow-up criteria.[5,10] CFA has already been previously compared to CAT titration values using a TS method,[8] which was described as showing promising comparability and intra-laboratory reproducibility. This study supports such findings by demonstrating parity between the two methods in determining passive and immune anti-D (Figure 2). Generally, where a high quantification result was reported, a high TS was also observed. Similarly, for low quantification, a low TS was observed. From

reviewing Figure 1, we were unable to demonstrate a linear correlation between the TS and the concentration of anti-D in IU/ml. However, there appear to be emerging risk groups that will be further analysed as part of an ongoing study. Such comparable clinical decision and risk correlations have already been observed between CFA anti-D concentrations and anti-D titres in other studies.[11]

Manual titration methods, both tube and column agglutination indirect antiglobulin test (IAT), have been associated with inter-laboratory variation due to the preparation of the reagents and serial dilutions, as well as the visual interpretation of the end result.[12] However, ORTHO BioVue Column Agglutination has been shown to be an appropriate replacement for tube IAT in antibody titration[13] by reducing some of the variability factors. Utilising an automated analyser to perform titration serial dilutions further improves standardisation and turnaround time and reduces the risk of errors associated with manual testing and interpretation.[14] Internationally, a titre endpoint may be used routinely as a clinically actionable result; however, it has also been widely critiqued as poorly correlating with regard to the severity of HDFN.[8] Titre endpoint is thought to be a semi-quantitative estimate and does not evaluate the strength of the reaction obtained,[8] whereas TS has been shown to be sensitive to a wide range of antibody levels as determined by CFA and takes into account analytical variation.[7,8]

Methodology on its own cannot be used to determine the nature of anti-D but should be used in conjunction with confirmed clinical history that includes evidence of anti-D prophylaxis date and dose.[3] With the use of a TS of >35 as a cut-off for CFA referral, 10 of 196 (5.1%) results were false positives. These samples would have been referred when they were in fact prophylactic in nature. In routine clinical practice, these samples would still have been referred under the current BSH guidelines, representing no clinical impact.

In addition, 11 of 196 (5.6%) samples were false negatives. These samples would not have been referred based on the TS alone. When taking into account patient clinical history, none of these patients had evidence of anti-D prophylaxis and therefore would all have been referred for CFA for the duration of the pregnancy, all being classified as immune in nature. Looking in detail at these 11 samples, 6 (3% of 196) were taken from one patient who consistently showed a low TS (6–16) with a low-risk category CFA result (0.4–1.2 IU/ml) throughout the duration of the pregnancy. In addition, four (2% of 196) would also have been predicted to be passive in nature based on CFA result. One patient was early in gestation with known anti-D and anti-G. In the absence of evidence of prophylaxis, all 11 samples would have been referred for CFA regardless, and as such, there is no clinical risk associated with this finding.

A decision-making algorithm should accompany any implementation of this method to aid interpretation and clinical decision-making. An example algorithm has been included in Appendix 1.

6 | CONCLUSION

In this study, the ORTHO VISION fully automated platform has provided the ability to standardise the TS methodology across multiple sites and systems, thereby removing variability inherent to manual titration techniques.

Using a TS cut-off value of >35, there was no additional clinical risk when facilitating conformity to BSH guidelines, thereby reducing the number of samples referred for CFA quantification and supporting the prevention of incidents related to the mis-categorisation of the nature of anti-D, as noted in the 2012 SHOT report.[6]

Some hospitals involved in this study are currently in the process of implementing this method, which will be used to support decision-making algorithms for appropriate referral for CFA. This could be beneficial to the obstetric department by reducing turnaround times, offering potential financial savings and improving patient care.

The next phase of the study will involve gathering further data on women with immune anti-D, correlating the automated TS with CFA and clinical risk categories and outcomes. This will include inter-laboratory variation studies.

Disclaimer: The data and conclusions above are specific for the ORTHO VISION platform and ORTHO VISION technology. Other methodologies may not yield equivalent results and should be validated appropriately.

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AUTHOR CONTRIBUTIONS

Michelle L. Evans, Benjamin Holmes, Kerry Dowling, Tracey Lofting, Matthew R. Barnett, Nicolette Heydon and **Tracy Clarke:** Performed the research. **Michelle L. Evans, Benjamin Holmes, Kerry Dowling, Tracey Lofting, Matthew R. Barnett, Nicolette Heydon, Tracy Clarke, Christopher Hall, Sophie C. I. Callsen**, and **Wim Malomgre:** Designed the study. **Michelle L. Evans, Benjamin Holmes, Kerry Dowling, Tracey Lofting, Matthew R. Barnett, Nicolette Heydon, Tracy Clarke, Christopher Hall, Sophie C. I. Callsen, Wim Malomgre** and **Eva-Maria Surmann:** Analysed the data, wrote and revised the paper. **Christopher Hall, Sophie C. I. Callsen, Wim Malomgre** and **Eva-Maria Surmann:** Contributed essential reagents and tools.

CONFLICT OF INTERESTS

The authors have no competing interests.

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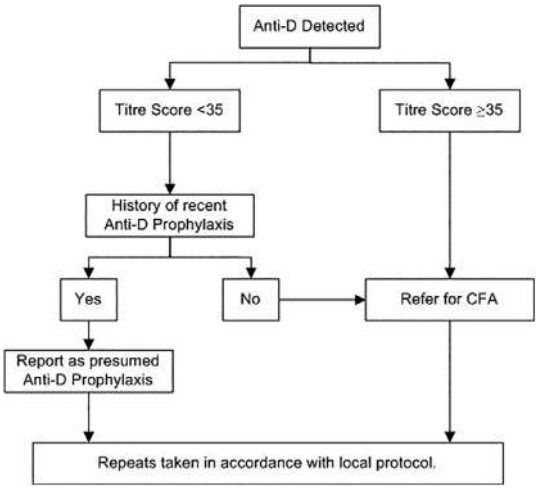
APPENDIX 1: An example of a laboratory decision algorithm for Anti-D nature interpretation using a TS cut off at 35



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D variants in the population of D-negative blood donors in the north-eastern region of Croatia

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Abstract

Objectives: The aim of this study was to determine RHESUS D GENE (*RHD*) allelic variants among Croatian D-negative blood donors and compare our results with respective data from other European countries.

Background: Altered or reduced D antigen expression can result in D variants, which can be mistyped and can lead to the alloimmunisation of the blood recipient. *RHD* genotyping can distinguish D variants: weak D, partial D and DEL, thus preventing alloimmunisation.

Material/methods: A total of 6523 samples obtained from D-negative Croatian donors were screened for the presence of *RHD* using the real-time polymerase chain reaction (PCR) method. PCR-SSP was performed for D variant genotyping by using commercial genotyping kits (Inno-Train, Kronberg, Germany). Genomic DNA sequencing for all 10 exons of the *RHD* was performed when the genotyping kits failed to assign a D variant.

Results: *RHD* molecular screening revealed 23 (0.35%) *RHD*-PCR positive samples, all C/E positive, in decreasing frequency: 11 hybrid *RHD-CE (2-9) D-CE* variants, 4 weak partial D type 11 and 2 weak D type 2. Six samples remained unresolved and were sequenced. For 12 of 23 samples (excluding large hybrids), an adsorption/elution of anti-D serum was performed, confirming that all 12 were RhD+. The calculated frequency of clinically significant D alleles in RhD-negative blood donors was 1:543 (0.18%) or 1:53 (1.89%) in C/E blood donors.

Conclusion: Data on the significant frequency of D variants among serologically D-negative blood donors in the north-eastern region of Croatia could help in introducing *RHD* molecular screening of blood donors in a routine workflow.

KEYWORDS

D antigen, D variant, partial D, *RHD* genotyping, weak D

1 | INTRODUCTION

The Rh system, with a large number of antigens, the five most important of which are C, c, D, E and e, is one of the most polymorphic and immunogenic blood group systems and the cause of most alloimmunisations following blood transfusions.[1,2] More than 50 Rh antigens have been described to date, all encoded by two closely linked genes, RHESUS D GENE *(RHD)* and *RHCE*, located on chromosome 1p3611.[2,3] RhD antigens can strongly stimulate the immune response and formation of anti-D antibodies in D-negative individuals, which may lead to strong transfusion haemolytic reactions and haemolytic disease of the newborn. The presence or absence of the

RhD protein on the erythrocyte membrane differentiates the D-positive (RhD+) from the D-negative (RhD−) phenotype, contributing to the high immunogenicity of D. The frequency of the D+ phenotype is reported to be 85% in Caucasians, 95% in sub-Saharan Africans and more than 99.5% in eastern Asians.[1] Although most Caucasians are RhD+ or RhD−, there are variations in D antigen expression. Around 0.2% to 1% of Europeans are carriers of aberrant *RHD* alleles, categorised as weak D, partial D and DEL phenotypes.[4] This altered D antigen expression can result in D typing discrepancies.[5] To date, a significant number of *RHD* alleles responsible for weak and partial D phenotypes have been identified, and their frequencies vary among ethnicities.[6]

The least expressed D variant, DEL, contains fewer than 30 D antigens sites on the red blood cell (RBC) membrane, compared to a normal RhD-positive RBC that expresses about 10 000 to 30 000 D antigens per cell and a weak D RBC with antigen densities between 60 and 4000.[1] Detection of DEL, that are usually serologically mistyped as D negative, is only possible through an adsorption/elution of anti-D reagents, and anti-D alloimmunisation has been reported after transfusion of such D-negative RBC units.[7-9] Different molecular mechanisms (mostly nucleotide changes in *RHD* and genetic recombination) responsible for D variants that can lead to altered D epitopes and/or decreased antigen copies per RBC have been described. Each country has its own policy and guidelines for the D typing of blood donors, and the proper reagents are used to determine most of the D variants. Routine serologic testing, however, has limitations and thus may not detect all D variants. *RHD* molecular screening, on the other hand, could detect them between presumed D-negative blood donors as such blood units pose a risk of the anti-D alloimmunisation of the transfusion recipient. This is why some blood services in Europe (Germany, Denmark, Austria and Switzerland) introduced routine molecular screening for *RHD* in seemingly D-negative blood donors; however, their strategies still vary.[10-13]

The aim of this study was to determine the type and frequency of D variants among the D-negative blood donor population in the north-eastern region of Croatia and to compare it with respective data from other European countries. Apart from the possibility of finding new genetic alterations, such data could help in the adaptation of *RHD* molecular screening of blood donors as part of a routine workflow.

2 | MATERIAL AND METHODS

2.1 | Blood donor samples

This study was carried out in the Department of Molecular Diagnostics, Croatian Institute of Transfusion Medicine (CITM). EDTA-anticoagulated blood samples were collected over a 1-year period, and all were typed as D-negative on standard serologic testing, including an indirect antiglobulin test (IAT). The age range of blood donors was between 18 and 65 years old (the blood donor age limit in Croatia), and there were no excluding criteria. The study was approved by the Ethics Committees of the CITM and School of Medicine, University of Zagreb. Serologic testing of the D antigen was performed using the direct serologic micromethod on the semi-automatic system Tecan-Genesis-Medusa (Tecan Trading AG, Männedorf, Switzerland) with monoclonal reagents RUM1 (CITM, Zagreb, Croatia) and MS 201 (Bioscot Serological, Livingston, United Kingdom) according to the manufacturer's instructions. D antigen was determined by DiaMed polyspecific microcards (BioRad, Cressier, Switzerland) using IAT with an IgM/IgG monoclonal reagent by combining clones D415 1E401/172-2 (Immucor-Gamma, Dreieich, Germany) according to the manufacturer's instructions.

2.2 | DNA extraction and molecular testing

Around 200 μL of each sample was first pooled in batches of 20, followed by DNA extraction, *RHD* screening by real-time polymerase chain reaction PCR and *RHD* genotyping using a PCR with sequence-specific priming (PCR-SSP).

Genomic DNA extraction from 200 μL of each pool was performed by a commercial QIAamp DNA Blood Mini kit on the QIAcube device (Qiaqen, Hilden, Germany). *RHD* molecular screening was followed by the qPCR method, with the amplification of exons 7 and 10, on the 7500 Real-Time PCR system (Applied Biosystems, Foster City, California). The sequences of the primers and probes were selected according to a previously described method.[14] In the case of qPCR exon 7- and/or exon 10-positive pool, we resolved the pool to the individual samples. *RHD* genotyping was performed from an individual D+ sample using the PCR-SSP genotyping kits Ready Gene D weak, Ready Gene D weak screen, Ready Gene CDE and Ready Gene D AddOn (Inno-Train, Kronberg, Germany), followed by electrophoresis and interpretation of the results.

2.3 | Adsorption/elution method

For DEL phenotype detection, an adsorption and elution test was performed using an IgM/IgG monoclonal reagent by combining clones D415 1E401/172-2 (Immucor-Gamma).

2.4 | Sequencing of the RHD gene

In cases where the *RHD* allele could not be identified by the genotyping kits, DNA sequencing was performed for all 10 exons[15] of the *RHD* gene at the Bristol Institute for Transfusion Sciences incorporating the International Blood Group Reference Laboratory.

2.5 | Statistical analysis

The Poisson 95% confidence interval (CI) for the numbers shown in Table 2 and 95% CI for the incidence rate in Table 1 have been

TABLE 1 *RHD* alleles established in this study, including estimated population frequency and upper and lower limits of 95% confidence interval (CI)

D variant	International Society of Blood Transfusion nomenclature	Structure	Genotype	Fenotype	Frequency (n = 6523 D-) 95% CI	Frequency (n = 640 D-, C/E+)
RHD (M295I)	*RHD*11* *RHD*weak partial 11*	885G>T (M295I)	*Dd Cc ee*	DEL	4 (0.06%) 1:1630 1.089-10.241	0.63% 1:160
Weak D type 2	*RHD*01W.2* *RHD*weak D type 2*	1154G>C (G385A)	*Dd Cc Ee*	DEL	2 (0.03%) 1:3261 0.242-7.225	0.31% 1:320
Weak D type 28	*RHD*01W.28* *RHD*weak D type 28*	1152A>C (T384T)	*Dd Cc Ee*	DEL	1 (0.015%) 1:6523 0.025-5.571	0,16% 1:640
RHD (1027delT)	*/*	Exon 7: 1027delT (premature stop codon at amino acid position 358—M358*)	*Dd cc Ee*	DEL	1 (0.015%) 1:6523 0.025-5.571	0.16% 1:640
No mutation #	*RHD*01*		*Dd Cc ee*	DEL	4 (0.06%) 1:1630 1.089-10.241	0.63% 1:160
RHD-CE(2-9)D	*RHD*01N.03*	Large hybrid allele	*Dd Cc ee*	D–	11 (0.17%) 1:593 5.491-19.68	1.72% 1:58
			In Total		23 (0.35%) 1:284 14.58-34.51	3.60% 1:28
			Clinically significant alleles		12 (0.18%) 1:543 6.20-20.96	1.88% 1:53

TABLE 2 Country differences in RHD-positive and significant D+ incidence rates

Country	Number of first-time donors screened	Number of RHD positive	RHD-positiveincidence rate (%)	95% confidence interval for RHD-positive incidence rate	Number of clinically significant D+	Clinically significant D+ incidence rate (%)	95% confidence interval clinically significant D+ incidence rate
Germany[a]	46 133	96	0.208	0.169-0.254	47	0.102	0.075-0.135
Austria[b]	23 330	94	0.403	0.326-0.493	74	0.317	0.249-0.398
Switzerland[c]	25 370	120	0.473	0.392-0.566	37	0.146	0.103-0.201
Croatia	6523	23	0.353	0.224-0.529	12	0.184	0.095-0.321

RHD-positive incidence rate comparison

	Difference	95% confidence interval for difference	χ^2	df	*P*
Croatia versus Germany	0.14%	0.018 to 0.325	5.144	1	.021
Croatia versus Austria	−0.05%	−0.141 to 0.198	0.326	1	.567
Croatia versus Switzerland	−0.12%	−0.072 to 0.270	1.674	1	.195

Clinically significant D+ rate comparison

	Difference	95% confidence interval for difference	χ^2	df	*P*
Croatia versus Germany	0.084%	−0.004 to 0.221	3.430	1	.064
Croatia versus Austria	−0.116%	−0.019 to 0.246	3141	1	.076
Croatia versus Switzerland	0.038%	−0.058 to 0.181	0.488	1	.484

[a]Reference 16.
[b]Reference 12.
[c]Reference 11.

calculated for *RHD*-positive findings and clinically significant D+ findings. Differences in rates between our data and the compared countries were analysed with a χ^2 test. All *P* values below 5% were considered significant. MedCalc Statistical Software version 19.2 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2020) was used in all statistical procedures.

3 | RESULTS

A total of 6523 D-negative blood donor samples were collected, and 325 pools of 20 samples and 1 pool of 23 samples were analysed. Of 6523 D-negative samples, 23 (0.35%) were qPCR positive. The *RHD*01N.03* (*RHD-CE (2-9) D-CE)* large hybrid allele was determined in 11 (47.8%) of 23 samples, which were only exon 10 positive on *RHD* molecular screening. Those are considered D-negative by International Society of Blood Transfusion and are thus clinically irrelevant. The allele *RHD**weak partial 11-*RHD*(M295I) was detected in four (17.4%) samples, whereas *RHD*01W.2 (RHD**weak D type 2) was detected in only two (8.7%) samples. Another six *RHD* qPCR-positive samples remained unidentified and underwent further characterisation by DNA sequencing. A novel mutation was determined in one sample, exon 7—1027delT, resulting in a premature stop codon at amino acid position 358 (M358*), most likely causing the synthesis of the truncated protein and, consequently, the DEL phenotype. One sample was categorised as *RHD*01W.28* (weak D type 28), with the reported nucleotide alteration 1152A>C (T384T). For the remaining four samples, no mutations were found in the *RHD* gene, indicating the genotype *RHD*01* (normal D). All of the qPCR-positive samples were also found to be C/E- RhC /RhE antigen C/E positive. The D variant alleles and frequencies detected in our donor population are listed in Table 1. For 12 of 23 samples (excluding 11 specimens defined as large hybrids), an adsorption/elution of anti-D serum was performed, confirming that all 12 were D+ and, thus, clinically relevant. The calculated frequency of clinically significant *RHD* alleles was 1:543 (0.18%) in the examined population of D-negative blood donors, that is, 1:53 (1.89%) in C/E+ blood donor group. All of them were heterozygotes for *RHD* gene zygosity testing.

We did not perform a post-genotyping donation follow up, nor do we have information on post-transfusion antibody screening in the blood recipients of the previously donated *RHD*-positive blood units. After *RHD* screening and genotyping, the blood group was changed from D-negative to D+ in all blood donors who were found to have the D variant, and donors were notified of their testing result.

We compared our data with the other European countries, and the differences in *RHD*-positive incidence and clinically significant D+ rates were calculated and are listed in Table 2.

4 | DISCUSSION

The role of *RHD* genotyping in determining D variants with the potential to alloimmunise D-negative blood recipients has been of major interest in transfusion medicine lately. The availability of molecular methods has enabled a better knowledge and understanding of *RHD* polymorphism, initiating a new era of blood donor typing.[4] Many reports have been published on the detection of D variants in order to reduce the residual risk of alloimmunisation to the D antigen.[9,17] To date, many population studies have provided data on *RHD* molecular background and have shown that the prevalence of D variants varies by races and ethnicity, even within the same country.[1,18-22] Therefore, each country must conduct its own population study on the prevalence of D variants and adjust typing strategies according to the most prevalent *RHD* alleles. The *RHD* molecular screening of blood donors is already being routinely performed in several European countries.[11,12,23]

The aim of this study was to determine the type and frequency of *RHD* alleles in D-negative blood donors in the north-eastern region of Croatia. In order to obtain the most D variants with *RHD*-specific polymorphism, we decided to screen for the presence of the *RHD* exons 7 and 10, which correlates with other studies.[13,16,19,23,24] To minimise the financial impact and to save time, we decided to perform testing by pooling samples from D-negative blood donors, which proved to be very practical and efficient.[23,25] Molecular screening of 326 pooled samples determined 0.35% *RHD* PCR-positive blood donors. Similar results have been reported—0.21, 0.4 and 0.47% in German, Austrian and Swiss blood donor populations, respectively.[11,12,16] Further PCR-SSP genotyping defined 17 of 23 samples, with the *RHD-CE (2-9)-D-CE hybrid (RHD*01N.03*) allele having the highest prevalence, followed by *RHD* weak partial 11 (RHD*11)* and *RHD* weak D type 2 (RHD*01W.2).* The most prevalent hybrid, *RHD-CE (2-9)-D-CE*, is not clinically relevant as carriers of this allele lack the D antigen on the RBC membrane. The second most prevalent allele, *RHD (M295I)* (borderline DEL/weak D tip 11), can induce anti-D alloimmunisation, and such units should be redefined as D+.[26] The least prevalent, weak D type 2, can be immunogenic, as shown in other studies.[13] Six *RHD*-positive samples undifferentiated by genotyping were sent for genome sequencing, which defined weak D type 28 *(RHD*01W.28*) in one sample and the novel mutation *RHD* (1027delT) (premature stop codon at amino acid position 358—M358*) in another sample, whereas four samples indicated genotype *RHD*01* (normal D). There is no explanation yet for the aberrant D expression in those samples. In the hope of finding an explanation with the advent of additional testing techniques, we will archive those samples.

The frequency of clinically significant D variants in the north-eastern region of Croatia was 1:543 (0.18%) or 1:53 (1.89%) in C/E blood donors. Our data are in concordance with the above-mentioned European studies, 0.1%, 0.3% and 0.15%, as seen in Table 2.[11,12,16] The differences in the named frequencies are likely related to sampling size and testing strategies.

The incidence of the DEL phenotype in Caucasians is around 0.1%.[27,28] The most common DEL phenotype in Caucasians is *RHD* weak partial 11* RHD (M2951), which has also been identified in our study. Finally, the incidence of *RHD*-positive blood donors in the serological RhD-negative, C/E-positive blood donor group is 3.60%;

reports from Central European countries show similar values, ranging from 2.7% to 5.23%.[10,12,19,23]

To the best of our knowledge, the present study is the first in our country to determine the frequency of *RHD* alleles in D-negative blood donors on a much larger scale. The results of the identified D variants will help in introducing *RHD* molecular screening of D-negative blood donors and in establishing optimal testing strategies for future use, leading to safer blood transfusions.

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CONFLICT OF INTEREST

The authors have no competing interests.

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ORIGINAL ARTICLE

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Blood group P1 prediction using multiplex PCR genotyping of *A4GALT* among Thai blood donors

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Summary

Objectives: This study aimed to investigate single-nucleotide variants (SNVs) associated with P1 expression among Thai blood donors and develop a genotyping method using multiplex polymerase chain reaction (PCR) to predict P1 blood group status.

Background: The α1,4-galactosyltransferase (*A4GALT*), also called Gb3/CD77 synthase or P1/P^k synthase enzyme, is encoded by the *A4GALT* gene and catalyses the transfer of galactose from uridine diphosphate-galactose to lactosylceramide, creating the P^k antigen (Gb3). The same enzyme synthesises the P1 antigen by adding terminal galactose to paragloboside. The *A4GALT* transcripts are elevated in P_1, and different SNVs in transcription factor-binding regions of *A4GALT* correlate with P_1 and P_2 phenotypes.

Material and Methods: A total of 218 blood samples from Thai blood donors at the Thammasat University Hospital were tested for the P1 antigen using the conventional tube technique. Genomic DNA was extracted, and non-coding regions of *A4GALT* were sequenced and analysed. A multiplex PCR assay was developed and validated to identify P1-associated SNVs and was subsequently tested on 1022 Thai DNA samples of unknown P1 antigen status.

Results: In the tested cohort (n = 218), P_1 and P_2 phenotypes were found in 24.77% and 75.23% of donors, respectively. Moreover, three SNVs—rs8138197 (C/T), rs2143918 (T/G) and rs5751348 (G/T)—correlated 100% with both phenotypes. Finally, findings agreed with serological phenotyping and DNA sequencing results, confirming their validity for predicting P1 antigen positivity.

Conclusions: This study confirmed that three SNVs also correlated with P_1/P_2 phenotypes among Thais, as expected. A multiplex PCR found that SNVs rs2143918 (T) and rs5751348 (G) predicted blood group P1 and is an accurate, reproducible, cost-effective and less time-consuming alternative to traditional methods.

KEYWORDS
A4GALT, blood group P1 prediction, multiplex PCR, Thai blood donors

1 | INTRODUCTION

The P1 (formerly called P) antigen was first discovered in 1927 by Landsteiner and Levine.[1] Currently, the P1PK blood group system (International Society of Blood Transfusion, ISBT no. 003) comprises P1, P^k and NOR antigens, whereas globoside (P antigen) and PX2 antigens are assigned to the GLOB system (ISBT no. 028). The high-prevalence LKE antigen belongs to the 901 series.[2] The P1, P^k and P antigens are synthesised from precursor glycosphingolipids by the sequential addition of monosaccharides by glycosyltransferases. In the

neolacto-paragloboside synthesis pathway, a galactose residue is added to paragloboside (lacto-*N*-neotetraosyl ceramide), giving rise to the P1 antigen. The presence and absence of the P1 antigen determines P_1 and P_2 phenotypes, respectively. The P_1^k phenotype lacks the P antigen, whereas the P_2^k phenotype lacks both the P and P1 antigens. On the other hand, the p phenotype lacks the P^k, P and P1 antigens.[3]

Weak P1 antigen expression is found at 12 weeks' gestation and takes up to 7 years before reaching full expression.[4,5] The strength of the antigen expression shows person-to-person variation.[6,7] The frequency of the P_1 phenotype varies in different populations, ranging from 90% among Africans and 80% among Caucasians to 20% among Asians,[3,8] whereas this frequency is around 26.97% among healthy Thai blood donors.[9]

Anti-P1 can be isolated from anti-PP1P^k of p individual by adsorption with P_2^k cells, and anti-P1P^k was mostly IgG. In contrast, the anti-P1 of P_2 individuals is usually IgM.[10] However, a case of haemolytic transfusion reaction (HTR) mediated by IgM anti-P1 reactive at 37°C has been reported,[11] and HTRs caused by IgG anti-P1 reactive at 37°C and/or indirect antiglobulin (IAT) phase(s) have been reported either as acute or delayed and range from mild to severe.[12-15] The anti-P, anti-P1 and anti-P^k components of anti-PP1P^k are separable through adsorption and have been reported to react against antigens on the placental tissues, resulting in the destruction of the placenta and early pregnancy loss or multiple abortions, and can be confused with anti-P1 antibodies.[10]

P1 and P^k antigen formation requires the α1,4-galactosyltransferase (also called Gb3/CD77 synthase, P1/P^k synthase) enzyme, which is encoded by the *A4GALT* gene located on chromosome 22 and consists of four exons spanning ~26.6 kb of genomic DNA.[16] Mutations in the *A4GALT* open reading frame may cause a loss of enzymatic activity, resulting in the p phenotype. However, no mutations in the coding region of this gene could be correlated to the P_1 and P_2 phenotypes. Furthermore, P_1 individuals express elevated levels of *A4GALT* transcripts, suggesting that P1 antigen expression is influenced by *A4GALT* gene regulation.[17,18]

Previous studies have found that a single-nucleotide variant (SNV) in *A4GALT* (rs8138197) correlated with P1 expression in 99.5% and 100% of Swedish[18] and Taiwanese[19] blood donors, respectively. Moreover, two other SNVs (rs2143918 and rs5751348), which are tightly linked to rs8138197, showed 100% correlation with P1 expression in different populations.[20-22] In agreement with the aforementioned studies, it was recently reported that four SNVs (rs66781836, rs8138197, rs2143918 and rs5751348) correlated with P_1 and P_2 phenotypes.[21] Studies examining the gene regulation of those SNVs showed that rs5751348 contains potential transcription factor-binding sites for runt-related transcription factor 1 (RUNX1) and early growth response 1 (EGR1), suggesting that these proteins play a role in creating P_1 and P_2 phenotypes.[22,23]

Despite progress, the molecular genetic basis of the P_1 phenotype in Thai blood donors remains to be elucidated. Therefore, this study aimed to first investigate the SNVs associated with P1 expression among Thai blood donors and, second, develop a multiplex PCR-based genotyping method to predict P1 blood group status.

2 | MATERIAL AND METHODS

2.1 | Subjects

This study was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Thammasat University, Pathumtani, Thailand (COE No. 002/2563). EDTA-anticoagulated samples were collected from the peripheral blood of 218 unrelated healthy Thai blood donors at the Blood Bank, Thammasat University Hospital, Pathumtani, Thailand, between May and July 2020. Genomic DNA from these samples was extracted using the DNeasy Blood & Tissue Kit according to the manufacturer's instructions (QIAGEN) and was then stored at −20°C until genotyping. An additional 1022 DNA samples from unrelated healthy Thai blood donors obtained from the National Blood Centre, Thai Red Cross Society (NBC-TRCS), Bangkok, Thailand, were used for multiplex PCR implementation experiments.

2.2 | P1 antigen typing by conventional tube technique

A total of 218 blood samples from Thai blood donors were tested for P1 antigen positivity. Briefly, a 2%–3% red blood cell (RBC) suspension in isotonic saline was prepared. One drop of RBC suspension and one drop of monoclonal anti-P1 (Clone 650, CE-Immundiagnostika, Eschelbronn, Germany) were added to the appropriate tube. The mixture was incubated for 5 min at room temperature, centrifuged and macroscopically observed for agglutination. The agglutination reactions were graded 4+, 3+, 2+, 1+ and negative according to standard guidelines.[6]

2.3 | SNV analysis by DNA sequencing

Genomic DNA samples were PCR-amplified for SNVs present in the non-coding regions of *A4GALT*, particularly rs66781836, rs8138197, rs2143918 and rs5751348.[20-23] Primer pair sequences for each amplicon are shown in Table 1. For each PCR reaction, 3 μl of genomic DNA (50 ng/μl) was amplified in a total volume of 50 μl using 3 μl of 10 μM forward primer and 3 μl of 10 μM reverse primer for each reaction. The PCR was performed with 25 μl of 2× PCR reaction mixture (Phusion High-Fidelity PCR Master Mix, New England BioLabs) and 16 μl of sterile distilled water in a T100 Thermal Cycler (Bio-Rad Laboratories, Inc.).

PCR conditions were as follows: initial denaturation at 98°C for 2 min; 10 cycles of denaturation at 98°C for 10 s and annealing/extension at 69°C for 60 s; 25 cycles of denaturation at 98°C for 30 s and annealing at 62°C 60 s and extension at 72°C for 30 s; and final extension at 72°C for 5 min. PCR products were separated on a 1.5% agarose gel containing SYBR Safe DNA Gel Stain (Invitrogen), electrophoresed in 1× Tris borate ethylenediaminetetraacetic acid buffer at 100 V and visualised under a blue light transilluminator. Thereafter, amplicons were purified using a gel extraction kit (GeneJET Gel Extraction Kit, Thermo Scientific), and eluted fragments were then sequenced (U2Bio DNA Sequencing Services) using these PCR primers.

TABLE 1 Primer sequences used in this study

Name of primer	Sequence of primer (5′ to 3′)	PCR product size, bp	Name of SNVs	Position	Modification
SEQ-1-F	GTCTCCCTCATCAGAAAGCACA	1000	rs8138197	chr22:43114551 (exon 2a)	C/T
SEQ-1-R	CCGAAAGAAGTAGGAAAAACAATTC		rs2143918	chr22:43114020 (intron 1)	T/G
			rs5751348	chr22:43113793 (intron 1)	G/T
SEQ-2-F	ACATATTTTGTAGATGGCTGTCCA	500	rs66781836	chr22:43115523_43,115 520 (intron 1)	delAAAG
SEQ-2-R	CAGGGTCCCATAGCAGAGTG				
2 143 918-F	TCATACGCACTGATTACCAGGTTA	754	rs2143918	chr22:43114020 (intron 1)	T
P1-R	ACCCTTGTCCAGATCCCAGA				
5 751 348-F	GCACGCACAAACACACCC	975	s5751348	chr22:43113793 (intron 1)	G
P1-R	ACCCTTGTCCAGATCCCAGA				
HGH-F	GCCTTCCCAACCATTCCCTTA	1070	NA	*HGH* gene	NA
HGH-R	GTCCATGTCCTTCCTGAAGCA				

Abbreviations: HGH, human growth hormone; NA, not applicable; SNVs, single-nucleotide variants.

2.4 | Blood group P1 prediction by multiplex PCR

A single-tube multiplex PCR for P1 antigen prediction amplified unique targets for SNVs rs2143918 and rs5751348. A target for the human growth hormone (*HGH*) gene was used as an internal control, as shown in Table 1. The PCR reaction mixtures consisted of 10 μl of the 2× PCR reaction mixture (GoTaq Hot Start Colourless Master Mix, Promega), 1 μl (50 ng/μl) of genomic DNA, 1 μl of each 10 μM SNV-specific forward primers (2143918-F and 5 751 348-F), 2 μl of 10 μM common reverse primer (P1-R), 1 μl of 3 μM HGH primers (HGH-F and HGH-R) and 3 μl of PCR grade water in a final volume of 20 μl.

Multiplex PCR amplification was performed in a T100 Thermal cycler (Bio-Rad Laboratories, Inc.). Thermocycling and amplicon separation and visualisation were performed as described above (see Section 2.3, SNV analysis by DNA sequencing).

All samples, including the 1022 samples of unknown P1 typing, were genotyped using this multiplex PCR method. To increase the validity and reliability of the newly developed multiplex PCR, technicians were blinded from P1 typing and DNA sequencing results. Any discrepancies between multiplex PCR genotyping and phenotyping results were confirmed by standard DNA sequencing. Multiplex PCR sensitivity, specificity, positive and negative predictive values and accuracy were calculated and compared with P1 typing. The sensitivity of the multiplex PCR was performed using known P1 positive and negative samples with concentrations ranging from 10 to 300 ng/μl. Finally, multiplex PCR genotyping was repeated on 100 randomly selected samples to ascertain reproducibility.

2.5 | Statistical analysis

The prevalence of observed and predicted P1 antigens was described using descriptive statistics. Results were expressed in percentages and 95% confidence intervals (CI), and differences between the prevalence of the studied populations[9,24-32] were assessed using Pearson's Chi-square (χ^2) test. All statistical analyses were conducted using SPSS, Version 16.0 (SPSS Inc.), and p values ≤0.05 were considered statistically significant.

3 | RESULTS

3.1 | P1 antigen typing among 218 Thai blood donors

The distribution of P1 antigen typing among 218 Thai blood donors was studied. The P_1 phenotype was observed in 24.77% of donors (n = 54/218, 95% CI, 0.1919-0.3105), whereas the P_2 phenotype was found in 75.23% of donors (n = 164/218, 95% CI, 0.6895-0.8081). Agglutination strength was determined in the 54 P_1 samples. We found that 37 samples (68.5%) scored a 4+, 16 (29.6%) scored a 3+, and only 1 (1.9%) scored a 2 + .

3.2 | SNV analysis

To find useful markers for analysing P1 antigen expression in our Thai donor study cohort, a genomic non-coding region of *A4GALT* (encompassing exon 2a and intron 1) was screened by DNA sequencing. Four SNVs, including rs66781836, rs8138197, rs2143918 and rs5751348, were identified and compared to the P1 antigen typing and agglutination strength test results (Table 2). In this study, rs66781836 was not polymorphic in any of the samples, irrespective of P_1 typing status. In contrast, rs8138197, rs2143918 and rs5751348 showed an association with P_1 and P_2 phenotypes. rs8138197 (T/T), rs2143918 (G/G) and rs5751348 (T/T) were highly associated with P_2 phenotypes and present in 100% of P_2 donor samples. Of the 54 P_1 samples, 6 samples with 4+ strength of agglutination demonstrated rs8138197 (C/C), rs2143918 (T/T) and rs5751348 (G/G) genotypes. The remaining P_1 samples showed

TABLE 2 P1 antigen typing and DNA sequencing results among 218 Thai blood donors

P1 antigen typing			Observed number			
			rs66781836	rs8138197, rs2143918, and rs5751348		
Result	Strength of agglutination	*N* (%)	delAAAG	C/C, T/T, and G/G	C/T, T/G, and G/T	T/T, G/G, and T/T
Positive	4+	37 (16.97)	0	6	31	0
	3+	16 (7.34)	0	0	16	0
	2+	1 (0.46)	0	0	1	0
	1+	0 (0.00)	0	0	0	0
Negative	0	164 (75.23)	0	0	0	164
Total		218 (100.00)	0	6	48	164

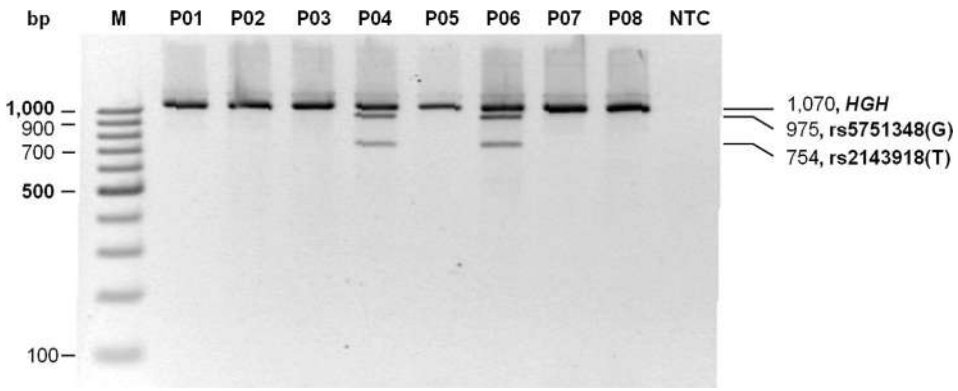

FIGURE 1 Gel electrophoresis of the multiplex PCR products. A 1070-bp fragment of *HGH* is present in all lanes. Blood group P1 prediction was deduced from the presence or absence of the 975 and 754 bp amplicons for rs5751348(G) and rs2143918(T), respectively. From left to right: Lane M: 100-bp DNA molecular weight marker. Lanes P01, P02, P03, P05, P07 and P08 correspond to P_2 phenotype of P^2/P^2 individuals, and P04 and P06 correspond to P_1 phenotype of P^1/P^2 and P^1/P^1 individuals, respectively. NTC-non-template control

heterozygous genotypes at rs8138197 (C/T), rs2143918 (T/G) and rs5751348 (G/T).

3.3 | Development and validation of multiplex PCR for blood group P1 prediction

The results of a single-tube multiplex PCR were used to distinguish SNVs rs2143918 and rs5751348. Importantly, the multiplex assay differentiated between P^1 alleles for rs5751348 (G) and rs2143918 (T), producing amplified PCR product sizes of 975 and 754 bps, respectively. In each reaction, the *HGH* internal control produced an expected band of 1070 bp, confirming optimal PCR amplification (Figure 1).

The multiplex PCR assay was validated using DNA samples from known P_1 and P_2 phenotypes, whereas a non-template control (water) assessed multiplex reaction contamination. Multiplex PCR was performed on our 218 donor DNA samples, and each corresponding amplicon was evaluated alongside P1 antigen typing by conventional tube technique (CTT) and DNA-sequencing results. To test our assay for reproducibility, multiplex PCR was repeated on 100 randomly selected DNA samples. Results from our reproducibility test showed identical findings to those observed in the initial test. Multiplex PCR sensitivity was determined using positive and negative DNA controls and using DNA concentrations ranging from 10 to 300 ng/μl. Specific amplicons were accurately identified in samples with DNA concentrations as low as 10 ng/μl. Therefore, our multiplex PCR assay exhibited robust sensitivity, specificity and reproducibility in the cohort of 218 Thai donor DNA samples.

3.4 | Implementation of multiplex PCR and comparison of P1 antigen among populations

The validated multiplex PCR was applied on 1022 central Thai donor DNA samples of unknown P_1 phenotype, and the observed genotyping results were compared to predicted P_1 phenotypes (Table 3). We found that multiplex PCR predicted P_1 in 288 samples (28.18%; 95% CI, 0.2544–0.3105) and predicted P_2 in 734 samples (71.82%; 95% CI, 0.6895–0.7456).

The observed and predicted P_1 phenotype frequencies among Thai donors were compared with those published for other populations,[9,24-32] as shown in Table 3. The observed P_1 phenotype frequencies of central Thai blood donors were similar to the predicted

TABLE 3 Frequencies of observed and predicted P_1 phenotype among different populations

Population	N	P_1		P_2		χ^2, DF = 1	p value
		N	%	N	%		
Observed Thai (serology)	218	54	24.77	164	75.23	-	-
Predicted Thai (genotyped)	1022	288	28.18	734	71.82	1.046	0.307
Thai9	456	123	26.97	333	73.03	0.370	0.543
Thai[24]	200	60	30.00	140	70.00	1.438	0.230
Lao[25]	464	88	18.97	376	81.03	3.032	0.082
Maldivian[26]	123	35	28.46	88	71.54	0.554	0.457
Taiwanese[27]	1000	324	32.40	676	67.60	4.868	0.027[a]
Indian[28]	115	74	64.35	51	44.35	40.260	<0.0001[a]
Han Chinese[29]	1412	534	37.82	878	62.18	13.942	0.0002[a]
Iranian[30]	522	346	66.28	176	33.72	106.705	<0.0001[a]
Saudi Arabian[31]	100	85	85.00	15	15.00	101.072	<0.0001[a]
Malay Malaysian[32]	200	80	40.00	120	60.00	11.107	0.001[a]
Chinese Malaysian[32]	274	85	31.02	189	68.98	2.341	0.126
Indian Malaysian[32]	120	82	68.33	38	31.67	61.079	<0.0001[a]

Note: "-" denotes not tested.
Abbreviations: DF, degree of freedom; *N*, Number.
[a]Frequencies differed from those among observed Thais ($p < 0.05$).

Thai donors ($\chi^2 = 1.046$, DF = 1, $p > 0.05$) and those found in two Thai reports, Lao, Maldivian and Chinese Malaysian populations.[9,24-26,32] In contrast, the observed P1 antigen frequencies of Thai donors significantly differed ($p < 0.05$) from those of Taiwanese, Indian, Han Chinese, Iranian, Saudi Arabian and Malay and Indian Malaysian populations.[27-32]

4 | DISCUSSION

Although anti-P1 is present in up to 67% of P_2 individuals[33] and is frequently reported in cases of mild-delayed HTRs, it is rarely found in severe HTRs among populations.[6,15,34] A previous study from the NBC-TRCS of multi-transfused Thai patients found that anti-P1 was present in about 2.87% of patients.[35] However, anti-P1 has not been reported to cause HTRs in Thai patients.

Serological techniques, including hemagglutination, are currently considered the "gold standard" for P1 antigen typing. However, these tests cannot overcome clinical challenges, such as recent transfusions and IgG-coated RBCs.[6] Genotyping presents a possible alternative to predict P_1 typing; however, the genetic markers for P_1 were unknown. In 2011, the rs8138197 showed 99.5% concordance with P_1 Swedish donor cohort[18] and, 3 years later, reported the other two SNVs (rs2143918 and rs5751348), tightly linked to rs8138197 to show 100% correlation in the four populations.[20] In 2019, four SNVs in *A4GALT*—rs66781836, rs8138197, rs2143918 and rs5751348—were reported to correlate with P_1 and P_2 phenotypes.[21] In this study, the SNV rs66781836 located at chr22:43115523_43,115520delAAAG, a 4-nucleotide deletion, was not found in all Thai donors, and it was thus excluded from further PCR development. The other three SNVs were 100% present among Thai blood donors and showed 100% correlation with P_1 and P_2 phenotypes. When we compared P1 expression (measured by CTT) with the three SNVs under investigation, we found that agglutination tests for six homozygous individuals (P^1/P^1 zygosity) all scored 4+. The present study confirmed the observation that P^1 zygosity partially describes the well-known inter-individual variation in P1 strength.[16] However, some P^1/P^2 heterozygous genotypes could identify 4+ in P1 antigen strength. This may result from characteristics of mouse monoclonal antibody used in this study, such as high affinity and specificity to P1 and P^k antigen epitope recognitions.

Although we identified a 100% correlation between SNV rs8138197 and P^1 and P^2 alleles similar to reported findings in Taiwanese (100%) and Swedish (99.5%) cohorts,[18,19] this SNV was not included in multiplex PCR development. The two SNVs included in multiplex PCR development (rs2143918 and rs5751348) were in linkage disequilibrium to rs8138197. Another group has shown that rs5751348 is located within *A4GALT* intron and contains a DNA-binding site for the well-characterised haematopoietic transcription factor, RUNX1, in P^1 blood group allele.[22] In agreement with this finding, another study found EGR1-binding sites at this locus in P^1 and P^2 blood group alleles, resulting in altered *A4GALT* transcript levels.[23] Therefore, rs5751348 should be used as the genetic determinant to predict P1 antigen expression.[22] In a recent study, a new P^1/P^2-allelic discrimination assay based on rs5751348 was validated, and the results were confirmed to be correlated to the P^1/P^2 status.[36]

In this study, we developed a single-tube multiplex PCR to resolve SNVs rs2143918 (T) and rs5751348 (G) in predicting the P1 antigen. We suggested that a compound genetic marker combining the *A4GALT* locus with tightly linked SNVs is more informative than any single polymorphism. Our developed multiplex PCR test works with

high fidelity, as supported by high sensitivity, specificity and 100% concordance with P1 antigen typing and standard DNA sequencing. In addition, we obtained accurate results even at low DNA concentrations (10 ng/μl). Although the preferential multiplex PCR amplification of one allele (P^1) relative to another in a heterozygous sample could produce an ambiguous genotype, P^1/P^1 and P^1/P^2 genotypes showed the same P1 phenotypic manifestations.

Implementation of the multiplex PCR on 1022 central Thai donor samples showed predicted P_1 phenotypes, and the frequencies were similar to the observed phenotypes obtained from serological testing. This implies that the multiplex PCR accurately identifies the presence of the P^1 allele from samples belonging to the same ancestral population.[9,24] Moreover, the above findings of P1 antigen frequencies among populations support the racial variability in the expression of the P1 antigen.[9,24-32]

In conclusion, this study confirmed that three SNVs also correlated with P_1 and P_2 phenotypes in the Thais, which was expected but still not previously investigated. We developed a reproducible, cost-effective and efficient multiplex PCR that accurately identified SNVs rs2143918 (T) and rs5751348 (G) for P1 antigen prediction. This multiplex PCR test offers a simple and accurate alternative technique for routine testing, which is necessary to provide safer blood transfusions.

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CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTIONS

Jigme Thinley: Performed the research, interpreted the data and analysed the data. **Oytip Nathalang:** Helped interpret the data and wrote and reviewed the manuscript. **Sarisa Chidtrakoon:** Helped perform the research. **Kamphon Intharanut:** Designed the study, performed the research, analysed the data and wrote the manuscript.

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ORIGINAL ARTICLE

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Effect of patient blood management system and feedback programme on appropriateness of transfusion: An experience of Asia's first Bloodless Medicine Center on a hospital basis

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Abstract

Background: Patient blood management (PBM) programmes minimise red blood cell (RBC) transfusion and improve patient outcomes worldwide. This study evaluated the effect of a multidisciplinary, collaborative PBM programme on the appropriateness of RBC transfusion in medical and surgical departments at a hospital level.

Methods/Materials: In 2018, the revised PBM programme was launched at the Korea University Anam Hospital, a tertiary hospital with 1048 hospital beds and the first Asian institution where a new computer PBM programme was implemented. Monthly RBC usage and adequacy were analysed from January 2018 to December 2019. The trend of adequacy over time was assessed.

Results: A total of 2 201 021 patients were hospitalised and visited an outpatient clinic. The number of RBC units transfused per 10 000 patients decreased from 139.8 for 2018 to 137.3 for 2019. The proportion of patients with Hb <7 g/dL receiving RBC transfusion increased significantly: 29.1%, 34.5%, 40.4% and 40.6% for periods 1, 2, 3 and 4, respectively ($p < 0.001$). The appropriateness of RBC transfusion significantly increased for medical (35.2%, 41.5%, 49.6% and 74.3% for periods 1, 2, 3 and 4, respectively [$p < 0.001$]) and surgical (37.8%, 33.3%, 45.5% and 71.1% for periods 1, 2, 3 and 4, respectively [$p < 0.001$]) departments.

Conclusion: Implementation of a PBM programme through a multidisciplinary clinical community approach increased the appropriateness of RBC transfusion in medical and surgical departments. Therefore, expanding publicity and PBM education to health care providers is important to maintain the appropriateness of blood transfusion.

KEYWORDS
appropriateness, feedback programme, patient blood management, transfusion

1 | INTRODUCTION

Patient blood management (PBM) is a patient-centred multidisciplinary, multimodal, individualised approach with evidence-based medical and surgical interventions designed to minimise red blood cell

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(RBC) transfusion and maintain patients' own blood mass with the goal of improving patient outcomes.[1,2] PBM relies on three pillar approaches to detect and treat perioperative anaemia, minimise surgical blood loss and optimise physiological tolerance of anaemia.[3,4] A hospital-wide system is required for the assessment of the three-pillar approach,[5] including the development of an electronic clinical decision support system for blood product ordering supported by education and physician feedback and improvement in compliance with the recommended restrictive transfusion guidelines.[6]

The World Health Organization proposed the implementation of the PBM programme in 2010 and suggested a new paradigm to maintain the quality and safety of blood transfusion (WHA63.12).[7] Implementing a health system-wide PBM programme resulted in the reduction of allogeneic RBC transfusions with savings of blood acquisition cost.[8,9] PBM has been integrated into the regulatory criteria. For example, the Joint Commission set the PBM standards, which have been employed to create the performance measures for PBM programmes since 2005 in the United States.[10,11] In about two-thirds of European countries, PBM has been incorporated into routine practice in 2012.[1] In Asia, only a few countries, hospitals and medical staff introduced the PBM programme,[12] and the possibility of over-utilisation of allogeneic RBC may be high compared with the amount of blood collected. Some studies reported that the transfusion appropriateness of Southeast Asia is lower than that in Western countries.[13,14]

In Korea, 2 million units of packaged RBCs had been used every year,[15] 30%–40% of which were used during the perioperative period.[16]

The difference in the clinical policy and urgency of clinical settings between the perioperative period and the general medical situation can be experienced by physicians in the medical department and those in the surgical department. Koren et al[17] reported that physicians in the medical departments are more familiar with the restrictive blood transfusion policy than surgeons. However, a majority of RBC transfusions were performed in the non-surgical departments.[17] This study aimed to investigate the impact of our PBM monitoring and feedback programme on allogeneic RBC transfusion and evaluate the RBC transfusion appropriateness between the medical department and surgical department at a hospital level.

2 | MATERIALS AND METHODS

2.1 | Implementation of blood transfusion management programme

A computerised blood transfusion management program at the Korea University Anam Hospital, Seoul, Republic of Korea, was developed in September 2013. However, the programme was not widely utilised due to a lack of proper monitoring, which led to the establishment of a minimal blood transfusion task force team in January 2018. The task force team revised the blood transfusion management programme with updates based on the guidelines[18] in April 2018. The updated guidelines[18] supported restrictive transfusion practices, and the guidelines[18] provided the detailed transfusion indications for blood products, including RBC transfusion indication, which was incorporated into both the education and intervention programmes as authorised transfusion indications. Dissemination of the updated blood transfusion guidelines by the task force team was performed via educational programmes at the workshops held within the Anam Hospital in 2018. From March to July in 2018, there were six separate sessions of workshops providing the education programmes to the physicians at the hospital, approximately over 500 personnel. The education programme employed evidence-based approaches using the data from the guidelines and clinical trials for physicians to acquire updated knowledge and information on key points of the guideline to minimise unnecessary blood transfusions in the medical and surgical departments. For continuation of concerted efforts of improving blood transfusion management, a Bloodless Medicine Center was established at the Anam Hospital in October 2018. From November 2018 to February 2019, the enhanced educational blood transfusion programme was presented at conferences for the departments of surgery and medicine at the Anam Hospital. Moreover, individual education sessions on the blood transfusion programme were provided to the new resident physicians as a part of the orientation programme in March 2019. In May 2019, five medical staff members from the Bloodless Medicine Center completed the short-term on-site training of the Bloodless Medicine Program at the Englewood Hospital, New Jersey, United States, to learn advanced features and practices of blood management for further improvement of the education programme.

After the successful administration of the blood transfusion education programme, an intervention programme on prospective audit and feedback for blood transfusion was implemented in May 2019. The intervention programme was designed to alert health care providers regarding the systemic method of administering blood transfusions and to provide feedback. If a prescribing physician was found to have ordered more than 10 cases of unnecessary or improper blood transfusion per month based on the guidelines,[18] then an email with evaluations of appropriateness of blood transfusion will be sent to the prescribing physician by staff at the Bloodless Medicine Center. Furthermore, a clinical decision support advisory model was implemented in a computerised provider order entry system in July 2019. The model required an additional step to order blood transfusion before a physician could proceed with the task of blood transfusion, in which the prescribing physician needed to click a pop-up window and choose one of the authorised blood transfusion indications based on the guidelines (Figure 1). This feature was implemented to provide educational information to the prescribing physicians and to increase adherence to the PBM programme. Additional educational programmes reflecting the newly incorporated model were presented at the conferences held at the Anam Hospital in November and December 2019 for further dissemination of the programme.

2.2 | Patient population and definitions

A hospital-based retrospective study was conducted at the Korea University Anam Hospital, Seoul, Republic of Korea, which is classified as

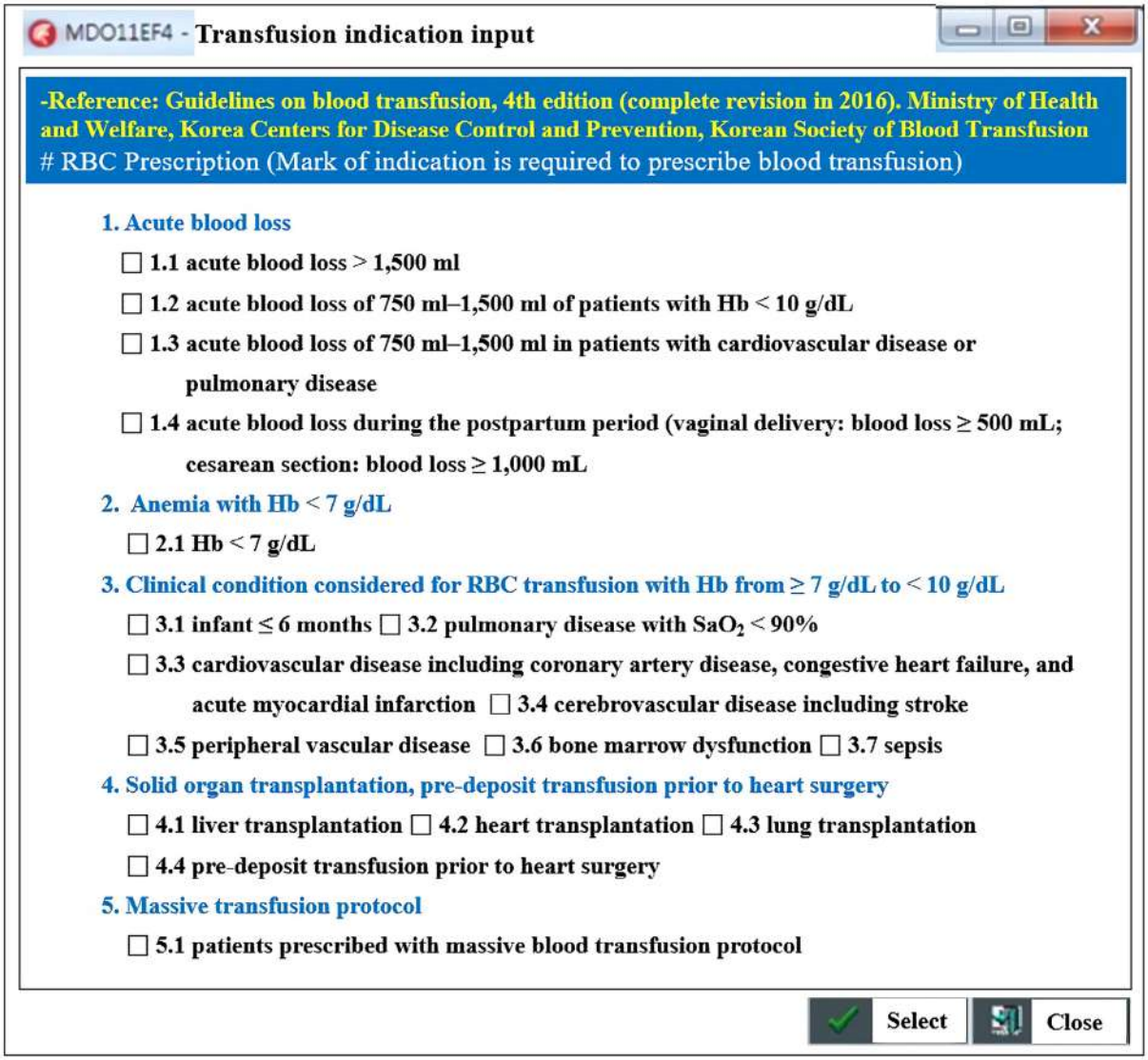

FIGURE 1 A pop-up window to choose one of the authorised red blood cell transfusion indications based on the guidelines (English version) [Color figure can be viewed at wileyonlinelibrary.com]

a tertiary care hospital with 1048 hospital beds. All patients who received RBC transfusion at the hospital, including outpatients, inpatients and emergency room patients, between January 2018 and December 2019 were reviewed by the staff at the Bloodless Medicine Center and enrolled in the study. Transfusion-related data, including the number of RBC transfusion in units, haemoglobin (Hb) concentrations and related clinical conditions incorporated in the clinical decision support advisory model before RBC transfusion, were collected.

As shown in Figure 1, the authorised blood transfusion indications based on the guideline[18] were as follows: (1) acute blood loss: 1.1 acute blood loss >1500 ml, 1.2 acute blood loss of 750–1500 ml in patients with Hb < 10 g/dL, 1.3 acute blood loss of 750–1500 ml in patients with cardiovascular disease or pulmonary disease and 1.4 acute blood loss during the postpartum period (vaginal delivery: blood loss ≥500 ml; caesarean section: blood loss ≥1000 ml); (2) anaemia with Hb < 7 g/dL; (3) clinical condition considered for RBC transfusion with Hb from ≥7 g/dL to <10 g/dL: 3.1 infant ≤6 months, 3.2 pulmonary disease with SaO_2 < 90%, 3.3 cardiovascular disease (including coronary artery disease, congestive heart failure and acute myocardial infarction), 3.4 cerebrovascular disease including stroke, 3.5 peripheral vascular disease, 3.6 bone marrow dysfunction and 3.7 sepsis; (4) solid organ transplantation, pre-deposit transfusion prior to heart surgery: 4.1 liver transplantation, 4.2 heart transplantation, 4.3 lung transplantation and 4.4 pre-deposit transfusion prior to heart surgery; and (5) massive transfusion protocol. The appropriateness of RBC transfusion was calculated from the following process: (1) identification of appropriate RBC transfusion by the computerised transfusion audit programme with the algorithm in accordance with the guideline[18] (Figure 2) from a retrospective review of medical records, (2) calculation of identified appropriate RBC transfusion from results of the computerised transfusion audit programme by the staff at the

Bloodless Medicine Center and (3) verification of appropriate RBC transfusion by the director of the Bloodless Medicine Center and the laboratory physician from the department of laboratory medicine. Therefore, RBC transfusion deemed to be performed according to the indication above was classified as an appropriate RBC transfusion. Inappropriate RBC transfusion was defined as RBC transfusion that was performed out of the authorised indications. Since the blood transfusion management programme has been implemented with significant updates and expansion of educational activities semi-annually from 2018 to 2019, the appropriateness of RBC transfusion was assessed in four periods (period 1: January–June 2018, period 2: July–December 2018, period 3: January–June 2019 and period 4: July–December 2019) stratified according to the department where the procedure was performed (surgical and medical). The medical departments included the Department of Internal Medicine, Cancer Center, Department of Emergency Medicine, Department of Rehabilitation, Department of Family Medicine and Department of Pediatrics. The surgical departments included the Department of General Surgery, Department of Orthopedic Surgery, Department of Neurosurgery, Department of Oral and Maxillofacial Surgery, Department of Plastic Surgery, Department of Urology and Department of Obstetrics and Gynecology. This study was approved by the Institutional Review Board (IRB) at the Korea University Anam Hospital (IRB number: 2020AN0256).

2.3 | Statistical analysis

Data were recorded using Microsoft Excel (Microsoft Corp, Redmond, WA) and analysed using SPSS software, version 18.0 for Windows (SPSS Inc., Chicago, IL). Dichotomous variables were compared using the Pearson χ^2 test or Fisher's exact test. The trend of the RBC transfusions in four periods was analysed using the Mantel-Haenzsel test for trend. A p value of <0.05 was considered statistically significant.

3 | RESULTS

A total of 2 201 021 patients were hospitalised and visited an outpatient clinic during the study period (1 094 105 patients in 2018 and 1 106 916 patients in 2019). A total of 15 300 units of RBCs were transfused in 2018 and 15 193 units in 2019. The number of RBC transfusions in units per 10 000 patients decreased over the study period: 139.8 in 2018 and 137.3 in 2019. The proportion of patients with Hb <7 g/dL receiving RBC transfusion significantly increased over the study period: 29.1%, 34.5%, 40.4% and 40.6% for periods 1, 2, 3 and 4, respectively ($p < 0.001$). However, the proportion of patients with 7 g/dL ≤ Hb < 10 g/dL receiving RBC transfusion decreased over the study period: 64.4%, 60.2%, 53.8% and 54.1% for periods 1, 2, 3 and 4, respectively ($p < 0.001$). Of note, the proportion of patients with Hb ≥10 g/dL receiving RBC transfusion was different over the study period: 6.5%, 5.3%, 5.8% and 5.4% for periods 1, 2, 3 and 4, respectively ($p = 0.004$) (Table 1). The rate of appropriate RBC transfusions gradually increased from January 2018 to May 2019. However, the rate has significantly increased after the implementation of an intervention programme on prospective audit and feedback for blood transfusion in May 2019. As shown in Figure 3 and Table 2, the appropriateness of RBC transfusion significantly increased over the study period: 36.0%, 39.2%, 48.5% and 73.2% for periods 1, 2, 3 and 4, respectively ($p < 0.001$).

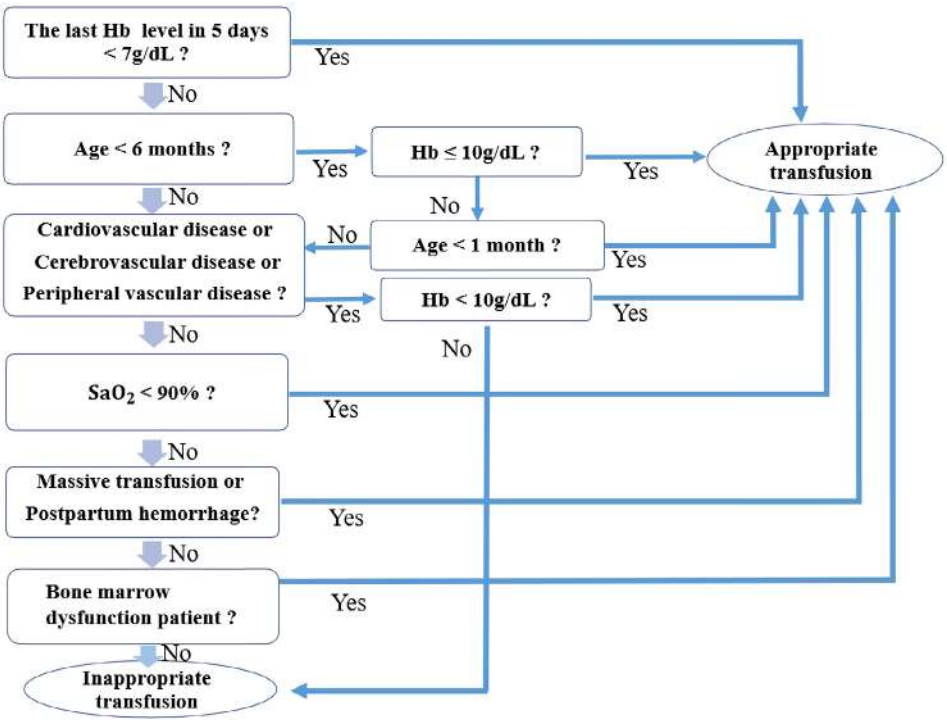

FIGURE 2 The algorithm for determination of appropriateness of red blood cell transfusion. Hb, haemoglobin; SaO2, arterial oxygen saturation [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Red blood cell transfusion in units used for the patients during the study period stratified by pre-transfusion haemoglobin levels and departments

	Overall medical and surgical departments			
	Haemoglobin < 7 g/dL	**7 g/dL ≤ Haemoglobin < 10 g/dL**	**Haemoglobin ≥ 10 g/dL**	**Total (units)**
January–June 2018 (n=)	2317 (29.1%)	5128 (64.4%)	517 (6.5%)	7962 (100.0%)
July–December 2018	2606 (34.5%)	4549 (60.2%)	398 (5.3%)	7553 (100.0%)
January–June 2019	3073 (40.4%)	4099 (53.8%)	440 (5.8%)	7612 (100.0%)
July–December 2019	3128 (40.6%)	4167 (54.1%)	414 (5.4%)	7709 (100.0%)
	Medical departments			
	Haemoglobin <7 g/dL	**7 g/dL ≤ Haemoglobin <10 g/dL**	**Haemoglobin ≥10 g/dL**	**Total (units)**
January–June 2018	1573 (28.8%)	3540 (64.8%)	346 (6.3%)	5459 (100.0%)
July–December 2018	1854 (35.2%)	3171 (60.2%)	241 (4.6%)	5266 (100.0%)
January–June 2019	2321 (41.7%)	2933 (52.7%)	313 (5.6%)	5567 (100.0%)
July–December 2019	2303 (44.8%)	2591 (50.4%)	251 (4.9%)	5145 (100.0%)
	Surgical departments			
	Haemoglobin <7 g/dL	**7 g/dL ≤ Haemoglobin <10 g/dL**	**Haemoglobin ≥10 g/dL**	**Total (units)**
January–June 2018	744 (29.7%)	1588 (63.4%)	171 (6.8%)	2503 (100.0%)
July–December 2018	537 (25.9%)	1378 (66.5%)	157 (7.6%)	2072 (100.0%)
January–June 2019	752 (36.8%)	1166 (57.0%)	127 (6.2%)	2045 (100.0%)
July–December 2019	825 (32.2%)	1576 (61.5%)	163 (6.4%)	2564 (100.0%)

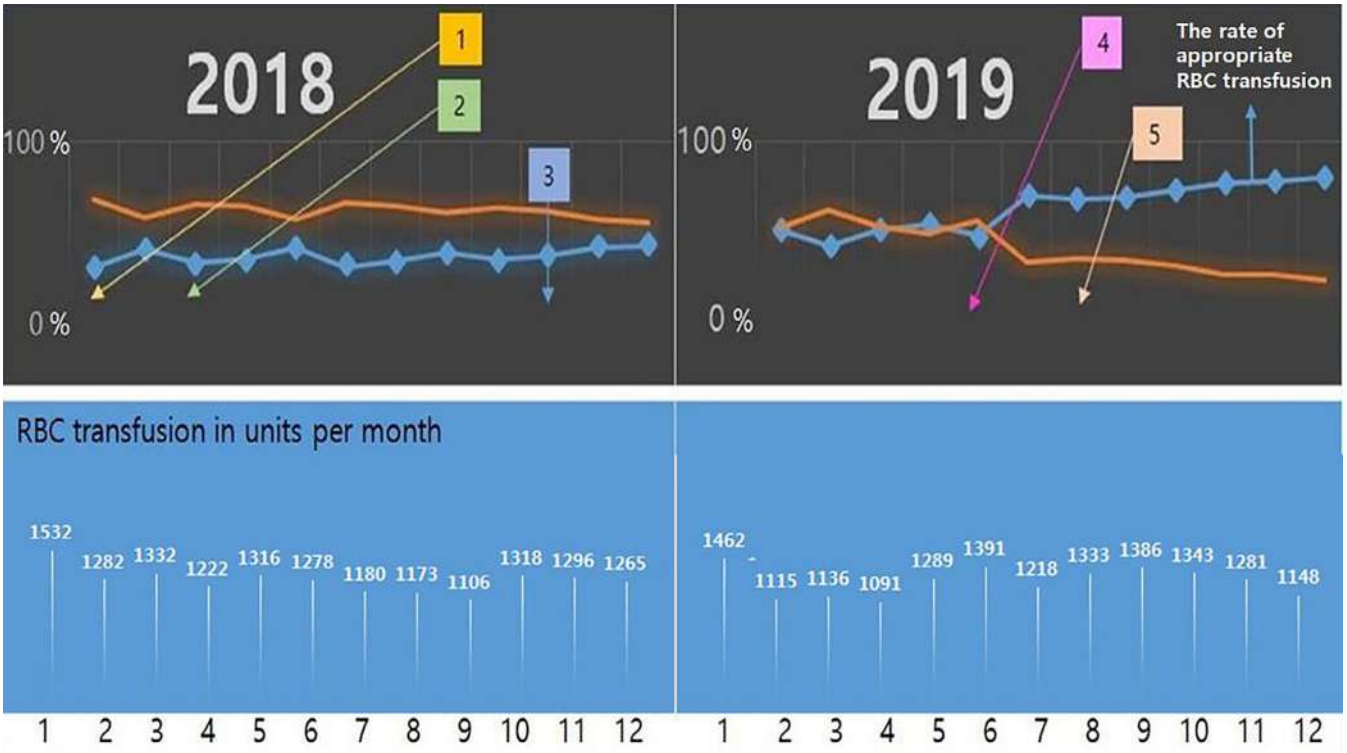

FIGURE 3 Monthly rate of appropriate red blood cell transfusion during the study period with a series of updates in the blood transfusion programme. RBC, red blood cell, 1. The implementation of a minimal blood transfusion task force team in January 2018. 2. Revision of the blood transfusion management programme with updates based on the guidelines in April 2018. 3. The establishment of a Bloodless Medicine Center at the Anam Hospital in October 2018. 4. The implementation of an intervention programme of prospective audit and feedback for blood transfusion in May 2019. 5. The implementation of a clinical decision support advisory model in a computerised provider order entry system in July 2019 [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Appropriateness of red blood cell transfusion by the study periods

	Overall medical and surgical departments				
	Study period 1 (January–June 2018)	**Study period 2 (July–December 2018)**	**Study period 3 (January–June 2019)**	**Study period 4 (July–December 2019)**	***p***
Inappropriate transfusion, n (units) (%)	5105 (64.0)	4464 (60.8)	3923 (51.5)	2064 (26.8)	<0.001
Appropriate transfusion, n (units) (%)	2867 (36.0)	2874 (39.2)	3690 (48.5)	5645 (73.2)	
	Medical departments				
	Study period 1 (January–June 2018)	**Study period 2 (July–December 2018)**	**Study period 3 (January–June 2019)**	**Study period 4 (July–December 2019)**	***p***
Inappropriate transfusion, n (units) (%)	3539 (64.8)	3081 (58.5)	2808 (50.4)	1323 (25.7)	<0.001
Appropriate transfusion, n (units) (%)	1920 (35.2)	2185 (41.5)	2760 (49.6)	3822 (74.3)	
	Surgical departments				
	Study period 1 (January–June 2018)	**Study period 2 (July–December 2018)**	**Study period 3 (January–June 2019)**	**Study period 4 (July–December 2019)**	***p***
Inappropriate transfusion, n (units) (%)	1566 (62.2)	1383 (66.7)	1115 (54.5)	741 (28.9)	<0.001
Appropriate transfusion, n (units) (%)	947 (37.8)	689 (33.3)	930 (45.5)	1823 (71.1)	

For medical departments, the numbers of patients hospitalised and who visited an outpatient clinic over the study period were 345 246, 348 373, 353 143 and 353 089 for periods 1, 2, 3 and 4, respectively. The numbers of RBC transfusion units over the study period per 10 000 patients were 158.1, 151.2, 157.6 and 145.7 for periods 1, 2, 3 and 4, respectively. The proportion of patients with Hb <7 g/dL receiving RBC transfusion increased over the study period: 28.8%, 35.2%, 41.7% and 44.8% for periods 1, 2, 3 and 4, respectively ($p < 0.001$). However, the proportion of patients with 7 g/dL ≤ Hb < 10 g/dL receiving RBC transfusion decreased over the study period: 64.8%, 60.2%, 52.7% and 50.4% for periods 1, 2, 3 and 4, respectively ($p < 0.001$). The proportion of patients with Hb ≥10 g/dL receiving RBC transfusion was significantly different over the study period: 6.3%, 4.6%, 5.6% and 4.9% for periods 1, 2, 3 and 4, respectively ($p < 0.001$) (Table 1). Furthermore, the appropriateness of RBC transfusion significantly increased over the study period: 35.2%, 41.5%, 49.6% and 74.3% for periods 1, 2, 3 and 4, respectively ($p < 0.001$) (Table 2).

For surgical departments, the numbers of patients admitted in the hospitals and who visited the outpatient department over the study period were 193 146, 207 340, 191 342 and 209 342 for periods 1, 2, 3 and 4, respectively. The numbers of RBC units transfused over the study period per 10 000 patients were 129.6, 99.9, 106.9 and 122.5 for periods 1, 2, 3 and 4, respectively. The proportion of patients with Hb <7 g/dL receiving RBC transfusion increased over the study period: 29.7%, 25.9%, 36.8% and 32.2% for periods 1, 2, 3 and 4, respectively ($p < 0.001$). However, the proportion of patients with 7 g/dL ≤ Hb < 10 g/dL receiving RBC transfusion decreased over the study period: 63.4%, 66.5%, 57.0% and 61.5% for periods 1, 2, 3 and 4, respectively ($p = 0.001$). There was no significant difference in the proportion of patients with Hb ≥10 g/dL receiving RBC transfusion over the study period: 6.8%, 7.6%, 6.2% and 6.4% for periods 1, 2, 3 and 4, respectively ($p = 0.277$) (Table 1). However, the appropriateness of RBC transfusion significantly increased over the study period: 37.8%, 33.3%, 45.5% and 71.1% for periods 1, 2, 3 and 4, respectively ($p < 0.001$) (Table 2).

4 | DISCUSSION

This study showed that the appropriateness of RBC transfusion significantly increased over the study period in both medical and surgical departments. In addition, the number of RBC transfusions per patient decreased after the application of PBM. Thus, a reduction in the proportion of patients receiving RBC transfusion was likely a result of an increase in the appropriateness of transfusion through the PBM and feedback programme. Several studies have shown that the incidence of over-utilisation of blood products was reduced through the implementation of PBM.[8,19-22] As Frank et al[8] pointed out, the three important factors in the implementation of this PBM are physician leadership, project management support and data analytics, and these three are the reasons why our organisation is successfully implementing them.[8] In particular, the implementation of an intervention programme on prospective audit and feedback for blood transfusion was very effective, which is similar to the reports of other recent study.[23] In a prospective, interventional cohort study by Mehra

et al,[23] the PBM monitoring and feedback programme was applied. They reported that, within the first year of introduction, transfusion of all allogeneic blood products per 1000 patients was reduced by 27%. The number of blood products transfused per patient was also significantly reduced. The introduction of our PBM monitoring and feedback programme reduced the transfusion probability by 14% (odds ratio: 0.86, confidence interval [CI]: 0.82–0.91, $p < 0.001$).

Although a previous study[17] reported that neither medical nor surgical doctors had a high level of knowledge on transfusion in non-operative-related matters, our study showed that PBM could be applied to medical physicians and surgical physicians to increase the appropriateness of blood transfusion. Thus, our study results reaffirm the effectiveness of improving appropriateness of blood transfusion through the implementation of monitoring and feedback programmes. In addition, this is the first study to analyse the medical and surgical sectors separately through the hospital-level PBM. However, a previous study[24] evaluated the application of PBM in the intensive care unit. Among the critically ill patients, PBM can be particularly effective given the extremely high prevalence of anaemia, variable and unjustified transfusion practices, high frequency of coagulation disorders and avoidable sources of blood loss such as unnecessary diagnostic blood draws.[24]

With regard to the surgical department, a recent meta-analysis of 17 studies comprising 235 779 surgical patients showed that a comprehensive PBM programme addressing all three PBM pillars (comprehensive anaemia management, minimisation of iatrogenic [unnecessary] blood loss and harnessing and optimisation of the patient-specific physiological tolerance of anaemia) is associated with the reduced need for RBC transfusion, lower complication and mortality rate and subsequent improvement in clinical outcomes.[25] In the meta-analysis, implementation of PBM significantly reduced the transfusion rates by 39% (risk ratio [RR]: 0.61, 95% CI: 0.55–0.68, $p < 0.00001$), 0.43 RBC units per patient (mean difference: −0.43, 95% CI: −0.54 to −0.31, $p < 0.00001$), hospital length of stay (mean difference: −0.45, 95% CI: −0.65 to −0.25, $p < 0.00001$), total number of complications (RR: 0.80, 95% CI: 0.74–0.88, $p < 0.00001$) and mortality rate (RR: 0.89, 95% CI: 0.80–0.98, $p = 0.02$).

The method of building the PBM programme has been reported before; however, this is the first study to report the results of the systemically implemented PBM programme incorporated in the electronic medical record system at a hospital level in Asia. It is also the first study to report the appropriateness of transfusion by separating the medical and surgical systems. As interest in proper blood transfusions is relatively low in Asia compared with that in Western countries, and the overuse of blood transfusions is expected to be relatively high, this study is meant to increase interest in terms of the proper utilisation of blood products in Asia, which accounts for more than half of the world's population, and to discuss timely topics because blood donations are expected to decrease gradually. However, our study has some limitations. First, the present study did not use plasma and platelet transfusion as part of the measurement of appropriate transfusion due to a shortage of data. Although our PBM programme has focused on RBC transfusion, we expect to expand the scope of the program to control plasma and platelet transfusion in the future, which will lead to a more comprehensive assessment of the program. Nonetheless, the most frequently performed transfusion in the hospital setting is RBC transfusion. Thus, we believe that our data analysis using RBC transfusion likely reflects the trend of transfusion, which may well serve as the measurement of appropriate transfusion. Second, there might have been information bias due to the retrospective nature of our study. In addition, confounding effects from unmeasured variables such as possible changes in types of surgeries or patients' demographics over the study period might have affected our analyses. However, we used consistent definitions for data collection to minimise potential bias. Third, our study and PBM programme have been based on the Korean guideline,[18] which recommends different RBC transfusion thresholds for patients with Hb ≥7 g/dL and pre-existing cardiovascular disease compared to other guidelines, such as the American Association of Blood Banks (AABB)[26] (the Korean guideline Hb from ≥7 g/dL to <10 g/dL vs. the AABB Hb 8 g/dL). Thus, the degree of effectiveness of the PBM programme might vary, especially for patients with Hb ≥7 g/dL and pre-existing cardiovascular disease, depending on the clinical settings and interpretation of the adopted guidelines. Fourth, the computerised provider order entry system may need improvement to further increase the appropriateness of RBC transfusion. Some physicians were noted to bypass the system by selecting the incorrect transfusion indication in the computerised provider order entry system for ordering RBC transfusion, which might have contributed to the inappropriateness of RBC transfusion in our study. Fifth, there might have been some seasonality component to the transfusion data as there was a higher rate of RBC transfusion among patients with Hb ≥ 10 g/dL in periods 1 and 3. Certain medical conditions that may predispose to requiring blood transfusions, such as cerebral haemorrhage, acute aortic syndrome and fracture from fall, are well known to occur more frequently in winter months, particularly from January to February in Korea.[27-29] Although a time series analysis would be helpful in uncovering such trends, our results of the benefits of the PBM programme over four separate 6-month periods can still be useful for demonstrating the impact of the PBM programme.

An analysis of cost saving, such as return on investment, is necessary in the future. As all blood donations have been made by voluntary blood donors, and the blood supply for transfusions is managed by the Blood Management Act in Korea,[30] the price of RBC is lower in Korea than in other countries.[31] However, a low birth rate and aging population in Korea will likely contribute to a decrease in blood donations, resulting in a risk of a shortage of blood products.[27] Thus, judicious use of blood products, particularly RBC, through the implementation of the PBM programme will be of great benefit for the safety of patients.

In conclusion, we observed that the appropriateness of transfusion increased after PBM implementation. In addition, publicity of PBM programme and its education for medical staff may play an important role. In particular, when the guidelines for appropriate transfusion were individually provided to the clinical workforce, the appropriateness of transfusion increased significantly. Therefore, expanding publicity and PBM education to health care providers is important to maintain the appropriateness of blood transfusion in the hospital.

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CONFLICT OF INTEREST

The authors have no competing interests.

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SHORT COMMUNICATION

TRANSFUSION MEDICINE WILEY

Screening of at-risk blood donors for Chagas disease in non-endemic countries: Lessons from a 2-year experience in Tuscany, Italy

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Abstract

Background: Chagas disease (CD) is caused by the protozoan parasite *Trypanosoma cruzi* and is transmitted by blood-sucking triatomine insects in endemic areas of Latin America. Transmission can also occur via blood transfusion and is a major cause of CD in non-endemic areas.

Objectives: The aim of the study was to assess the prevalence of anti-*T. cruzi* antibodies in blood donors at risk of infection in Tuscany, Italy, following the introduction of blood safety Italian legislation.

Material and methods: Donors (N = 1985) were tested in 2016 to 2018 for anti-*T. cruzi* IgG using an immunochromatographic test (ICT). Chemiluminescent immunoassay (CLIA) was performed on ICT-positive donors to exclude CD, whereas enzyme-linked immunosorbent assay and western blot were performed in case of discordant results. All assays were performed on CD patients (N = 10) for validation.

Results: Ten blood donors had a positive ICT result, with a resulting *T. cruzi* seroprevalence of 0.5% but demonstrated negative results to CLIA, as well as to the other serological assays. The comparison of serological assays suggested a lower relative sensitivity of ICT.

Conclusion: The results of this study confirm the significance of serological testing in the screening strategy for CD. However, they provide evidence for discontinuing the use of ICT as a screening test and suggest that a sensitive, specific and multi-sample format assay should be used at the national level for uniformity of results.

KEYWORDS

blood donors, Chagas disease, screening, serology, transfusion

1 | INTRODUCTION

Chagas disease (CD), or American trypanosomiasis, is caused by the protozoan parasite *Trypanosoma cruzi* and is transmitted by blood-sucking triatomine insects (mainly *Triatoma*, *Panstrongylus*, *Rhodnius*) in endemic areas of continental Latin America.

Transmission of the parasite can also occur vertically from mother to child, via transfusion with blood and blood products, through the

transplant of solid organs or haematopoietic stem cells, consumption of food or drinks contaminated by vectors or their faeces and via laboratory accidents.[1]

The acute phase of the infection, although mostly asymptomatic, may present with fever, inflammation at the inoculation site (chagoma), unilateral palpebral oedema (Romaña sign), lymphadenopathy and hepatosplenomegaly. After 4–6 weeks, the acute phase resolves spontaneously, and blood parasitaemia reduces substantially, but patients remain chronically infected, and in 30%–40% of them, chronic disease may occur up to 30 years later, with cardiomyopathy and/or megaviscera as the most common clinical manifestations.

In endemic countries, due to multinational control initiatives, the epidemiological situation has dramatically improved, with a significant decline in the incidence of the disease in the last decade. For example, in Argentina, one of the most affected countries in Latin America, a decrease of the infected population has been estimated from 23.0% in 2005 to 5.2% in 2010.[1]

In contrast, in non-endemic countries, a dramatic increase in the number of CD cases has been reported in the last decades, changing the scenario of the disease into a worldwide public health concern.[2]

For example, in the United States and in Canada, more than 325 000 and between 4000 and 6000 cases of CD have been estimated, respectively. As for Europe, in Spain and Italy, the two countries with the highest number of Latin American immigrants, around 70 000 to 76 000 and 90 000 to 10 000 infected individuals have been estimated, respectively.[1]

According to the World Health Organization (WHO), for the diagnosis of CD, at least two serological tests based on different principles should be performed to detect anti-*T. cruzi* antibodies.[3]

Different strategies have been adopted in order to reduce the risk of transfusion-transmitted (TT) CD. Currently, in endemic areas, all donations should be analysed for *T. cruzi* antibodies. In non-endemic countries, interventions are different: exclusion of at-risk donors (Sweden), *T. cruzi* serology screening of at-risk blood donors (Portugal, Spain, France and—more recently—Italy) or one-time serological testing of all donors (United States)[4] (Angheben, personal communication). Where serological screening is performed, a single test is considered sufficient to decide on exclusion from donation.[1] For its part, the WHO recommends the use of a single enzyme-linked immunosorbent assay (ELISA) for blood bank screening.[3]

The aim of this study was to assess the seroprevalence of *T. cruzi*-specific antibodies in a population of at-risk blood donors from Tuscany, Italy, in a 2-year period following the introduction of the Italian National Blood Centre regulation in November 2015 (L.219, D.M. 02/11/2015). This requires the identification of at-risk candidate donors (those born in Latin America, born from Latin American mother or travellers with history of rural or outdoor activities in endemic areas) through a questionnaire, followed by testing for anti-T. cruzi antibodies with immunological techniques, without any further specification on the characteristics of the test itself. Therefore, samples from both at-risk blood donors and CD patients were tested with different serological methods in order to provide evidence for the amelioration of the current screening strategy.

2 | METHODS

2.1 | Study area and population

2.1.1 | Blood donors at-risk for *T. cruzi* infection

The Parasitology laboratory at Pisa University Hospital is responsible for the serological testing of serum samples collected from blood donors identified as at risk for *T. cruzi* infection by the Transfusion Centres of the Provinces of Livorno, Lucca, Massa-Carrara and Pisa (northwest area of the Tuscany Regional health system). At-risk blood donors were identified through a questionnaire and serologically tested, as required by the current legislation. Between 15 February 2016 and 31 December 2018 the laboratory has performed n = 2042 immunochromatographic (ICT) tests (Chagas Quick Test, Cypress Diagnostics, Belgium; the manufacturer reports 100% sensitivity and 100% specificity based on 91 sera samples from Chile and comparison with ELISA and IFA assays) on serum samples from n = 1985 candidate blood donors. The choice of serological method used was constrained by availability for acquisition through the Tuscany regional health system (ESTAR). ICT was therefore used despite the WHO not recommending the use of rapid tests for blood-screening purposes.[3]

2.1.2 | Samples from CD patients

The Department of Infectious—Tropical Medicine and Microbiology of the IRCCS Sacro Cuore Don Calabria Hospital in Negrar, Verona, has an in- and out-patient service, and more than 400 individuals have been diagnosed and offered treatment for CD in the last decades. Samples of blood from 10 patients born in Bolivia who were recruited during screening surveys of Latin American communities between 2012 and 2015 and underwent serology diagnosis for CD through two ELISA assays (details provided below) have been selected as controls for the current study. Nine individuals tested positive to both tests, chronic CD was diagnosed (indeterminate phase), and they were therefore used as positive controls. One patient tested negative for both tests and was therefore considered a negative control.

2.2 | Serological methods

Six different assays currently present in the Italian market were performed for the detection of anti-*T. cruzi* antibodies in serum samples from ICT-seropositive candidate donors, as well as from control patients, to compare the performance of methods.

2.2.1 | Immunochromatographic test

The ICT method Chagas Quick Test (Cypress Diagnostics) was performed according to manufacturer's instructions. The ICT has a

strip format, and it is based on *T. cruzi* recombinant antigen including nine different epitopes and requires 10 μl of serum. After 30 min, results can be read on the strip. A sample is considered positive if both test and control lines appear on the strip, negative if only the control line is present and invalid if the control line is absent.

2.2.2 | Chemiluminescent immunoassay

The chemiluminescent immunoassay (CLIA) method Architect System Chagas (Abbott) was performed following manufacturer's instructions using the instrument Architect i2000 SR (Abbott). The CLIA is based on four recombinant proteins of *T. cruzi* (FP3, FP6, FP10, TcF) and requires 100 μl of serum. At the end of the reaction (∼60 min), the chemiluminescent signal (relative light units, RLUs) is read. A calibrator sample is assayed along with the test samples, and a cut-off value (CO) is obtained as the mean of three values of the calibrator. The positivity is defined by the ratio between the RLUs of the sample (S) and the RLUs of the cut-off (CO). A sample is considered positive if S/CO ≥ 1, negative if S/CO ≤ 0.8 and uncertain if 0.8 < S/CO < 1.

2.2.3 | Enzyme-linked immunosorbent assay 1

The ELISA method Chagatest ELISA lisado (Wienerlab) was performed according to manufacturer's instructions. The ELISA has a 96-well plate format; it is based on *T. cruzi* lysate antigen and requires 20 μl of serum. At the end of the reaction (∼180 min), the absorbance (OD) is measured using a spectrophotometer (Seac ELISA-Reader Sirio S, Radim Diagnostic) at a wavelength of 450 nm. A negative control sample is assayed along with test samples, and a CO is obtained as the mean of three negative control values +0.200. A sample is considered positive if OD ≥ CO and negative if OD < CO.

2.2.4 | Enzyme-linked immunosorbent assay 2

The ELISA method Bioelisa Chagas (Biokit) was performed following manufacturer's instructions. The ELISA has a 96-well plate format, uses a *T. cruzi* recombinant antigen including four immunodominant epitopes and requires 10 μl of serum. At the end of the reaction (∼180 min), the absorbance (OD) is measured using a spectrophotometer (Seac ELISA-Reader Sirio S, Radim Diagnostic) at a wavelength of 450 nm. A negative control sample is assayed along with test samples, and CO is obtained as the mean of three negative control values +0.300. A ratio between the samples' OD (S) and the CO is calculated. A sample is considered positive if the S/CO ratio is equal or greater than 1, negative if the ratio is lower than 0.9 and uncertain if the ratio is in the 0.9–1.0 range.

2.2.5 | Western blot 1

The Western blot (WB) method Chagas IgG Lineblot (Novatec) was performed according to manufacturer's instructions. The WB, in a strip format is based on *T. cruzi* TcF recombinant antigen and requires 10 μl of serum. At the end of the reaction (∼180 min), results were read with the naked eye on the strip. The strip contains three control lines: Sample Load Control (SLC), Conjugate Control (CC) and CO; the assay is valid if the colour intensity of the control lines is SLC ≥ CC > CO. A sample is considered positive if the intensity of the test line is greater than CO, negative if the intensity of the test line is lower than CO and uncertain if the intensity of the test line is equal to CO.

2.2.6 | Western blot 2

The WB method Chagas Western Blot IgG (LDBIO Diagnostic) was performed according to manufacturer's instructions. The WB, in a strip format, is based on *T. cruzi* lysate antigens and requires 10 μl of serum. A positive control sample is assayed along with test samples to assess the validity of the assay. At the end of the reaction (∼3.5 h), results were read with the naked eye on the strip, according to manufacturer's instructions. A sample is considered positive if at least two clearly defined bands among five couples of bands (P15-16, P21-22, P27-28, P42, P45-47) in the molecular weight range of 10–200 kDa were present. A sample is considered negative if the two bands were absent.

3 | RESULTS

In the 2016–2018 period, 1985 blood donors at risk for *T. cruzi* infection were identified through a questionnaire at transfusion centres in the northwest area of Tuscany. The average proportion of at-risk blood donors in the whole population was 2.4% in this region (personal communication from the directors of the transfusion centres). The number of blood donors at risk whose sera samples were tested for anti-*T. cruzi* antibodies were: 985 in 2016, 512 in 2017 and 488 in 2018, with 216 from Livorno, 340 from Lucca, 564 from Massa-Carrara, 233 from Pisa and 597 from Viareggio.

Ten blood donors had a positive result at the screening test (ICT), with a resulting *T. cruzi* seroprevalence of 0.5% (*N* = 10/1985, 95% CI = 0.3%–0.9%). The seropositive subjects were excluded from blood donation, in agreement with the current legislation. The introduction of laboratory testing in the screening strategy has therefore resulted in safe blood donation from 1975 healthy subjects, with an impressive reduction in the potential loss of blood supply.

Table 1 shows the distribution of demographic characteristics (gender, age group, country of birth) among the total population of at-risk donors screened and among the group of donors with a seropositive result, as well as the frequency of seropositive donors. Females,

TABLE 1 Characteristics of blood donors at risk for *Trypanosoma cruzi* infection and of *T. cruzi* seropositive blood donors

Demographic characteristic		*N* donors at risk (%)	*N* seropositive donors (%)	% Seropositivity (95% CI)
Gender	Female	786 (39.6)	6 (60.0)	0.8 (0.4–1.7)
	Male	1199 (60.4)	4 (40.0)	0.3 (0.1–0.9)
Age group (years)	18–29	361 (18.2)	5 (50.0)	1.4 (0.6–3.2)
	30–39	435 (21.9)	1 (10.0)	0.2 (0.0–1.3)
	40–49	563 (28.4)	3 (30.0)	0.5 (0.2–1.6)
	50–69	439 (22.1)	0 (0.0)	0.0 (0.0–0.9)
	60–71	156 (7.9)	1 (10.0)	0.6 (0.1–3.5)
	Unknown	31 (1.6)	0 (0.0)	0.0 (0.0–11.0)
Continent of birth	Europe	1534 (77.3)	8 (80.0)	0.5 (0.3–1.0)
	Latin America	205 (10.3)	2 (20.0)	1.0 (0.3–3.5)
	Other	19 (1.0)	0 (0.0)	0.0 (0.0–16.8)
	Unknown	227 (11.4)	0 (0.0)	0.0 (0.0–1.7)
Total		1985 (100)	10 (100)	0.5 (0.3–0.9)

Note: The table shows the number (*N*) and percentage (%) of study subjects stratified by gender, age group and country of birth in the total population of blood donors at risk of *T. cruzi* infection and in the group of donors seropositive for anti-*T. cruzi* antibodies, together with the percentage of seropositive individuals (% Seropositivity) and its 95% CI.

TABLE 2 Results of different serological methods for the detection of *Trypanosoma cruzi* antibodies in ICT-seropositive blood donors

Donor ID	ICT result	CLIA result	CLIA index	ELISA1 result	ELISA1 index	ELISA2 result	ELISA2 index	WB1 result	WB2 result
2716	pos	neg	0.02	neg	0.11	neg	0.02	neg	neg
2190	pos	neg	0.01	neg	0.67	neg	0.04	pos	neg
4190	pos	neg	0.01	…	…	…	…	neg	neg
3189	pos	neg	0.15	neg	0.20	neg	0.05	neg	neg
9472	pos	neg	0.09	neg	0.84	neg	0.05	neg	neg
1180	pos	neg	0.02	…	…	…	…	neg	neg
7190	pos	neg	0.07	neg	0.19	neg	0.06	neg	neg
3971	pos	neg	0.01	neg	0.21	neg	0.01	neg	neg
1597	pos	neg	0.02	neg	0.06	neg	0.20	neg	neg
2993	pos	neg	0.06	neg	0.08	neg	0.08	neg	neg

Note: The table shows the results of different serological methods for the detection of *T. cruzi* antibodies in ICT-positive, at-risk blood donors (*N* = 10). "…" denotes data not available because of lack of sera.
Abbreviations: ELISA1, ELISA using recombinant antigens; ELISA2, ELISA using lysate antigen; WB1, WB using TcF recombinant antigen; WB2, WB using lysate antigen.

individuals aged 18–29 years old and individuals born in Latin American countries seem over-represented among seropositive donors with respect to the total population of donors at risk, although differences in seropositivity are not statistically significant.

A second test (CLIA) is performed on sera samples of ICT-seropositive subjects as part of the diagnostic algorithm for chronic CD. All 10 subjects with an ICT-positive result had a CLIA-negative result.

WHO guidelines for the diagnosis of chronic CD recommend performing a third serological test in case of discordance of the first and second tests.[3] As it is not yet established which assay should be used as a confirmatory test, all commercially available assays at the time of the study were performed for evaluation: two different ELISA tests and two different WB tests (Table 2).

Both ELISA tests and the WB test based on *T. cruzi* lysate antigen gave a negative result for all sera samples, whereas the WB test based on TcF recombinant antigen gave a negative result for nine samples and a positive result for one sample. Taken together, these results indicate that, there is no evidence of chronic CD for any of the at-risk blood donors with a positive ICT result for *T. cruzi* antibodies.

To evaluate the concordance and relative sensitivity of the different serological tests (ICT, CLIA, ELISA1, ELISA2, WB1, WB2), those were performed on control sera samples from CD patients (*N* = 10). One patient had a negative result for all tests, and eight patients had a

TABLE 3 Results of different serological methods for the detection of *Trypanosoma cruzi* antibodies in CD patients

Patient ID	ICT result	CLIA result	CLIA index	ELISA1 result	ELISA1 index	ELISA2 result	ELISA2 index	WB1 result	WB2 result
4278	neg	neg	0.02	neg	0.04	neg	0.30	neg	neg
4277	neg	pos	1.23	pos	3.05	pos	1.43	pos[a]	pos[b]
4293	pos	pos	9.37	pos	9.34	pos	3.42	pos	pos
4658	pos	pos	9.91	pos	6.75	pos	3.36	pos	pos
4287	pos	pos	11.22	pos	9.52	pos	3.80	pos	pos
4522	pos	pos	12.05	pos	8.21	pos	4.13	pos	pos
4302	pos	pos	12.79	pos	9.58	pos	3.67	pos	pos
4306	pos	pos	13.07	pos	9.31	pos	4.57	pos	pos
4666	pos	pos	13.28	pos	9.77	pos	3.80	pos	pos
4660	pos	pos	13.46	pos	9.52	pos	3.57	pos	pos

Note: The table shows the results of different serological methods for the detection of *T. cruzi* antibodies in CD patients (N = 10).
Abbreviations: ELISA1, ELISA using recombinant antigens; ELISA2, ELISA using lysate antigen; WB1, WB using TcF recombinant antigen; WB2, WB using lysate antigen.
[a]Sample result equal to cut-off.
[b]This sample did not showed reactivity to lower molecular weight bands 21–22 and 15–16.

positive result for all tests, whereas one patient had a positive result for all tests but ICT (Table 3). These results would suggest a lower sensitivity of ICT compared to other serological methods and therefore provide evidence for the need to replace ICT as the screening test.

Taken together, comparison of results of different assays performed on samples for ICT-seropositive donors and on samples from CD patients indicates no agreement between ICT and other assays (agreement = 45%–50%; Cohen k = 0) but demonstrates almost perfect to perfect agreement of other assays among themselves (agreement = 94%–100%; Cohen k = 0.88–1.00).

4 | DISCUSSION

When individuals from CD endemic countries migrate to non-endemic countries and act as donors (blood or other cellular products), there is a need to prevent transmission through transfusion or transplantation.[5]

In this way, it is possible to guarantee the safety of blood and its products, simultaneously maintaining the blood supply from candidate donors,[6] as shown in the United States where selective *T. cruzi* screening is nearly equally effective as universal screening, but at a reduced cost.[7]

In this study, a low prevalence of seropositive individuals (0.50%) has been observed among blood donors identified to be at risk for *T. cruzi* infection after an appropriate interview. This frequency is much lower than the 3.9% previously reported from a different Italian hospital in the Lazio region among a small number of donors (n = 128)[8]—possibly due to different criteria used to identify at-risk donors via questionnaire as this study anticipated the introduction of national legislation—but is in the range of results reported from other European countries such as Spain (1.91%), France (0.31%), Switzerland (0.08%), the United Kingdom (0.50%) and the Netherlands (nul).[1]

Comparison of results of different serological methods on seropositive donors and CD patients suggested lower sensitivity of ICT. This observation confirms results published when the present study was ongoing, which showed that the sensitivity of ICT was not optimal (82.8%) in non-endemic countries.[9] It is therefore important to acknowledge the fact that, in light of the non-optimal sensitivity of the ICT assay used for screening, the observed prevalence of seropositive at-risk donors might be underestimated. Furthermore, the fact that ICT-positive results obtained on seropositive donors were not confirmed by other assays also raises concerns regarding this test's specificity.

An ELISA test based on lysate antigen would be the preferred choice as a screening test based on simplicity of the procedure, the multi-sample format and on the need to use different antigens with respect to the second test (CLIA based on recombinant antigens).

5 | CONCLUSION

The present study, although limited by the small number of samples used for comparison of serological methods, provides evidence that supports the need to reconsider the diagnostic algorithm for CD at reference laboratories in Italy. We suggest, for blood donor screening, that an ELISA assay based on *T. cruzi* lysate could be the first test used, and as second test, a CLIA assay based on *T. cruzi* recombinant antigens should be carried out. It is worth noting that all CD sera that tested positive with CLIA were confirmed positive with both WB tests. As the third, or confirmatory, test, we suggest a WB assay based on crude antigen.

More generally, there is a recommendation to discontinue the use of ICT as a screening test given its limited sensitivity and specificity. It is envisaged that a sensitive and multi-sample format assay be used as a screening test and that this should be adopted at the national level for uniformity of results; a second test, as well as a confirmatory one, should be available at reference laboratories for the rapid and safe exclusion of CD in seropositive blood donors.

In addition to donor selection, other strategies may increase transfusion safety, such as pathogen inhibition methods.[10,11] These methods would lower the risk not only of TT CD but also of other parasitic diseases such as malaria, babesiosis and leishmaniosis whose importance in transfusion medicine is often neglected.[12]

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CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTIONS

Valentina D. Mangano and Fabrizio Bruschi conceived the study. Marco Prato and Giovanna Moscato performed the experiments. Valentina D. Mangano performed data analyses. Valentina D. Mangano, Marco Prato and Fabrizio Bruschi wrote the manuscript. All authors revised and approved the final version of the manuscript.

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SHORT COMMUNICATION

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Association of four-factor prothrombin complex concentrate with subsequent plasma transfusion: A retrospective cohort study

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Abstract

Objective: To assess whether patients prescribed four-factor prothrombin complex concentrate (4FPC) received less plasma during the following 24-hour period than those treated for the same indications who received only plasma.

Introduction: It is unclear whether 4FPC is associated with a reduction in subsequent plasma transfusion. This is important for minimising transfusion-associated risks and for inventory management.

Materials and Methods: We retrospectively studied patients treated for bleeding or coagulopathy. Individuals receiving 4FPC were matched by indication to patients treated with only plasma. Blood products received during 24-hour follow up were compared between 4FPC and plasma-only patients.

Results: There was no difference in the number of patients receiving additional plasma (19 (21%) 4FPC patients vs 31 (34%) plasma-only patients, $P = .07$) nor in the median number of additional plasma units received (0 units for both groups, interquartile range [0, 0] for 4FPC patients vs [0, 1] for plasma-only patients, $P = .09$). Subgroup analysis comparing patients who received 4FPC for on-label vs off-label indications found no difference in the number of patients receiving plasma nor in the median number of plasma units received.

Conclusion: 4FPC was prescribed to a diverse set of patients, and administration was not associated with reduced plasma transfusion at our institution.

KEYWORDS

coagulation factor concentrate, plasma, transfusion therapy

1 | INTRODUCTION

Four-factor prothrombin complex concentrate (4FPC) is prepared from human plasma and contains the vitamin K-dependent coagulation factors II, VII, IX and X, in addition to proteins S and C, heparin and antithrombin III. In 2013, the Food and Drug Administration (FDA) approved 4FPC for the urgent correction of vitamin K antagonist (VKA) therapy in patients with acute major bleeding. Historically, agents for warfarin correction have included vitamin K and plasma. Plasma is cheaper per dose and provides a more physiological blend of coagulation factors compared to 4FPC. However, its transfusion requires ABO matching, its effect on international normalised ratio (INR) is slow, and large volumes are required to achieve meaningful doses of coagulation factors. Plasma transfusion also introduces the risk of complications such as transfusion-associated circulatory overload, transfusion-associated lung injury, transfusion-transmitted infections, transfusion-related immune modulation and allergic reactions. 4FPC obviates these risks but remains more expensive per dose. The

average wholesale price for 4FPC in the United States was recently quoted at $3 per unit in the Micromedex Red Book,[15] whereas the most recent National Blood Collection and Utilization Survey of blood collection and transfusion in the United States found that hospitals paid a median price of $54 per unit of plasma.[7] This means that, for a 70-kg (kg) patient with an INR of 3, one dose of 4FPC would cost $5250 (70 kg × 24 units/kg × $3/unit) using dosing based on the FDA-approved prescribing information.[5] Conversely, one dose of plasma for a 70-kg patient would cost $151.20 (70 kg × 10 mL/kg × 1 unit/250 mL × $54/unit) using minimum dosing of 10 mL/kg based on a recent multicentre study of plasma use in the United States.[24]

The safety and efficacy of 4FPC for urgent VKA correction in bleeding patients have been demonstrated in a prospective, multicentre, randomised controlled trial.[21] Other studies have examined the use of 4FPC in a variety of patient populations, such as those requiring VKA correction prior to urgent surgical or invasive procedures,[9] those undergoing liver transplant,[1] those recovering from cardiac surgery[4,8] and those on warfarin[20,23] or direct factor Xa inhibitors[10] who have intracranial haemorrhage. Meta-analyses have concluded that 4FPC is safe and effective for VKA correction in the context of bleeding[3] and that 4FPC reduced transfusion requirements in patients undergoing cardiac surgery.[18] In contrast, a Cochrane review examining 4FPC for VKA correction in both bleeding and non-bleeding patients showed that 4FPC does not appear to reduce mortality or transfusion requirement but does demonstrate the possibility for reversing VKA-induced coagulopathy without requiring plasma.[12] Overall, the impact of 4FPC on resource utilisation has generated mixed conclusions.[13,16]

In this study, we aimed to determine whether 4FPC was associated with a clinically important reduction in plasma transfusion compared to plasma alone for similar indications. We hypothesised that patients receiving 4FPC would receive less subsequent plasma than individuals receiving only plasma for a similar indication.

2 | MATERIALS AND METHODS

After approval by our institutional review board, we conducted a retrospective chart review of patients who received 4FPC (Kcentra, CSL Behring, King of Prussia, PA) between March 1, 2016 and June 15, 2016 (Figure 1). Demographic information, clinical characteristics, laboratory data and the number and types of blood products administered were obtained, as well as whether vitamin K was given. For patients whose charts indicated a diagnosis of liver disease, Model for End-stage Liver Disease (MELD) scores, which predict survival using a formula that incorporates serum bilirubin, creatinine, sodium and INR values,[14] were calculated. A note was made of patients who died during admission or within 30 days of admission. A single indication for 4FPC administration was assigned to each patient based on a list of indication codes developed by one of the authors (SJR) after review of pilot data (Appendix S1).

Control subjects were identified by matching patients who received 4FPC to individuals who were treated only with plasma for the same indication between October 1, 2015 and December 31, 2016. This time frame was expanded beyond the time frame used for the 4FPC patients due to the large number of potential control patients needed to identify a sufficient number of plasma-only patients with matching indications. We recorded blood product transfusion for the 24 hours following the administration of either 4FPC administration (4FPC cohort) or after two units of plasma (plasma-only cohort). For plasma-only patients, blood products were only counted after the first two units of plasma so that both cohorts received a similar initial dose of coagulation factors. Thus, when comparing plasma transfusion in the 24 hours following intervention, the intervention for the 4FPC patients was the administration of 4FPC, and the intervention for the plasma-only patients was the administration of the first two units of plasma, per our study protocol (Figure 1).

In order to evaluate whether the indication for 4FPC administration impacted subsequent plasma transfusion, we performed a subgroup analysis examining the same parameters but comparing two groups within the 4FPC cohort. These groups were based on whether patients received 4FPC for an "on-label" indication vs an "off-label" indication, with "on-label" indications considered to be those listed in the FDA package insert for 4FPC (Appendix S1).

To summarise demographic and baseline characteristics, we used count and percentage (n,%) for categorical variables. For normally distributed continuous variables, we used mean and SD (mean ± SD), and for non-normally distributed continuous variables, we used median and interquartile range (median, [Q1, Q3]). For comparing these values between the 4FPC patients and plasma-only patients, we used chi-squared tests, *t*-tests or Mann-Whitney *U* tests, as appropriate. In addition, the number of patients requiring transfusions and the median plasma received in the 24-hour follow-up period were also compared using chi-squared and Mann-Whitney U tests, respectively. We used similar tests for the same variables in the subset comparing the "on-label" and "off-label" uses of 4FPC. *P*-values less than .05 were considered statistically significant. All statistical analysis was conducted in R version 3.5.1[19]

3 | SUBJECTS STUDIED

Ninety patients received 4FPC and were matched to a corresponding patient who received plasma for the same indication. We were unable to match six additional 4FPC patients (five patients received 4FPC for warfarin correction for intracranial haemorrhage and one for coagulopathy correction prior to cardiothoracic surgery). Five of the 90 4FPC patients received two doses of 4FPC within 24 hours of the initial dose, and one patient received three doses. Dosing of 4FPC is based on weight and pre-treatment INR per the FDA-approved prescribing information ([5]) and, in our group, ranged from 1096 to 3354 units (2402 ± 783 units).

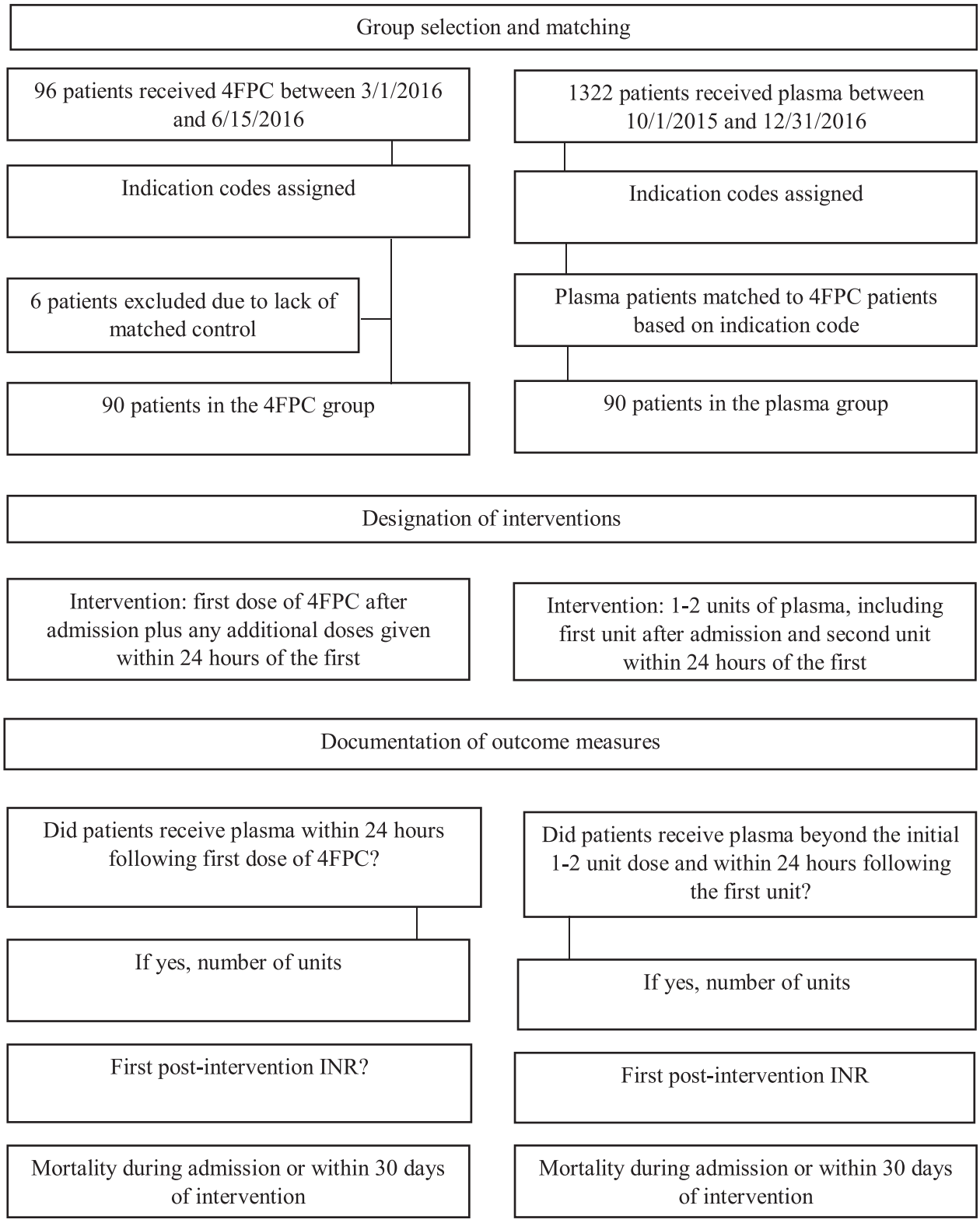

FIGURE 1 Workflow of study group selection and data collection

4 | RESULTS

Demographic and baseline clinical and laboratory characteristics were largely similar between groups (Table 1) except that 4FPC patients were older (66 ± 16 vs 62 ± 14 years, $P = .02$), and for those who had MELD scores calculated, 4FPC patients had higher scores (39 ± 7, n = 21 vs 26 ± 9, n = 25, $P < .01$). The number of patients who received vitamin K in addition to 4FPC or plasma was not significantly

TABLE 1 Demographic and baseline clinical and laboratory characteristics

Characteristic	4FPC group		Plasma group		
	Mean	SD	Mean	SD	*P* value
Age (yr)	66 (n = 90)	16	62 (n = 90)	14	.02
Body weight (kg)	86	22	85	23	.69
	Number	Percent	Number	Percent	
Gender (female)	35	38.9	35	38.9	1.00
Warfarin use	45	50.0	47	52.2	.88
History of liver disease	21	23.3	27	30.0	.40
Received vitamin K	43	47.8	45	50.0	.88
	Median	IQR	Median	IQR	
INR prior to intervention	2.8[a]	1.6	2.2[a]	1.4	.09
Hgb within 2 h prior to intervention (g/dL)	10.0[b]	4.0	9.7[b]	3.4	.78
Platelet count within 2 h prior to intervention (# x10^9/L)	167[c]	168	187[c]	131	.86
MELD score	39[d]	7	26[d]	15	<.01

Abbreviations: 4FPC, 4-factor prothrombin complex concentrate; Hgb, haemoglobin; INR, international normalised ratio; IQR, interquartile range; MELD, model for end-stage liver disease.
[a]4FPC n = 85, plasma n = 85.
[b]4FPC n = 44, plasma n = 31.
[c]4FPC n = 39, plasma n = 27.
[d]4FPC n = 21, plasma n = 25.

different between groups (43 (47.8%) for 4FPC patients vs 45 (50%) for plasma-only patients, *P* = .88).

4.1 | Plasma transfusion in the 24 hours following intervention

Less than half of patients in each group received additional plasma in the 24 hours following either 4FPC or the equivalent dose of plasma (19 (21%) 4FPC patients vs 31 (34%) plasma-only patients, *P* = .07). Of the 59 plasma-only patients who only received the initial two-unit dose of plasma and did not require additional plasma transfusion, 19 received a total of one unit, and 40 received a total of two units, including the first unit after admission and a second unit within 24 hours of transfusion of the first. The median number of units of additional plasma received by both the 4FPC and plasma groups was 0 units ([0, 0] for 4FPC patients vs [0, 1] for plasma-only patients, *P* = .09). The 4FPC group included two outliers that received 18 and 31 units of plasma in the 24 hours following 4FPC administration (Figure 2). The largest number of additional plasma units received in the plasma group was 11. No statistically significant differences were noted between groups in terms of how many patients required red blood cell transfusion (25 (28%) 4FPC patients vs 35 (39%) plasma-only patients, *P* = .15) or platelet transfusion (20 (22%) 4FPC patients vs 24 (27%) plasma-only patients, *P* = .60) in the 24 hours following intervention, nor in terms of the median numbers of units of transfused red blood cells (0, [0, 1] for 4FPC patients vs 0, [0, 2] for plasma-only patients, *P* = .22) or platelets (0, [0, 1] for 4FPC patients vs 0, [0, 1] for plasma-only patients, *P* = .47).

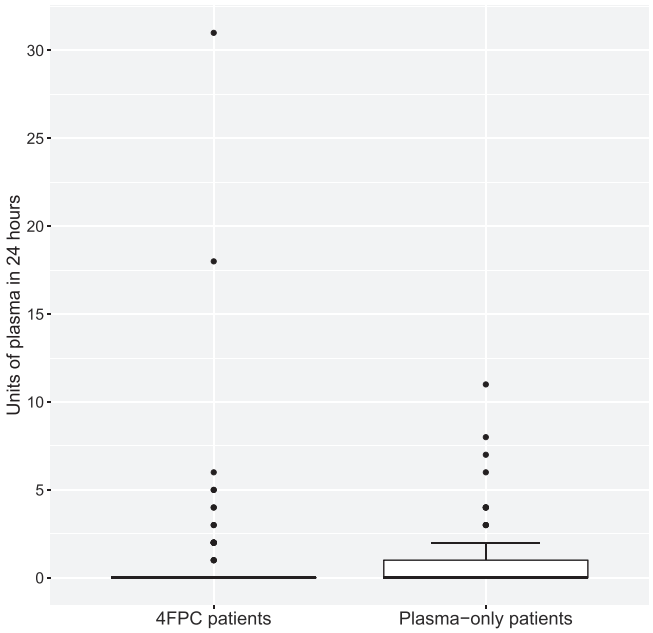

FIGURE 2 Number of units of plasma received by 4FPC patients vs plasma-only patients in the 24 hours following either 4FPC or the equivalent dose of plasma. The median number of units received during the 24-hour follow-up period was the same for both groups. The distribution differed somewhat; more plasma-only patients than 4FPC patients received 1 to 2 additional plasma units, whereas two 4FPC patients received massive plasma transfusion (18 and 31 units). Bolded line = median; box = interquartile range; whisker = greatest non-outlier observation; dot = outlier (any point less than 25th percentile minus 1.5 times the interquartile range or greater than 75th percentile plus 1.5 times the interquartile range)

4.2 | First post-intervention INR and mortality

The 4FPC patients (n = 78) had a lower median first post-intervention INR compared to the plasma-only patients (n = 66) (1.5, [1.3, 2.0] vs 1.7, [1.5, 2.1], $P < .01$). There was no difference in overall mortality between groups (33 (37%) 4FPC patients vs 21 (23%) plasma-only patients, $P = .07$).

4.3 | Subgroup analysis

Subgroup analysis comparing patients who received 4FPC for on-label indications (n = 22, 24%) to patients who received 4FPC for off-label indications (n = 68, 76%) demonstrated no statistically significant difference in the percentage of patients who received plasma in the 24 hours following 4FPC administration (4 (18%) on-label patients vs 15 (22%) off-label patients, $P = .93$). The median number of units of plasma received by both the groups was 0 units ([0, 0] for on-label patients vs [0, 0] for off-label patients, $P = .64$). Significantly more patients in the on-label group received vitamin K in addition to 4FPC (17 (77%) vs 26 (38%), $P < .01$). Although patients in both groups had similar INR values prior to 4FPC administration (2.5, [2.3, 3.0], n = 22 on-label patients vs 2.8, [2.3, 3.7], n = 63 off-label patients, $P = .95$), patients who received 4FPC for on-label indications had a lower median first post-intervention INR (1.3 [1.2, 1.5], n = 21 vs 1.6 [1.3, 2.1], n = 57, $P = .01$). The off-label group had a considerable prevalence of liver disease (21 [31%]), whereas the on-label group did not include any patients with liver disease.

5 | DISCUSSION

We did not find a reduction in subsequent plasma usage at our institution for individuals who received 4FPC compared to patients treated with two units of plasma for a similar indication. A total of 79% of 4FPC patients and 66% of plasma-only patients did not receive additional plasma following administration of either 4FPC or an equivalent dose of plasma. Although most patients in both groups did not require additional plasma, 19 4FPC patients did receive plasma in addition to 4FPC. These patients may have demonstrated progressive coagulopathy, requiring additional intervention within 24 hours of 4FPC administration. Two patients in the 4FPC group were massively transfused in the 24 hours following 4FPC administration. Both patients had a history of cirrhosis. One was admitted for toxic epidermal necrolysis and received 4FPC and plasma transfusion in the setting of haemorrhagic shock following a dressing change. The other received 4FPC and plasma transfusion intraoperatively during liver transplant surgery. These patients demonstrate that 4FPC is administered in a variety of clinical scenarios and patient groups, complicating its association with plasma transfusion. No institutional protocols governing the administration of 4FPC were in effect during the time frame of this retrospective review; varying clinical practice patterns may have contributed to subsequent plasma transfusion in some 4FPC patients.

Prior studies have reported conflicting conclusions on whether 4FPC is associated with decreased plasma transfusion. Several studies have suggested that 4FPC administration may decrease plasma transfusion in specific clinical scenarios, including in the setting of warfarin-related intracranial haemorrhage[2] and in the perioperative period surrounding heart transplant[8] and other surgeries requiring cardiopulmonary bypass.[6] Although a Cochrane review examining the use of 4FPC for VKA correction in bleeding and non-bleeding patients also supported the potential for 4FPC to reduce plasma use, it did not find sufficient evidence to support the claim that 4FPC lowers overall transfusion requirements.[12] A published audit of plasma use concluded that plasma use remains "inappropriately high" in the setting of warfarin correction, despite the implementation of 4FPC.[22] Our study reviewed 4FPC use for a wide variety of clinical indications and found that, when examining multiple indications together, 4FPC did not appear to be associated with reduced plasma transfusion at our institution.

Of the patients with an INR documented in the medical record following administration of 4FPC or the equivalent dose of plasma, the 4FPC patients had a median first post-intervention INR of 1.5, compared to 1.7 in the plasma-only patients. Several prior studies have reported a higher percentage of patients in the 4FPC group achieving an INR ≤1.3 at 0.5 hours post-intervention when compared to patients in the plasma-controlled group ([9,17,21]). One study of 4FPC use during cardiopulmonary bypass found that a higher percentage of patients in the 4FPC group achieved a target INR of ≤1.5 at 15 minutes but that there was no difference in the percentage achieving target INR at 1 hour,[6] whereas another study of 4FPC use for intracranial haemorrhage found a difference in INR values between 4FPC and plasma groups for up to 6 hours post-intervention.[2] Most of our patients had first post-intervention INR values documented more than 0.5 hours post-intervention, and our findings support that the lower INR noted in the 4FPC patients is sustained beyond 0.5 hours post-intervention.

Notably, when 4FPC patients were divided into subgroups based on whether 4FPC was received for on-label vs off-label indications, it became apparent that the vast majority, over three-fourths, received 4FPC for off-label indications. Subgroup analysis comparing patients who received 4FPC for on-label indications to patients who received 4FPC for off-label indications did not demonstrate a significant difference in plasma transfusion between groups, with approximately one-fifth of patients in both on-label and off-label groups receiving plasma in addition to 4FPC. This finding was unexpected based on the assumption that, for on-label indications, 4FPC would be used instead of plasma rather than in addition to it. The absence of an institutional protocol to standardise transfusion of plasma subsequent to 4FPC administration may have led to variations in clinical practice, possibly contributing to the lack of a difference in plasma transfusion between groups.

We did find a difference both in percentage of patients who received vitamin K and in first post-intervention INR between groups. A greater percentage of patients in the on-label group received vitamin K in addition to 4FPC. This finding may reflect more standardised

practice patterns in the on-label group; that is, clinicians prescribing 4FPC for on-label indications were more likely to co-administer vitamin K, as outlined in the package insert. However, as described above, a similar percentage of patients in the on-label and off-label groups received plasma in addition to 4FPC, which may belie variation in clinical practice patterns in both the on-label and off-label groups. Although patients in both groups had similar baseline INR values, those in the on-label group had a median first post-intervention INR of 1.3, compared to 1.7 in the off-label group. The lower median INR noted in the on-label group may be attributed to the more frequent co-administration of vitamin K. Alternatively, this finding may suggest that 4FPC is more efficacious for INR correction for certain indications (Appendix S1). To our knowledge, no other studies have directly compared INR in patients who receive 4FPC for on-label vs off-label indications. However, the efficacy of 4FPC for many of the on-label indications has been supported individually in several studies examining single indications. These indications include VKA correction in the setting of acute haemorrhage,[21] specifically for intracranial haemorrhage,[20,23] and VKA correction prior to surgery or invasive procedures,[9] intraoperatively during liver transplantation[1] and postoperatively following cardiac surgery.[4,8]

Approximately one-third of the off-label group comprised patients with liver disease, compared to no patients with liver disease in the on-label group. These patients with liver disease may have contributed to the higher post-intervention INR noted in the off-label group. A recent study examining the safety and efficacy of 4FPC in patients with liver disease found that these patients demonstrated suboptimal coagulopathy correction and haemostasis compared to patients without liver disease.[11] As most of the patients who received 4FPC received it off-label, studies are needed to determine if its off-label use provides benefits and what the appropriate dose is, if any, or if its off-label use should be reconsidered.

Several limitations to our study warrant consideration. Our study was conducted at a single institution, which may limit the generalisability of our findings. Inherent to our retrospective study design is a limited ability to control for potential confounders. Furthermore, our study did not directly assess the severity of patients' clinical scenarios. Although our study captured mortality information, we did not explore bleeding and clotting complications and how these may have contributed to mortality as these factors were outside the scope of our study. Another limitation is the fact that most of our plasma-only patients received only 1 to 2 units of plasma, indicating that plasma was not always dosed according to weight-based dosing of at least 10 mL/kg as supported by a recent study.[24] Finally, the high incidence of off-label prescribing led to a small sample size for on-label use, which may explain why our results differ from prospective randomised controlled trials.

In conclusion, we did not find evidence that 4FPC administration was associated with reduced plasma use at our institution. We did find that 4FPC was prescribed to a diverse patient population, including a subset with liver disease and high MELD scores, which may have complicated our study of the association between 4FPC and subsequent plasma transfusion. We also found that the vast majority of patients received 4FPC for off-label indications. Other possible contributors to the lack of association between 4FPC and subsequent plasma transfusion might include the absence of an institutional protocol for 4FPC use and individual patient scenarios demonstrating progressive coagulopathy or haemorrhage. Our findings highlight the need for stricter institutional guidelines and clear corresponding policies for administering 4FPC to optimise its use for on-label indications. Further studies are warranted to clarify the effectiveness and proper use of 4FPC for both on- and off-label indications.

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CONFLICT OF INTEREST

The authors have no competing interests.

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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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