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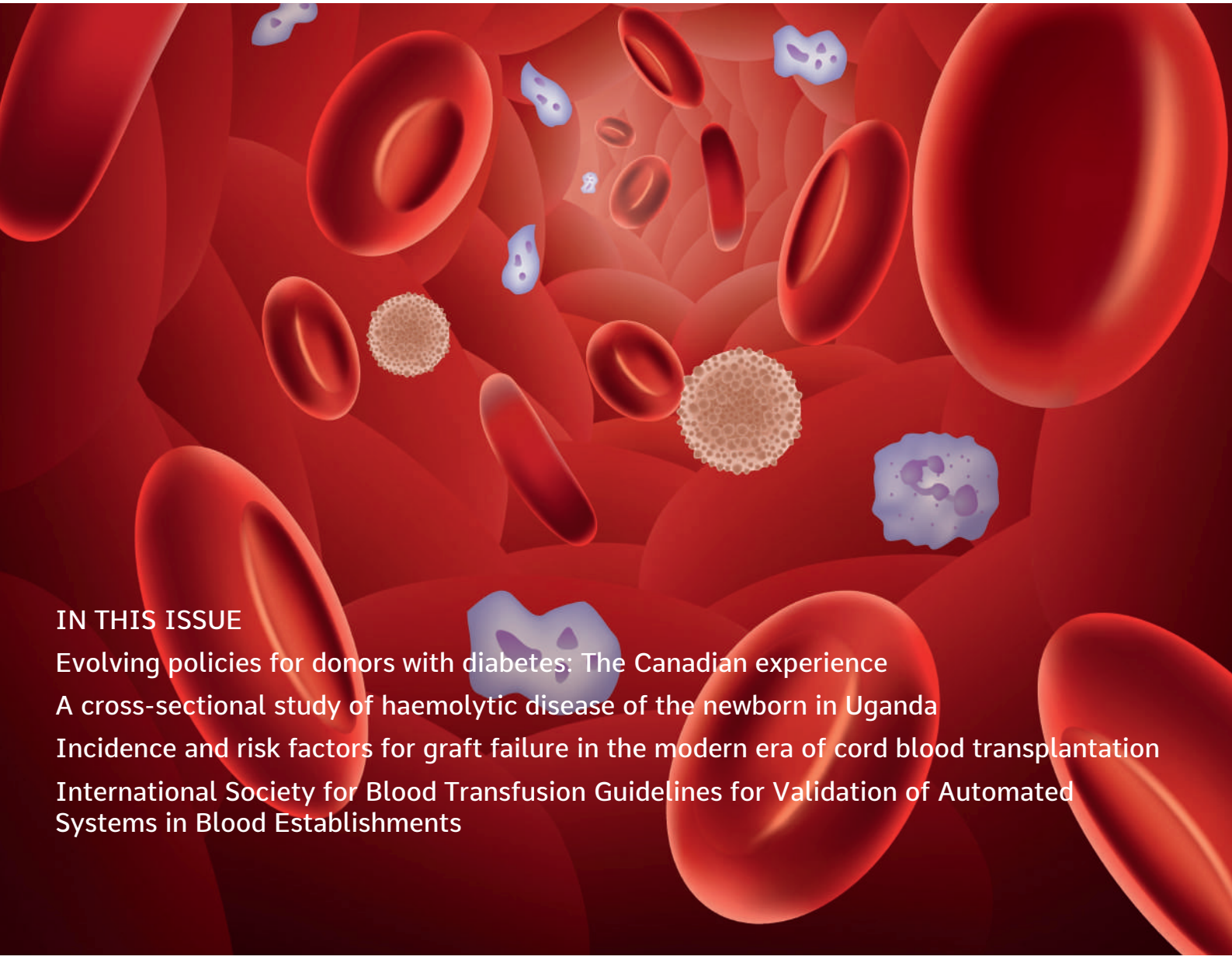

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ISBT International Society of Blood Transfusion

Vox Sanguinis

International Journal of Blood Transfusion

Official Journal of the International Society of Blood Transfusion

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Vox Sanguinis

International Journal of Blood Transfusion

Aims and Scope

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

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

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

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ORIGINAL ARTICLE

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Red cells manufactured from lipaemic whole blood donations: Do they have higher haemolysis?

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Abstract

Background and Objectives: Lipaemia in blood donations is thought to influence haemolysis in stored red blood cell (RBC) components. Higher lipid concentrations are believed to increase red cell fragility, exacerbating haemolysis during collection and subsequent red cell storage. This study aimed to investigate associations between lipoproteins in plasma and haemolysis of red cells stored in saline–adenine–glucose–mannitol (SAGM).

Materials and Methods: Fifty-four plasma and matched RBCs were obtained from lipaemic whole blood donations. Plasma was tested for coagulation factors, triglycerides and cholesterol. Haemolysis, glucose, lactate, extracellular potassium, lactate dehydrogenase and adenosine triphosphate (ATP) were measured in RBC on Days 7, 21 and 42 of storage. Additionally, 20 plasma and matched RBCs from non-lipaemic donations were tested as controls.

Results: Lipaemic plasma had significantly higher triglyceride concentrations compared with non-lipaemic plasma. However, there was no significant difference in plasma cholesterol between the two groups. There were no significant differences in glucose, extracellular potassium or ATP concentrations in RBC from either group. There was no significant difference in haemolysis at expiry in lipaemic-derived and control RBC, with a weak correlation between haemolysis and either triglycerides or cholesterol.

Conclusion: There was no significant difference in haemolysis in RBC manufactured from lipaemic and non-lipaemic whole blood donations when stored in SAGM; however, the proportion of RBC from lipaemic donations with higher haemolysis was greater than in the controls. There was a weak correlation between red cell haemolysis and plasma triglycerides. Therefore, RBCs derived from lipaemic donations are suitable for blood bank inventories.

Keywords

BMI, haemolysis, lipaemic plasma, red cell concentrates, triglycerides

Highlights

- Lipaemic plasma has a fourfold higher triglyceride concentration than control plasma.
- There is no significant difference in haemolysis in red blood cells manufactured from lipaemic and non-lipaemic whole blood donations, when stored in saline–adenine–glucose–mannitol.
- There was no correlation between red cell haemolysis and plasma cholesterol, with a weak correlation between haemolysis and plasma triglycerides.

INTRODUCTION

Lipaemia is the presence of abnormally high concentrations of emulsified lipids in the blood, with triglycerides being the most common. A donation may be lipaemic if the donor has consumed a high-fat meal prior to donating, as it only takes 30–60 min following gastrointestinal absorption for the triglycerides to appear in the circulation [1]. Alternatively, lipaemia can be due to underlying medical conditions, such as diabetes, or caused by hormone-altering medications [1, 2], which all increase plasma lipid concentration. Additionally, a donor's health and lifestyle can affect their lipid levels, including body mass index (BMI), physical activity, smoking, alcohol and cardiovascular health [3–6].

The clinical impact of transfusing lipaemic plasma is not fully understood, although it is believed not to affect the therapeutic value of the blood components. The major concern with lipaemic plasma is turbidity caused by the lipoproteins in the plasma. Turbidity interferes with laboratory testing, as lipoproteins scatter light, thus disturbing light transmission and leading to inaccurate results [1, 7]. Although most common in spectrophotometric methods, it may also interfere with coagulation assays and haematological testing [1, 8].

In Australia, donated plasma is visually assessed for lipaemia by holding a segment of plasma in front of a grid; if the grid is not visible, the plasma is recovered for fractionation or discarded. Severely lipaemic whole blood donations are also discarded when mandatory infectious disease testing cannot be completed due to interference from turbidity. While highly lipaemic plasma is discarded, the red blood cell (RBC) component can still be issued as a clinical component, provided the mandatory testing can be completed. Similarly, the guidelines for Blood Transfusion Services in the United Kingdom recommend that platelets or plasma should not be produced from lipaemic donations [9]. The Canadian Blood Services also uses a visual assessment guide, stating that all lipaemic blood components are acceptable for transfusion unless there is excessive lipaemia, which interferes with testing, whereby lipaemic components are discarded [1, 10].

Recent studies have reported higher haemolysis in RBC processed from lipaemic whole blood donations [11–14]. Haemolysis is the release of free haemoglobin (Hb) from ruptured or damaged RBCs [15–18]. Transfusion of free Hb can have detrimental effects, including kidney damage, and is fatal in extreme cases [15, 16]. Haemolysis occurs during RBC processing and storage from bacterial haemolysis, complement lysis and biochemical and biomechanical changes in the RBC [16, 17]. The introduction of leucocyte depletion has reduced haemolysis of stored RBC [15, 18] as have improvements in RBC additive solutions [15, 19]. The American Association of Blood Banks (AABB) guidelines in the United States and Council of Europe guide to the preparation of blood components set upper limits of acceptable haemolysis at less than 1% and 0.8%, respectively [20, 21]. A number of studies have attempted to address the differences in RBC haemolysis, attributing the differences to donor characteristics, such as BMI, testosterone and donation frequency [22–25].

With increasing rates of lipaemia in whole blood donations, the aim of this study was to investigate the in vitro quality of RBC prepared from lipaemic whole blood donations and stored in saline–adenine–glucose–mannitol (SAGM) additive solution for 42 days. In particular, haemolysis was compared in RBC from non-lipaemic donations. The relationship between lipoproteins in the plasma and RBC haemolysis at expiry was also investigated.

MATERIALS AND METHODS

Whole blood collections

This study was approved by the Australian Red Cross Lifeblood Human Research Ethics Committee. Whole blood donations (470 ml) were collected into citrate–phosphate–dextrose collection bags (Macopharma, Polonia Sp. Z Oo, Poland) and held at 20–22°C for up to 18 h before centrifugation (5000 × *g*; 10 min) and separation using an automated blood extractor (MacoPressSmart; Macopharma, Arcore, Italy). All RBCs were leukodepleted by in-line filtration and stored in SAGM at 2–6°C.

Component selections

Lipaemic ($n = 54$) and non-lipaemic ($n = 20$; controls) plasma components were selected based on visual inspection (milky/turbid or clear), frozen in a rapid plasma freezer (Arrowsmith and Grant Refrigeration, Dandenong South, VIC, Australia) and stored below −30°C. The blood group was not considered a determining factor for lipaemia, and therefore, components from all blood groups were included. Controls were matched for donor sex and age brackets (18–35 , 36–55 and >55 years). The RBC manufactured from the same donation as each of the lipaemic and control plasma components were also obtained.

In vitro plasma quality

Plasma was thawed in a 37°C water bath, visually inspected and aliquoted for testing. Plasma was tested immediately for coagulation factors (factor VIII [FVIII], factor IX, factor X, von Willebrand factor antigen and fibrinogen) using an automated coagulation analyser (STA Compact; Diagnostica Stago Ltd., Asnieres, France) as per manufacturer's instructions and previously described [26]. A 12-ml plasma sample was refrigerated for 24 h to determine the presence of chylomicrons [27], and the height of the chylomicron layer was measured. The remaining plasma samples were frozen at −80°C for subsequent measurement of coagulation factors (factor II, factor V and antithrombin III; STA Compact analyser) and triglyceride, cholesterol, high-density lipoprotein and total protein measurements using a biochemistry analyser (VITROS XT3400; Ortho Clinical Diagnostics, Raritan, NJ, USA). The turbidity score was also measured using a biochemistry analyser and used to grade the severity of lipaemia in the plasma.

In vitro red cell quality

RBCs were sampled on Days 7, 21 and 42 post-collection. Hb content, haematocrit (Hct), red blood cell count (RBCC), mean cell volume, red blood cell distribution width (RDW), mean corpuscular Hb and mean corpuscular Hb concentration were measured using a haematology analyser (CELL-DYN Ruby; Abbott Diagnostics, Abbott Park, IL, USA). A spun Hct was also determined by centrifugation (12,000 × *g*; 5 min) using a micro-Hct centrifuge (C2 Series; Centurion Scientific, Chichester, UK). The pH at room temperature was measured using a pH meter (Seven Multi; Mettler Toledo, Schwerzenbach, Switzerland).

Supernatants from red cells for all subsequent assays were prepared by centrifugation at 5000 × *g* for 8 min (Allegra X-15R; Beckman Coulter, Indianapolis, IN, USA) and were stored at −80°C until tested. Free Hb in the supernatant was measured using a plasma/low Hb system (HemoCue AB, Ängelholm, Sweden). Subsequently, haemolysis was calculated from the supernatant Hb and expressed as a percentage of the total Hb in the red cell suspension [28]. Glucose, lactate, potassium and lactate dehydrogenase (LDH) were measured using a biochemistry analyser (VITROS XT3400). Microparticles (MPs) were dual-labelled using CD235a-allophycocyanin (APC) and annexin-V fluorescein (FITC) and measured using a flow cytometer (FACSCanto II; BD Biosciences, San Jose, CA, USA) as previously described [29].

Red cells were deproteinized with 10% trichloroacetic acid, and the supernatants were neutralized with potassium carbonate as described previously [29] and stored at −80°C until tested. The intracellular concentrations of adenosine triphosphate (ATP) were measured using ATP bioluminescence (Roche, Mannheim, Germany).

Statistical analysis

Results are expressed as the mean ± standard deviation (SD). Unpaired *t*-tests (two-tailed) were used to compare lipaemic and control plasma using MS Excel (Microsoft Office, Redmond, WA, USA). A two-way repeated measure analysis of variance was used to compare data from red cells derived from lipaemic whole blood donations and control red cells using Prism 7 (GraphPad Software, Inc., La Jolla, CA, USA). Post hoc Bonferroni multiple comparisons were performed to determine the specific differences between each group and time point. Correlations between haemolysis and various parameters in the study were determined using linear regression, with a linear line of best fit. Pearson's correlation was used to calculate *r*- and *p*-values. A chi-squared (Fisher's exact) test was used to determine whether there was a difference in the proportion of RBC with haemolysis above 0.4% (Day 42) between lipaemic and control groups. A haemolysis value of 0.4% was chosen as this was the cut-off value used in a Dutch study [30] and enabled a comparison of data. A *p*-value <0.05 was considered significant.

RESULTS

Lipaemic whole blood collections

Donor characteristics, including sex, age and BMI, for the lipaemic and control groups are reported in Table 1. The mean BMI of donors whose whole blood donations were lipaemic was not significantly different to that of the control donors: 29.2 ± 4.1 kg/m^2 and 28.6 ± 6.7 kg/m^2, respectively ($p = 0.6746$). Only three of the lipaemic whole blood donations were collected before 12 PM, and the remaining 51 were afternoon collections. The mean collection time was between 2 and 4 PM. Four of the 20 control units were also collected before 12 PM, 10 collections between 12 and 4 PM and 6 collections after 4 PM.

TABLE 1 Donor sex, age and body mass index (BMI)

Parameter	Lipaemic ($n = 54$)	Control ($n = 20$)
Sex		
Male	47 (87%)	14 (70%)
Female	7 (13%)	6 (30%)
Age (years)		
Mean ± SD	39 ± 12	42 ± 15
18–34	24 (44%)	8 (40%)
36–54	26 (48%)	8 (40%)
Over 55	4 (8%)	4 (20%)
BMI (kg/m^2)		
Mean ± SD	29.2 ± 4.1	28.6 ± 6.7
Healthy: 18.5–24.5	4 (7%)	5 (25%)
Overweight: 25–29.5	33 (61%)	12 (60%)
Obese: Over 30	17 (32%)	3 (15%)

Note: BMI classifications as per World Health Organization for the general population [33].

TABLE 2 Coagulation factors in lipaemic and control fresh frozen plasma (mean ± SD)

Parameter	Lipaemic ($n = 54$)	Control ($n = 20$)	*p*-value[a]
Antithrombin III (%)	100.9 ± 10.7	102.6 ± 14.6	0.5910
Factor II (%)	98.2 ± 13.2	94.7 ± 12.2	0.3029
Factor V (%)	101.0 ± 15.9	98.7 ± 18.2	0.5893
Factor VIII (%)	108.0 ± 28.5	121.6 ± 29.3	0.0738
Factor IX (%)	117.2 ± 25.4	124.6 ± 28.3	0.2848
Factor X (%)	100.6 ± 16.4	102.4 ± 14.0	0.6791
Fibrinogen (g/L)	2.8 ± 0.5	2.8 ± 0.8	0.9963
von Willebrand factor (%)	99.1 ± 32.0	109.1 ± 26.6	0.2154
Total protein (g/L)	57.7 ± 3.7	58.1 ± 2.9	0.5926

[a]*p*-value determined using unpaired *t*-tests.

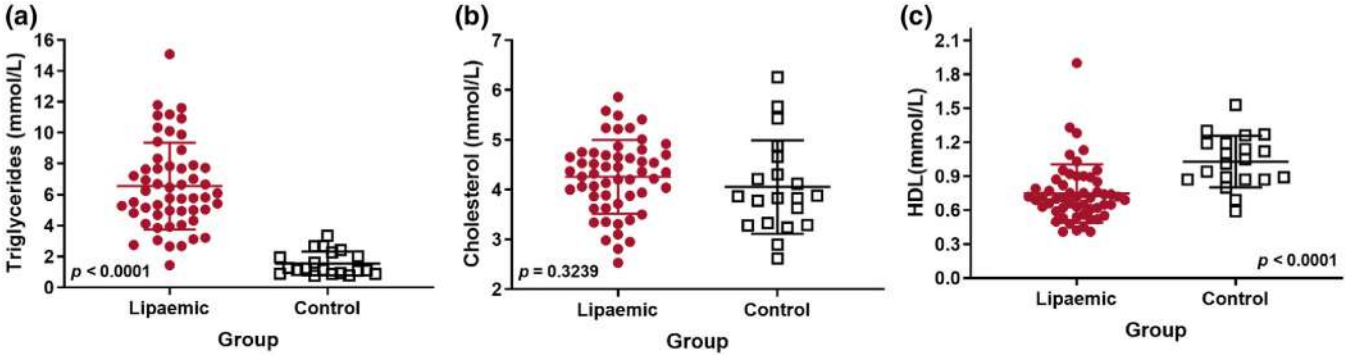

FIGURE 1 Triglyceride and cholesterol concentrations in lipaemic and control plasma. (a) Triglyceride, (b) cholesterol and (c) high-density lipoprotein (HDL) concentrations in lipaemic and control plasma. Triglyceride, cholesterol and HDL concentrations in plasma were measured using a biochemistry analyser. Dots represent individual units; lipaemic, $n = 54$; control, $n = 20$. Horizontal lines are the mean ± SD. The p-value was determined using an unpaired t-test.

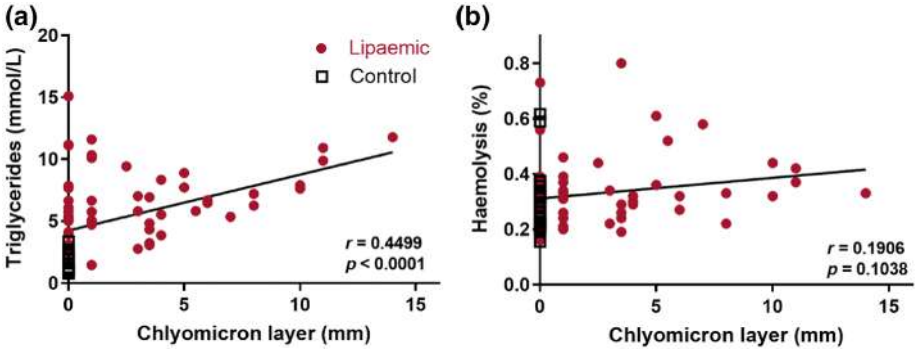

FIGURE 2 Correlation between chylomicrons layer, plasma triglycerides and red cell haemolysis. Correlation between chylomicron height and (a) triglyceride concentration and (b) red cell haemolysis at expiry. The presence of chylomicron was determined after overnight refrigeration, and the height of the layer was measured. Triglyceride concentrations in plasma were measured using a biochemistry analyser. Dots are individual units; lipaemic, $n = 54$; control, $n = 20$. The solid line represents a simple linear regression line of best fit, and a Pearson's correlation was used to calculate r- and p-values.

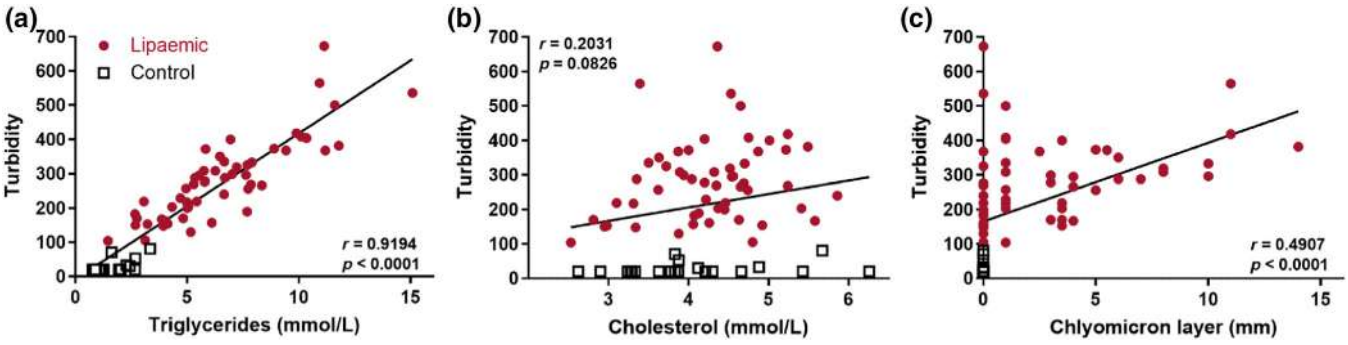

FIGURE 3 Turbidity score of lipaemic plasma, and correlations with triglycerides, cholesterol and chylomicrons. Correlation between lipaemic plasma turbidity score and (a) triglyceride concentration, (b) cholesterol concentration and (c) chylomicron layer. Turbidity score, triglyceride and cholesterol concentrations in plasma were measured using a biochemistry analyser. Chylomicron presence was determined after overnight refrigeration, and the layer was measured. Dots represent individual components; lipaemic, $n = 54$; control, $n = 20$. The solid line represents a simple linear regression line of best fit, and a Pearson's correlation was used to calculate r- and p-values.

Plasma

There were no significant differences in the coagulation properties of the lipaemic and control plasma (Table 2). The FVIII concentration was lower in the lipaemic plasma group than the control plasma; however, this was not significant ($p = 0.0738$). The blood groups of the donors in the lipaemic group were 30 non-Group O, 20 Group O and 4 unknown, as the donation was too lipaemic for blood typing to be

TABLE 3 Red cell indices and quality markers during storage (mean ± SD)

Parameter	Lipaemic ($n = 54$)			Control ($n = 20$)			p-value[a]
Volume (ml)	260 ± 15			260 ± 13			0.9172
RBCC (10^{12}/L)	6.79 ± 0.37			6.55 ± 0.32			0.0100
Haemoglobin (g/unit)	52.2 ± 5.3			51.0 ± 5.0			0.3656
Haematocrit (L/L)	0.601 ± 0.019			0.580 ± 0.023			0.0815
Day	**7**	**21**	**42**	**7**	**21**	**42**	**p-value[b]**
MCV (fL)	88.8 ± 5.1	89.8 ± 5.3	90.2 ± 5.5	88.8 ± 3.7	89.2 ± 3.8	89.7 ± 3.9	0.7730
RDW (%CV)	12.4 ± 0.9	12.3 ± 0.9	12.3 ± 0.9	12.9 ± 0.8	12.9 ± 0.8	12.6 ± 0.8	0.0447
Glucose (mmol/L)	24.2 ± 1.5	20.0 ± 1.4	16.3 ± 1.8	24.0 ± 1.1	20.5 ± 1.2	17.0 ± 1.5	0.3590
Lactate (mmol/L)	11.1 ± 1.4	20.2 ± 2.5	26.4 ± 3.1	10.7 ± 1.3	18.6 ± 2.2	24.4 ± 2.9	0.0157
Potassium (mmol/unit)	5.1 ± 1.2	9.8 ± 1.5	13.5 ± 1.7	4.5 ± 1.1	9.5 ± 1.7	13.5 ± 1.8	0.5047
pH at 22°C	7.0 ± 0.1	6.8 ± 0.1	6.6 ± 0.1	7.0 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	0.8366
ATP (μmol/g Hb)	4.8 ± 0.6	4.6 ± 1.2	2.5 ± 0.5	5.1 ± 0.6	4.4 ± 0.5	2.6 ± 0.5	0.7570
Microparticles (10^6/unit)	46 ± 25	106 ± 114	486 ± 406	122 ± 153	307 ± 791	511 ± 620	0.1859

Abbreviations: ATP, adenosine triphosphate; Hb, haemoglobin; MCV, mean corpuscular volume; RBCC, red blood cell count; RDW, red cell distribution width.

[a]p-value determined using an unpaired t-test.

[b]p-value determined using a repeated measures analysis of variance.

completed. All donors in the control group had blood Group A. Therefore, the difference in FVIII concentrations was most likely due to the higher proportion of Group O donors in the lipaemic group rather than lipaemia per se. The mean triglyceride concentration in the lipaemic plasma was fourfold higher than the control plasma, while there was no difference in the cholesterol concentration between the two groups (Figure 1a,b). Thirty-six of the 54 lipaemic plasma donations (67%) had chylomicrons, while they were absent in all of the control plasma samples. The proportion of chylomicrons did not correlate with either the triglyceride concentration or the RBC haemolysis (Figure 2). The turbidity scores in the lipaemic plasma strongly correlated with the triglyceride concentration but not with cholesterol or the height of the chylomicron layer (Figure 3). Fifteen of the 20 control plasma had turbidity scores below limit of detection of the analyser (<20), and therefore were assigned a value of 20 to enable inclusion in statistical analyses.

Red cells

Red cell indices and quality markers measured during storage of RBC are reported in Table 3. RBC derived from lipaemic whole blood donations had significantly higher RBCC and lower RDW than the control RBC. There was no difference in the glucose concentrations between

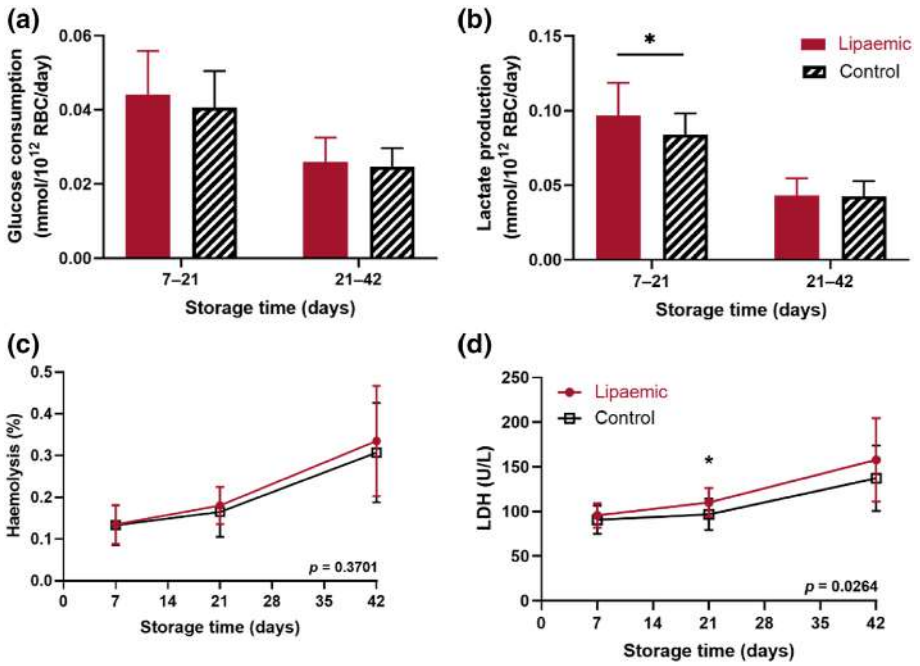

FIGURE 4 Metabolism and cell damage of lipaemic and control red cells over storage. (a) Glucose consumption and (b) lactate production in lipaemic and control RBC over storage. (c) Haemolysis and (d) lactate dehydrogenase (LDH) concentrations in lipaemic and control RBC over storage. Glucose, lactate and LDH concentrations in RBC supernatants were measured using a biochemistry analyser. Data are the mean ± SD; lipaemic, $n = 54$; control, $n = 20$. *$p < 0.05$ as determined using a two-way repeated measures analysis of variance.

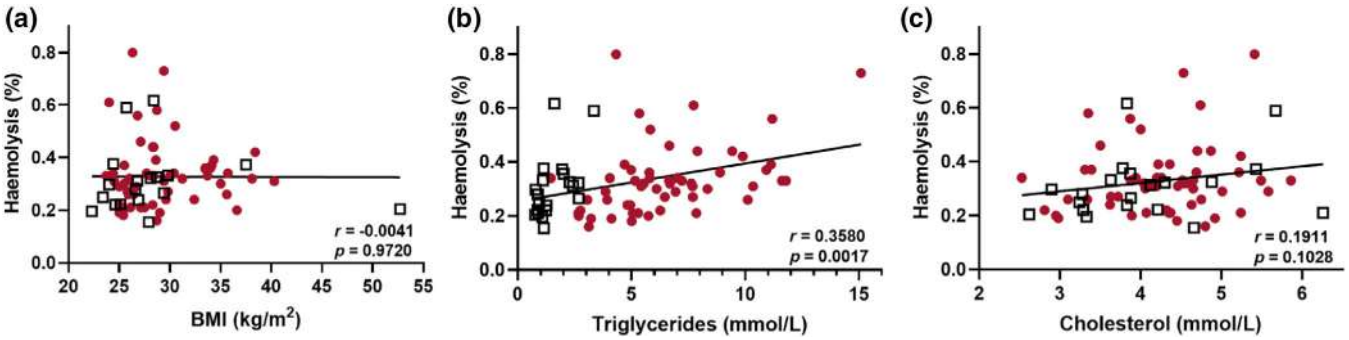

FIGURE 5 Correlations of haemolysis with donor body mass index (BMI), triglyceride and cholesterol. Correlation between red cell haemolysis at expiry (Day 42) and (a) donor BMI, (b) triglyceride concentration and (c) cholesterol concentration. Triglyceride and cholesterol concentrations in plasma were measured using a biochemistry analyser. Dots represent individual components; lipaemic, $n = 54$; control, $n = 20$. The solid line represents a simple linear regression line of best fit, and a Pearson's correlation was used to calculate *r*- and *p*-values.

groups; however, the lactate concentration was higher in the lipaemic group than in the control group (Table 3). To account for differences in the RBCC, glucose consumption and lactate production rates were calculated (Figure 4a,b). Lactate production rates were significantly higher in the lipaemic-derived RBC compared to controls between Days 7 and 21 but not from Days 21 to 42. The mean haemolysis was not significantly different between the two groups (Figure 4c), and neither was the proportion of RBC components with haemolysis above 0.4%, as determined using Fisher's exact test ($p = 0.494$). However, the LDH concentration in RBC prepared from lipaemic whole blood donations was significantly higher than in control RBC on Day 21 of storage (Figure 4d). By expiry, there was no longer a significant difference in LDH, primarily due to the larger error bars at this time point. There were no significant differences in potassium release, ATP and MP numbers in the lipaemic and control RBC (Table 3).

RBC haemolysis at expiry did not correlate with donor BMI (Figure 5a); however, there was a weak but significant correlation with triglyceride concentration in the corresponding plasma samples (Figure 5b) and a weak but not significant correlation with plasma cholesterol concentrations (Figure 5c).

DISCUSSION

This study compared the in vitro quality of RBC stored in SAGM prepared from lipaemic and non-lipaemic whole blood donations and examined correlations between lipoproteins in the plasma and RBC haemolysis at expiry. There was no significant difference in RBC haemolysis between the two groups; however, the LDH concentration was significantly higher in lipaemic RBC on Day 21 compared to the control RBC. The triglyceride concentration in plasma derived from lipaemic whole blood donations was fourfold higher than the control plasma, which weakly but significantly correlated with haemolysis in the matched RBC.

A number of recent reports have suggested an association between lipaemia and higher RBC haemolysis [11, 13]. Our data demonstrate that there was only a weak correlation between haemolysis and plasma triglycerides, haemolysis and cholesterol, and haemolysis and plasma turbidity in RBC stored in SAGM. This concurs with a previous study, where there was no direct relationship between plasma triglycerides and the degree of haemolysis [30]. It has been suggested that chylomicrons may contribute to higher haemolysis [11], however, there was no significant difference in RBC haemolysis from donations where chylomicrons were present or absent in the associated plasma.

Specifically, the study by Laleijne-Liefting et al. (2018) showed that the majority of red cells from lipaemic donations had haemolysis levels greater than 0.4% compared to haemolysis in control donations, which was less than 0.4% [30]. In our study, only 10 of the 54 lipaemic-derived RBCs had haemolysis greater than 0.4%, and only two control units also had haemolysis greater than 0.4%. While the haemolysis measurements in both groups were not normally distributed, it is an accurate representation of haemolysis measurements from routine processing and quality control at our facility. Further, the proportions of RBC from lipaemic and control groups with haemolysis above and below 0.4% were not significantly different. Both studies had more male donors than female donors. However, their mean donor age was higher (50 years), compared to 39 years in our study, and our mean donor BMI was higher (29) compared to 26 in their study. The two studies used different methods for collecting supernatant and measuring haemolysis, that is, spectrophotometry versus HemoCue technology, which may account for the observed differences in haemolysis. These may have been further compounded by differences in the donor demographics between the two studies. Interestingly, Sanquin Blood Service decided not to use lipaemic whole blood donations for the production of blood components as a result of their study [30].

More recently, red cell haemolysis has been linked to donor attributes such as BMI, donor age and donation frequency [22, 23, 25, 31]. There is a rising occurrence of obesity in Western countries, and although blood donors are generally healthy, the mean donor BMI is increasing [32, 33]. Obesity is thought to alter RBC metabolism, whereby RBCs become more susceptible to damage. Obesity has been correlated with increased RBC aggregation, which may compromise blood flow and oxygenation, and an increased RDW correlating with inflammatory markers [23]. However, in our study, the RDW in RBC from control donors was greater than that in RBC derived from lipaemic donations.

The mean BMI in our study was 29.2 ± 4.1 kg/m^2 and 28.6 ± 6.7 kg/m^2 for the lipaemic and control whole blood donors, respectively, suggesting that BMI is not a predictor of lipaemia in our population. The proportion of control donors with a healthy BMI was higher, and while the proportion of lipaemic donors with an obese BMI was higher, the differences were not significant. Further, there was no correlation between haemolysis and donor BMI for lipaemic whole blood donations, nor when combining the lipaemic and control donations in our study ($R^2 < 0.0001$). A recent evaluation of the effect of donor obesity on RBC haemolysis conducted in the United States found a significant positive association between donor BMI and haemolysis. Even with a small sample size ($n = 18$), their data showed a significant increase in both storage and osmotic haemolysis in donors with BMI > 30 kg/m^2, compared with overweight and healthy BMI donors, and an increased rate of degradation of red cells over storage in the obese donors. Some of the notable differences in the studies were that RBCs in our study were stored in SAGM, while theirs were stored in Additive Solution Formula 3 (AS-3); the citrate in AS-3 has been shown to assist in balancing osmotic pressure [34]. There were also differences in the methods for measuring haemolysis.

Many other health and lifestyle factors influence RBC quality. In particular, factors that cause oxidative stress, such as diet, exercise, alcohol consumption, smoking and hormone replacement therapies, are thought to influence haemolysis, which are not recorded prior to blood donation [22]. In Australia, donors are requested to drink lots of water and have plenty to eat prior to donating. However, there is no recommendation as to what foods should be consumed. Others have reported a reduction in discards due to lipaemia (reduced by 6% in 4 years) following a campaign to educate donors on the impact of diet and exercise before blood donation [35]. Our study showed that the

time of donation is a key factor in the occurrence of lipaemia in our donor centres, as only 3 of the 54 lipaemic donations were collected before midday. This concurs with other studies where significant differences in lipaemia were observed in donations made before and after 2 PM [36].

RBCs at our facility were produced from top–bottom separation after an 18-h hold at ambient temperature. Each jurisdiction differs in its processing methods and holding times, and therefore, RBCs produced in other centres may have longer exposure to lipaemic plasma prior to separation, potentially exacerbating any effects. With the impending removal of Diethylhexyl phthalate (DEHP) from blood bags, there has been speculation that lipaemia may have more of an effect on RBC haemolysis [30], and further investigation of the effects of lipaemia on RBC stored in non-DEHP packs is warranted.

Some of the limitations of this study include differences in sample size between groups, control donations from one blood type (Group A) and a skewed distribution in BMI with limited numbers in the healthy range (19–25). While an alternative approach would have been to pair each lipaemic donation with age, sex and BMI control, this was not feasible, and a smaller control sample ($n = 20$) was selected to cover each age group for male and female categories.

The data from this study show that there were some small but significant differences in lactate production and LDH in RBC prepared from lipaemic and non-lipaemic whole blood donations, and these differences are unlikely to be biologically relevant. Although there is a slight trend toward higher haemolysis in RBC from lipaemic donations, this was not significant. As such, RBCs produced from lipaemic whole blood donations are still suitable for transfusion, providing mandatory infectious disease screening can be completed.

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CONFLICT OF INTEREST

D.C.M. has received research funding from Macopharma and Cryogenics Holdings in the past 2 years. Other authors have no conflict of interests to declare.

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ORIGINAL ARTICLE

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High false discovery rate of the Architect anti-HCV screening test in blood donors in Uganda and evaluation of an algorithm for confirmatory testing

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Abstract

Background and Objectives: Adequate supplies of donor blood remain a major challenge in sub-Saharan Africa. This is exacerbated by a lack of confirmatory testing for transfusion-transmitted infections by blood transfusion services (BTS), leading to significant blood disposal owing to putatively high seroprevalence rates amongst Ugandan blood donors. We aimed to ascertain the false discovery rate of the Architect anti-hepatitis C virus (HCV) screening assay and categorize screen-reactive samples into three groups: presumed false positive, active and past infection, and develop an algorithm for confirmatory testing.

Materials and Methods: A total of 470 screen-reactive HCV blood donations were retested using the Architect anti-HCV assay, an alternative antibody test (SD Biosensor) and a core antigen (cAg) test. signal-to cut-off (S/CO) ratios and pre-analytical factors (centrifugation speed, haemolysis check, time between collection and testing) were recorded. Based on the S/CO ratio evaluation, we propose a testing algorithm to guide supplemental tests.

Results: The false discovery rate of the Architect anti-HCV assay was 0.84 as 395/470 (84%) screen-reactive samples had no evidence of HCV infection (SD Biosensor and cAg negative) (presumed false positive), 38/470 (8.1%) were antigenaemic, and 32/470 (6.8%) had evidence of past infection. The median S/CO ratios of the presumed false-positive and active infection samples were 1.8 and 17.3, respectively. The positive predictive value of HCV positivity in samples with ratios above 12 was 91.8%. On retesting, 104/470 (22.1%) samples became negative.

Conclusion: The Architect anti-HCV assay has a very high false discovery rate in Ugandan BTSs, leading to excessive blood disposal. Pre-analytical factors likely contribute to this. An introduction of confirmatory testing using an algorithm based on S/CO ratio evaluation could limit unnecessary blood wastage and donor deferral.

Keywords
blood transfusion services, donor blood, false positive, Hep C virus, screening, transfusion-transmitted infections

Highlights

- Eighty-four per cent of screen-reactive hepatitis C virus (HCV) blood donations in Ugandan blood transfusion services were identified as presumed false positive.
- Active HCV infection is only present in 8.1% of screen-reactive HCV samples.
- Introduction of confirmatory testing using an algorithm based on signal-to cut-off ratio evaluation could limit unnecessary blood wastage and donor deferral.

INTRODUCTION

Providing safe blood for transfusion in sub-Saharan Africa (sSA) remains a challenge in the context of limited resources and infrastructure, suboptimal diagnostics and a high prevalence of transfusion-transmissible infections (TTIs) [1]. The epidemiology and true burden of hepatitis C virus (HCV) in sSA is unclear and overestimated due to prevalence estimates often relying solely on serology without confirmatory testing [2]. Furthermore, there is a substantial reported biological false-positive rate in screening serology tests, particularly in sSA, where it is hypothesized that persistent exposure to infections, such as malaria, schistosomiasis, syphilis or HIV, as well as the presence of chronic disease and malnutrition may be exacerbating the issue [3, 4].

The mean HCV seroprevalence rate in blood donors attending Ugandan blood transfusion services (BTS) over the last 5 years is 1.8% (O. Lucey, personal communication); however, the rate ranges between 0% and 8% depending on geographical region and season. National HCV seroprevalence rates for the general population are estimated at 2.7% (0.4–7.0) [5, 6]. There is limited recent literature on rates in blood donors specifically [7]. The two-step screening process for HCV, endorsed by CDC and WHO, involves the detection of antibodies against HCV (anti-HCV) followed by either nucleic acid testing (NAT) or core antigen (cAg) testing to confirm the presence of active infection [8–10]. However, in Ugandan BTS centres, samples testing positive on the FDA-approved Architect anti-HCV screening assay (Abbott Diagnostics) do not undergo supplementary testing due to the lack of funding. Without the ability to confirm HCV infection status, the BTS are obliged to discard donations that are anti-HCV initially reactive (IR) (regardless of duplicate testing results on the same assay). Reporting results without supplemental testing causes unnecessary anxiety for donors and reduces the donor pool due to donor deferral in the context of scarcity of blood for transfusion [11].

The barriers to the implementation of NAT-based testing are well described and significant in low- and middle-income countries (LMICs) [12]. HCV cAg testing is an alternative confirmatory test, which, although not extensively evaluated in blood donor populations, has proved an attractive alternative [13–15]. Unpublished data from retesting quarantined donor blood that was subsequently discarded by Mbale BTS (Eastern Uganda) during a multi-site transfusion trial in Uganda showed that 45/50 (90%) of seropositive blood units were, in fact, RNA-PCR negative, suggesting a high false-positive rate in the Architect anti-HCV screening assay. Sommese et al. and Candotti et al. also found 10% RNA positivity amongst seroreactive donor samples from anti-HCV screening assays suggesting either high false-positive rates or cleared infection [16, 17].

This study aimed to determine the false discovery rate of the anti-HCV screening assay used in two Ugandan BTS centres by retesting screen-reactive samples with supplemental tests. It aimed to understand the true prevalence of HCV amongst quarantined HCV screen-reactive blood and categorize this into three groups: active HCV infection, past cleared infection and presumed false-positive results. Secondary aims were to understand the significance of signal-to-cut-off (S/CO) ratios of the Architect anti-HCV assay, propose an HCV testing algorithm and investigate pre-analytical factors affecting results, including haemolysis, time delay and centrifugation speed.

METHODS

Sample collection and storage

A total of 470 consecutive anti-HCV reactive serum samples from voluntary non-remunerated blood donors were identified between February 2019 and January 2020 in two BTS centres in Uganda: 235 from Nakasero National BTS, Kampala, and 235 from Mbale Regional BTS, Mbale. These samples had been collected, transported, and stored in the respective BTS laboratories, according to routine Ugandan BTS procedures. They had undergone TTI screening as per the National BTS algorithm by anti-HCV chemiluminescent microparticle immunoassay (CMIA) on the Architect i2000SR analyser (Abbott Diagnostics, Germany) according to BTS Standard Operating Procedures that follow the manufacturer's instructions. A S/CO of ≥1.00 was considered IR and retested in duplicate as per the manufacturer's guidance. The S/CO ratio results for the first and duplicate runs for each sample were recorded directly from the automated system. Both IR and repeatedly reactive (RR) samples were included in the study and are termed 'screen-reactive'. Samples with S/CO ratios of 1.00–1.99 were described as borderline reactive in the analysis. A visual haemolysis check was performed using a colour chart on all samples as a proxy for sample quality. The date of blood collection and

screening was documented. A delay between sample collection and testing was defined as more than 2 days. Screen-reactive samples from both sites were prepared into serum aliquots for storage at −80°C. The 235 samples from Mbale underwent an extra centrifugation step (ultracentrifugation) using a microcentrifuge at 10,000 *g* for 10 min before storage to ascertain whether this affected the S/CO ratios on retesting.

Supplementary testing

All 470 sera were thawed (after a single freeze–thaw cycle), mixed thoroughly by low-speed vortexing and retested using the same Architect anti-HCV assay. S/CO ratios of the repeat testing were recorded to observe any differences in S/CO ratios between the BTS screening and repeat testing, particularly following ultracentrifugation in the Mbale samples.

In order to discriminate presumed false-positive from true-positive results (both active infection and past infection), samples underwent two supplementary tests: an alternative antibody-based test and a test to confirm active infection (HCV cAg). The antibody-based test was the STANDARD Q HCV Ab Test (SD Biosensor, Korea), a rapid chromatographic immunoassay for the qualitative detection of specific antibodies to HCV, prequalified by WHO in 2020 with a quoted sensitivity and specificity of 99.4% (96.6–100) and 99.7% (98.3–100), respectively [18]. The Architect HCV Ag assay was performed on the automated Architect i2000SR CMIA system. The cut-off value is 3.00 fmol/L; thus, samples with values of <3.00 fmol/L are considered non-reactive, and those >10.00 fmol/L are considered reactive. Samples between 3.00 and 10.00 fmol/L are considered grey zone. These supplementary tests were performed (within 48 h of sample thaw) in the National BTS laboratory according to the manufacturer's instructions.

HCV infection category definitions after analysis

All samples included in the study were screen-reactive, that is, were either IR or RR on BTS Architect anti-HCV screening. Samples also positive for HCV cAg were considered to represent active HCV infection (antigenaemic). Those negative for HCV cAg, with positive SD Biosensor results, were interpreted as past, resolved HCV infection. Samples negative for both SD Biosensor and HCV cAg were regarded as presumed false-positive anti-HCV results with no evidence of HCV infection. Samples that fell within the grey zone for the cAg assay or were invalid were categorized as unconfirmed.

HCV testing algorithm

Based on S/CO ratio evaluation of the Architect anti-HCV assay, we propose a testing algorithm to guide supplemental tests on IR samples. We applied our dataset to the proposed algorithm.

Ethics

Ethical approval was sought and granted by the Imperial College and Ugandan Research Ethics Committees.

Statistics

Statistical analysis was performed in GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). Median with interquartile range (IQR) was used for S/CO ratios. Median differences in S/CO ratios between BTS screening and repeat testing were analysed using the Wilcoxon signed-rank test. The presence of haemolysis by time delay between collection and testing was analysed using χ^2 for categorical variables. Significance was defined as a p value of <0.05.

RESULTS

HCV infection category results after analysis

Following anti-HCV retesting and additional testing, 395/470 (84.0%) of the screen-reactive samples tested negative by SD Biosensor and HCV cAg, confirming these as presumed false positives (Figure 1). In these two BTS centres, the false discovery rate of the screening Architect anti-HCV test was, therefore, 0.84 (395/470), which translates to a positive predictive value of 0.16 (16%). Only 38/470 (8.1%) samples were antigenaemic with evidence of active HCV infection. A further 32/470 (6.8%) samples had evidence of past cleared HCV. Of the five unconfirmed samples, one had an invalid cAg result, and four had a cAg result between 3 and 10 f/mol (grey zone); two of which had positive SD Biosensor results, and two were negative. These were not retested in duplicate due to a lack of reagents and cannot be classified without further testing. Of the 444 samples across both sites, 433 (97.5%) were RR on the first BTS screening (26 samples did not have duplicate runs due to lack of reagent). Therefore, the calculated false discovery rate is estimated based on 470 screen-reactive samples that included a small proportion (2.5%) of only IR samples.

HCV results in relation to S/CO ratio

A S/CO ratio of >15 correlated with a positive cAg in 35/41 (85.4%) samples and a positive SD Biosensor result in 39/41 (95.1%) samples (Table 1). In the highest S/CO ratio category (>15), all 35 cAg-positive samples also tested positive by SD Biosensor. Thirty-six (94.7%) of the 38 positive cAg samples were samples with S/CO ratios of above 12. Thirty-six (73.5%) of the 49 samples with S/CO ratio > 12 were cAg positive, and 45/49 (91.8%) were also positive by SD Biosensor.

In three (3/38; 7.9%) cAg-positive samples, the SD Biosensor was negative, suggesting false-negative SD Biosensor results. The anti-HCV S/CO ratios of these three samples were 1.4, 3.1 and 17.5 (with cAg values of 500, 5043 and 690 fmol/L, respectively). Two (2/345;

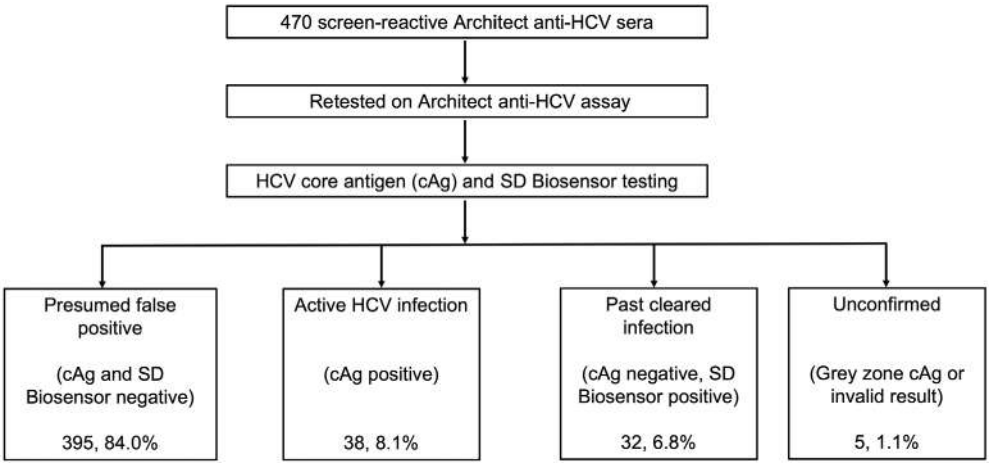

FIGURE 1 Hepatitis C virus (HCV) results following repeat (Architect anti-HCV) and further (HCV core antigen and SD Biosensor) testing

TABLE 1 Additional hepatitis C virus (HCV) test results according to Architect anti-HCV signal-to-cut-off (S/CO) ratio following repeat and further testing

S/CO ratio	Total (*n*, %)	SD Biosensor positive		HCVc Ag negative (>10 fmol/L)	
<1.00	**104 (22.1%)**	**1**	**1.0%**	**0**	**0.0%**
1.00–1.99	121 (25.7%)	2	1.7%	1	0.8%
2.00–4.99	141 (30.0%)	10	7.1%	1	0.7%
5.00–9.99	46 (9.8%)	11	23.9%	0	0.0%
10.00–11.99	9 (1.9%)	1	11.1%	0	0.0%
12.00–14.99	8 (1.7%)	6	75.0%	1	12.5%
15–18.99	41 (8.7%)	39	95.1%	35	85.4%
Total	470	70		38	

TABLE 2 Comparison of the distribution of signal-to-cut-off (S/CO) ratios between blood transfusion services (BTS) Architect anti-hepatitis C virus (HCV) screening and repeat Architect anti-HCV testing

Architect anti-HCV S/CO ratio	BTS screen-reactive samples (*n*, %)	Retest (*n*, %)
<1.00	0 (0%)	104 (22.1%)
1.00–1.99	256 (54.5%)	121 (25.7%)
2.00–4.99	135 (28.7%)	141 (30.0%)
5.00–9.99	30 (6.4%)	46 (9.8%)
10.00–14.99	23 (4.9%)	17 (3.6%)
≥15.00	26 (5.5%)	41 (8.7%)
Total	470	470

0.6%) of the samples with S/CO ratios less than four had confirmed cAg presence. A significant proportion (113/134; 84.3%) of samples with S/CO ratios of 3.00–11.99 were classified as presumed false positives. The median S/CO ratio of the presumed false-positive samples was 1.8 (IQR 1.0–3.2), whereas that of the cAg-positive samples was 17.3 (IQR 16.3–18.1). The median ratio of the samples displaying past cleared infection was 5.7, with a wider IQR of 3.7–11.3.

Pre-analytical factors

On the first anti-HCV screen, a large proportion of samples (256/470; 54.5%) were borderline reactive (S/CO ratios 1.00–1.99) (Table 2). One-hundred and four (22.1%) of the 470 screen-reactive samples became negative (S/CO ratio < 1.00) following retesting, with 73/104 (70.2%) of these occurring in samples from Mbale Regional BTS where an extra centrifugation step had occurred. To note, 7/104 (6.7%) of these samples were not RR on the first BTS screen. The median S/CO ratios of these samples on the first BTS screen were 1.22 and 1.98 in Nakasero and Mbale, respectively, with median decreases in S/CO ratios (between the first screen and retesting) of 1.42 in Mbale ($Z = 7.42$, p value > 0.001) and 0.41 in Nakasero ($Z = 6.12$, p value > 0.001). Following retesting of all samples, a statistically significant median increase in S/CO ratio of 0.45 was seen in all samples from Nakasero ($Z = -8.00$, p value > 0.001), whilst a non-statistically significant median decrease of 0.15 was observed ($Z = 1.48$, p value = 0.14) in Mbale.

Overall, 262/458 (57.2%) samples showed some level of visual haemolysis, with a higher proportion of Mbale samples showing evidence of haemolysis compared to Nakasero samples (95/235 [40.4%] vs. 167/223 [74.9%]). The median time between donor sample

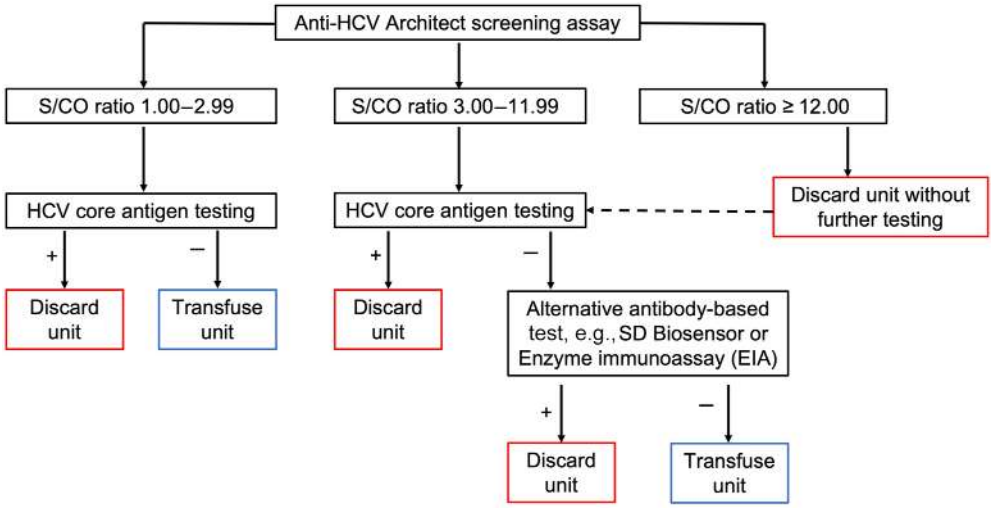

FIGURE 2 Proposed hepatitis C virus (HCV) confirmatory testing algorithm for initially reactive (IR) samples by Architect anti-HCV assay. All IR samples with signal-to-cut-off (S/CO) ratios between 1.00 and 11.99 would undergo core antigen (cAg) testing. Samples with low positive S/CO ratios (1.00–2.99) could be used for transfusion based on a negative cAg only, but samples with S/CO ratios between 3.00 and 11.99 would require an alternative antibody test if the cAg were negative

TABLE 3 Application of proposed hepatitis C virus (HCV) testing algorithm to our dataset

Architect anti-HCV S/CO ratio after analysis	Total no. of samples (366)	No. of core antigen (cAg) tests (positive/total performed)	No. of SD Biosensor tests (positive/total performed)	No. of incorrectly identified samples (*n*, %)	No. of units recommended for transfusion (*n*, %)
1.00–2.99	183	1/183	NA	5/183 (2.7%)[a]	182/183 (99.5%)
3.00–11.99	134	1/134	19/133	0	114/134 (85.1%)
≥12	49	NA	NA	4/49 (8.2%)[b]	0/49 (0%)

Note: Total number of samples is 366, as 104 samples became negative after retesting. NA: not applicable according to the algorithm.
Abbreviation: S/CO, signal-to-cut-off.
[a]Cleared, past infection (cAg negative, SD Biosensor positive).
[b]Presumed false positive (cAg negative, SD Biosensor negative).

collection and initial screening was 4 days (range 0–33 days) across both sites. There was a significant difference in the proportion of samples with haemolysis in those that were processed after a delay (>2 days) (185/298; 62.1%) versus those that were processed without delay (0–2 days) (77/157; 49.0%) (p value = 0.0075).

Testing algorithm

Figure 2 shows a proposed HCV testing algorithm based on S/CO ratios of the Architect anti-HCV screening assay. Through the application of the algorithm to our dataset (Table 3), 317 cAg tests and 133 SD Biosensor tests would be required to ascertain whether the 366 reactive samples (S/CO ratio > 1.00 following repeat anti-HCV testing) were confirmed positive or presumed false reactive. Of the samples, 70/366 (19.1%) units would have been discarded based on either a S/CO ratio > 12 or a positive cAg or SD Biosensor result amongst IR samples with a S/CO ratio between 3.00 and 11.99, 49/70 units (those with S/CO ratio > 12) would have been discarded without the need for any supplemental testing, 45/49 of these had either active or past infection (Table 1). Following supplemental tests, 296/366 (80.9%) units would have been recommended for transfusion following supplemental tests, including five anti-HCV positive, cAg-negative (past infection) samples in the 1.00–2.99 category. No cAg-positive samples would have been recommended for transfusion.

DISCUSSION

This study has shown the Architect anti-HCV assay to have an alarmingly high false discovery rate in the context of a BTS laboratory in sSA. This, coupled with the lack of supplemental or confirmatory testing, has led to significant blood wastage. Our data confirm that 395/470 blood units (84%) IR for HCV were discarded unnecessarily. The positive predictive value (PPV) of 0.16 is unacceptably low for a screening test. The BTS report that each year, on average, 224,350 units are donated and screened, with 4038 units discarded on the basis of Architect anti-HCV positivity (O. Lucey, personal communication). Assuming 84% of these are, in fact, false positive, an estimated 3392 units are discarded unnecessarily each year. Many of

these donors were therefore preliminarily classified as HCV seropositive necessitating post-donation counselling, retesting and donor deferral at a significant economic and personal cost to the BTS and donor, respectively.

False positivity in anti-HCV screening immunoassays is well described and multi-factorial, with reported false positivity rates of between 10% and 60% [19, 20]. Populations with a lower prevalence of HCV such as blood donors are more affected by false positivity [20, 21]. The need for high sensitivity in screening assays can come at a cost in terms of specificity, although CMIA is reported to have better specificity than enzyme immunoassays (EIAs) [22, 23]. There remains uncertainty in how to interpret anti-HCV test results with a borderline S/CO ratio, particularly without confirmatory testing [24]. The lower PPV of the Architect anti-HCV assay discovered in this study compared to existing literature may reflect pre-analytical factors in the context of a BTS rather than a research laboratory [20, 23, 25]. We suggest this may be more generalizable to other LMIC settings where transport delays, inadequate funding and laboratory infrastructure pose greater challenges than in high-income countries and may affect test results. We demonstrated that 22% (104/470) of samples were anti-HCV negative upon repeat testing following sample thaw. The extra centrifugation process in Mbale may have contributed to the difference in S/CO ratios seen between the first screen and retesting in this site, although the reduction was only statistically significant in the samples that became negative. Samples screened after a delay of more than 2 days were more likely to be haemolysed. We acknowledge that additional factors contributing to the S/CO ratio differences by site may have been present, for example, differences in sample collection and handling, including the presence of haemolysis, centrifugation equipment and differences in donor populations, for example, prevalence of chronic or infectious diseases [3, 4]. These pre-analytical factors are likely contributing to the high false-positive rate and should be further investigated and monitored through regular laboratory quality assessment, but achieving 'perfect' conditions can be extremely challenging in sSA [26].

Studies have shown a relationship between the S/CO ratio of the anti-HCV test and the infection status of the individual [14, 16, 19, 27]. There is a correlation between high S/CO values and serological confirmation with anti-HCV positivity, and in some studies, also with viraemia [23]. However, false positives and cleared infections are difficult to distinguish as both may have low S/CO values. Our data show that donors with active infection (cAg positive) had samples with median S/CO values that were almost 10-fold higher than the presumed false positives (cAg and SD Biosensor negative). Our results are in keeping with other studies, which demonstrated significant differences in median S/CO ratios between RNA-negative and RNA-positive results [20] and cAg positivity rates of 83.6% in samples with S/CO ratios above 10 [14]. Knowledge of threshold ratios can help guide whether supplemental testing is required [28]. Ha et al. found that using logistic regression, a S/CO value threshold of 3.13 on Architect predicted HCV positivity (defined as anti-HCV or RNA positive) with 95% probability, suggesting that samples with ratios above this could be reported as positive without supplemental testing. Other studies have reported 5.0 or 5.6 as the optimal cut-off threshold based on the receiver operator characteristic curve analysis [20, 21, 28, 29]. Applying a cut-off threshold of 3.13 to our samples would result in 110 samples (presumed false positives) being discarded unnecessarily. Optimal threshold ratios differ depending on HCV prevalence and laboratory settings. By setting the threshold at 12 in our dataset, the PPV of anti-HCV positivity is much higher (45/49; 91.8%) and prevents unnecessary disposal of units.

We believe that our proposed testing algorithm based on S/CO ratio evaluation presents a pragmatic approach to providing safe blood whilst maintaining judicial use of supplemental tests in LMICs. Importantly, when applied to our dataset, no cAg-positive samples would have been transfused, and only four false reactive samples (in the S/CO ratio > 12 groups) would have been discarded. The main drawback of omitting confirmatory tests in samples with higher S/CO ratios is that donors would not receive comprehensive results on infection status. By limiting the use of alternative antibody tests to samples with ratios of 3.00–11.99, there is a small but appreciable risk that samples in the lowest ratio category (1.00–2.99) may be anti-HCV positive (cAg negative) suggesting past cleared infection. These donors would not be accepted for donation in Uganda under current guidelines. We identified 5/183 (2.7%) antibody-positive cAg-negative samples in the 1.00–2.99 group, which would have been recommended for transfusion. The risks of transfusing antibody-positive blood would need to be balanced against resource scarcity, and we acknowledge this as a controversial area. One case of active infection occurred in a sample with a borderline reactive anti-HCV screening test (S/CO ratio 1.4). This can occur during acute HCV seroconversion or in immunocompromised hosts [14], and strengthens the argument for confirmatory testing. The current regulatory requirement in Uganda to discard screen-reactive samples due to a lack of confirmatory testing would need to be reviewed to enable the implementation of the algorithm.

Currently, all donors with screen-reactive (IR or RR) results are deferred, either temporarily or permanently, depending on repeat testing results 3–6 months later and the availability of an alternative anti-HCV test. The cost of permanent deferral is ~$81 (O. Lucey, personal communication from UBTS) and includes phlebotomy, initial TTI screening, retesting, post-donation counselling and blood unit disposal. Using the proposed algorithm with supplemental tests (at $10/cAg test and $1/SD Biosensor) to confirm or refute positivity for the 366 donor samples was $1647 compared to the potential cost of permanent deferral of $29,646. In addition, accurate results through confirmatory testing would facilitate linkage to care where appropriate, avoid unnecessary anxiety and return visits for donors and enable those testing falsely positive to return to the donor pool. Furthermore, incorrect seroprevalence data generated as a result of poor diagnostics have repercussions on the accuracy of HCV epidemiology, surveillance, and linkage to care, and therefore, elimination strategies [5, 21, 30].

As described above, both a strength and limitation of this study is that screen-reactive samples were identified for inclusion after BTS screening; therefore, the process by which they were taken and screened was not according to a research protocol, as such, samples

were potentially subject to pre-analytical errors such as sample haemolysis and testing delays. This may have affected the validity of the assays used in both BTS screening and subsequent testing. It may be a contributing factor to the high false discovery rate. We argue that it is important to present real-world data in an effort to understand how tests are performing in LMIC laboratory conditions, and therefore, how to improve processes and tailor algorithms appropriately.

Our results and the proposed algorithm rely on the alternative antibody test possessing a similar sensitivity to the Architect anti-HCV assay to reliably categorize screen-reactive samples as either false positive or past infection. A recent large meta-analysis showed that RDTs showed comparable sensitivity and specificity profiles compared to EIAs in diverse populations [31], and specifically, SD Biosensor achieved a sensitivity of 99.2% in a large sample size [32]. We acknowledge that the use of a rapid test, albeit a WHO prequalified test with comparable quoted sensitivity to Architect anti-HCV, is not considered a gold standard test to detect anti-HCV. Although using a combination of alternative antibody assays, including two EIAs and immunoblot testing, would achieve greater certainty in categorizing the anti-HCV positive samples, this would not be financially or logistically feasible within the structure of Ugandan BTS. Three samples were falsely negative by SD Biosensor, suggesting that the test may have a poorer sensitivity compared to the Architect, assay or may be affected by genotypic differences or pre-analytical factors [32].

The gold standard test to detect HCV RNA is NAT. We chose HCV cAg as the confirmatory test as NAT requires a dedicated sample to be taken to maintain RNA integrity, which was not feasible in this study. It is, therefore, conceivable that due to the slightly higher limit of detection of cAg, active infections could theoretically be missed. However, cAg testing is endorsed by WHO as a confirmatory test [8] and has several advantages over NAT; namely, it is less expensive, can be used on the existing i2000SR analyser, demands less personnel expertise and is less susceptible to contamination and degradation of samples [14]. Moreover, it has been shown to have good diagnostic accuracy and correlation with RNA, and although the analytical sensitivity is lower than NAT, more than 90% of patients with HCV have viral loads above 3000 IU/ml [5], making it an affordable, appropriate and an excellent alternative to NAT in LMIC settings where the realistic alternative at present is no confirmatory test.

In conclusion, due to both a high false discovery rate of the Architect anti-HCV screening assay in this setting and the lack of access to confirmatory testing, a significant amount of blood donated is discarded unnecessarily. Since blood for transfusion is relatively scarce, focusing available resources on obtaining accurate HCV results would result in economic gains from limiting donor deferral and reducing unnecessary blood disposal. Optimization of pre-analytical factors, including introducing ultra-centrifugation before testing may reduce false-positive rates, especially in samples with low positive S/CO ratios. Knowledge of Architect threshold S/CO ratios in predicting anti-HCV positivity may be helpful in updating testing algorithms and guiding the supplemental tests with which to perform in screen-reactive samples. We suggest that the algorithm we developed should be validated in other sSA BTS where HCV detection is higher than anticipated in low-risk blood donor populations. The introduction of confirmatory testing in BTS settings is paramount for accurately identifying HCV-positive donors who require follow-up care and confidently distinguishing false-positive and true-positive anti-HCV results to limit blood wastage and unnecessary donor deferrals.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Research partnerships between blood services and public health authorities: An international, cross-sectional survey

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Abstract

Background and Objectives: The COVID-19 pandemic has brought to the fore how blood services can partner with public health (PH) authorities to inform decisions. Yet the scope of partnerships between blood services and PH authorities is inadequately documented. We explored how blood services partner with PH authorities outside the scope of COVID-19.

Materials and Methods: On 19 January 2022, survey was sent to employees of blood services located throughout the world. Survey questions mainly pertained to partnerships with PH authorities, including how blood specimens are used and collected.

Results: Twenty-seven recipients—4 (14.8%) in Africa, 3 (11.1%) in Asia, 9 (33.3%) in Europe, 6 (22.2%) in North America, 2 (7.4%) in Oceania and 3 (11.1%) in South America—completed the survey. Fifteen recipients (55.6%) indicated their blood service was directly or indirectly supervised by PH authorities. Twenty-four recipients (88.9%) indicated currently using or planning to use blood donor data or samples for PH research or pathogen surveillance. A substantial proportion of respondents reported using samples or results from non-routine tests for the surveillance of non-transfusion-transmitted infectious disease pathogens ($n = 13$ [48.1%]); samples or results of non-routine tests for PH research unrelated to pathogens ($n = 10$ [37.0%]); donor data for PH research unrelated to pathogens ($n = 12$ [44.4%]) and donor data for PH research unrelated to transfusion safety ($n = 11$ [40.7%]). Fourteen (51.9%) had established (or planned to establish) longitudinal cohorts and 19 (70.4%) biobanks.

Conclusion: The majority of responding blood services were already involved in or planned to be involved in PH research or pathogen surveillance.

Keywords

blood collection, blood donation testing, donors, public health, transfusion-transmitted infectious diseases

Highlights

- Nearly 90% of blood services that participated in this international survey indicated currently using or planning to use blood donor data or samples for public health research or transfusion-transmissible pathogen surveillance.

- Most participating blood services had established or planned to establish a longitudinal cohort or biobank from blood donors.
- The pandemic has revealed the potential for expanded scope of partnerships between blood services and PH authorities.

INTRODUCTION

Blood services primarily collect blood for recipients in need of transfusions, but the SARS-CoV-2 pandemic has brought to the fore a secondary (yet important) role: partnering with public health (PH) authorities to inform decision-making.

For years, blood donations have been leveraged to study transfusion-transmitted infectious diseases (TTIDs) and emerging pathogens in collaboration with PH authorities [1, 2]. For example, the population-based Scandinavian Donations and Transfusions database holds several decades of complete donor and recipient data from Sweden and Denmark, allowing for the study of TTIDs and the impact of donor and recipient characteristics on transfusion safety, among other research goals [3]. Another example is the West Nile virus outbreak that occurred in the early 2000s, for which blood services and PH authorities rapidly developed seasonal testing to protect blood recipients and inform PH decisions on this emerging pathogen [4]. Blood donations have also been used to estimate the seroprevalence associated with emerging pathogens, such as *Babesia microti* [5], Hepatitis E [6], Zika [7] or *Coxiella burnetii* [8]. Blood services regularly report positive results to PH authorities, often required as part of reportable disease laws, and also share samples containing strains of various pathogens to understand the genotype distribution and molecular epidemiology.

Although less common, blood donations have also been leveraged to address research questions related to PH outside the scope of TTIDs, emerging pathogens and blood donation. For example, the Danish Blood Donor Study (DBDS) is a large, prospective blood donor cohort initially set up to understand donor health and determinants of donation frequency [9]. However, the DBDS also aims to provide a platform to explore many other research questions [10], such as the association between obesity and infection [11], and the genetic determinants of human health [12]. The Danish Blood Donor *Staphylococcus aureus* Carriage Study has established a prospective cohort and biobank investigating the colonization of *S. aureus* among healthy individuals for research into the health consequences of colonization [13]. Other large studies, such as INTERVAL from the United Kingdom, was a randomized controlled trial designed to answer a relatively narrow research question (i.e., what is the optimal frequency of whole-blood donation?) [14, 15], but participant data and samples were later used to study coronary heart disease [16], congenital heart defects [17], schizophrenia [18] and primary sclerosing cholangitis [19]. Blood donors were also used as a data source to study the association of blood groups with coronary heart disease, cerebrovascular disease and peripheral vascular disease [20].

The SARS-CoV-2 pandemic likely expanded the scope of these partnerships with PH authorities. Throughout the pandemic, blood services have collaborated with PH authorities to document a population's history of COVID-19 infection, fatality rates, high-risk subgroups, correlates of protection and the immune responses to infection and vaccination [21]. In a previous international survey, 73% of countries had ongoing or planned seroprevalence studies, most of which aimed to inform PH policies [22].

Yet the scope of partnerships between blood services and PH authorities is not well documented, particularly for projects unrelated to SARS-CoV-2. Therefore, we conducted an international survey among blood services to explore how they engage in partnerships with PH authorities outside the scope of SARS-CoV-2.

MATERIALS AND METHODS

Participating in blood services

Survey recipients were members of the International Society of Blood Transfusion—TTID Working Party and the European Blood Alliance—Emerging Infectious Disease Monitoring Working Group. All recipients were senior employees of blood services located anywhere throughout the world. No eligibility criteria were otherwise applied.

Survey

The link to the survey was e-mailed on 19 January 2022, and one reminder was sent on 23 March 2022 (after 71 days). Survey questions focused on the following themes: (1) donations and donor characteristics in 2019 (i.e., before the pandemic); (2) partnerships involving PH authorities, including how blood specimens are used and collected for these partnerships; (3) specific research initiatives involving PH authorities, including longitudinal cohorts and biobanks; (4) sharing of data and samples with PH authorities and (5) consent and ethical considerations. Respondents were instructed to focus on non-SARS-CoV-2-related partnerships. They were free to skip certain questions if they could not or did not want to answer them. The full survey is available in the supplemental material (Data S1). Ethical review was not needed for this study because it did not involve human participation or the collection of personal data, and there was no secondary use of data.

RESULTS

Participating in blood services

Of the 79 targeted blood services, 27 (34.2%) completed the survey. Respondents were well distributed across the world with 4 (14.8%) in Africa, 3 (11.1%) in Asia, 9 (33.3%) in Europe, 6 (22.2%) in North America, 2 (7.4%) in Oceania and 3 (11.1%) in South America (Table 1). On median (interquartile), respondents reported 101,538 (13,706–433,450) blood donors in 2019 (i.e., before the pandemic), of which 49.0% ± 20.7% were females.

TABLE 1 Characteristics of participating blood services

	$N = 27$
Region, *n* (%)	
Africa	4 (14.8%)
Asia	3 (11.1%)
Europe	9 (33.3%)
North America	6 (22.2%)
Oceania	2 (7.4%)
South America	3 (11.1%)
Number of donors,[a] mean ± SD (range)	367,109 ± 661,404 (2130–2,950,579)
Proportion of female donors,[a] mean ± SD (range)	49.0% ± 20.7% (20.0%–95.2%)
Proportion of new donors,[a] mean ± SD (range)	28.6% ± 25.2% (1.4%–99.0%)
Number of donations,[a] mean ± SD (range)	656,524 ± 1,125,602 (1912–4,793,467)

Abbreviation: SD, standard deviation.
[a]In 2019.

Partnerships between blood services and PH authorities

A majority of blood services are supervised (directly or indirectly) by PH authorities. Irrespective of PH supervision, a clear majority of blood services collaborate to some extent with PH authorities. Fifteen (55.6%) blood banks were directly ($n = 6$ [22.2%]) or indirectly ($n = 9$ [33.3%]) supervised by PH authorities, and 12 (44.4%) were not supervised by PH authorities (Figure 1).

Twenty-two (81.5%) blood services indicated currently using blood donor data or samples for PH research or transfusion-transmissible pathogen surveillance, and 2 (7.4%) planned to do so in the future; however, only 5 (18.5%) reported receiving external or joint funding to initiate those projects.

Surveillance for TTID in the general population was the most common research activity conducted by participating blood services. Approximately 75% of blood services used routine donor screening test results and/or samples or non-routine donor screening test results for surveillance of TTID in the general population. From 37% to 48.1% of respondents collaborated to some extent with PH authorities on studies of non-transfusion-transmitted infectious disease-

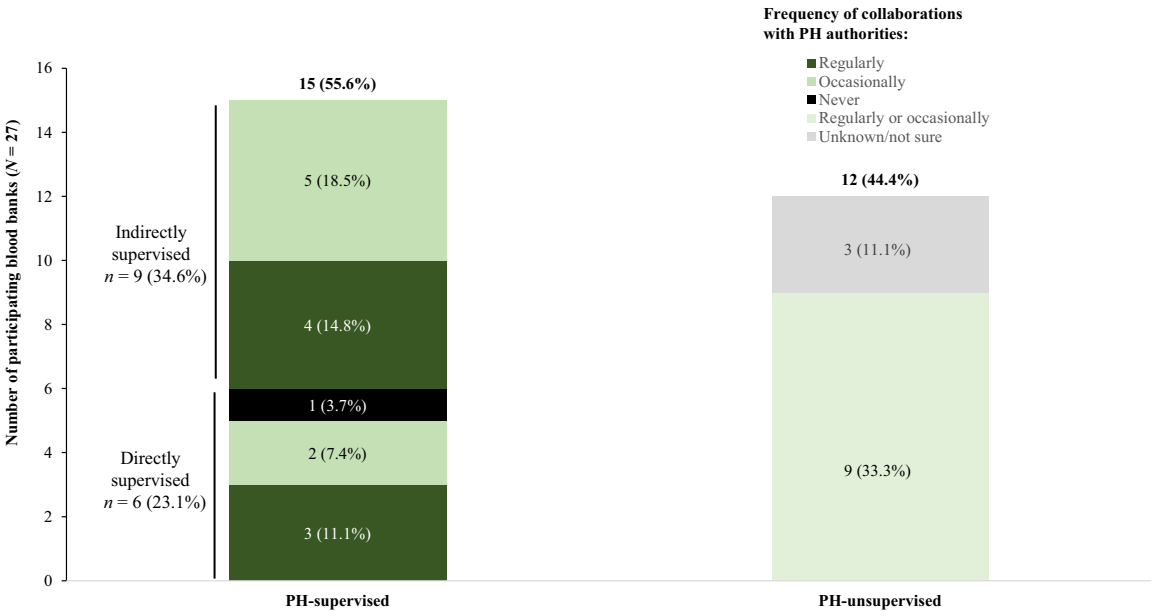

FIGURE 1 Supervision of blood banks by public health (PH) authorities and frequency of collaborations with PH authorities. 'Regulatory or occasionally' refer to whether they work collaboratively with PH (i.e., yes or no), irrespective of whether this collaboration is regular or occasional

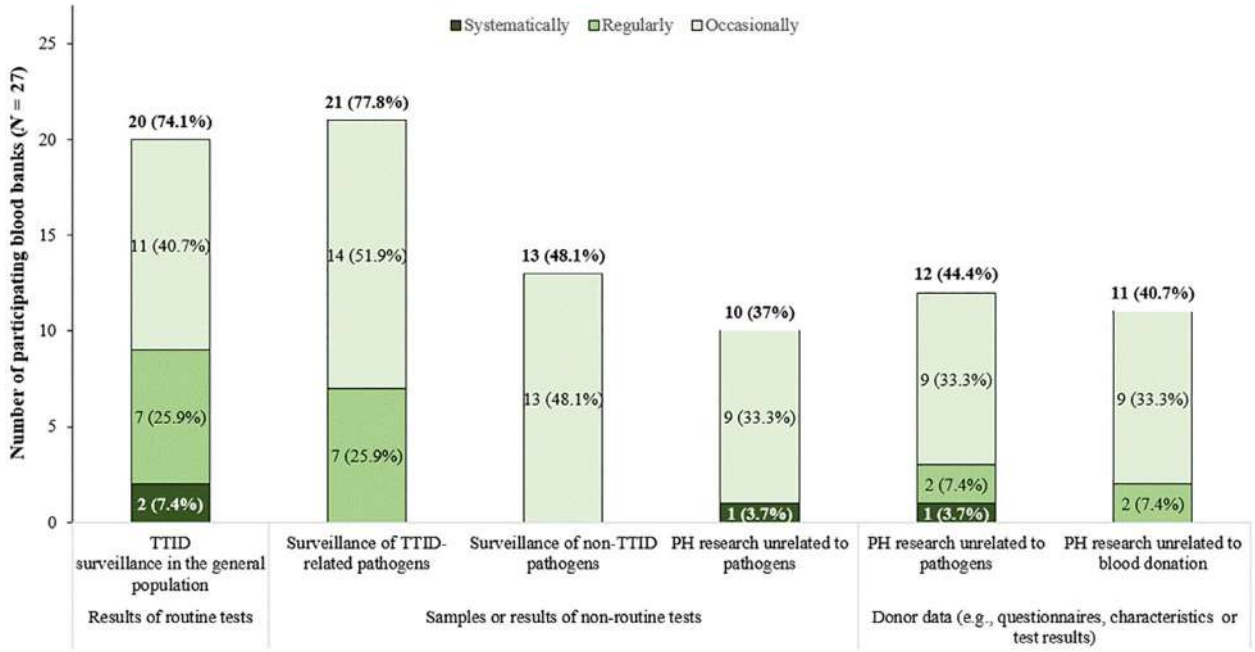

FIGURE 2 Use of donor data, samples and test results.
PH, public health; TTID, transfusion-transmitted infectious disease

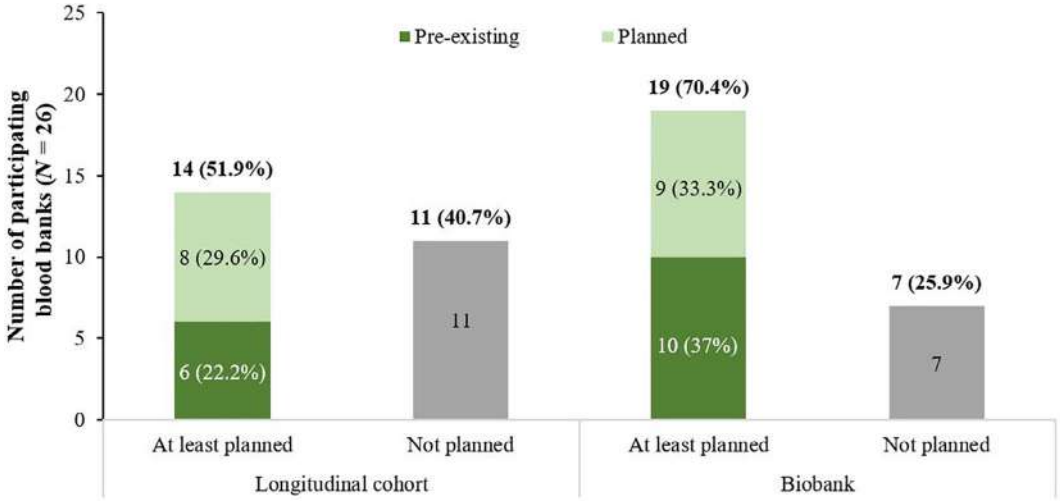

FIGURE 3 Respondents who had established or planned to establish a longitudinal cohort or biobank

related pathogens or on other questions of PH interest (Figure 2). Examples include assessments of iron deficiency or anaemia, blood pressure, pulse, phthalate and cholesterol levels in donors as proxies for general population health [23, 24].

Longitudinal cohorts and biobanks based on blood donors

Six (22.2%) blood services have established longitudinal cohorts of blood donors, and 8 (29.6%) were planning to do so (Figure 3). Donors included in these cohorts were mainly TTID- or SARS-CoV-2-positive donors, established with the intent of investigating rates of infection, reinfection and immunological markers of disease progression.

Furthermore, 10 (37.0%) blood services have established a biobank based on blood donors, and 9 (33.3%) were planning to do so later. Donors included in pre-existing biobanks were also mainly TTID- or SARS-CoV-2-positive donors.

Data and sample sharing

Twenty-three (85.2%) respondents reported sharing donor data with PH authorities at least occasionally, whether as required by law

($n = 10$ [37.0%]), by a collaborative agreement ($n = 5$ [18.5%]) or both ($n = 8$ [29.6%]). Among these respondents, 16 (69.6%) shared only aggregate data, and 20 (87.0%) shared aggregate data or de-identified, individual-level data. Twelve (44.4%) blood services reported (at least occasionally) sharing samples with PH authorities, 9 (33.3%) of which de-identified samples before sharing them.

Consent and ethical considerations

Eighteen (66.6%) blood services indicated that their routine donor consent form at the time of donation included a statement on the use of donor data and samples for PH research. Nineteen (70.4%) blood services also reported having an ethics advisory board that approves research activities outside the scope of routine TTID screening and PH emergency responses to pathogens, such as SARS-CoV-2. Finally, six (22.2%) blood services have a data-sharing agreement with PH agencies.

DISCUSSION

The results of this survey indicate that blood services collaborate extensively with PH authorities on a wide variety of research goals. The vast majority of respondents (i.e., 88.9%) currently use or were planning to use blood donor samples for PH research or pathogen surveillance. Furthermore, most respondents have already established or were planning to establish a longitudinal cohort or biobank from blood donors; however, we do not know if the process has been started or if it is only in concept planned.

The participation of blood services around the world in seroprevalence studies to inform PH policy for SARS-CoV-2 was unprecedented. Our survey highlights the collaborative role that blood services play for other pathogens and health issues and provides insights into the potential for expanding the scope of collaborations between blood services and PH authorities after the pandemic. More than a third of respondents have indicated using samples, test results or data to conduct PH research unrelated to pathogens or transfusion safety. The DBDS is one of the few examples of a systematic effort to establish such initiatives before the pandemic, and may be viewed as a model of how blood services can collaborate with PH authorities to further our understanding of infectious and non-infectious health conditions [9, 10]. Notably, donor data collected by the DBDS include questionnaire data, data from PH registries and genetic data, thus enabling the sharing of comprehensive data from consenting donors [9, 10]. Moreover, a French longitudinal biobank using paired plasma specimens from blood donors has the ability to estimate the impact of influenza A (H1N1) and implement appropriate prevention and response strategies [25].

Although not evaluated in our survey, the many logistic advantages of blood services probably helped spur collaborations with PH authorities. Blood services have pre-existing infrastructures, trained personnel, and quality-control mechanisms, thereby substantially alleviating the start-up costs associated with setting up prospective cohort research initiatives that are typically resource-intensive [10]. Blood donors also facilitate the study of large cohorts, as questionnaires and laboratory data are readily available at a minimal cost [10]. Notably, longitudinal analyses are feasible since a large proportion of donors are repeat donors [10, 26]. Furthermore, minimal recruitment efforts are necessary since the pool of eligible participants (i.e., repeat donors) who present at blood drives can trigger the collection of longitudinal data. Finally, blood donors are generally willing to give blood for biomedical research [10, 27], preferably in the form of a small, extra blood sample collected at the same time as their regular donation [27]. Therefore, participation rates are expected to be high (e.g., >95% in the DBDS) [11].

Blood donors are broadly representative of the healthy adult population, but researchers must be aware of possible selection bias when using them as a data source for PH research. First-time donors provide a better approximation of the health status of the general population. Nonetheless, researchers have found that low-income, ill and less educated persons, as well as minorities and females, may be under-represented among blood donors [28, 29]. Other groups are excluded by eligibility criteria (e.g., persons with sexual exposure risk or who have travelled to areas with endemic infections known to be TTID). Certain geographic regions within a country or blood collection agency's service area may also be underrepresented, depending on the presence of fixed collection centres and whether blood drives are organized in rural regions. Most of these factors can, however, be accounted for using statistical adjustment techniques, such as reweighting- and regression-based techniques. Furthermore, alternative data sources—such as establishing a prospective cohort from scratch—may not meaningfully reduce some of these biases in addition to being resource-intensive. For example, participants in the UK Biobank cohort tend to be older, include more females and live in more affluent neighbourhoods [30]. Relative to the general population, they also included a lower proportion of persons with obesity, smokers, and daily alcohol users [30]—consistent with a 'healthy volunteer' bias similar to that observed among blood donors. These selection biases may be reduced as blood collectors strive to make blood donation more inclusive, for example, through outreach efforts to recruit donors in underrepresented groups.

This study is subject to some limitations. First, the respondent's ability to understand English was not assessed, and translations of the survey in local or national languages were not available for respondents from non-English-speaking countries. This may have hindered the participation of some respondents or their understanding of survey questions, particularly those in non-English-speaking countries. Furthermore, the rate of participation (i.e., 32.9%) was relatively low compared with previous surveys of blood collection agencies [22, 31]. This low participation rate may be related to the fact that the questionnaire was sent during a surge of COVID-19 infections in many countries or because of the comprehensiveness/size of the questionnaire. Blood services with established PH partnerships may have been more enthusiastic about participating in our study, leading to response bias. Regardless, respondents were well-distributed throughout the world.

Donors' perspectives on PH research would also be interesting to further investigate [32]. While collaboration between blood services

and PH seems obvious for some donors, others may be more reluctant to share their information. As donors may already be regularly solicited for blood donation, additional emails or onsite questionnaires might be perceived as too intrusive, and some of them turned down. Finally, an extensively detailed consent form might create confusion with the donation process itself.

This survey also highlights a broader limitation revealed by the findings reported herein: in many jurisdictions, there are already established working relationships between blood services and PH authorities, yet these relationships are not widely known. This lack of knowledge and awareness represents missed opportunities for collaborative research between blood services, PH and other health service researchers. As part of an effort to document available data and resources, the TTID Surveillance, Risk Assessment and Policy subgroup is developing a communications toolkit to provide an information resource for researchers and/or blood centres who want to gain PH commitment for new research or surveillance programs and to increase awareness about the role of blood donors in PH.

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A.L. and S.F.O. conceived and designed the study. A.L. collected the data and analysed the data. C.O., C.E., B.C., C.R., P.T., A.R., R.L.K. and S.F.O. helped interpret the results. A.L. drafted the manuscript, and C.O., C.E., B.C., C.R., P.T., A.R., R.L.K. and S.F.O. critically revised it for important intellectual content. All authors approved the final version to be published.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Management of blood transfusion services in low-resource countries

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Abstract

Background and Objectives: Enabling universal access to safe blood components should be a key component of every country's national healthcare strategy. This study aimed to assess the current status of infrastructure and resources of blood transfusion services (BTS) in low- and middle-income countries.

Materials and Methods: A cross-sectional survey was designed to gather information on blood donations, components, redistribution, testing resources and quality management systems (QMSs). The survey was distributed to the International Society of Blood Transfusion members between October 2021 and November 2021.

Results: A total of 54 respondents from 20 countries responded to the survey. This included hospital-based BTS/blood centres (46%), national blood centres (11%)and national and regional blood services (11%). Voluntary non-remunerated, replacement and paid donors accounted for 94.2%, 84.6% and 21.1% of donations, respectively. Apheresis donation was available in 59.6% of institutions. National/regional criteria for redistribution of blood components were reported by 75.9% of respondents. Blood components incurred payment charges in 81.5% of respondents' institutions, and payments were borne by patients in 50% of them. Testing methods, such as

manual (83%), semi-automated (68%) or fully automated (36.2%), were used either alone or in combination. QMSs were reported in 17 institutions, while accreditation and haemovigilance were reported in 12 and 8 countries, respectively.

Conclusion: QMS was implemented in most of the countries despite the common use of paid donations and the lack of advanced testing. Efforts to overcome persistent challenges and wider implementation of patient blood management programmes are required.

Keywords

accreditation, blood transfusion services, low- and middle-income countries, quality management systems

Highlights

- In a survey among blood centres located in low- and medium-income countries, two thirds of the 20 respondents had blood redistribution programmes in use and 17 had quality management systems implemented
- Paid donations were reported by 21% of respondents but achieving 100% voluntary blood donation remains a top priority.
- More than half of the respondents use slide technology for blood grouping and cross-matching and rapid testing for transfusion-transmitted infections (alone or in conjunction with advanced testing). Most respondents report a lack of patient and donor haemovigilance programmes.

INTRODUCTION

The World Health Organization (WHO) reported 118.5 million blood donations worldwide in 2018, ranging from 31.5/1000 people in high-income countries (HICs) to fewer than 5/1000 in low- and middle-income countries (LMICs) [1]. Blood transfusion services (BTS) in LMICs have reportedly been inadequate in terms of blood availability, basic testing methods, clinical transfusion guidelines and quality management services [2, 3]. Reported reasons included ineffective regulatory and professional oversight, lack of appropriate legislation and policies and their implementation, insufficient quality and safety programmes for donor screening, testing, monitoring (haemovigilance) and inadequate quality improvement systems (e.g., accreditation, quality assurance) [4, 5]. Cost and availability of critical equipment, reagents and consumables, as well as structural constraints, such as limited space and irregular electricity supply, are additional challenges [6]. Furthermore, the cost of blood and blood components, as well as inadequate government funding, are likely to have impeded the provision of adequate transfusion care [7]. Becauseof the lack of transportation, resources for continuous temperature monitoring and tough geographic conditions, redistribution of blood components is challenging and requires the implementation of enabling policies at the ground level [8]. The WHO recommends strengthening national BTS to ensure a safe blood supply [9]. In 2018, almost a quarter of countries had no national blood policy, and 61% of LMICs had no specific legislation covering the safety and quality of blood transfusion [1].

Despite the numerous obstacles that LMICs face, significant progress has been made in the field of transfusion medicine, with several initiatives in hospital-based blood banks, blood establishments (BEs) and transfusion services. This study aimed to assess the current status of infrastructure and resources in BEs and BTS in LMICs and to explore their challenges and future plans for improvement.

MATERIALS AND METHODS

A cross-sectional survey was conducted using a self-administered questionnaire designed by a multidisciplinary group of the International Society of Blood Transfusion (ISBT) (Data S1). The survey questions were captured electronically using the Survey Monkey® software and were piloted among the group members for content validity and identification of any ambiguity. Forty-five survey questions covered five main sections namely: (1) respondents' demographics ($n = 8$), (2) donation and components manufacturing ($n = 12$), (3) transfusion services ($n = 13$), (4) management systems ($n = 10$) and (5) future plans for improvement ($n = 2$). The donor and components section (second) aimed to address the type of donors and donations, existing component manufacturing facilities and donor recruitment strategies. The transfusion services section (third) explored existing facilities for testing, component storage and redistribution and the cost of blood components. The management system section (fourth) covered documentation, accreditation and quality and existing patient blood management (PBM) and haemovigilance programmes. Finally, the fifth section, sought plans for improvements. The survey was then disseminated by the ISBT office to 1136 ISBT members from all WHO regions. Participation was voluntary, and consent was obtained by completing the survey. The

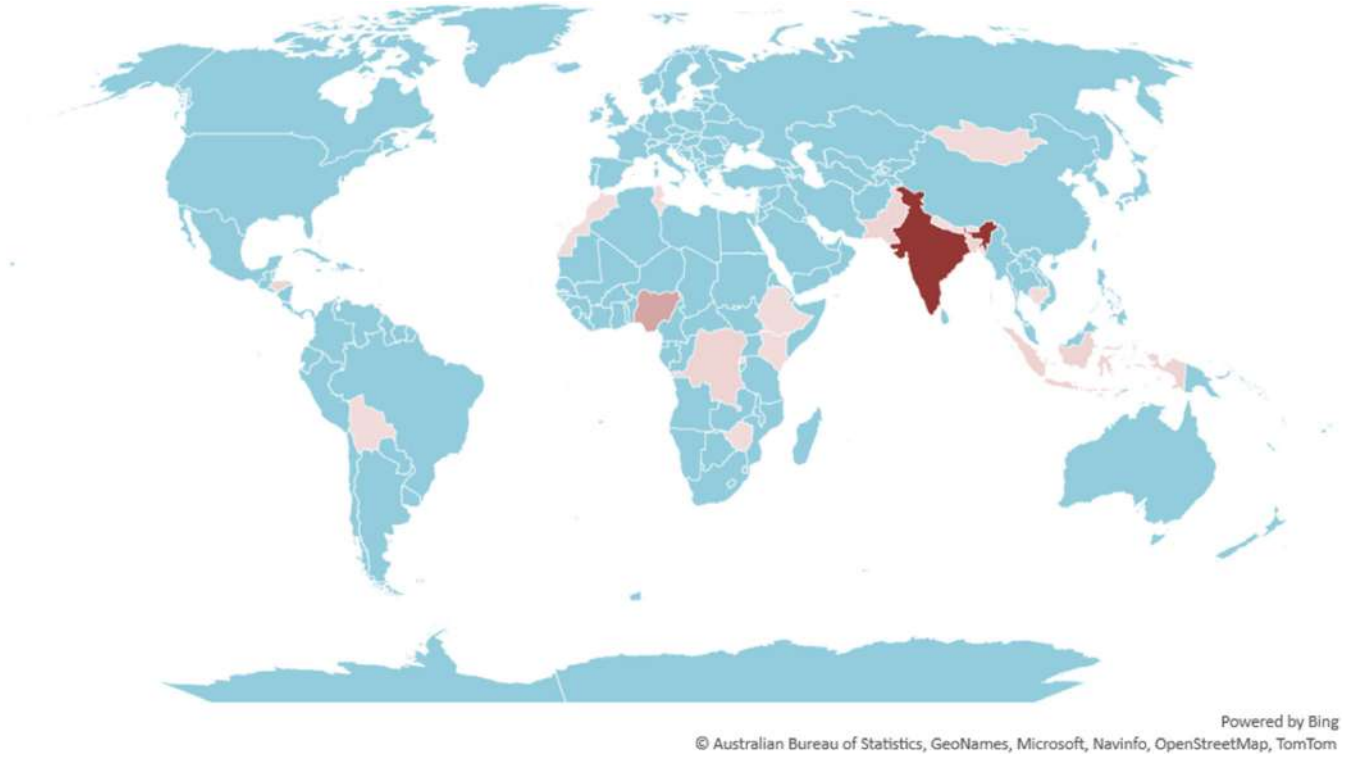

FIGURE 1 Geographic distribution of survey respondents from low- and middle-income countries ($n = 54$)

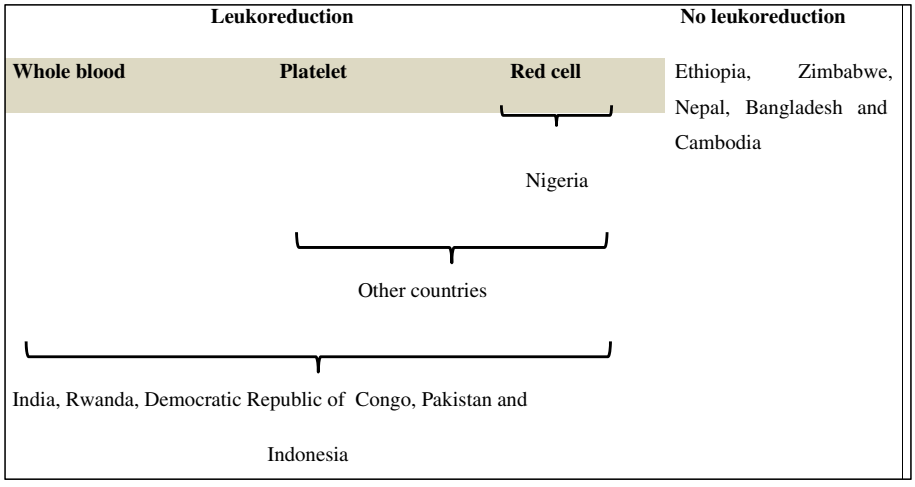

FIGURE 2 Component leukoreduction in respondent's institutions

survey was opened between 14 October 2021 and11 November 2021. Respondents were asked to address both the institution and the country's blood transfusion and management service where possible. Descriptive statistics were applied, and the reported variables were expressed in numbers and percentages.

RESULTS

A total of 54 respondents from 20 LMICs countries completed the survey (response rate 5%). The largest number of respondents were from India (42%), followed by Nigeria (15%). (Figure 1 and Table S1) The majority of respondents worked in hospital-based BTS ($n = 25$, 46.3%). The rest worked in national blood centres ($n = 6$, 11.2%), national BTS ($n = 5$, 9.3%), regional BTS ($n = 1$, 1.8%), regional blood centres ($n = 8$, 14.8%), hospital-based BEs ($n = 1$, 1.8%) and others ($n = 8$, 14.8%), such as thalassemia centres.

BTS in the respondent's countries were broadly categorized into centralized services in national blood centres (9%, $n = 5/54$), decentralized services (50%, $n = 27/54$) and a hybrid of both (40.7%, $n = 22/54$). Ethiopia, Bolivia and Rwanda have centralized

TABLE 1 Donor recruitment and types of incentives/remunerations given to voluntary blood donors ($n = 52$)

		n	%
Recruitment strategies	Motivational speeches in schools, colleges, universities and organizations	43	82.7
	Distribution of motivational and educational leaflets	41	78.8
	Phone calls to blood donors	40	76.9
	Radio/television announcements	36	69.2
	Social media motivational posts	35	67.3
	Campaigns by community leaders, celebrities and/or influencers	33	63.5
	Mass text messages to blood donors	27	51.9
	Emails to blood donors	15	28.8
	Other[a]	4	7.7
Types of incentives/remunerations	Token of appreciation or gifts <25 USD in value	20	38.5
	Day-off from work	16	30.8
	None	15	28.8
	Other (please specify)	14	26.9
	Free transportation passes	6	11.5
	Gift's worth >25 USD in value	3	5.8
	Money/cash	3	5.8

[a]Recognition by Minister, State Governors or National Blood Service, recognition on World Blood Donor Day, or snacks and refreshments.

BTS, while India, Bhutan, Bangladesh and Pakistan have decentralized BTS.

Blood donors and blood donation

The majority of the respondents' institutions performed collection of blood 96.3% ($n = 52/54$) and from different donor sources within the same institution. Voluntary non-remunerated, replacement (family or non-family) and paid donors accounted for 94.2% ($n = 49$), 84.6% ($n = 44$) and 21.2% ($n = 11$), respectively. Exclusive voluntary donations were reported in Ethiopia, Mongolia and Zimbabwe (5.8%), while the rest of the respondent institutions collected blood from both voluntary and replacement donors. Directed and autologous donations were offered in 59.6% ($n = 31$) and 51.9% ($n = 27$) of the respondents' institutions.

The majority of the blood collecting institutions ($n = 52$) applied national criteria for donor acceptance (80.8%, $n = 42$), followed by WHO (42.3%, $n = 22$) and institutional (36.5%, $n = 19$) criteria. Majority of the 52 institutions with blood collection facilities applied a combination of donor selection criteria, that is, national, WHO and institutional criteria (41, 78.6%). Seven institutions utilized national criteria solely (13.5%), while one used it in combination with WHO criteria. Institutional criteria were applied in two institutions in combination with WHO criteria in one of them. National donor eligibility criteria were applied in institutions in India, Zimbabwe, Mongolia, Bhutan, Nepal, Moroccoand the Lao People's Revolutionary Party (Lao PDR).

Different measures were utilized for donor recruitment. Donor incentives were in use in 71% ($n = 37/52$) of the participant institutions (Table 1). Respondents from Ethiopia, Nepal, Bangladesh, Cambodia and Morocco reported no incentives for the donors.

Blood component processing

Most respondents' institutions had whole blood (100%) and apheresis donation services 59.6% ($n = 31/52$), including plateletpheresis, plasmapheresis, red cell apheresis and leukapheresis. The lack of apheresis facilities was reported by institutions in Bhutan, Cambodia and Lao PDR.

Component separation facilities were available in 94.2% ($n = 49/52$) of the institutions. The percentages of whole blood inventory kept on the shelf for clinical use were variable (Table S2). Whole blood inventory accounted for up to 80% of the total inventory in respondent institutions in Nigeria, Cambodia, Honduras and Indonesia. Leukoreduced blood components were available in 51.9% ($n = 27/52$). According to 67.3% of the respondents, leukoreduced blood components were available in other institutions in their country, and 19.2% reported a lack of leukoreduction resources (Figure 2).

Out of all 54 institutions, blood component storage facilities were available in all the institutions except Zimbabwe, where a few blood centres did not have adequate storage facilities. Adequate storage facilities for red cells were reported by 96.3% ($n = 52/54$), while sufficient storage for platelet and plasma components was reported by 90.7% ($n = 49/54$) of respondents.

BLOOD REDISTRIBUTION PROGRAMME

National/regional guidelines for the redistribution of blood and blood components were reported to be available by 90.7% of the respondents ($n = 49/54$). These were, however, reported unavailable by

respondents from Zimbabwe, Bhutan, Cambodia, Morocco and Tunisia.

Continuous temperature monitoring during transportation was performed using manual methods, temperature charts (25.9%, $n = 14/54$), data loggers (7.4%, $n = 4/54$) or a combination of both (20.4%, $n = 11/54$). However, 24.1% ($n = 13/54$) of respondents reported a lack of temperature monitoring. Other methods of temperature monitoring were reported by 22.2% ($n = 12/54$), including the use of vaccine carriers with in-built thermometers or checking the temperature at the time of shipment and receipt of blood products. The most frequent transportation distance and time between blood centres were 5–25 km (44.4%, $n = 24/54$) and 1–4 h (42.6%, $n = 23/54$), respectively (Figure S1).

While the most frequent means of blood transportation reported were car (75.9%, $n = 41/54$), or dedicated transport ambulance (57.4%, $n = 31/54$), 33.3% also reported the use of public transportation, for example, buses and motorbikes. Air transportation was reported to be available by 22.2% ($n = 12/54$), and drone use was reported by respondents from Mongolia and Rwanda.

Cost of blood and blood components

A majority of respondent institutions (81.5%, $n = 44/54$) reportedly charged user fees for blood components, with responsibility for payment residing with either the patient (50%, $n = 22/44$) or the government for specific patients' categories, such as thalassemia or haemato-oncology patients (38.6%, $n = 17/44$). Respondents from Ethiopia, Bhutan, Cambodia, Bolivia and Kenya indicated that blood components were provided free of charge in their countries.

Participants from India, Lao PDR and Honduras reported that the government paid for treatment in public hospitals while patients used private insurance funds to access treatment in private hospitals. In Honduras, patients made payments through social security accounts. In countries where patients paid user fees for blood components, the charges ranged from 3 to 40 euros for whole blood and 6 to 30 euros for red blood cells. For platelet concentrates and fresh frozen plasma, the charges ranged from 1 to 50 euros. For plateletpheresis procedures, the charges ranged from 14 to 678 euros.

Testing facilities

Basic testing on blood donor units, including ABO and Rh blood grouping, and testing for transfusion-transmitted infections (TTIs), was reported by 47 respondents (Table S3). Reagents for antibody screening and identification on donor units were reported to be available by 57.4% of respondents ($n = 27/47$), while bacterial screening on platelet units before being issued was reported to be available by 10.6% ($n = 5/47$) of respondents only.

Methods used for blood grouping and cross-match testing were manual with tube and slide technology (83%, $n = 39/47$), semi-automated testing with column agglutination technology (68%, $n = 32/47$), and fully automated testing with solid-phase red cell adherence assay or erythrocyte-magnetizing technology (36.2%, $n = 17/47$). Full automation was reported to be available in some centres in India, Nigeria, Morocco, Honduras and Pakistan.

TTIs screening testing methods reported to be used in respondent's institutions were enzyme-linked immunosorbent assay (ELISA) (82.9%, $n = 39/47$), rapid tests (68.1%, $n = 32/47$), chemiluminescence testing (61.7%, $n = 29/47$) and nucleic acid amplification testing (NAAT) (36.2%, $n = 17/47$).

Quality management systems

Implementation of the quality management system (QMS) was reported in 17 out of the 20 countries surveyed. Respondents from institutions in Magnolia, Rwanda and Tunisia reported the lack of a well-established QMS. Internal and external quality assurance programmes were reported in most of the respondents' institutions (65.9%, $n = 31/47$), while others had either internal (23.4%) or external (10.6%) QMS (Table 2). BTS records were maintained by manual and electronic systems (76.6%, $n = 36/47$), manual/paper-based systems only (19.1%,

TABLE 2 Quality assurance program and proficiency testing in respondent countries ($n = 47$)

S.no.	Programmes	Types	Countries
1.	Quality assurance programme	Internal ($n = 11$, 23.4%)	Rwanda, Demographic Republic of Congo, Nepal, Morocco and Lao PDR
		External ($n = 5$, 10.6%)	Bhutan
		Both ($n = 31$, 65.9%)	India, Ethiopia, Zimbabwe, Bangladesh, Nigeria, Cambodia, Honduras, Pakistan, Indonesia, Bolivia and Kenya
2.	Proficiency testing samples	Local ($n = 15$, 31.9%)	Demographic Republic of Congo, Nepal, Bangladesh, Morocco, Pakistan and Lao PDR
		External ($n = 25$, 53.2%)	Ethiopia, Zimbabwe, Bhutan, Cambodia, Honduras, Indonesia, Bolivia and Kenya
		Both ($n = 7$, 14.9%)	India and Nigeria

Abbreviation: Lao PDR, Lao People's Revolutionary Party.

TABLE 3 Types of accreditation programme in respondent's institutions ($n = 39$)

S. no.	Type of accreditation programme	Number of respondents
1.	National accreditation programmes	26 (70.3%)
2.	International Organization for Standardization (ISO)	11 (29.7%)
3.	Association for the Advancement of Blood and Biotherapies (AABB)	3 (8.1%)
4.	College of American Pathologists (CAP)	3 (8.1%)
5.	Others (African Society for Blood Transfusion [AfSBT], and College of Physicians accreditation programmes)	8 (21.6%)

Note: Four institutions had multiple accreditation programmes: one had national + ISO + AABB + CAP, one had ISO + AABB + CAP, one had national + ISO + AABB and one had national + ISO.

$n = 9/47$) or digital/electronic systems (4.3%, $n = 2/47$). The accreditation programmes were available in a majority of respondents' institutions (81.3%, $n = 39/48$) in 12 countries (Table 3).

Haemovigilance and PBM

Haemovigilance programmes were available in eight out of the 20 countries surveyed, unavailable in eight countries and respondents from four countries were unaware of the existence of such programmes. The majority of the countries ($n = 6$) with haemovigilance programmes had both patient and donor programmes, while one had only a recipient programme and the other maintained only a donor programme. PBM was reported to be implemented in one third of institutions (34%, $n = 16/47$); in 50%, it was not implemented, and the others were not aware of the same.

Future planningfor improvement

The respondents shared their institutions' improvement plans over the next 5–10 years. The most common plans were strengthening haemovigilance, improving quality assurance and quality managementand the achievement of 100% voluntary blood donation (Table 4).

DISCUSSION

This survey highlights the available resources in BTS of LMICs in terms of donor management, testing facilities, blood cost, redistribution policies, quality management services and future planning for improvement.

Almost 25% of the countries surveyed reported decentralized BTS. Decentralization is associated with many risks in the provision of services. As per the WHO guidance document, centralization of key functions of the BTS, such as testing and processing of blood donations, can overcome shortcomings that often exist in decentralized blood systems [10]. Hosseinifard et al. found that centralized BTS led to reducing the blood shortage and expiry of blood units by 40% and 21%, respectively, and also decreased the average age of issued blood to the patients [11].

Our survey showed that blood donation remains a major challenge in LMICs healthcare systems, with high reliance on replacement blood donations. This is in concordance with other reported literature [9, 12]. We also showed the use of paid donors and incentives in some of these countries. According to WHO, paid donations account for more than half of the blood supply in 72 countries, 64 of which are LMICs. Previous studies suggested the role of non-cash incentives, such as tokens of appreciation (medals and certificates), goods and gifts (T-shirts, mugs, food or vouchers to stores or restaurants) as a possible means of navigating between the two established theoretical frameworks for donation—altruism and payment [13, 14]. Our survey also showed variations in the application of donor eligibility criteria. One institution surveyed did not use national or WHO donor selection criteria, possibly due to a lack of national guidelines, inability to access or a lack of awareness of international/WHO resource materials.

We also found variability in access to component manufacturing, leukoreduction, apheresis and supportive services of component redistribution. According to WHO reports, approximately 25% of LMICs do not have facilities for manufacturing banked blood into components [15]. Leukoreduction helps in the prevention of febrile non-haemolytic transfusion reactions, alloimmunization and leucocytes virus transmission [16]. Therefore, recipients of blood in such environments are more at risk of adverse events and infection. Blood component redistribution helps reduce pressure on inventory levels by decreasing overall discard rates through the transfer of near-expiry blood units to needy blood centres [17]. In LMICs, geographical constraints and resource scarcity are major challenges to the effective implementation of redistribution policies.

In this survey, around 85% of respondents reported that transfusion was payable by patients. Divkolaye et al. reported in a 2019 survey that in LMICs, the total or partial costs of blood were mostly recovered directly from the patients [7]. Testing of blood components accounts for the majority of the charges. According to WHO, 32% of countries had specific governmental budgets for BTS, 16% had a cost recovery system, and 33% reported having both, while the remaining 11% reported neither a specific budget nor a cost recovery system [9].

More than 80% of the responding institutions used manual testing methods for blood grouping and cross-matching. Non-availability of screening equipment and reagents might be one of the major reasons for the lack of advanced testing. In addition, almost more than 40% of respondents reported a lack of antibody investigation tests. The lack of availability of advanced testing may result in adverse transfusion reactions, such as haemolytic transfusion reactions due to alloantibodies or septic transfusion reactions due to bacterial contamination of platelet components [18]. Limited supply or access to test

TABLE 4 Future improvement plans reported by respondents in their institutions and countries

S. no.	Country	Future planning
1.	India	• Centralization of BTS • Universalization of guidelines, leukoreduction, NAAT, accreditation, PBM, and pricing of blood and blood components • Molecular blood grouping • Electronic cross-matching • Pathogen inactivation • Cellular and gene therapy • Rare blood donor registry • Radio-frequency identification system
2.	Bhutan	• National strategies for consolidation of blood donation and TTI testing • Hospital transfusion committee • Accreditation • Centralized blood transfusion information system • Universal component separation • Apheresis services
3.	Nepal	• Qualified person to run the BTS • Development of antibody screening and identification programme
4.	Bangladesh	• Implementation of automated services in blood centres
5.	Indonesia	• Build a new building for blood transfusion centre
6.	Pakistan	• Improve utilization of blood components • Introduction of NAAT
7.	Morocco	• Involvement of an autonomous public establishment in blood banking services • PBM • Apheresis cell therapy • NAAT and chemiluminescence testing for TTI
8.	Tunisia	• Implement electronic record maintenances
9.	Ethiopia	• Improve necessary infrastructure • Resource mobilization • Accreditation • Plasma fractionation • Tissue banking • Organ transplantation
10.	Zimbabwe	• Improve testing of blood donor samples by implementation of NAAT
11.	Democratic Republic of Congo	• Development of national blood transfusion centres with advanced equipment • Easy availability and accessibility of blood to all
12.	Nigeria	• Increase national availability and accessibility of safe blood, regionalization of BTS • Improved collaboration with regional centres • Universal component separation • Leukoreduction • Automated blood grouping • Automated cross-matching • Electronic record maintenances • Universal antibody screening of blood donors and patients • Reference laboratories • Rare blood donor registry • PBM, • Accreditation programme • Hospital transfusion committee
13.	Kenya	• To initiation of antibody screening and molecular testing
14.	Honduras	• Planning of better legislation and regulations for BTS • Standardizations of blood donor acceptance criteria and blood bank protocols • Centralization of testing and NAAT testing
15.	Bolivia	• Easy availability of high-quality blood components
16.	Cambodia	• Development of centre of excellence in accreditation programme
17.	Lao PDR	• Increase plasma collection and decrease the burdens to thalassemia patients

Abbreviations: BTS, blood transfusion services; Lao PDR, Lao People's Revolutionary Party; NAAT, nucleic acid amplification testing; PBM, patient blood management; TTI, transfusion-transmitted infection.

kits, as well as basic training of the laboratory technicians, were common barriers [19].

We also found that 70% of respondent institutions performed rapid testing exclusively or in conjunction with ELISA, chemiluminescence and NAAT facilities. Only 18% of these used rapid testing and ELISA testing in combination, while the rest used rapid testing with all the tests, such as ELISA, chemiluminescence and NAAT. In 2008, as many as 39 countries were unable to screen all donated blood for one or more of the infections: HIV, hepatitis B, hepatitis C and syphilis [20]. This difference leads to an enormous gap in residual risks of TTIs between developed and developing countries [20].

Safe and good-quality blood requires an established QMS. In our study, we found that 17 out of 20 respondent countries had QMS in use, of which 11 countries had both internal and external quality assurance programmes. As per WHO, external quality assessment monitoring is reportedly available in only 34% LMICs compared to 81% of HICs [8]. This was in keeping with our report. In 2016, up to 5 million deaths in LMICs were ascribed to poor-quality health services [21]. Safe, quality BTS are a vital component of quality health systems.

In our study, we found that more than 70% of respondent institutions implemented national accreditation programmes, while the rest implemented international accreditation programmes. Many LMICs have developed national accreditation programmes for hospitals, but the lack of financial resources remains a key constraint to the success of accreditation and its sustainability [22–24]. For example, in 2009, the 'Yellow Star' accreditation programme in Uganda was suspended by the government after development partner funding from the United States Agency for International Development was cut [25]. In Zambia, the programme was stopped [26].

We discovered that only eight respondent countries had implemented haemovigilance programmes, with only six having both donor and recipient programmes. Haemovigilance programmes play a major role in quality improvement in BTS. In a recent study of 10 sub-Saharan African countries, it was found that a lack of a comprehensive legal framework, a lack of clear understanding, distinction of the function of the blood service and a lack of human resources were major constraints to the implementation and performance of haemovigilance systems [27].

This survey revealed that only one third of the respondents' institutions have fully implemented PBM. Ironically, PBM is especially relevant for countries such as LMICs that do not have enough blood supply [28].The application of PBM helps to establish restrictive blood transfusion policies and utilizes pharmacological alternatives for allogeneic blood transfusion. Insufficient training on transfusion medicine, the lack of available clinical transfusion guidelines or the ineffective implementation of any existing guidelines could be the reasons behind its limited utilization in LMICs [29, 30].

Over the next 5–10 years, nearly all the respondents plan to achieve 100% voluntary blood donations, improve quality assurance and QMSs and strengthen their haemovigilance programmes. Within blood banking and transfusion medicine, gaps are known in LMICs with respect to competent training programmes and leadership development [31]. Universal guidelines and implementation of advanced techniques, along with improvements in basic infrastructure and manpower, are key potential areas for improvement of any BTS.

To the best of our knowledge, this is the first study that highlighted the current state of BTS/BEs in LMICs with future plans for improvement in their institution or country. Our study had some limitations, one of which was its reliance on email as the method of distribution, which made generalization difficult. Individual participant responses could not be extrapolated to represent uniform practices across any given country. We also observed diversity within a country with more than one response. There was a wide range of respondents (1–24) from different countries. Many respondents did not answer the entire questions, which led to different denominators in different response calculations. Some phrases, such as 'adequate storage facilities,' were not defined in the survey to shorten the length of the survey, which may have resulted in subjective bias. Selection, recall and desirability bias may have also influenced the responses.

In conclusion, despite the numerous challenges, the majority of LMICs had well-established QMSs and accreditation programmes. The implementation of quality systems was identified as essential for blood and transfusion safety, but without regulatory oversight, it is unlikely to be achieved.

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G.K.P. and J.T. conceptualized the study, G.K.P and Y.D. prepared the survey questionnaire, A.O., H.V., K.B., R.G.R., C.S.O and A.Z.A.R. reviewed the survey questionnaire and finally approved. G.K.P. and Y.D. compiled the survey results, analysed and prepared the initial draft of the manuscript. A.O. and A.Z.A.R. reviewed the draft manuscript and finalized the manuscript. J.T. H.V., K.B., R.G.R. and C.S.O supervised the research and reviewed and edited the manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Factors affecting need for blood transfusion in paediatric patients undergoing open surgery for hip dysplasia

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Abstract

Background and Objectives: The management of intraoperative blood loss in the surgical treatment of paediatric hip dysplasia is resource intensive. There are numerous clinical factors that impact the need for intraoperative transfusion. Identification of patient and surgical factors associated with increased blood loss may reduce the unnecessary use of resources. This study aimed to identify factors predictive of intraoperative transfusion in children undergoing hip dysplasia surgery.

Materials and Methods: This is a single-centre retrospective review of patients undergoing surgery for hip dysplasia from 1 January 2012 to 15 April 2021. Patient demographic factors, anaesthetic, surgical and transfusion histories were reviewed. Multivariable logistic regression analysis was performed to identify factors predictive of allogeneic red blood cell transfusion requirements during the intraoperative period.

Results: This study includes 595 patients who underwent open surgery for hip dysplasia, including 297 (52.6%) classified as developmental dysplasia (DD) and 268 (47.3%) as neuromuscular (NM) with a mean age of 9.1 years (interquartile range 3–14). Intraoperative allogeneic transfusion was identified in 26/297 (8.8%) DD and 73/268 (27.2%) NM patients. Adjusted factors associated with increased odds of intraoperative transfusion were NM (odds ratio [OR] = 2.96, 95% confidence interval [CI] [1.76, 5.00]) and the number of osteotomies performed (OR = 1.82/osteotomy, 95% CI [1.40, 2.35]). Adjusted factors that reduced the odds of transfusion were the use of antifibrinolytics (OR = 0.35, 95% CI [0.17, 0.71]) and regional anaesthesia (OR = 0.52, 95% CI [0.29, 0.94]).

Conclusion: For children undergoing surgery for hip dysplasia, the number of osteotomies performed is predictive of the need for allogeneic blood transfusion. Antifibrinolytics and regional anaesthesia are associated with reduced risk for allogeneic blood transfusion. Blood management initiatives, such a preoperative optimization of haemoglobin and the use of antifibrinolytics, could target patients at increased risk of intraoperative bleeding and transfusion.

Keywords

antifibrinolytics, blood, hip dysplasia, hip instability, intraoperative, paediatrics, transfusion

Highlights

- For children undergoing surgery for hip dysplasia, the number of osteotomies performed and neuromuscular disease are strong predictors of the need for allogeneic blood transfusion.
- Antifibrinolytics and regional anaesthesia were associated with a reduced risk of the need for allogeneic blood transfusion.

INTRODUCTION

The management of blood loss in the surgical treatment of paediatric hip dysplasia is resource intensive. Physicians must anticipate and monitor for blood loss before and during the surgery and then adequately address any clinically significant blood loss that does occur to ensure optimal patient outcomes. Patients are usually required to undergo preoperative laboratory testing that can be both costly and onerous (particularly for young children and those with developmental delays), which often leads to no changes in the management strategy. Intraoperative blood transfusion can be reduced through the use of antifibrinolytics, preoperative optimization of haemoglobin and treatment of iron deficiency anaemia, administration of allogeneic blood to replace losses, or by the return of autologous blood using cell salvage. Advances in technology for autologous blood salvage (cell salvage) and the widespread availability of antifibrinolytics have been shown to reduce blood loss in different paediatric surgical populations [1, 2]. Transfusion of red blood cells (RBCs) is not without risks, including haemolysis, transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO) and transfusion-related immunomodulation [3]. Transfusion-related complications, TACO and TRALI, have been reported to occur at rates of 26.6 and 28.2 per 100,000 transfusions in paediatric patients, respectively [4]. RBC transfusion (≥25 ml/kg) has also been demonstrated to be associated with increased surgical site infections and wound dehiscence in paediatric perioperative patients [5]. As such, the implementation of patient blood management (PBM) protocols is increasingly recommended, particularly for adult surgical patients. Not all patients will, however, benefit from the interventions included in those strategies (e.g., preoperative iron supplementation, prophylactic antifibrinolytic administration and use of cell salvage) [6–9]. Although the cost of transfusions and the need for judicious use of blood products and resources (e.g., preoperative blood work) have been discussed previously (even more so during the COVID pandemic), the implementation of resource reduction strategies is also associated with economic and logistical challenges [10, 11]. For optimizing the cost-effectiveness of those programmes, preoperative identification and stratification of patients at higher risk of requiring transfusions is a priority. Hip dysplasia surgery patients are a heterogenous group with regard to underlying medical comorbidities. Most specifically, patients are classified as having idiopathic hip dysplasia (e.g., developmental dysplasia [DD]) or neuromuscular (NM) dysplasia (e.g., disorders that result in dysplasia cerebral palsy). This distinction is also likely to contribute to the need for blood transfusion, given the increase in medical and surgical complexity. Thus, the aim of this study is to determine the patient and surgical characteristics associated with the need for allogeneic blood transfusion in patients undergoing open surgery to treat hip dysplasia with the intent to create a risk stratification model to guide intraoperative blood management.

MATERIALS AND METHODS

After obtaining approval from the institutional review board at the Baylor College of Medicine (#H-44822) with a waiver of written consent, we conducted a retrospective review of anaesthetic records extracted from the electronic medical record (EPIC, Verona, WI, USA) at Texas Children's Hospital, a large paediatric quaternary care academic centre. All patients undergoing surgical correction of hip dysplasia requiring osteotomy for hip dysplasia from 1 January 2012 through 15 April 2021 were reviewed. We included patients with hip dysplasia related to both developmental and neuromuscular causes. Exclusion criteria were hip dysplasia surgery in combination with additional non-hip dysplasia procedure, absence of osteotomy, previous hip surgery on the ipsilateral side (i.e., redo operations), post-traumatic reconstructions and patients with known coagulation disorders. Each surgical encounter was considered a separate event such that patients having two procedures at different surgical encounters would be considered as two events.

The following demographic information was collected: patient age, weight, gender and American Society of Anesthesiologists physical status classification. Medical information collected included aetiology of hip dysplasia (neuromuscular or developmental), hospital length of stay and history of prematurity as <32 weeks post-conceptual age and 32–37 weeks post-conceptual age or full term. The following characteristics related to surgery were collected: primary surgeon, operative time and the number of individual osteotomies performed as reviewed from both the operative report and post-operative radiography. Osteotomies performed were defined as the number of bones cut (e.g., periacetabular osteotomy [PAO] = three bones cut [ilium, ischium and pubis], Dega = one bone cut [ilium only]). It meant that a patient who underwent PAO and femoral osteotomy would have four bones cut. The following characteristics related to anaesthesia care were collected from the anaesthetic record: intraoperative blood product allogeneic RBCs, fresh frozen plasma (FFP), platelets and cryoprecipitate administration, use of cell salvage, antifibrinolytic administration, and intravenous fluid administration. Transfusion given during surgery or in the immediate post-anaesthesia care unit period was considered. Intraoperative cell salvage was used at our institution for the duration of the cohort, while we do not perform autologous pre-donation or acute normovolemic

haemodilution. Institutionally, until 2017, patients were transfused when the haemoglobin was less than 9.0 g/dl. After 2017, transfusion was reserved for when haemoglobin was less than 7.0 g/dl. Transfusion of autologous blood from cell salvage was performed in cases where a sufficient yield was obtained. Antifibrinolytic administration for hip dysplasia surgery was instituted for all patients by a protocol in January 2018 and included tranexamic acid as a bolus (10 mg/kg) followed by continuous infusion (5 mg/kg/h) intraoperatively. Before January 2018, tranexamic acid administration was at the discretion of the anaesthesiology provider.

Data analysis

The sample size was determined by the number of patients undergoing reconstructive surgery, including osteotomy for hip dysplasia, at our institution from January 2012 through April 2021. Categorical variables were described by frequencies and percentages, and continuous variables by means and standard deviations. The exception was the volumes of intraoperative allogeneic transfusions are reported as medians and interquartile ranges due to this variable's potential for being skewed. To determine factors associated with intraoperative blood transfusion, we used the Student's *t*-test for continuous variables and the χ^2 test for categorical variables unless a cell count was <5 and therein, we used Fisher's exact test.

Logistic regression with robust standard errors clustered at the patient level was used to assess the association of predictors on the need for intraoperative transfusion. This method was used as some patients had multiple surgeries at different time periods of the study, and this technique adjusts for possible serial correlation within subjects [12]. We, a priori, decided that the treatment group (DD vs. NM) would be in any final model and considered clinically plausible variables stratified by outcome with an unadjusted p-value < 0.2 as a candidate for entry into a multivariable logistic regression model. We further tested for interactive effects with the treatment group and each variable in the final model, and a priori, decided to include any interaction if it was statistically significant. Otherwise, the final model would have main effects only. To measure model performance, we report the area under the ROC curve for discrimination and the Hosmer–Lemeshow statistic for calibration. All p-values < 0.05 were considered statistically significant. All analyses were done using Stata/MP 15.1 for Windows (StataCorp, College Station, TX, USA).

RESULTS

Data on 679 patient encounters were available and screened, with 84 patients meeting the exclusion criteria (Figure 1). Our cohort for analysis had 565 patient encounters who underwent open surgery for hip dysplasia at our institution from 1 January 2012 to 15 April 2021. This number included 71 patients (12.6%) with more than one eligible surgery during the study period. For our analyses, other than multivariable logistic regression modelling, we considered the encounter as a unique patient. The cohort included 297 (52.6%) classified as DD and 268 (47.3%) as NM. The demographic characteristics of these patients are summarized in Table 1. There were 14 unique surgeons and 77 anaesthesiologists treating these patients during the study period.

With respect to intraoperative transfusion of RBCs, 26/297 (8.8%) of DD patients required transfusion as compared to 73/268 (27.2%) of NM patients (p < 0.001). When intraoperative allogeneic transfusion was required, the median (25th percentile, 75th percentile) volumes (in milliliters per kilogram) administered were 8.5

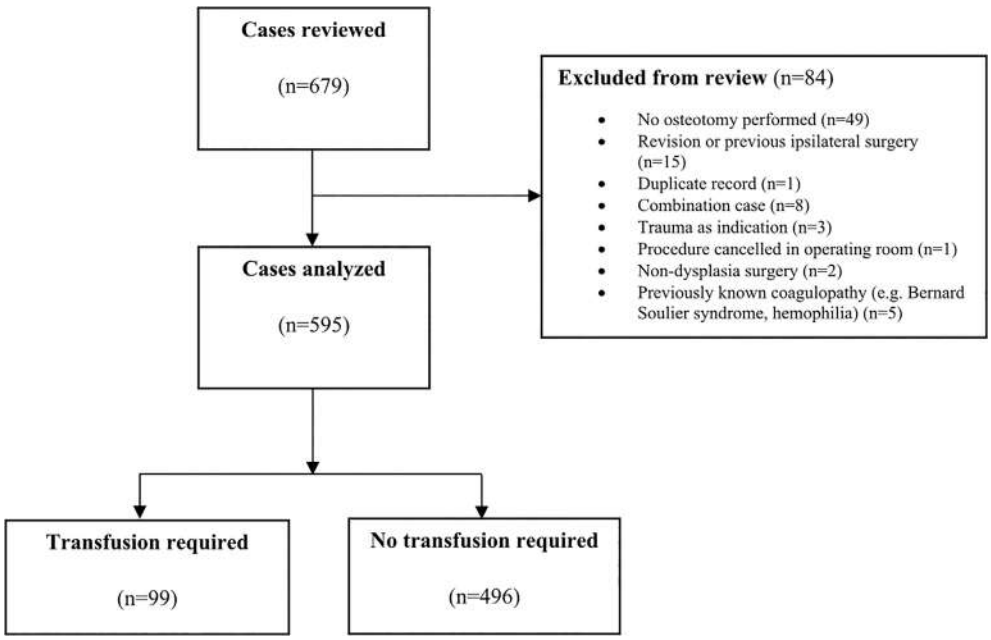

FIGURE 1 Attrition table for study patients

TABLE 1 Patient characteristics stratified by intraoperative transfusion

Variables for all patients	No intraoperative transfusion, $N = 466$ (82.48%)	Intraoperative transfusion, $N = 99$ (17.52%)	*p*-value
Age (mean, SD)	9.8 (5.8)	9.3 (5.8)	0.453
Weight (kg) (mean, SD)	37.1 (26.8)	37.0 (28.2)	0.996
% Female	280 (60.09%)	53 (53.54%)	0.229
Length of stay (days) (mean, SD)	4.1 (3.2)	4.0 (3.8)	0.962
Procedure time (min) (mean, SD)	239.9 (85.0)	240.0 (81.9)	0.994
ASA physical status			
1	52 (11.16%)	10 (10.10%)	0.419
2	182 (39.06%)	45 (45.45%)	
3	215 (46.14%)	43 (43.43%)	
4	17 (3.65%)	1 (1.01%)	
Prematurity			
0	301 (64.59%)	76 (76.77%)	0.086
1	80 (17.17%)	8 (8.08%)	
2	80 (17.17%)	8 (14.14%)	
Unknown (adopted)	5 (1.07%)	1 (1.01%)	
Arterial line; yes	205 (43.99%)	41 (41.41%)	0.639
Regional anaesthesia			
No	65 (13.95%)	23 (23.23%)	<0.001
Epidural	229 (49.14%)	57 (57.58%)	
Caudal	59 (12.66%)	18 (18.18%)	
Lumbar plexus	109 (23.39%)	1 (1.01%)	
Pericapsular nerve group block (PENG) block	4 (0.86%)	0 (0.00%)	
Any regional anaesthesia given	401 (86.05%)	76 (76.77%)	<0.001
Cell saver given; yes	208 (44.73%)	34 (34.34%)	0.058
Starting Hct (%) preop (mean, SD)	38.9 (3.8); $n = 406$	39.1 (4.6); $n = 99$	0.714
Number of osteotomies performed (mean, SD)	2.3 (1.0)	2.9 (1.0)	<0.001
Antifibrinolytics given (yes)	130 (27.90%)	14 (17.17%)	0.004
Treatment group			
Idiopathic group	271 (58.15%)	26 (26.26%)	<0.001
Neuromuscular	195 (41.85%)	73 (73.74%)	

Abbreviations: ASA, American Society of Anesthesiologists; Hct, haematocrit.

(5.1, 20.6) and 12.2 (9.4, 16.7) for DD and NM, respectively. Post-operative transfusion of RBCs was required by 14/297 (4.7%) and 72/268 (26.9%) of developmental and neuromuscular patients, respectively, $p < 0.001$.

The number of osteotomies involved in the surgeries was positively associated with the risk of receiving an intraoperative transfusion (Table 1, Figure 2). Moreover, patients in the NM group were more likely to have more bones involved (i.e., a greater number of osteotomies performed) when compared with DD patients. Figure 3 shows how intraoperative transfusion rates vary by antifibrinolytic status and treatment group. We observed reductions in transfusions among those receiving antifibrinolytics in both treatment groups. These unadjusted reductions were statistically significant in the idiopathic patients ($p = 0.04$) but not in the neuromuscular/syndromic patients ($p = 0.15$). There was no difference in antifibrinolytic use between DD and NM patients.

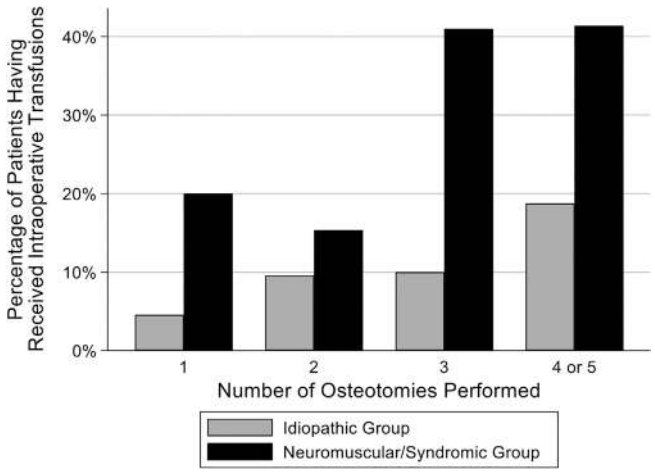

FIGURE 2 Number of osteotomies performed by the treatment group

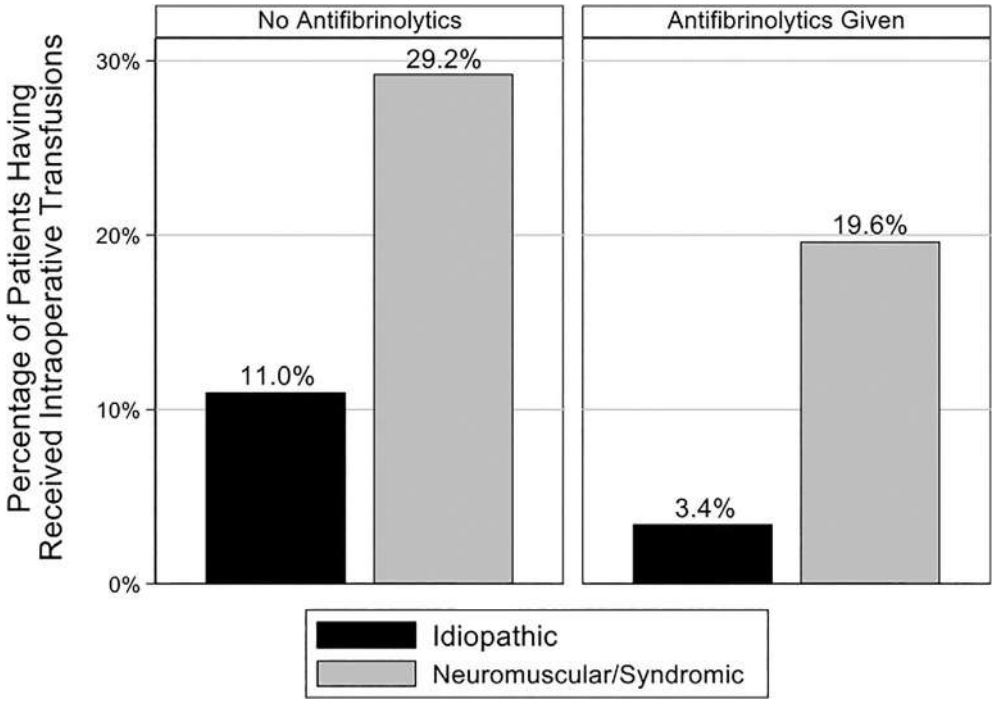

FIGURE 3 Intraoperative transfusion rates by antifibrinolytic status and treatment group

TABLE 2 Logistic regression model with robust standard errors

Covariate	Odds ratio	95% confidence interval	$p > \|z\|$
Group			
Idiopathic*	1		
Neuromuscular	2.96	(1.76–5.00)	<0.001
Osteotomies performed; per every osteotomy	1.82	(1.40–2.35)	<0.001
Antifibrinolytics given (yes)	0.34	(0.17–0.71)	0.004
Epidural status			
None*	1		
Any regional epidural	0.52	(0.29–0.94)	0.030

Note: Outcome = intraoperative transfusion.
*denotes reference category.

The final multivariable logistic regression model is in Table 2. Adjusted factors associated with increased odds of intraoperative transfusion were NM status (vs. DD status) (odds ratio [OR] = 2.96, 95% confidence interval [CI] [1.76, 5.00]) and the number of osteotomies performed (OR = 1.82 per osteotomy, 95% CI [1.40, 2.35]). Adjusted factors that reduced the odds of transfusion were the use of antifibrinolytics (OR = 0.35, 95% CI [0.17, 0.71]) and regional anaesthesia (OR = 0.52, 95% CI [0.29, 0.94]). The model had both good discrimination (area under ROC curve = 0.75) and calibration (Hosmer–Lemeshow statistic = 8.35, p = 0.30). Interaction effects between the treatment group (NM vs. DD) and the other three predictors (number of osteotomies performed, antifibrinolytic status, and regional anaesthesia status) were tried in separate models and no interaction effect was statistically significant (results not shown). One patient experienced an acute haemolytic transfusion reaction for which the transfusion was terminated immediately. There were no adverse events from tranexamic acid identified.

DISCUSSION

We examined 565 paediatric surgical encounters for hip dysplasia surgery, including 297 (52.6%) classified as DD and 268 (47.3%) as NM dysplasia. Our findings with regard to intraoperative blood loss are similar to those previously reported [13]. Sherrod et al. reported similar rates for transfusion: 9.8% and 31.1% for DD and NM patients, respectively, from a query of the National Surgical Quality Improvement Program database from cases performed in 2012–2013 [13]. In

comparison, our rate of transfusion for both the DD and NM was lower than previously reported and may represent the benefit of using tranexamic acid, which was not used in paediatric anaesthesia during their study years. Transfusion of RBC was also associated with increased length of stay in paediatric patients with NM and other underlying comorbidities undergoing procedures for hip dysplasia [10].In our study, however, we did not identify transfusion to be associated with an increased mean length of stay for DD or NM patients. For the DD patients, the mean (SD) length of stay was increased (4.1 [3.5] days without transfusion vs. 4.6 [6.7] days with transfusion; $p = 0.49$), likely owing to a more extensive surgical procedure. Similarly, multiple studies have identified a decrease in the rate of allogeneic packed red blood cells (PRBC) transfusion in children over time, likely owing to more conservative thresholds in transfusion guidelines [14–17].

Based on our data, NM disease and the number of osteotomies performed were both significantly associated with greater odds of allogeneic blood transfusion. Notably, having NM disease meant that the odds of receiving an allogeneic transfusion were almost 3 times higher than in children with DD, while every additional osteotomy involved in the surgery increased the likelihood of an allogeneic transfusion by approximately 1.8 times. To put this in context holding all other predictors at their means, the marginal probability of receiving an allogeneic transfusion was 6.6% with one osteotomy, 11.2% for two osteotomies, 18.3% for three osteotomies and 28.6% for four osteotomies.

Additionally, it appears that using the antifibrinolytic of tranexamic acid was associated with a significant reduction in allogeneic transfusion. Institutionally, routine use of tranexamic acid and the reduction of our transfusion threshold to <7 g/dl of haemoglobin has resulted in a significant reduction in transfusion for hip dysplasia surgery. With these changes, rates of transfusion dropped from 11.0% to 3.4% and 29.2% to 19.6% in the DD and NM groups, respectively. Our model also identified a reduction in intraoperative transfusion with the use of regional anaesthesia, including epidural and lumbar plexus blocks. Regional anaesthesia, particularly epidural blocks been shown to reduce bleeding and transfusion requirements during a number of procedures [18–20]. Physiologically, these regional approaches reduce arterial and venous blood pressures in part from the sympathectomy that occurs, which may reduce surgical losses [18].

Patients with NM disorders have more challenging anatomy, including contractures, undergo more extensive surgical correction and take medications that impact coagulation. Consequently, they undergo longer and more extensive operations. Increased blood loss and higher rates of transfusion need have also been observed for NM disorder patients undergoing posterior spinal fusion for scoliosis [16, 21, 22]. Paediatric scoliosis patients with NM (spastic quadriplegia) have been demonstrated to have higher prothrombin and activated partial thromboplastin times at baseline than their idiopathic counterparts, although these remained within normal limits [23]. Kannan et al. identified a prolongation of prothrombin time and a decrease in Factor VII in patients with NM disease undergoing scoliosis surgery, suggesting activation of the extrinsic pathway and depletion of coagulation factors to a greater extent than observed in idiopathic scoliosis patients [21]. In our cohort, one patient required cryoprecipitate, one required platelets and three patients required FFP transfusions for coagulopathy, all in the NM group (one of these patients required both platelets and FFP and did not receive antifibrinolytics).

Blood loss during hip dysplasia surgery, while often significant, is generally not sudden. For patients at low risk of requiring transfusion (e.g., single osteotomy, DD patients), providers should consider monitoring haematocrit using point-of-care testing or venous blood gas sampling. Providers should consider the overall risk of requiring banked blood transfusion when ordering preoperative testing and performing invasive lines. In cases where the indication for an arterial line is not clear, one or both arms can be left untucked and available to place an arterial line or draw a venous sample during the course of the surgery. Type and cross-match may be sent after induction of anaesthesia for patients at higher risk of requiring blood transfusion. Patients with a history of a previous transfusion should, however, undergo preoperative type and cross-match preoperatively due to the increased risk of antibodies, which may preclude blood availability on the day of surgery. Additionally, there are costs and risks associated with the use of allogeneic blood [3, 24]. Toner et al. reported the direct cost per unit to be $210.74, with a charge of $343.63 [25] to the patient. While the cost of cell savage has been reported to be around $315 per patient [9]. Thus, the ability to predict transfusion requirements can reduce unnecessary preoperative testing, use of invasive blood pressure monitoring and improve cost efficiency by anticipating the likelihood of requiring allogeneic blood transfusion.

Our study has several limitations. First, this is a single-centre study, so our results may not be generalizable to hospitals and patients different from our own. That said, this study reports the outcomes from 14 surgeons and 77 anaesthesiologists. Since our study covered 9 years, practice guidelines have changed over time at our institution. Patients having surgical procedures before 2017 were transfused at higher levels of haemoglobin compared to the current practice of reserving transfusion for levels below 7 g/dl. A review of anaesthetic records, however, revealed that nearly 60% of patients that were transfused had intraoperative haemoglobin of <7 g/dl, with the remainder being below 8.5 g/dl. Also, patients only routinely received antifibrinolytics starting January 2018. Additionally, we could not assess for preoperative anaemia in our cohort, a known risk factor for transfusion [16, 26, 27]. For patients with preoperative blood work, 8/505 (1.6%) had a starting haematocrit of <30.0%, with four patients requiring blood transfusion (of which four had NM disease and three received tranexamic acid). Additionally, as with all multivariable logistic regression models, there may be other confounders that were not assessed. We also note that the multivariable model was not validated and so its predictive ability on patients outside this study is unknown. Last, we did not assess the use of medications that can influence the coagulation system, and thus, the need for transfusion [28].

In conclusion, this study helps provide clinical guidance when performing surgery for paediatric hip dysplasia. Less than 10% of DD and 30% of NM disease patients required blood transfusion. The number of osteotomies planned and NM status should guide anaesthetic and intraoperative blood management. Modifiable factors, such as the

administration of antifibrinolytic therapy and regional anaesthesia, appear warranted as they are associated with a reduced risk of blood transfusion. Preoperative type and cross-match, and arterial line placement are not necessary for all patients undergoing surgery for hip dysplasia but should be employed for cases with a high risk of blood loss. Based on the increasing recommendation to implement PBM protocol in paediatric surgical patients, we suggest that our risk stratification model could be used to guide the implementation of these initiatives (e.g., preoperative identification and treatment of anaemia, routine use of antifibrinolytics and cell salvage) in patients that would benefit the most from the measures implemented.

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A.C.A. and A.C. conceptualized and designed the study, drafted the initial manuscript and reviewed and revised the manuscript. B.E.B. and H.-Y.N. helped with data collection and manuscript revision. B.H.N. provided statistical analysis and manuscript revision. L.A.H.H. and S.B.R. helped in drafting the manuscript, reviewed and revised the manuscript.

CONFLICT OF INTEREST

The authors have nothing to disclose.

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

Blood transfusion is associated with increased mortality for neonates with congenital diaphragmatic hernia on extracorporeal membrane oxygenation support

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Abstract

Background and Objectives: Blood transfusion is frequently needed to maintain adequate haemostasis and improve oxygenation for patients treated with extracorporeal membrane oxygenation (ECMO). It is more so for neonates with immature coagulation systems who require surgical intervention such as congenital diaphragmatic hernia (CDH) repair. There is growing evidence suggesting an association between blood transfusions and increased mortality. The aim of this study is to evaluate the association of blood transfusions during the peri-operative period of CDH repair, among other clinical parameters, with mortality in neonates undergoing on-ECMO CDH repair.

Materials and Methods: We performed a single centre retrospective chart review of all neonates with CDH undergoing on-ECMO surgical repair from January 2010 to December 2020. Logistic regression was used to investigate associations with survival status.

Results: Sixty-two patients met the inclusion criteria. Platelet transfusions (odds ratio [OR] 1.42, 95% confidence interval [CI]: 1.06–1.90) in the post-operative period and ECMO duration (OR 1.17, 95% CI: 1.05–1.30) were associated with increased mortality. Major bleeding complications had the strongest association with mortality (OR 10.98, 95% CI: 3.27–36.91). Gestational age, birth weight, Apgar scores, sex, blood type, right versus left CDH, venovenous versus venoarterial ECMO and duration of ECMO before CDH repair and circuit change after adjusting for ECMO duration were not significantly associated with survival.

Conclusion: Platelet transfusion in the post-operative period and major bleeding are associated with increased mortality in CDH neonates with surgical repair. The data suggest a need to develop robust plans for monitoring and preventing coagulation aberrancies during neonatal ECMO support.

Keywords

blood transfusion, CDH, ECMO, haemorrhage, mortality

Highlights

- Congenital diaphragmatic hernia (CDH) is the most common indication for extracorporeal membrane oxygenation (ECMO) in neonates, which requires anticoagulation to maintain the normal function of ECMO and prevent thrombotic complications.
- To balance coagulopathy and the need for anticoagulation, red blood cell and platelet transfusions are often needed.
- Major bleeding and platelet transfusions in the post-operative period were associated with increased mortality for neonates on ECMO for CDH repair.

INTRODUCTION

Congenital diaphragmatic hernia (CDH) affects 2.6 per 10,000 total births with significant morbidity and mortality of about 37.7% [1]. CDH results from embryologically incomplete fusion of the diaphragm, allowing foetal abdominal viscera to migrate into the thoracic cavity, resulting in lung hypoplasia and persistent pulmonary hypertension. If conventional treatment with gentle ventilation and optimized vasoactive medication fails to stabilize patients, extracorporeal membrane oxygenation (ECMO) may be required. CDH is the most common indication for ECMO in neonates [2].

Initiation of ECMO in neonates can lead to coagulopathy through multiple mechanisms. These include inflammatory and prothrombotic responses secondary to exposure to foreign surfaces, mechanical stress, turbulent flow, and so on, with a subsequent risk of clotting the circuit components [3]. The dilutional effects of the extracorporeal blood volume, in conjunction with an immature coagulation system, pose an additional challenge in maintaining haemostasis. Anticoagulation is necessary to maintain the normal function of the ECMO circuit and prevent thrombotic complications. For balancing coagulopathy and the need for anticoagulation, blood transfusions are often needed, to prevent and mitigate major bleeding complications.

Transfusion adds to the financial burden of patient care and carries potential complications. Greater volumes of red blood cell (RBC) transfusions were reported to be independently associated with increased mortality in infants with ECMO support [4]. Increased mortality has also been reported with platelet transfusion for paediatric patients on ECMO support [5, 6].

Optimal transfusion thresholds for paediatric patients with ECMO support have not been well established. At our institution, transfusion medicine is directly involved with the transfusion and coagulation management of ECMO patients. We have developed institutional guidelines for platelet and fibrinogen targets and the heparin therapeutic range. Multiple assays, including Heparin level via anti-factor Xa (anti-Xa) assay, antithrombin, prothrombin time/International Normalized Ratio (INR), and activated partial thromboplastin time (aPTT), are used to guide transfusion and coagulation management. Coagulation status is routinely monitored using an ECMO coagulation panel, including the above-mentioned parameters [7]. In this study, we reviewed the transfusion record of CDH patients undergoing surgical repair while on ECMO support over a period of 11 years. Our aim was to investigate the relationship between peri-operative blood transfusion and mortality before hospital discharge.

MATERIALS AND METHODS

Neonates with CDH requiring ECMO support at Texas Children's Hospital in Houston between 1 January 2010 and 31 December 2020 were identified through the institutional ECMO database. Patients with ECMO support discontinued before or initiated after CDH repair surgery were excluded. All patients having CDH repair while on ECMO support were included, regardless of gestational age or birth weight. Patient information was gathered from electronic medical records. No patient was known to have a congenital bleeding disorder. Transfusion records were obtained from blood bank transfusion audit software. The institutional review board at Texas Children's Hospital approved this study.

Our institution-specific criteria for ECMO support in CDH patients are severe hypoxic respiratory failure characterized by an oxygenation index >40 on two separate measurements, pO_2 persistently <40 mmHg or serum lactate >3.0 mmol/L. Both venovenous (VV) and venoarterial (VA) ECMO were utilized. VA ECMO was utilized when there was underlying cardiac dysfunction necessitating myocardial support or when VV cannulation was not feasible due to the small vessel size.

The CDH severity was classified as mild, moderate, or severe based on prenatal magnetic resonance imaging (MRI) indices, including observed to estimated total foetal lung volume and percentage of intrathoracic liver herniation, as reported previously [8].

Blood priming was used for all circuits. RBCs used for ECMO circuit priming were leukoreduced and less than 3 days old. For RBCs transfusion, occasionally, more than 1 week old but less than 14 days old RBCs were used to minimize the numbers of donor exposure. All platelets used were single donor apheresis units, suspended in donor plasma with no additive solution, neither were they pathogen reduced. The plasma used was either fresh frozen plasma or thawed plasma. Our coagulation targets and management have not changed significantly over the 11-year study period. We do not have a universal haematocrit target, although typically, haematocrit is maintained at >40%. Transfusion of RBCs is at the discretion of the attending physician. Other transfusion targets are as follows: platelet count > 100,000/μl, fibrinogen > 200 mg/dl, heparin level (anti-Xa) 0.2–0.5 IU/ml, antithrombin > 80%, INR < 1.6 and aPTT

between 60–90 s [7]. Antithrombin was replaced according to the target level. Occasionally, bivalirudin was used instead of heparin if there was a high dosage requirement for heparin (>40 U/kg/h) or thrombotic complications despite having therapeutic heparin levels. Our institutional guideline on the volume of blood transfusion in the neonatal period is between 5–10 ml/kg for RBCs and platelets, 10–15 ml/kg for plasma and 1–2 units/10 kg for cryoprecipitate. Blood transfusion data were collected as transfusion events instead of volume, and the date of the transfusion events relative to the ECMO initiation date and CDH repair surgery date was recorded.

Transfusion that occurred on the day of ECMO initiation to the day prior to CDH repair surgery is defined as the 'pre-operative (pre-op)' period. Transfusion that occurred on the day of surgery to 3 days post-surgery is defined as the 'post-operative (post-op)' period. Major bleeding is defined as (1) bleeding requiring surgical intervention, (2) Grade III or IV intracranial haemorrhage, or (3) at least two transfusion events directly necessitated by the bleeding episode [9, 10]. Major bleeding episodes were recorded during the entire hospital stay, regardless of whether the patient was on or not on ECMO when it happened. Major bleeding is categorized as follows: (1) Surgery-related bleeding, which is defined as bleeding at surgical sites within 3 days post-operatively; (2) Severe intracranial haemorrhages, which are defined as grade III or IV intracranial haemorrhages. Aside from these two major categories of bleeding, other bleeding events are grouped by sites in addition to two cases of bleeding after ECMO de-cannulation. Epsilon aminocaproic acid (Amicar™) or tranexamic acid was routinely administered to all patients for 24–48 h peri-operatively. Anticoagulation was turned down approximately 2 h before the surgical incision and resumed 4–6 h after surgery at a lower rate. This was determined based on intraoperative events at the medical team's discretion.

Statistics

SAS version 9.4 (SAS Institute, Cary, NC) was used for data analysis. Categorical variables were described as frequencies and percentages. Continuous variables were described using the mean ± SD if they followed a normal distribution; otherwise, the median and interquartile range (IQR) was used for variables exhibiting a significant departure from normality. The Shapiro–Wilk test was used to assess normality. Quantitative variables were compared between groups using the Wilcoxon rank-sum test, and associations between quantitative variables were analysed using Spearman's rank correlation. Logistic regression analysis was used to investigate associations of individual predictors with survival status. Additionally, multivariable logistic regression models were used to investigate associations of pre-op blood product transfusions with mortality after controlling for ECMO duration. For logistic regression analysis, one predictor can be included in the multivariable regression model for every 10 events in the smallest outcome category [11]. Since 39 patients survived to discharge and 23 died in this study, there were more than 10 observations in the smallest outcome category, so the sample size was adequate to include two predictors in the logistic regression model.

TABLE 1 Patient characteristics and associations with mortality

Characteristic	Survived ($n = 39$)	Deceased ($n = 23$)	Odds ratio (95% CI)	p-Value
Gestational age[a] (weeks)	37.7 (35.6, 39.1)	38.1 (37.0, 39.1)	1.18 (0.91–1.52)	0.22
Birth weight[b] (kg)	2.9 ± 0.7	2.6 ± 0.6	0.53 (0.77–1.21)	0.13
Female sex[c]	14 (35.9)	7 (30.4)	1.78 (0.26–2.35)	0.66
1 min Apgar[a]	3 (1, 6)	3 (3, 6)	1.19 (0.92–1.55)	0.19
5 min Apgar[a]	7 (5, 8)	7 (6, 8)	1.20 (0.88–1.65)	0.24
Blood type[c]				
A/AB	15 (38.4)	8 (34.8)	0.80 (0.26–2.47)	0.89
B	6 (15.4)	3 (13.0)	0.75 (0.16–3.59)	0.82
O	18 (46.2)	12 (52.2)		
VA ECMO mode[c]	31 (79.5)	18 (78.3)	0.93 (0.26–3.27)	0.91
Left-sided CDH[c]	30 (76.9)	20 (87.0)	2.0 (0.48–8.31)	0.34
ECMO duration before repair surgery[a] (day)	1 (1, 2)	2 (1, 2)	1.7 (0.89–3.24)	0.11
Total duration of ECMO[a] (day)	7 (5, 11)	13 (9, 16)	1.17 (1.05–1.30)	0.005
Circuit change[c,d]	13 (33.3)	14 (60.7)	3.11 (1.07–9.07)	0.038
Bleeding during CDH repair surgery[a]	15 (5, 30)	20 (12.5, 45)	1.01 (0.99–1.03)	0.33
Major bleeding during hospital stay[c]	8 (20.5)	17 (73.9)	10.98 (3.27–36.91)	<0.001

Abbreviations: CDH, congenital diaphragmatic hernia; CI, confidence interval; ECMO, extracorporeal membrane oxygenation; VA, venoarterial.
[a]Median (interquartile range), logistic regression p-value.
[b]Mean ± SD, logistic regression p-value.
[c]Frequency (%), logistic regression p-value.
[d]Circuit change not associated with survival after adjusting for ECMO duration.

RESULTS

During the study period, 62 neonates with CDH surgical repair while on ECMO were identified. All but two CDH repairs occurred within 3 days after ECMO cannulation. Two repairs occurred on Day 4 of ECMO cannulation. All CDH repairs occurred within 3 weeks of birth. All but one patient had transabdominal Gore-Tex patch repair. This patient was repaired without a patch. MRI-based CDH severity classification was as follows: 4/62 (6.5%) mild, 22/62 (35.5%) moderate, 32/62 (51.6%) severe. MRI indices were not available for four patients. Overall, the vast majority of patients were in the moderate to severe CDH category based on MRI indices. Thirty-nine out of 62 patients (62.9%) survived till hospital discharge.

Patient characteristics and their associations with mortality are shown in Table 1. Longer ECMO duration (odds ratio [OR] 1.17, 95% confidence interval [CI]: 1.05–1.30), major bleeding (OR 10.98, 95% CI: 3.27–36.91) and circuit change (OR 3.11, 95% CI: 1.07–9.07) were associated with increased mortality. However, longer ECMO duration predisposes to circuit change. After adjusting for ECMO duration, circuit change was not associated with survival (OR 1.17, 95% CI: 0.30–4.63). No other patient characteristics were associated with survival status.

Blood transfusions in the pre-operative period were not associated with survival when adjusted for pre-op ECMO duration (Table S1). Only platelet transfusion in the post-operative period (from the day of surgery to 3 days after surgery) was associated with decreased survival (OR 1.42, 95% CI: 1.06–1.90). In the pre-op period, increased cryoprecipitate transfusions were associated with decreased survival before adjusting for pre-op ECMO duration (0–4 days) (OR 2.15, 95% CI: 1.06–4.38, Table 2). Transfusion events on the day of surgery were not associated with survival. No other blood component was associated with survival in the post-op period. A similar analysis was done for the duration from the day of surgery to 1 day and 2 days post-op, respectively. And similarly, only increased platelet transfusion was associated with decreased survival (Table S1).

Overall, major bleeding occurred in 40.3% (25 out of 62) patients. The median (IQR) time of major bleeding relative to the day of CDH surgery was Day 3 (0.5–8). Forty-four percent (11 out 25) of major bleeding episodes occurred from the day of surgery to 3 days post-surgery. The

TABLE 2 Transfusion and associations with mortality

Blood product[a]	Survived ($n = 39$)	Deceased ($n = 23$)	Odds ratio (95% CI)	p-Value
Pre-op RBC	4 (4, 6)	5 (4, 9)	1.15 (0.97–1.37)	0.11
Pre-op platelets	2 (1, 3)	2 (1, 3)	1.31 (0.98–1.75)	0.07
Pre-op plasma	2 (1, 4)	3 (2, 4)	1.12 (0.92–1.37)	0.27
Pre-op cryoprecipitate	1 (0, 1)	1 (1, 2)	2.15 (1.06–4.38)	0.03
Op day RBC	2 (2, 4)	2 (2, 4)	1.07 (0.81–1.42)	0.62
Op day platelets	2 (1, 2)	2 (1, 3)	1.78 (0.93–3.39)	0.08
Op day plasma	1 (1, 3)	2 (1, 3)	1.11 (0.83–1.49)	0.48
Op day cryoprecipitate	0 (0, 1)	0 (0, 1)	1.36 (0.70–2.64)	0.36
Op + 3 days post-op RBC	7 (5, 9)	7 (6, 13)	1.06 (0.94–1.19)	0.38
Op + 3 days post-op platelet	5 (4, 6)	7 (5, 8)	1.42 (1.06–1.90)	0.02
Op + 3 days post-op plasma	2 (1, 5)	3 (2, 7)	1.14 (0.98–1.33)	0.10
Op + 3 days post-op cryoprecipitate	1 (0, 2)	1 (0, 2)	1.29 (0.79–2.10)	0.31

Abbreviations: CI, confidence interval; RBC, red blood cell.
[a]Median (interquartile range), logistic regression p-value.

TABLE 3 Categories of major bleeding events and managements

Category	Count (%)	Management
Severe intracranial haemorrhage	8 (32.0%)	All cases led to subsequent ECMO decannulation
Surgery-related bleeding	7 (28.0%)	Four cases by medical management and transfusion, three cases by exploratory laparotomy
Abdominal bleeding	3 (12.0%)	One case by medical management and transfusion, two cases by exploratory laparotomy
Thoracic bleeding	3 (12.0%)	One case by medical management and transfusion, one case by thoracotomy, one case of pulmonary haemorrhage by bronchoscopy
Pericardial bleeding	1 (4.0%)	One case by pericardiotomy and sternotomy
ET tube and mouth	1 (4.0%)	One case by medical management and transfusion
Post-ECMO decannulation	2 (8.0%)	One case of haemothorax needing chest tube placement and transfusion, one case of tracheostomy site bleeding by medical management and transfusion
Total	25 (100%)	

Abbreviations: ECMO, extracorporeal membrane oxygenation; ET, endotracheal.

different categories of major bleeding and their management are listed in Table 3. Severe intracranial haemorrhage (32.0%) and surgery-related bleeding (28.0%) account for 60.0% of all cases with major bleeding. Interestingly, gestational age ($p = 0.81$) and birth weight ($p = 0.88$) of the patients with severe intracranial bleeding do not differ from the rest of the patients, suggesting that prematurity is not a significant contributor to severe intracranial haemorrhage in our study cohort.

Since both major bleeding and post-op platelet transfusion was associated with survival, we analysed whether increased post-op platelet transfusion was secondary to haemorrhages. However, the association between post-operative platelet transfusion and major bleeding was not statistically significant (OR 1.30, 95% CI: 0.94–1.73).

The severity of CDH was not significantly associated with mortality (OR 1.31, 95% CI: 0.54–3.12), major bleeding (OR 1.60, 95% CI: 0.65–3.93), or ECMO duration ($r = 0.07$, $p = 0.62$). Among the pre-op and post-op blood transfusions, only the number of post-op platelet transfusions was weakly associated with CDH severity ($r = 0.263$, $p = 0.046$).

DISCUSSION

In this study, we investigated the association between peri-operative blood transfusions and survival of neonates with on-ECMO CDH repair. This is a vulnerable patient population as both ECMO and repair with a patch (except for one patient) were necessary, predisposing them to a higher risk of surgical complications [12]. And yet, the overall survival rate before hospital discharge was at 62.9%, comparable to or even superior to previously reported data with early on-ECMO CDH repair [13, 14].

Notably, patients with major bleeding had nearly 11 times higher odds of death. The inverse relationship between major bleeding and survival in CDH patients has been reported in multiple studies [12, 15, 16]. Of those with major bleeding, the most common were severe intracranial haemorrhage (32.0%) and surgical bleeding (28.0%). The incidence of surgery-related bleeding in the entire study sample was 11.3% (7/62), comparable to published data on CDH repair while on ECMO support [12, 17, 18]. The overall rate of severe intracranial haemorrhage was 12.9% (8/62), also comparable to published data [19, 20]. Once major bleeding occurs, there is often a vicious cycle of transfusion, fluid overload, haemodynamic swings and increased risk of secondary complications. Here, when significant bleedings happened, aPTT, as well as anti-Xa, were adjusted towards the lower end of the target range, with or without raising platelet and fibrinogen targets toward 150,000/μl and 300 mg/dl, respectively, depending on the clinical condition. Recombinant factor VIIa or prothrombin complex concentrates were not used while patients were on ECMO. In addition, severe intracranial bleeding often leads to ECMO de-cannulation and re-direction of care. All these factors contribute to the association between major bleeding and increased mortality.

The association between increased post-op platelet transfusion and decreased survival was statistically significant. Some platelet transfusions may reflect increased platelet needs secondary to major bleeding. It is also possible that increased platelet transfusion further contributes to increased mortality. A recent multicentre randomized control trial compared the effect of prophylactic platelet transfusion in preterm infants with severe thrombocytopenia; 660 preterm infants were assigned to receive prophylactic platelet transfusion at a platelet count threshold of 50,000/μl (high-threshold) or 25,000/μl (low-threshold). Surprisingly, the high-threshold group had a significantly higher rate of death and major bleeding within 28 days after randomization than the low-threshold group [21]. Similarly, Kumar et al. [22] found that in thrombocytopenic preterm neonates, liberal platelet transfusion to maintain platelet count >100,000/μl was associated with increased Intraventricular hemorrhage compared with restrictive platelet transfusion (platelet count > 20,000/μl with active bleeding). Although the primary role of platelets is to maintain haemostasis, platelets also have immunologic and inflammatory effects [23]. Inflammatory consequences, as well as haemodynamic shifts related to platelet transfusion and fragility of germinal matrix, were proposed to contribute to the increased risk of haemorrhage. The studies mentioned above did not include patients on extracorporeal life support. For critically ill patients requiring ECMO support with anticoagulation, the risk for bleeding and platelet transfusion threshold was higher.

Of note, we did not find a significant association between platelet transfusions and major bleeding complications. Here, the transfusion threshold for all patients was set at 100,000/μl, in contrast to the very different transfusion thresholds in the two study arms in the previous trials [21, 22]. The fact that platelet transfusion was associated with decreased survival independent of major bleeding further supports that platelet transfusions may have directly contributed to mortality. The multiple biological effects platelets have other than haemostasis may explain the association between platelet transfusions and poor patient outcomes. In addition, adult and neonatal platelets are not identical functionally. Healthy full-term neonates have in vitro platelet hyporeactivity and yet show enhanced primary haemostasis compared with adults. Higher haematocrits, higher von Willebrand factor (vWF) concentrations and fractions of ultralong vWF multimers together with hyporeactive platelets create a balanced haemostatic system for neonates [23]. Infusing adult donor platelets into neonates creates developmental mismatch, may lead to unexpected clotting events. It is even more complex for patients on ECMO support due to the extracorporeal activation and consumption of both platelets and vWF.

Only cryoprecipitate transfusions were associated with mortality in the pre-op period before adjusting for pre-op ECMO duration. Overall, there was little variation in the number of cryoprecipitate transfusion events (IQR 0–1.3). Such a small variation in cryoprecipitate transfusion is unlikely to alter survival status. But it suggests the possibility of subclinical intravascular coagulation or circuit consumption. The cryoprecipitate transfusion in this study was mostly driven by the failure to maintain the fibrinogen target of >200 mg/dl rather than clinical bleeding. Hence, pre-op cryoprecipitate requirement may be an early marker for failure to maintain a coagulation balance prior to surgery.

Of note, there was only one case of major thrombotic complication with thrombus formation in the atrium and aorta with subsequent patient death. This patient had biventricular dysfunction, a major risk factor for thrombus formation. For the rest of the cases, minimal to significant clot burden was limited to ECMO circuit or thrombus in line-associated sites without significant end organ damage. Circuit change was the major thrombotic complication observed. The decision for circuit change was based on extensive clotting in the arterial or venous circuit, clotting in the membrane oxygenator that affected gas exchange, and/or significant haemolysis with plasma haemoglobin >150 mg/dl. After adjusting for ECMO duration, circuit change was not associated with survival status. Although a significant risk is associated with circuit change, ECMO duration is a better predictor of survival status. Morbidity and mortality due to documented thrombotic events were low. Despite the same initial anticoagulation targets, some patients bled, and some did not, suggesting patient-specific factors in bleeding tendency. Future studies are warranted to explore risk factors for major bleeding and to identify anticoagulation targets that balance bleeding and thrombotic risk.

All on-ECMO CDH repairs were carried out early, ranging from 0 to 4 days of ECMO prior to surgery. Pre-op ECMO duration was not associated with survival, agreeing with other reports [13]. While there are studies showing that gestational weight, birth weight, VV versus VA ECMO and left-sided versus right-sided CDH are associated with CDH survival [24, 25], we did not find similar associations. MRI-based CDH severity was not associated with survival status either, as opposed to previous reports [8]. However, those studies had different patient populations, and CDH may not be repaired with ECMO support. Eighty-four percent of our patients required ECMO within the first day of birth, and 10% on Day 2 of birth. The need for ECMO shortly after birth and the fact that 87% of patients were in the moderate to severe CDH category indicate our patient population were at the more severe end of the CDH spectrum. For this specific population, the above-listed factors do not significantly affect mortality.

To the best of our knowledge, this is the first study that investigates peri-operative transfusions and their associations with mortality in a relatively homogenous patient population, where transfusion and anticoagulation are guided by uniform institutional targets. In addition, surgical interventions were performed in similar manners. Therefore, our study provides important novel insights into the relationship between transfusion and survival status in a well-standardized setting.

This retrospective study has several limitations. Transfusion events instead of transfused volume were used to measure the associations between transfusions and mortality. The transfused volume may not directly correlate with transfusion events, although the majority of our transfusions for ECMO patients approximated 10–15 cc/kg. Also, ECMO duration was counted as days rather than hours. And transfusion events were tallied daily instead of based on discrete time points. It is possible there were transfusion events that occurred when patients were not actually on ECMO. But that would only account for a small proportion of total transfusion events, and the time lapse is estimated to be within a few hours. Thirdly, only major bleeding events were analysed. Mild to moderate bleeding was common, such as minor surgical site bleeding and bleeding from endotracheal tube placement or Replogle tube. The significance of these bleeding events is unaccounted for in this study.

In conclusion, major bleeding strongly increased the odds of death, with severe intracranial bleeding as the most frequent major bleeding event. No blood component other than platelet transfusions had a major impact on survival in ECMO patients. The ideal platelet transfusion threshold for neonatal ECMO patients awaits to be established. Haemostasis in this patient population needs to be carefully monitored and managed.

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CONFLICT OF INTEREST

There are no conflicts identified.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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A cross-sectional study of haemolytic disease of the newborn in Uganda

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Abstract

Background and Objectives: Haemolytic disease of the newborn (HDN) is an immune haemolytic anaemia from maternal alloantibodies. Rh immunoglobulin (RhIg) prophylaxis can prevent alloimmunization to the D antigen. However, RhIg is not universally available in Uganda. ABO incompatibility also causes HDN. We determined the prevalence of HDN among newborn infants with jaundice in Uganda.

Materials and Methods: We conducted a prospective cross-sectional study at Kawempe National Referral Hospital, Kampala, Uganda. Infants aged 0–14 days with neonatal jaundice (or total bilirubin >50 μmol/L) were enrolled. Clinical evaluation and laboratory testing, including ABO, RhD typing and maternal antibody screen, were performed.

Results: A total of 466 babies were enrolled. The mean (SD) age was 3.4 (1.5) days. Of newborn babies with jaundice, 17.2% (80/466) had HDN. Babies with HDN had lower haemoglobin (SD); 15.7 (2.7) compared with those without HDN; 16.4 (2.4) g/dL, $p = 0.016$; and a higher bilirubin (interquartile range); 241 (200–318) compared with those without HDN; 219 (191–263) μmol/L, $p < 0.001$. One baby had anti-D HDN, while 46/466 had HDN from an ABO incompatibility (anti-A 43.5% and anti-B 56.5%); 82% of babies with HDN also had suspected neonatal sepsis or birth asphyxia. About 79.2% (57/72) of mothers did not have ABO/Rh blood group performed antenatally. All infants with HDN survived except one.

Conclusion: Among newborn infants with jaundice, HDN is not rare. The majority is due to ABO HDN affecting group A and group B babies equally. Ensuring routine ABO/Rh grouping for all pregnant women is an area for improvement.

Keywords

ABO incompatibility, alloantibodies, HDN, newborn

Highlights

- The majority of haemolytic disease of the newborn (HDN) in Uganda is due to ABO HDN, affecting group A and group B babies equally.
- Neonatal sepsis and birth asphyxia are common comorbidities in HDN in Uganda that is found in 82% of babies with HDN.
- About 79.2% of mothers of babies with HDN did not have ABO blood group testing during their antenatal care.

INTRODUCTION

Haemolytic disease of the newborn (HDN) is an immune haemolytic anaemia resulting from the destruction of fetal and/or newborn red blood cells (RBCs) by maternal alloantibodies [1]. Although several RBC alloantibodies have the potential to cause HDN, severe disease occurs most frequently with RhD antibodies among RhD-positive babies born to RhD-negative mothers—often alloimmunized during previous pregnancies. Maternal alloantibodies of the immunoglobulin G (IgG) class can cross the placenta and coat fetal RBCs, resulting in erythrophagocytosis in the reticuloendothelial system, leading to extravascular haemolysis, anaemia and hyperbilirubinemia of varying severity [1–4]. Severe anaemia in-utero results in hydrops fetalis. Neonatal hyperbilirubinemia overwhelms the conjugating capacity of the newborn's immature liver, putting some infants at risk of developing kernicterus [4].

The burden of HDN varies in different jurisdictions. This is largely explained by differences in the population distribution of blood groups, as well as prenatal care policies, which have evolved over the past five decades [4, 5]. In particular, the prenatal and perinatal administration of Rh immunoglobulin (RhIg), as prophylaxis against RhD alloimmunization among RhD-negative women, has greatly contributed to the reduction of HDN due to anti-D [1]. In countries with universal access to RhIg prophylaxis, recent estimates suggest the incidence of HDN due to anti-D is less than 0.1% of pregnancies [6]. In resource-poor settings, however, RhIg prophylaxis is neither widely available nor financially accessible, and HDN due to anti-D is more commonly seen [7].

ABO antibodies frequently cause HDN, especially among group O women with higher titers of anti-A and anti-B of the IgG class. ABO HDN occurs among ABO-incompatible mother–baby pairs [1, 3, 4]. Although ABO HDN disease may often be mild, its burden across different populations varies, being higher in African populations [1]. Other RBC antibodies of the IgG class also cause HDN. The prevalence of HDN not due to ABO or Rh antibodies is low at about 1/5000 births. Nevertheless, alloantibodies, such as anti-c, anti-K and anti-E, have been reported to cause severe HDN [4, 6, 8–10]. The burden of HDN among newborn infants in East Africa is not known. The objective of this study was to estimate the prevalence and characteristics of HDN among newborn infants with jaundice in an urban setting in Uganda.

MATERIALS AND METHODS

Study design

This was a descriptive cross-sectional study conducted at Kawempe National Referral Hospital, Kampala, Uganda, between March and December 2021.

Study setting

Kawempe National Referral Hospital is a public healthcare facility and one of the teaching hospitals for Makerere University College of Health Sciences. The hospital is located in the Kawempe division, about 5 km north of Kampala city. With a bed capacity of about 170, the facility provides maternal, newborn and child healthcare services to patients from Kampala and neighbouring districts, as well as referrals from across the country. The hospital has a labour ward where about 50–70 babies are birthed daily, a maternal and fetal unit, a postnatal ward, a gynaecology emergency ward, a general gynaecology ward, outpatient clinics, a paediatric ward and a newborn unit [11].

Screening and eligibility

Infants were pre-screened for potential study eligibility by checking their case notes. Pre-screening eligibility criteria included: admission to the neonatal intensive care unit (NICU) of Kawempe National Referral Hospital, age 0–14 days and a clinical diagnosis of neonatal jaundice (and/or total bilirubin >50 μmol/L). Initially, verbal consent was obtained from the parent to perform a direct antiglobulin test (DAT) on the infant's RBCs, haemoglobin level and bilirubin estimation on the newborn. Babies with neonatal jaundice whose DAT was positive were considered to have HDN. Written informed consents from the mothers of babies with positive DATs were subsequently obtained (this included consent for a blood draw from the mother). Mother–baby pairs with a positive DAT were evaluated. We excluded newborns who had received a blood transfusion prior to DAT testing.

Sample size

The sample size of 466 newborns was estimated using a sample size calculation for one-sample proportion based on confidence interval (CI) and margin of error (interval width) [12]. Using a presumed prevalence of 2.2% for mothers with alloantibodies capable of causing HDN from a study by Natukunda et al. [13] and a margin of error of ±1.5% around the 95% CI, the minimum required sample size was 368. An interval width of ±1.5% implied a lower and upper interval of the 95% CI at 1.7% and 3.7%. We favoured a wider interval since less information was known about the disease locally, plus an expected 10% loss to follow-up.

Study variables and data collection

Clinical evaluations were performed and recorded on a structured paper case-report form for both the baby and mother and included the past medical history, physical examination and laboratory testing. For the baby, the variables included: date, place and type of birth, age, sex, the onset of jaundice, presenting complaints, clinical diagnoses, treatment provided, discharge date and hospitalization outcome. Data for the mother included: prenatal history, past obstetric history, past medical history and social history.

Laboratory measurements

A quantity of 2 ml of blood was collected in an EDTA tube from the baby and 4–5 ml of blood in an EDTA tube from the mother. The following tests were performed. For the baby: A DAT was performed by the tube method, using a polyspecific murine monoclonal blend of anti-human globulin (anti-IgG, -C3d) (Immucor Inc., Norcross, GA, USA). The test was run in parallel with negative control and all negative test results were read microscopically or validated using commercial check-cells (whenever available); haemoglobin concentration using a point-of-care device (Hemocue® 201, Angelholm, Sweden); ABO (forward), and RhD blood typing (tube method) using commercial monoclonal antisera; transcutaneous bilirubin (TcB) using a point-of-care jaundice detector; MBJ20 (M&B Electronic Instruments Co., Ltd, Beijing, China); Lui-Freeze Thaw elution tests were performed on a subsample ($n = 20$) of suspected ABO HDN to confirm the presence of anti-A, or anti-B using pools of A cells and B cells prepared in-house. Maternal blood testing included ABO and RhD typing, and a 3-cell antibody screen (indirect antiglobulin test), using commercial reagent RBCs (Immucor Inc.). We considered an infant to have HDN if presented with neonatal jaundice, had a total bilirubin >50 μmol/L and a positive DAT.

Data management and statistical analysis

Data were entered into EPI-DATA version 3.1 software package (The EpiData Association, Odense, Denmark) and analysed using STATA v14.0 (Stata, College Station, TX, USA). The primary outcome was the proportion of jaundiced infants with a positive DAT. For descriptive statistics, proportions were used to show the distribution of demographic factors in affected infants. We present means (SD) of symmetrical continuous variables and medians (interquartile range) for asymmetrical continuous variables. Cross-tabulation was done for sub-group comparisons of the clinical characteristics of the disease. Using logistic regression, we examined categorical covariates associated with ABO HDN and assessed odds ratios and statistical significance. In all, 95% test-based CIs for odds ratios and p-values are presented. A $p < 0.05$ was considered statistically significant.

Ethics statement

We obtained ethical clearance from the Research and Ethics Committee of Makerere University School of Medicine (ref # 2020–214) and the Uganda National Council for Science and Technology (HS-1089ES).

RESULTS

We enrolled a total of 466 babies with jaundice. Overall, the mean age (SD) of babies was 3.4 (1.5) days. Of these, 17.2% (80/466) of newborns with jaundice had a positive DAT and were considered to have HDN. Babies with HDN had a mean (SD) haemoglobin of 15.7 (2.7) g/dl compared with 16.4 (2.4) g/dl among those without HDN, a difference, which was statistically significant, $p = 0.016$. Similarly, babies with HDN had higher bilirubin (μmol/L), median (interquartile range) of 241 (200.5–318) compared with 219 (191–263) μmol/L among those without HDN, $p < 0.001$ (Table 1).

Primary outcome

Of newborns with jaundice, 17.2% (80/466) had HDN. Eight of the mothers declined to have their blood drawn, leaving a total of 72 mother–baby pairs that we evaluated (Tables 2 and 3). Of note, neonatal sepsis and birth asphyxia were the most frequent comorbid

TABLE 1 Baseline characteristics of babies with neonatal jaundice ($n = 466$)

Characteristic	Overall	Positive DAT		p-value
		Yes	No	
Overall	466 (100)	80 (17.2)	386 (82.8)	
Age, in days				
Mean (SD)	3.4 (1.5)	3.2 (1.2)	3.5 (1.6)	0.230
Hgb (g/dl)				
Mean (SD)	16.3 (2.5)	15.7 (2.7)	16.4 (2.4)	0.016
Bilirubin (μmol/L)				
Median (IQR)	222 (192–273)	241 (200.5–318)	219 (191–263)	<0.001[a]
Maturity (column %)				
Term	461 (98.9)	80 (100.0)	381 (98.7)	0.594[b]
Preterm	5 (1.1)	0 (0.0)	5 (1.3)	

Abbreviations: DAT, direct antiglobulin test; Hgb, Haemoglobin; IQR, interquartile range; SD, standard deviation.
[a]Statistical significance at 5%.
[b]p-value from a Fisher's exact test.

TABLE 2 Baseline characteristics of babies with haemolytic disease of the newborn ($n = 72$)

Variable	Summary statistics
Age in days—mean (SD)	3.4 (1.2)
Birth weight (kg)—mean (SD)	3.2 (0.4)
Haemoglobin (g/dl)—mean (SD)	15.8 (2.8)
Bilirubin (μmol/L)—median (IQR)	236 (200–320)
Variable	**Number (%)**
Sex	
Male	45 (62.5)
Female	27 (37.5)
Type of birth	
Vaginal	37 (51.4)
Caesarean section	33 (45.8)
Instrumental	2 (2.8)
Major presenting complaint (history) (yes)	
Jaundice	71 (98.6)
Fever	11 (15.3)
Inability to suckle	28 (38.9)
Treatment received during hospitalization (yes)	
Antibiotics therapy	72 (100)
Anticonvulsants	13 (18.1)
Phototherapy	50 (69.4)
Primary diagnosis/comorbidity (besides jaundice)	
Neonatal sepsis	29 (40.3)
Birth asphyxia	30 (41.7)
RDS/TTN	2 (2.8)
Hyperbilirubinemia—unspecified	10 (13.9)
ABO blood type	
A	26 (36.1)
B	30 (41.7)
O	14 (19.4)
AB	2 (2.8)
RhD type	
Positive	70 (97.2)
Negative	2 (2.8)

Abbreviations: IQR, interquartile range; RDS, respiratory distress syndrome; SD, standard deviation; TTN, transient tachypnoea of the newborn.

TABLE 3 Characteristics of mothers of babies with haemolytic disease of the newborn ($n = 72$)

Variable	Summary statistics
Mother's age in years—mean (SD)	24 (4.5)
Variable	**Number (%)**
Marital status	
Married	60 (83.3)
Engaged	7 (9.7)
Single	5 (6.9)
Mother's occupation	
Unemployed/housewife	35 (48.6)
Self-employed or business/trader	27 (37.5)
Private worker	8 (11.1)
Civil servant	2 (2.8)
Mother's parity	
One	35 (48.6)
Two	20 (27.8)
Three	13 (18.1)
Four	3 (4.2)
Five	1 (1.4)
Number of antenatal visits during pregnancy	
≥Four	56 (77.8)
Three	6 (6.9)
Two	9 (12.5)
One	1 (1.4)
None	1 (1.4)
ABO typing performed during antenatal	
No	57 (79.2)
Yes	15 (20.8)
Mother's ABO blood type	
A	7 (9.7)
B	7 (9.7)
O	58 (80.1)
Mother's RhD type	
Positive	70 (97.2)
Negative	2 (2.8)
Antibody screening of mother's plasma	
Positive	1 (1.4)
Negative	68 (94.4)
Not performed (insufficient sample)	3 (4.2)

conditions in babies with a positive DAT (82%). The majority, $n = 50$ (69.4%), of babies with HDN evaluated received phototherapy.

One infant born to an RhD-negative and alloimmunized mother had anti-D HDN. This was a male term baby, with a 3.8 kg birth weight. Two days after birth, the baby was admitted to the NICU because of jaundice and birth asphyxia; Hb 19.7 g/dl, TcB 204 μmol/L and DAT 3+. The mother was B negative and the baby O positive. The 3-cell antibody screen of the mother's plasma was positive in a pattern consistent with anti-D. The baby received phototherapy, for two days, intravenous antibiotics, and recovered fully. At follow-up, 30 days following discharge, the baby was well. The mother was a 26-year-old woman, G3P2. Her two previous babies are alive, and neither had neonatal jaundice. Although she received prenatal care during pregnancy, no ABO/RhD typing or antibody screening was performed. Also, she did not receive RhIg during her prior pregnancies.

In contrast, ABO HDN was found in 46/466 jaundiced babies. The distribution of blood types A and B, among babies with ABO

TABLE 4 Baby's haemolytic disease of the newborn (HDN) disease category

HDN disease category	Number (%)
Anti-D HDN	1 (1.4)
ABO HDN	46 (63.9)
Blood type of ABO-incompatible babies	
Group A	20 (43.5)
Group B	26 (56.5)
HDN from unidentified alloantibody/ or HDN not confirmed	25 (34.7)

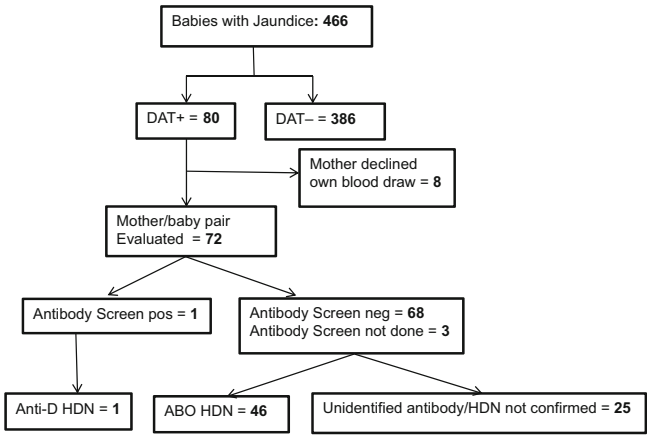

FIGURE 1 Flow diagram of study participants. DAT, direct antiglobulin test; HDN, haemolytic disease of the newborn

HDN was similar, 43.5% (20) and 56.5% (26), respectively, and not statistically different ($2 \times 2\ \chi^2$ test is 0.902; $p = 0.342$). The mothers' blood type of all ABO HDN babies was O. One infant with ABO HDN died during hospitalization, while the rest survived (Tables 2 and 4, and Figure 1).

The infant who died was admitted to the NICU with hyperbilirubinemia, difficulty in breathing and inability to suckle and cry; Hb 16.1 g/dl, TcB 232 μmol/L and DAT 2+. ABO/RhD types of mother and baby were O positive and A positive, respectively. The antibody screening of the mother's plasma was negative. The baby was born by vaginal birth, with a birth weight of 3.36 kg and an APGAR score of 3/10 at birth. The baby received phototherapy, oxygen, intravenous antibiotics and anticonvulsants but died on the fourth day of life. The mother was a 25-year-old woman, G2P2; her previous baby was alive and did not have neonatal jaundice. Although she received prenatal care during pregnancy, no ABO/RhD typing had been performed previously.

Although the majority of mothers had attended antenatal care, only 20.8% (15/72) had their ABO and Rh blood group tested during antenatal visits (Table 3).

Logistic regression (bivariable) analysis

Babies with ABO HDN were more likely to have a strong DAT; that is, DAT test score of >2+ than those in the category 'unidentified antibody/HDN not confirmed'; crude OR (95% CI), 13.3 (2.49, 36.39), $p < 0.001$. We found a 55.3 μmol/L (95% CI [−94.1, −16.4], $p = 0.006$) mean difference in bilirubin levels between ABO HDN and

TABLE 5 Bivariable associations with ABO haemolytic disease of the newborn (HDN)

Variable	Study category: ABO HDN $N = 46$	Study category: HDN not confirmed and probable unidentified antibody/C3d $N = 25$	Crude odds ratio (95% CI)	*p*-value
Haemoglobin: mean (SD)	15.7 (2.8)	15.8 (2.7)	n/a	0.945
Bilirubin: median (IQR)	282 (201–340)	219 (196–236)	n/a	0.010
Sex, *n* (%)				
Male	30 (68.2)	14 (31.8)	1.00	
Female	16 (59.3)	11 (40.7)	0.67 (0.25, 1.84)	0.304[a]
History of fever, *n* (%)				
No	37 (61.7)	23 (38.3)	1.00	
Yes	9 (81.8)	2 (18.2)	2.79 (0.55, 14.10)	0.174[a]
Inability to suckle, *n* (%)				
No	29 (67.4)	14 (32.6)	1.00	
Yes	17 (60.7)	11 (39.29)	0.37 (0.27, 2.01)	0.562
DAT strength				
≤1[a]	20 (47.6)	22 (52.38)	1.00	
≥2[a]	26 (89.6)	3 (10.3)	13.3 (2.49, 36.39)	<0.001

Abbreviations: CI, confidence interval; DAT, direct antiglobulin test; IQR, interquartile range; n/a, not applicable.
[a]*p*-value from Fisher's exact test.

the 'unidentified antibody/HDN not confirmed'. This was statistically significant; however, the mean difference in haemoglobin was not statistically significant; 0.05 g/dl (95% CI [−1.35, 1.44], $p = 0.945$) (Table 5).

DISCUSSION

The aim of this study was to determine the prevalence and characteristics of HDN among newborn infants with jaundice attending an urban national referral hospital in Uganda. We present findings on HDN in a setting where RhIg prophylaxis is not generally available.

The results of our study suggest that 17.2% of newborn babies with jaundice in Uganda had HDN—in a setting where approximately 22% of all newborn babies present with neonatal jaundice [14]. We found one baby with anti-D HDN, while 46/466 (i.e., 1 in 10) had ABO HDN. The ABO HDN cases were slightly more common in group B than in group A (26 vs. 20), but this was not statistically different. These findings are in contrast to studies among Caucasians that have reported fewer cases of ABO HDN among B infants [1]. Indeed our study findings align with the literature about ABO HDN in African populations [1]. A study in Zimbabwe found that 85% of HDN were from ABO incompatibility [7].

The disease categories, in particular anti-D HDN and ABO HDN, along with their breakdown in groups A and B, can be explained by the local distribution of RBC antigens. The proportion of RhD-negative individuals in Uganda is 5% [12], while that of blood groups A, B, AB and O in Uganda was 21%, 24%, 4% and 49%, respectively (Unpublished–Uganda Blood Transfusion Services). Our study identified only one baby with anti-D HDN in a setting where theoretically, we may expect a higher anti-D HDN because RhIg prophylaxis is not generally available. This finding can be explained by two factors: the evidence that ABO incompatibility between mother and baby can reduce the risk of alloimmunization to RhD [1] and that Uganda has a lower frequency of RhD-negative individuals.

Mainly, babies with HDN presented with lower haemoglobin, and higher total bilirubin levels, compared to those without HDN. These findings align with the pathophysiological mechanisms of the disease [4]; however, in 25/72 cases, the majority (22/25) with weak DAT (i.e., ≤1+ strength), we could neither identify an antibody nor confirm HDN. Some babies (30%) with HDN did not receive phototherapy, probably due to space and equipment restrictions at this busy national referral facility. Nevertheless, all babies with HDN recovered fully except one.

We found that although the majority of the mothers attended antenatal care, approximately 80% did not have their ABO/Rh blood group tested antenatally. These findings are similar to previous research in Uganda that has shown that basic ABO and RhD blood typing—the best opportunity to identify RhD-negative mothers, is not routinely performed for the majority of women attending prenatal services in Uganda [15]. This represents a policy-service gap in healthcare since, in its policy goal for maternal and newborn care, Uganda recommends blood group testing during antenatal visits [16]. Moreover, the same policy does not mention RhIg prophylaxis for RhD-negative mothers. Despite the strong evidence base for RhIg prophylaxis in preventing RhD-related HDN, for resource-poor countries, such as Uganda, such weaknesses in antenatal-care services and policies, compounded by the lack of universal access to RhIg prophylaxis and the high parity of African parturients, put many infants at risk of HDN.

In our study, 82% of infants with HDN also had neonatal sepsis and birth asphyxia—the two leading cause of neonatal morbidity and mortality in these settings [17]. The coexistence with these severe disease entities may mask HDN, resulting in its misdiagnosis and underreporting. In addition, these comorbidities can both contribute to hyperbilirubinemia, which may further complicate outcomes. The case in point is the newborn in the current study who died; this newborn had both birth asphyxia and HDN.

The literature suggests that HDN caused by ABO incompatibility is a mild disease. The current study findings, however, suggest that this concept needs to be understood in context and with caution, especially in resource-poor African settings, for two reasons. First, HDN caused by ABO incompatibility may coexist with any one of two lethal newborn diseases: neonatal sepsis or birth asphyxia. Second, ABO incompatibility is the commonest type of HDN.

A strength of our study is the inclusion of only babies with jaundice, as opposed to studying uncomplicated newborns.

This study had some limitations; namely, the frequency of HDN in Uganda may have been overestimated, given that the study was conducted at a referral facility. Similarly, the range of alloantibodies associated with HDN in the current study (that used the tube method for antibody screening) may not be exhaustive. Natukunda et al., have previously shown that 2.2% of women attending antenatal care at an upcountry regional hospital in Uganda have alloantibodies beyond anti-D [13]. They included anti-K, anti-Fy(b) and anti-Jk(a), which can cause HDN. In our study, eluate testing was performed only in a subsample of suspected ABO HDN. Also, we could not determine peak bilirubin levels because we did not perform serial bilirubin measurements.

In conclusion, among newborn infants with jaundice, HDN is not rare—the majority being from ABO incompatibility HDN. ABO HDN affects group A and group B babies equally. Neonatal sepsis and birth asphyxia are common comorbidities in HDN in Uganda.

We recommend strengthening the implementation of ABO/Rh grouping during antenatal care—in line with local policy goals for maternal and newborn care. This will facilitate the identification of RhD-negative mothers and identify pregnancies at risk for ABO HDN. Access to RhIg prophylaxis for RhD-negative pregnant women should be improved to reduce preventable HDN and newborn mortality (and morbidity) in general. Moreover, since RhD-negative individuals are relatively few in Uganda (about 5%), the overall cost to the system would be quite low, yet with the possibility of eradicating the risk of RhD HDN. In resource-poor countries, there is a need to address the weaknesses and gaps in antenatal care services and policies.

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CONFLICT OF INTEREST

The authors have no competing interests.

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ORIGINAL ARTICLE

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Incidence and risk factors for graft failure in the modern era of cord blood transplantation

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Abstract

Background and Objectives: Graft failure (GF) after cord blood transplant (CBT) has decreased with improved supportive care and cord selection strategies. We aimed to evaluate cord blood selection and factors associated with retransplantation on the incidence of GF, determine risk factors for GF including host antibodies to Kell antigen and evaluate survival after GF.

Materials and Methods: We retrospectively reviewed 84 patients who underwent CBT at the University of Oklahoma between 2000 and 2016 and compared outcomes in patients with/without engraftment by Day 28. The nonengraftment cohort was further divided into patients who underwent retransplantation. Kaplan–Meier curves with log-rank tests were calculated to assess the association between mortality and engraftment.

Results: Engraftment following CBT was high at 81%, with 52% engrafting by Day 28 and an additional 29% engrafting by a median of 36 days. Retransplantation led to 88% engraftment at a median of 53 days. Overall, 75% of the 40 patients who did not engraft by Day 28 died. Female sex and total nucleated cell count < 3.5/kg were significantly associated with lack of engraftment and higher mortality. Antibodies to Kell fetal antigen were not identified. Retransplantation by Day 28 for primary GF conferred a survival advantage.

Conclusion: This study demonstrates that failure to engraft by 28 days was associated with increased mortality, and risk was mitigated with early retransplantation. Female sex and low total cell dose were associated with increased mortality. Early identification of GF coupled with early retransplantation can reduce mortality in CBT.

Keywords
cord blood transplant, graft failure, haematopoietic stem cell, progenitor cell, transplantation

Highlights

- In cord blood transplantation, risk factors for graft failure include female sex and low total nucleated cell count.
- In people identified to have graft failure at Day 28, early retransplantation may confer mortality benefits.
- Identification of people who may experience graft failure and early retransplantation has the potential to decrease mortality associated with cord blood transplantation.

INTRODUCTION

Since the advent of cord blood transplantation (CBT) in 1989 [1], over 12,000 allogeneic transplantations have been performed for a variety of hematologic disorders. The advantages of cord blood donor source remain: (1) increased donor availability due to permissible HLA mismatches [2–4], (2) facile and rapid product acquisition, (3) lower rates of graft versus host disease with comparable antitumour effects [2, 5–7] and (4) long-term successful immune reconstitution. However, the use of cord blood as a cell source has been hampered by the increased rates of graft failure (GF), historically as high as 24% [8].

The rates of GF after cord blood have improved by implementing cord selection strategies to reduce the risk of nonengraftment, such as prioritizing cell number with double cord transplants if needed, lack of donor-specific antibodies and certified cord centers [6, 8–14]. Improvements in supportive care in recent decades should also have improved outcomes after CBT [8]. However, data on current rates of successful engraftments are scant and difficult to compare across studies, given the lack of consensus about how much time post-transplantation is sufficient to ascertain failure, ranging from 28 to 45 days after CBT [9, 15]. Further, the impact of these modern selection strategies on the risk of GF after CBT has not been evaluated. Prior risk factors for GF after CBT have included CD34 cell dose, TNC cell dose, elevated HLA antibodies and disease characteristics (SCD/myelofibrosis) [3, 16–18]. While previously untested, similar to HLA-specific antibodies, host antibodies to Kell, a fetal antigen lost during healthy maturation, could be a risk factor for GF after CBT as well [19].

We hypothesized that GF rates had declined in the modern era, and that different risk factors would be linked to GF, including host antibodies to the fetal antigen Kell and that early diagnosis of GF (by 28 days) and retransplantation for absent haematopoiesis would be associated with survival.

MATERIALS AND METHODS

We retrospectively and consecutively reviewed paediatric and adult patients who underwent CBT at the University of Oklahoma between 2000 and 2016 to compare outcomes in patients who did or did not engraft by Day 28 following CBT (OU IRB: OU201911). No patients in this study had positive anti-HLA antibodies that were donor specific. Patients with positive anti-HLA antibodies do not qualify for CBT at our institution. The nonengraftment cohort was further divided into patients who underwent second transplantation. All patients who did not engraft by Day 28 were evaluated for a second transplantation. Patients were chosen for second transplantation if they did not have active uncontrolled infection or significant organ dysfunction. Engraftment is defined as ANC > 500 cells/μL for three consecutive days. Transplantation, disease and patient characteristics were captured, including preparative regimen intensity, disease, age, sex, cord match (antigen level matching for A, B and C and allele matching DR), ABO mismatch, number of cords, cell dose, donor-specific antibody, bone marrow biopsy (BMBX) cellularity, evidence of relapse, length of survival and nonrelapse mortality. Descriptive statistics were calculated. The association between transplant and patient characteristics and engraftment by Day 28 was analysed using chi-square analysis and logistic regression. Kaplan–Meier curves with log-rank tests were calculated to assess the association between mortality and engraftment by Day 28, as well as second haematopoietic cell transplantation (HCT). Kell antibody IHC and ELISA testing were performed on marrow from 10 patients with marrow slides available (Abcam 116,891) to evaluate antibody presence. Cox proportional hazards models were calculated to evaluate the association between engraftment by Day 28 and death, adjusting for significant confounders. Among patients who had a BMBX, the proportion who engrafted among those with normal/hypercellularity versus hypocellularity were compared using Fisher's exact test. A significance level of α equal to 0.05 was used. Analyses were conducted using SAS statistical software, version 9.4 (SAS Institute, Cary, NC).

RESULTS

Of the 91 CBT recipients, two were excluded for missing data and five due to death before Day 28 after transplantation. Baseline characteristics of the remaining 84, including patients, cord units and transplantation, are summarized in Table 1. The median cell dose was 5.83×10^7 TNC/kg of the recipient's body weight (BW) and 57% had a five of six or six of six matches.

TABLE 1 Characteristics of the patients

Variable	N (%)
Sex	
Female	39 (46)
Male	45 (54)
BMBX	
Yes	29 (35)
No	55 (65)
Biopsy results ($n = 29$)	
Hypocellular	21 (72)
Normal/hypercellular	7 (24)
Relapse	1 (4)
Cellularity % among those with hypocellular results ($n = 21$)	
<5%	12 (57)
5%–15%	4 (19)
16%–60%	5 (24)
ABO mismatched	
Yes	64 (76)
No	20 (24)
Engraft by Day 28	
Yes	44 (52)
No	40 (48)
Diagnosis	
Myeloid	64 (76)
Lymphoma	7 (9)
Malignancy, other	1 (1)
Nonmalignant	12 (14)
Preparation category	
Full	65 (77)
Reduced	19 (23)
Number of cords in first transplant	
Single	50 (60)
Double	34 (40)
Number of HLA match	
4 of 6	36 (43)
5 of 6	40 (48)
6 of 6	8 (9)
Age in years, median (Q1−Q3)	21 (6.5–36)
Age categories	
<18 years	37 (44)
≥18 years	47 (56)
CD34 ($\times 10^5$)/kg BW, median (Q1−Q3)	5.63 (2.8–23.1)
CD34 ≥ 2.0×10^5/kg BW ($n = 78$)	69 (88)
TNC ($\times 10^7$)/kg BW, median (Q1−Q3)	5.83 (3.66–9.75)
TNC ≥ 3.5×10^7/kg BW ($n = 79$)	64 (81)

Abbreviations: /kg BW, per kilograms of the recipient's body weight; ABO, Landsteiner's blood grouping system; BMBX, bone marrow biopsy; CD34, haematopoietic progenitor cell antigen CD34; HLA, human leukocyte antigens; TNC, total nucleated cell count.

Engraftment was high at 81%, with 52% ($n = 44/84$) engrafting by Day 28 after CBT and an additional 29% ($n = 24$) at a median of 36 days (range 30–79). The remaining patients included 10% who received a second CBT (four adults, four children; all eight received a mismatched CBT), and 10% died without engraftment ($n = 8$) at a median of 45 (33–266) days following CBT. Of the 16 patients who did not engraft: seven had 95% recipient chimerism, six had 95% donor chimerism and three had nonevaluable samples (test failure). Among the eight patients who received a second CBT: five had 95% recipient chimerism and three had 95% donor chimerism. Among the eight patients who died without engraftment: two had 95% recipient chimerism, three had 95% donor chimerism and three had nonevaluable samples (test failure). Retransplantation led to 88% engraftment ($n = 7/8$) at a median of 53 days (38–119) following the second transplant and one died without evidence of engraftment. Overall, 63% ($n = 53/84$) of the patients have died; 52% ($n = 23/44$) among those who engrafted by Day 28 and 75% ($n = 30/40$) who did not engraft by Day 28.

There was no association between engraftment by Day 28 after CBT and the potential confounders of sex, age, preparative regimen intensity, ABO mismatch, degree of cord match, or relapse. Prior risk factors of double versus single cord, TNC < 3.5×10^7/kg of the recipient's BW and CD34 < 2.0×10^5/kg of the recipient's BW were also not associated with GF after CBT. As expected, a higher proportion of children had a TNC count ≥ 3.5×10^7/kg of the recipient's BW (94% children vs 80% adults, $p = 0.0073$). Children also experienced a higher 1-year survival compared to adults (58% children, 38% adults, $p = 0.0200$). Among all patients, the risk of death was 0.57 (95% confidence interval [CI]: 0.33–0.98, $p = 0.0425$) times lower for those who did engraft by Day 28 compared to those who did not engraft (Figure 1). Along with engraftment by Day 28, the variables age, sex, TNC < 3.5×10^7/kg of the recipient's BW and CD34 < 2.0×10^5/kg of the recipient's BW were individually statistically significantly associated with death. However, only engraftment by Day 28 (HR = 0.55, 95% CI: 0.31–0.98, $p = 0.0421$), female sex (HR = 2.76, 95% CI: 1.52–5.03, $p = 0.0009$) and TNC < 3.5×10^7/kg of the recipient's BW (HR = 5.50, 95% CI: 2.84–10.65, $p < 0.0001$) remained significant and were included in the final model. In this cohort, available anti-HLA (19 negative, 16 positive) was not associated with engraftment by Day 28 or survival. In total, 29 patients had a BMBX, and neither cellularity nor chimerism correlated with engraftment. Kell antibodies were not detected in any of the CBT recipients, thus this was not able to be assessed as a risk factor for GF.

Among those who did not engraft by Day 28, the risk of death was 0.24 (95% CI: 0.07, 0.79, $p = 0.0192$) times lower with a second HCT versus those without a second HCT (Figure 2). After controlling for sex and TNC < 3.5×10^7/kg of the recipient's BW, the HR for those with a retransplantation decreased to 0.21 (95% CI: 0.06–0.76, $p = 0.0177$). For those that did not engraft by Day 28, survival was impacted by sex, with female sex associated with an HR of 3.19 (95% CI: 1.37–7.42, $p = 0.073$) and TNC < 3.5×10^7/kg of the recipient's BW with an HR of 3.31 (95% CI: 1.27, 8.64) in the final model.

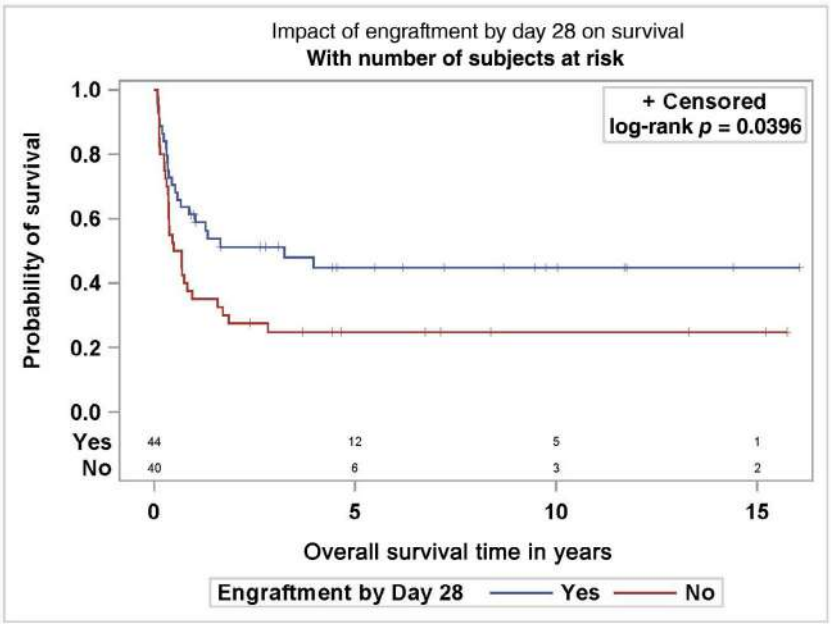

FIGURE 1 Impact of engraftment by Day 28 on survival. Kaplan–Meier estimates of impact of engraftment by Day 28 on overall survival. The *p*-value is shown using log-rank analysis.

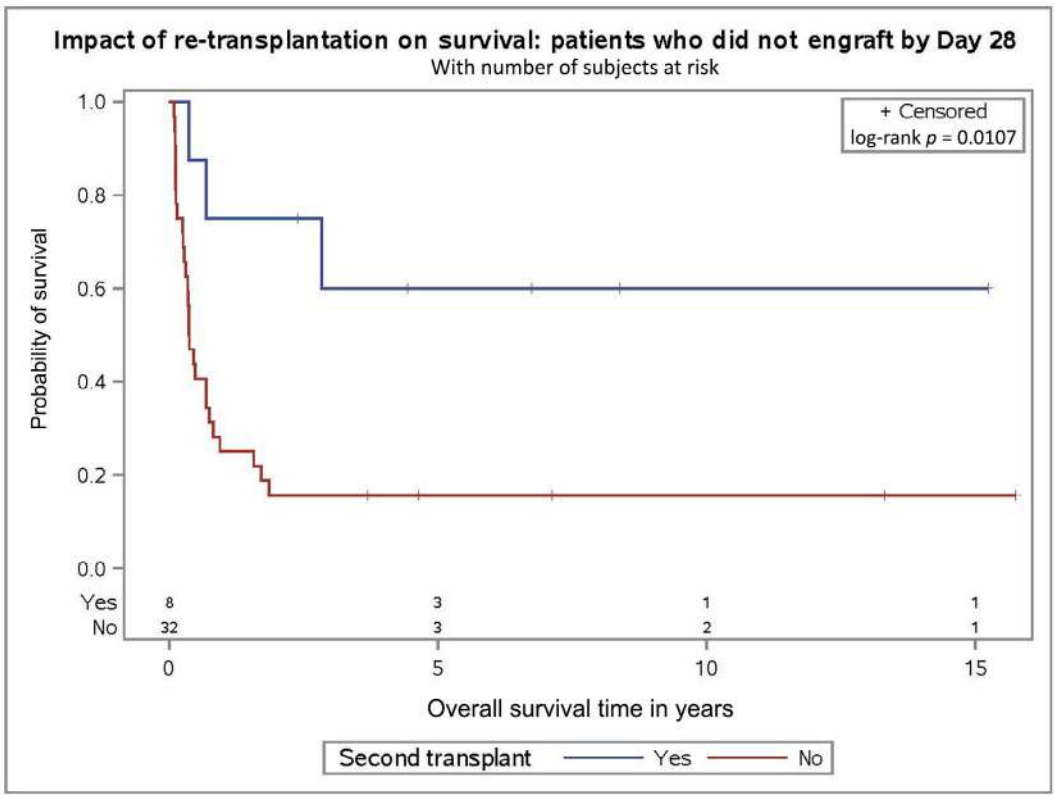

FIGURE 2 Impact of retransplantation on survival: Patients who did not engraft by Day 28. Kaplan–Meier estimates of impact of retransplantation on overall survival among patients who did not engraft by Day 28. The *p*-value is shown using log-rank analysis.

DISCUSSION

Our data demonstrate that optimized donor selection strategies and modern supportive care lead to low rates of GF, comparable to other alternative donors [20, 21]. Failure to engraft by 28 days was associated with increased mortality, though this was mitigated if early retransplantation was initiated. If confirmed in other studies, this would suggest that prompt initiation of retransplantation is beneficial

despite increased risks of additional chemotherapy with a second preparative regimen and delay of engraftment by using a second stem cell source. Our data did show that female sex and low total cell dose were also associated with increased mortality. This may reflect the role of T cells in CBT engraftment now that sufficient CD34 cell dose is commonly achieved with modern selection strategies [6, 22]. While not able to be tested in our study, impaired T cell function and/or number have been linked to increased GF rates. Our data reiterate that rigorous selection of robust cell number cord blood units is critical and that other subsequent methods, such as optimizing the conditioning regimen, would be beneficial [23]. The association with the female sex is less clear, although female lymphocytes are more prone to activation than male lymphocytes due to the presence of oestrogen receptors and simulation following oestrogen exposure. Thus, one could hypothesize that female recipient T cells could be more prone to activation and subsequent graft rejection. This would need to be assessed in a larger study to both determine if these risk factors are validated and to determine the causality. We had hypothesized that Kell antibodies would be a risk factor for GF; as these were not detected in any recipients, this hypothesis is unable to be assessed in this study. While this could represent the absence of Kell antibodies in cord blood recipients, it is also possible that the limit of detection of our assay was high enough to preclude sensitive assessment. Additionally, aspects of processing and storage may have impacted these results.

Our data demonstrate that the GF rate after CBT has improved with the modern adoption of cord selection techniques, 11% of the total cohort versus 24% historically [8]. GF was not higher in patients with risk factors of HLA disparity, presence of donor-specific anti-HLA antibodies and ABO mismatch when modern strategies were used to select grafts, consistent with previous trials [24]. We hypothesize that our data did not corroborate these previously published risk factors because of the optimization of modern strategies, including a minimum cell dose of CD34+ 2.0×10^5/kg of the recipient's BW, a total cell dose of 3.5×10^7/kg of the recipient's BW for single cord and the absence of anti-HLA antibodies present in patients at infusion.

Limitations of this data include that it is a retrospective study and performed at a single institution. However, it demonstrates that cord blood has the potential to provide comparable rates of engraftment in a similar length of time to other methods of transplantation, when optimal selection techniques are employed. Given the ability to rapidly acquire cord blood cells, tolerable mismatch and the excellent associated graft-versus-tumour effect, CBT remains an important option as a transplant source [6, 25, 26]. Our data support that this is especially true for children, who experienced higher overall survival, confirming the data of others [27]. In addition, this benefit continues to provide critical donor sources for those of non-Caucasian descent, for which the likelihood of identifying a matched unrelated donor is less common. For those who are not Caucasian, an 8/8 HLA-matched unrelated donor is found less than 53% of the time compared to 75% for Caucasians. In contrast, the likelihood of finding cord blood with at least a 4/6 HLA match is more likely for non-Caucasians, identifying a donor for 81% of African Americans and 91% for Hispanic people [28].

Our work serves as a benchmark time point for GF after CBT and may be beneficial for cord blood expansion protocols, showing the benefit of modern selection strategies and supportive care on engraftment rates after CBT. These data suggest many prior risk factors may no longer be significant in the modern era and that high survival can be achieved even in those with GF with expeditious retransplantation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SHORT REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

Risk of a blood donation contaminated with hepatitis E virus entering the blood supply before the implementation of universal RNA screening in France

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Abstract

Background and Objectives: The risk of a blood donation contaminated with hepatitis E virus (HEV) entering the blood supply before introducing universal HEV-RNA screening in France was estimated to assess the benefit of such a measure.

Materials and Methods: The results of selective HEV nucleic acid testing (HEV-NAT) performed in mini pool of six plasma donations between 2018 and 2020 were extrapolated to the whole blood donor (BD) population after adjustment on three variables: regional establishment, sex and age group.

Results: Among the 246,285 plasma donations collected from 172,635 BDs tested for HEV-RNA, 248 (10.1/10,000) were positive. The extrapolation to all BDs led to an estimated rate of 5.9/10,000 donations (95% confidence interval [CI]: 4.5–7.4) which would be positive to HEV-RNA and a prevalence of 9.9/10,000 BDs (95% CI: 7.5–12.3). This prevalence was 4.4 times higher in males than females (16.8/10,000 vs. 3.8/10,000, $p < 10^{-4}$). The highest prevalence was observed in males in the 30–39 age group (20.5/10,000) and the lowest in females in the 50–70 age group (2.8/10,000).

Conclusion: The risk of an HEV-RNA-positive donation entering the blood supply was estimated at 1 in 1682 donations. This risk does not translate directly to the risk of HEV transfusion transmission, which mainly depends on the total number of viral particles in the transfused blood component and the sensitivity of NAT.

Keywords

blood donors, HEV, NAT, risk, transfusion

Highlights

- We estimated the risk of hepatitis E-virus (HEV) in whole blood donations from data collected on plasma donations in France between 2018 and 2020.
- The estimated risk of a whole blood donation being positive for HEV-RNA was 1 in 1682 donations.
- In early 2023, universal HEV-RNA screening will be introduced in France.

INTRODUCTION

In Europe, hepatitis E-virus (HEV) infection is mainly acquired by the consumption of contaminated raw or undercooked meat. From the eight genotypes described to date, genotype 3, originating from animal reservoirs, is responsible for most infections in Western Europe. HEV infections generally cause asymptomatic or self-limiting acute hepatitis but severe forms such as chronic hepatitis in immunocompromised patients have been reported [1]. HEV is also transmitted by transfusion, and all types of blood components, including solvent detergent (SD)-treated plasma and components inactivated with amotosalen, have been involved [2–5]. In France, to reduce the risk of transfusion-transmitted (TT) infection, HEV nucleic acid testing (HEV-NAT) of plasma donors was introduced selectively in November 2012 in pools of 96 apheresis plasma donations for plasma intended for SD plasma production. After SD plasma production by the National Blood Service (Etablissement Français du Sang [EFS]) ended in 2014, testing in pools of 96 plasma donations was discontinued and HEV-RNA testing was applied in pools of 6 plasma donations to maintain a stock of approximately 30% of HEV-RNA negative plasma components for at-risk recipients [3].

Since the implementation of selective HEV-NAT screening, the French haemovigilance system has not reported any case of TT infection caused by plasma [3]. The risk of viral transmission with cellular blood components, however, persists and prompts the consideration of expanding HEV-RNA screening to all donations. Before introducing universal HEV-RNA screening, we evaluated the risk of a contaminated donation entering the blood supply by extrapolating HEV-NAT results obtained through selective screening of plasma donors over a 3-year-period (2018–2020) to the whole blood donor (BD) population of mainland France.

MATERIALS AND METHODS

HEV-RNA testing

Since 2018, plasma donations tested for HEV-RNA have been tested in MP-6 with the Procleix HEV-RNA assay on the Panther automated platform (Grifols, Barcelona, Spain), showing a 95% limit of detection (LOD) estimated at 24 IU/ml (95% confidence interval [CI]: 19–33) [6].

Risk assessment

The risk of a blood donation contaminated with HEV entering the blood supply corresponds to the rate of HEV-RNA-positive blood donations estimated in the whole BD population in the absence of NAT screening.

To calculate this risk, the prevalence of HEV-RNA in the plasma donor population tested between January 2018 and December 2020 was extrapolated to the total donor population by adjusting data on three variables, which are associated with the risk factors for infection:

TABLE 1 HEV-RNA data in the total blood donor (BD) population of mainland France estimated from HEV-RNA testing results in plasma donors, 2018–2020

HEV-RNA testing results in plasma donors					Total BD population		Estimates after extrapolation to the total BD population				
N plasma donations tested for HEV-RNA (A)	N plasma donations HEV-RNA positive (B)	HEV-RNA-positive rate per 10,000 donations (B)/(A)	N plasma donors tested for HEV-RNA (C)	N plasma donors HEV-RNA-positive[a] (D)	Prevalence of HEV-RNA per 10,000 plasma donors (D)/(C)	N donors (E)	N donations (F)	N donations HEV-RNA-positive[b] (G)	HEV-RNA-positive rate per 10,000 donations (G)/(F)	N donors HEV-RNA-positive[a] (H)	Prevalence of HEV-RNA per 10,000 donors (H)/(E)
246,285	248	10.1	172,635	248	14.4	4,556,301	7,601,606	4519 (95% CI: 3425–5614)	5.9 (95% CI: 4.5–7.4)	4519	9.9 (95% CI: 7.5–12.3)

Abbreviations: CI, confidence interval; HEV, hepatitis E-virus.
[a]Assuming that a given donor did not develop more than one infection.
[b]Estimation after adjustment on the region of donation, sex and age class of donors.

the regional EFS establishment (assumed to be the donor's region of residence), the sex and age group of donors. This extrapolation was based on the assumption that these risk factors were the same in tested plasma donors and all BDs, for whom the selection criteria are otherwise the same.

To perform the adjustment, the total number of HEV-RNA-positive plasma donations and their variance were estimated by the Horvitz–Thomson estimator [7]. A finite population correction was assumed, with a sampling fraction per stratum (EFS establishment × sex × age group) equal to the ratio of the number of plasma donations to the number of blood donations.

The risk of a blood donation testing positive for HEV-RNA over the study period was calculated as the estimated number of HEV-RNA-positive blood donations divided by the total number of donations collected during the same period. This calculation was done assuming that a given donor was HEV-RNA-positive only once during the study period.

Donors from overseas territories whose donations were not processed into plasma were excluded from the study.

RESULTS

A total of 7,601,606 blood donations were collected from 4,556,301 BDs in mainland France during the study period. Among them, 246,285 plasma donations of 172,635 donors (85% were males) were tested for HEV-RNA in MP-6. HEV-RNA was detected in 248 donations giving a positive rate of 10.1 per 10,000 plasma donations and a prevalence of 14.4 per 10,000 plasma donors (Table 1).

The extrapolation to the total BD population led to an estimated number of HEV-RNA-positive blood donations of 4519 (95% CI: 3425–5614) with a mean of 1506 (95% CI: 1142–1871) per year, and a prevalence of 9.9 per 10,000 donors (95% CI: 7.5–12.3). This prevalence was 4.4 times higher in males than females (16.8 per 10,000 vs. 3.8 per 10,000, $p < 10^{-4}$). The highest rate was observed in males in the 30–39 age group (20.5/10,000) and the lowest in females in the 50–70 age group (2.8/10,000) (Figure 1).

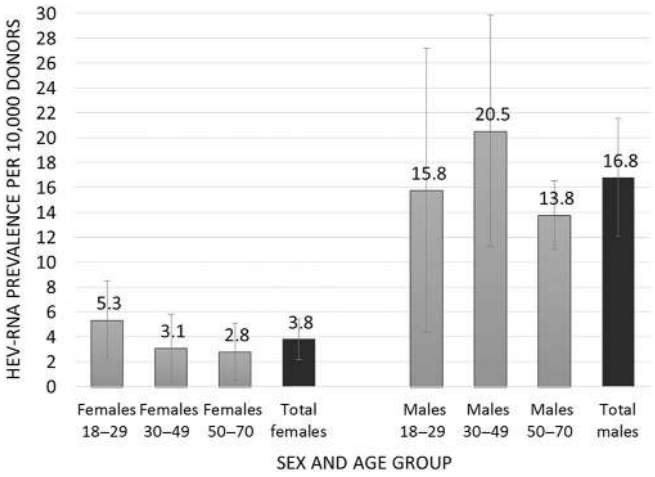

FIGURE 1 Estimates of hepatitis E virus-RNA prevalence in blood donors by sex and age group in mainland France, 2018–2020

After extrapolation, the rate of a blood donation testing positive for HEV-RNA in France over the 2018–2020 period in the whole BD population was estimated to be 5.9 (95% CI: 4.5–7.4) per 10,000 donations or 1 per 1682 donations (95% CI: 1:1354–1:2220).

DISCUSSION

To further reduce the risk of transmission of HEV by transfusion, the French health authorities decided to replace the current selective HEV-RNA testing performed in plasma donors with a universal HEV-RNA screening strategy, which will be implemented in early 2023 using HEV-NAT methods with similar analytical performance as those currently in place. To assess the benefit of such a measure, we evaluated the risk of a contaminated donation entering the blood supply by extrapolating HEV-NAT results obtained through selective screening of plasma donors over a 3-year-period (2018–2020) to the whole BD population of mainland France. Our results showed that a mean of 1506 HEV-RNA-positive donations would enter the blood supply each year. This number is double the estimated 788 per year (95% CI: 478–1098), reported in 2013 [8].

This increased prevalence could be explained by several factors. The change of testing method with improved sensitivity is the most likely cause for the increase in the estimated number of positive donations. In 2013, blood donations were tested in pools of 96 donations with a LOD 16 times higher than the current MP-6 NAT (2208 vs. 144 IU/ml) [9]. In our experience, 70% of blood donations tested with MP-6 between 2015 and 2018 had a viral load below 2208 IU/ml (data not shown). Assuming that the 788 donations estimated to have entered the blood supply in 2013 when using MP-96, had a viral load over 2.208 IU/ml and represented only 30% of HEV-positive donations, the expected number of HEV-positive donations that would have been detected if HEV-NAT testing had been performed in MP-6 instead of MP-96 could be estimated to be 2627 (788 × 100/30). Moreover, the estimates performed in 2013 were probably less accurate than those of the present study because of a four-times lower number of plasma donations tested (57,000 in 2013 vs. 246,285 in the present study) and because in 2013 several blood centres had very few (or no) donors tested, which necessitated extrapolation from data obtained in other centres. The increasing estimated rate of positive donors might not be related to epidemiological changes in the general population since, in France, the national reference centre noted a declining rate of individuals infected with HEV from 5% in 2013 to 3% in 2018 [10]. Notably, there is no dedicated surveillance system implemented at the national level allowing a proper evaluation of these trends.

Our study has however some limitations. First, the proportion of plasma donations tested for HEV-RNA among the total collected donations varied according to the regions (not shown in details). In regions where the number of tested donations was low, estimates by sex and age group were less robust. This may have led to biased

estimations when we extrapolated the results to the total BD population. As previous studies have reported seroprevalence geographical differences in France, it will be interesting to analyse the relationship between antibody prevalence and incidence data at the regional level after the introduction of universal HEV-RNA screening [11]. However, the rate of 0.059% HEV-RNA-positive blood donations estimated in our whole BD population is in line with the studies from Spain and Germany, suggesting the reliability of the adjustment [12, 13]. Furthermore, although reinfections remain possible, we assumed that a given donor could not be infected more than once during the study period.

In conclusion, we have estimated that the risk of a blood donation contaminated with HEV entering the blood supply prior to the implementation of universal RNA screening in France was 1 in 1682 donations between 2018 and 2020. This risk does not translate directly to the risk of HEV transfusion-transmission infection, as the viral load in the donor sample, the volume of plasma in the transfused blood component (i.e., the infectious dose) and other factors, such as the sensitivity of the test applied need to be considered [14]. In addition to risk mitigation, the introduction of universal HEV-NAT in the BD population planned in 2023 will provide a better understanding of the epidemiology of HEV infection in France and valuable information on viral exposure in the general population. Moreover, the clinical benefit of such a measure for transfused patients will be more accurately estimated through the haemovigilance reporting.

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J.P. designed the study, analyzed the data, contributed to the research and to the paper redaction; C.S. performed the risk assessment and statistical analysis; C.M., S.L. and C.P. acquired the data; J.F. reviewed and edited the manuscript; P.R. and P.M. contributed to the study design, reviewed and edited the manuscript; S.L. and P.G. designed the study, supervised the research and wrote the paper.

CONFLICT OF INTEREST

The authors declare no conflicts of interest for this study.

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SHORT REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

Evolving policies for donors with diabetes: The Canadian experience

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Abstract

Background and Objectives: Donor criteria for diabetes vary significantly. We describe our evolving policies for donors with diabetes, their contribution to the Canadian blood supply and their rate of syncopal reactions compared to other donors.

Materials and Methods: All donors are asked if they have diabetes and have taken medications in the last 3 days. We assessed donors with diabetes on various medications, the number deferred over time, and syncopal reactions in donors with diabetes and other donors in our donor reaction database.

Results: Policy changes allowing type 2 diabetic donors on oral hypoglycaemics alone, type 2 diabetic donors on oral medications and insulin and type 1 diabetic donors (all on insulin) to donate resulted in a decrease in deferrals from 450 to 22 donors annually. Of donors being treated with medication for diabetes, 11% are receiving insulin as part of their treatment. Syncopal reaction rates were low and not statistically different between diabetic and non-diabetic donors, although confidence intervals (CIs) are large.

Conclusion: Policies decreased deferrals while maintaining safety. A longer observation period would strengthen these observations.

Keywords

blood donation, diabetes, donors

Highlights

- Incremental changes in criteria for accepting whole blood donors with type 1 and 2 diabetes have led to reduced deferrals and increased successful donations.
- Donor syncopal reaction rates were similar in donors with and without diabetes over a 1-year observation period.

INTRODUCTION

Diabetes mellitus is a metabolic illness caused by impaired insulin secretion or action. Type 1 diabetes is an autoimmune disease characterized by the destruction of insulin-producing cells requiring lifelong treatment with insulin. Type 2 diabetes is a metabolic disorder related to tissue insulin resistance; treatment usually involves lifestyle modification and non-insulin antihyperglycaemic medications, usually given orally, more rarely, subcutaneously. Insulin may be added if glycaemic targets are not achieved [1].

Donor criteria for diabetes vary significantly among blood centres [2]. Donors with diabetes may be deferred for possible increased donor risk (syncopal or pre-syncopal reactions) and recipient risk (bacteraemia) [3]. Canadian Blood Services (CBS) collects, tests and

distributes blood components to all provinces in Canada except for Quebec. We describe our evolving policies for donors with diabetes, the contribution these donors make to the blood supply and adverse reactions in these donors.

MATERIALS AND METHODS

Donor health assessment and data collection

All donors are asked, "Do you have diabetes", and "Have you taken any medications, excluding birth control pills or vitamins in the last three days"; there is a unique code for diabetes (eProgesa, MAK Systems). Medications and their indications are added to the donation record. Donor deferrals with a diabetes code were extracted from eProgesa.

Denominator data on whole blood donors and donors answering yes to having diabetes were extracted from the National Epidemiology Donor Database (NEDD). Donors on insulin alone were considered to have type 1 diabetes; donors on hypoglycaemic agents with or without insulin or undergoing lifestyle modifications alone were considered to have type 2 diabetes.

Vasovagal reactions and injuries were extracted from our operational Reaction and Incident Report (RIR) database, which contains syncopal reactions that occurred on site and after donation if reported

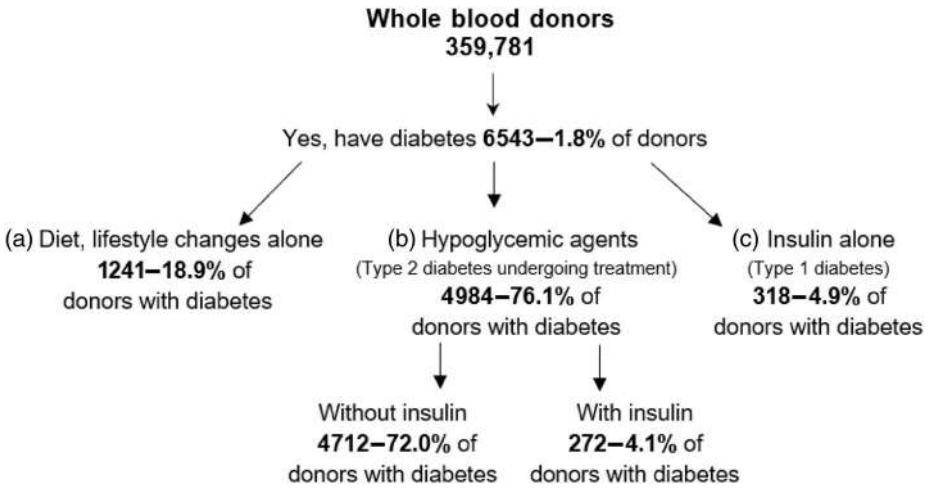

FIGURE 1 Successful whole blood donors and history of diabetes, 15 March 2021–15 March 2022

TABLE 1 Changes in criteria and deferrals for diabetes, Canadian Blood Services 2012–2022

Time period	Donors accepted	Deferrals/year: Donors with diabetes	Comments
Before Oct 2012	• Donors not currently on insulin	~450	• Dissatisfaction in longstanding donors ineligible once on insulin
Oct 2012–Aug 2018	• Donors with type 2 diabetes, stable insulin dose so hypoglycaemia unlikely, insulin started after 31 Dec 2006 rather than questioning donors about bovine insulin use	~140	• Insulin taken before 2007 may have been of bovine origin (vCJD risk) • Some donors still deferred due to small changes in insulin dosage
Aug 2018–15 Mar 2021	• Donors with type 2 diabetes, regardless of insulin dose change, insulin started after 31 Dec 2006	~98	• Advocacy from Diabetes Canada and individuals with type 1 diabetes to change this policy
15 Mar 2021, onwards	• Donors with type 2 diabetes, regardless of date of insulin use • Donors with type 1 diabetes if: - eaten in last 2 h - no acute event in last 3 months[a] - no regular episodes of dizziness on standing due to neuropathy - no leg or foot ulcers	~22	• Updated US FDA Guidance removed bovine insulin vCJD concern [4] • Only deferral of donors at greatest risk for hypoglycaemic episode, vasovagal reaction, or intermittent bacteraemia

Abbreviation: vCJD, variant Creutzfeldt-Jakob disease.
[a]Acute event defined as needing the assistance of another person to treat symptoms, such as a severe hypoglycaemic episode.

to CBS after donation but does not include minor (pre-syncopal) reactions.

Data cleaning and statistical analysis

Data for medication names, frequently spelt incorrectly, were cleaned (90% accuracy) using the statistical software 'R' with regular expressions, primarily Tidyverse (R Project, Tidyverse). Subsets were created for various diabetic donors (Figure 1).

Stata 17® (StataCorp LLC, Texas, USA) was used to calculate reaction rates and 95% CIs, the incidence-rate comparison calculator function was used to compare reaction rates. Fisher's exact test was used to determine statistical significance. $p < 0.05$ was considered statistically significant.

TABLE 2 Syncopal reaction rates per 10,000 donations by donation and diabetic status, 15 Mar 2021[a]–15 Mar 2022

	First-time donations (*N*)	Number of reactions	Reaction rate	95% confidence intervals
Type 1 diabetes[b]				
Male	83	1	120.5	[0.0, 355.2]
Female	142	1	70.4	[0.0, 208.0]
Total	225	2	88.9	[0.0, 211.5]
Type 2 diabetes[c]				
Male	550	0	0.0	N/A
Female	274	1	36.5	[0.0, 107.9]
Total	824	1	12.1	[0.0, 35.9]
Type 1 and 2 diabetes combined[d]				
Male	633	1	15.8	[0, 46.7]
Female	416	2	48.1	[0.0, 114.5]
Total	1049	3	28.6	[0.0, 60.9]
Non-diabetic				
Male	31,316	214	68.3	[59.2, 77.5]
Female	32,399	301	92.9	[82.5, 103.4]
Total	63,715	515	80.8	[73.9, 87.8]
	Repeat donations (*N*)[e]	**Number of reactions**	**Reaction rate**	**95% confidence intervals**
Type 1 diabetes[b]				
Male	82	0	0.0	N/A
Female	106	2	188.7	[0.0, 447.7]
Total	188	2	106.4	[0.0, 253.0]
Type 2 diabetes[c]				
Male	2905	3	10.3	[0, 22.0]
Female	1690	4	23.7	[0.0, 46.8]
Total	4595	7	15.2	[0.0, 26.5]
Type 1 and Type 2 diabetes combined[d]				
Male	2987	3	10.0	[0, 21.4]
Female	1796	6	33.4	[6.7, 60.1]
Total	4783	9	18.8	[6.5, 31.1]
Non-diabetic				
Male	423,340	285	6.6	[5.8, 7.3]
Female	340,701	597	17.6	[16.1, 19.0]
Total	764,041	882	11.5	[10.8, 12.3]

Abbreviation: N/A, not applicable.
[a]Non-diabetic donor data begin 1 March 2021.
[b]Group c in Figure 1.
[c]Groups b in Figure 1.
[d]Groups b and c in Figure 1.
[e]Repeat donors with type 1 diabetes were mainly donors who donated in the past but were deferred for several years before being allowed to donate again after 15 Mar 2021 (see Table 1).

Approval of our Research and Ethics Board was not required since no additional information was collected and data were aggregated.

RESULTS

Changes in eligibility criteria over time

Table 1 shows criteria changes and donor deferrals over time. Donors deferred for diabetes may have had other reasons for deferral, such as cardiovascular disease. In 2021–2022, about 600 donors (11% of all donors undergoing treatment with medication for diabetes) who would have previously been deferred for insulin use successfully donated.

Characteristics of donors with diabetes

Figure 1 shows the number of donors who answered yes to 'Do you have diabetes' and successfully donated between 15 March 2021 and 15 March 2022. Out of 768,202 donations from 359,781 donors, 6543 donors (1.8%) answered affirmatively to having diabetes. Of these, 3978 (61%) were identified as male and 2565 (39%) as female. The median age and interquartile range were 35 years (27–50) for donors with type 1 diabetes, 56 years (46–64) for donors treated with lifestyle modifications alone, 59 years (51–66) for donors treated with hypoglycaemic agents alone and 60 years (49–65) for donors treated with hypoglycaemic agents and insulin. Donors on insulin for type 2 diabetes and type 1 diabetes contributed 312 and 413 donations over the year, respectively.

Donors undergoing treatment for diabetes (Figure 1, groups b and c) were often on other medications, most commonly antihypertensives in 65.9% and lipid-lowering agents in 52.1%. Comparable rates are approximately 12% for antihypertensives and 7% for lipid-lowering agents in our entire donor population. None of these medications would result in a deferral.

Safety of donation process for diabetic donors and recipients

Table 2 shows the syncopal reaction rate for donors with type 1 diabetes, donors with type 2 diabetes undergoing treatment (Figure 1, groups b and c) and donors who did not answer affirmatively to having diabetes.

For both first-time and repeat donors, there was no statistically significant difference in syncopal reaction rates between donors with and without diabetes. This was also the case when comparing the rates of diabetics with non-diabetics overall, regardless of donation status. Donors with diabetes on treatment had a rate of syncopal reactions of 20.6 (95% CI: 8.95–32.2) per 10,000 versus 16.9 (CI: 16.0–17.8) per 10,000 in donors without diabetes ($p = 0.42$). There were four injuries, all mild, in donors with diabetes who had syncopal reactions.

There were no septic transfusion reactions associated with the transfusion of components from diabetic donors.

DISCUSSION

Deferral policies for donors with diabetes vary considerably, partly because of the lack of published studies addressing safety concerns [2]. Most US blood centres accept all donors with diabetes, and eligibility is not covered by the US Food and Drug administration (FDA) guidance. The New York Blood Centre/Innovative Blood Resources specifies that donors should not have had an acute event in the last 3 months, defined as needing the assistance of another person to treat symptoms, such as a severe hypoglycaemic episode [5]. In Europe, donors on insulin are deferred, as specified in the European Directive [6].

Over the course of the year since expanding eligibility to donors with type 1 diabetes, there were 225 first-time donors with this disease, with a median age of 35, more than 20 years younger than the median age of type 2 diabetics. This bodes well for the longevity of these new donors.

As donor populations age, the presence of chronic diseases becomes more frequent [7]. Both type 1 and particularly type 2 diabetes are increasing in frequency [8]. Insulin is now part of the treatment algorithm for some people with type 2 diabetes and is the cornerstone of treatment for type 1 diabetes [1].

Stainsby et al. performed a systematic review of donation safety in individuals treated for hypertension or type 2 diabetes who were not on insulin and found that these conditions were not associated with increased reactions; the level of evidence was very limited [9]. Large US studies analysing reaction risk factors did not identify diabetics as a high-risk group. However, it would be very hard to detect increased risk in a small subset of donors [10].

Our criteria were changed incrementally, starting with donors with type 2 diabetes on stable doses of insulin, many successfully donating for years on oral hypoglycaemic therapy; most were on a long-acting insulin analogue, with reduced risk of hypoglycaemia. Since no adverse effects were noted, this criterion was simplified. Finally, we extended eligibility to donors with type 1 diabetes, trying to mitigate adverse events. Donors are asked if they have eaten in the last 2 h and are given a snack if they have not to reduce the risk of a hypoglycaemic episode, which may have similar symptoms to a presyncopal reaction; similar to the New York Blood Center, donors who had a severe hypoglycaemic episode, requiring assistance from another person are deferred for 3 months. Donors with diabetic neuropathy leading to orthostatic hypotension are deferred, since they might be at higher risk for a syncopal reaction when standing up after donation. Finally, we ask donors if they have any chronic ulcers of the lower extremities to reduce the risk of bacteraemic donations. Although people with diabetes are at higher risk for accelerated atherosclerosis and increased cardiovascular disease, these conditions

are not uncommon in people without diabetes and are covered by specific criteria.

Data from our reaction database demonstrated that moderate and severe reaction rates in donors with diabetes were low and not significantly higher than in non-diabetics.

Our study has several strengths. Since we ask donors about diabetes and medication use and have a specific deferral code for diabetes, we were able to follow the impact of changes over time and evaluate reaction rates in diabetic donors. Our study also has several weaknesses. Since the number of donors with diabetes is relatively small and our reaction database only captures syncopal reactions, reaction frequency CIs are large. We also did not assess the quality of components from donors with diabetes, another interesting area of study [11].

In conclusion, incremental changes in eligibility criteria for donors with diabetes resulted in donation gain, with few adverse reactions. A longer observation period would improve the strength of these observations.

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S.F.O. and M.G. conceived the study and O.M. performed data collection. All authors assisted in analysis and write-up.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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GUIDELINES

Vox Sanguinis ISBT International Society of Blood Transfusion

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Contents

This document has been written by the International Society of Blood Transfusion's Working Party on Information Technology, Validation Task Force.

INTRODUCTION

The objective of validation of applications and qualification of infrastructure is to produce documented evidence that provides a high level of assurance that all parts related to the use of an automated system will work correctly and consistently. There are many ways this objective may be achieved by a blood establishment. Given the complexities and critical control functions of today's automated systems, it becomes particularly challenging to ensure thorough, but not excessive, validation/qualification practices.

Once a validated/qualified automated system is in use, it is essential to maintain the validated state by periodically testing and reviewing its performance.

These guidelines, produced by the ISBT Working Party on Information Technology (WPIT) provide insight into the development of appropriate practices in these areas. They were originally published in 2003 and again in 2010. While many concepts of validation have remained the same, the ISBT WPIT has updated the guidelines and expanded them in the following areas:

- infrastructure qualification;
- supplier qualification;
- qualification of virtual computerized systems;
- disaster recovery planning;
- periodic review;
- software patches/service pack installation and
- backup and recovery.

These guidelines do not advocate a particular validation/qualification methodology but do promote the Quality Risk Management approach advocated by GAMP® 5 [1–3] and ICH Q9 [4], a life cycle approach within the QMS and the use of risk assessments to define the validation/qualification strategy for critical systems. They are intended to be relevant and applicable to all blood establishments regardless of the approach adopted by each or the level of development and resources. To adapt the guidelines to an organization's needs and to be compatible with existing programmes such as the Africa Society for Blood Transfusion (AfSBT) Step Wise Accreditation Programmes (SWAP), consideration should be given to:

- the size and type of the organization;
- the probability and impact of risk to the organization;
- the diversity of activities taking place in the blood establishment;
- the dependence on the automated system for critical control and blood product quality;
- applicable regulations and standards;
- opportunities to leverage the supplier documentation and knowledge and
- the availability of needed resources.

Finally, the approach to validation/qualification used by a blood establishment must allow for the process to be scalable to the functionality of the system, for example, the validation of a centrifuge is less complex than that for a bespoke blood management system.

The WPIT, therefore, encourages each blood establishment to determine for itself appropriate policies and practices for the validation and maintenance of the validated state of its automated systems using these guidelines as a reference.

OVERVIEW

Every blood banking organization must have a Quality Management System (QMS). It is the management's responsibility to participate and approve sign off on the design, implementation, monitoring and maintenance of an effective QMS. It should include a section on validation (e.g., Validation Master Plan or Validation Policy) that describes the organization's policy regarding the validation of equipment, facilities, utilities, methods, processes and automated systems required during the procurement, production and use of blood components. An organization's validation policy should comply with the regulatory requirements applicable in the country of use.

The guidelines are not intended to present a new concept of validation but to be relevant and applicable to all blood establishments regardless of the approach to validation adopted by each. They were originally built upon other field validation experiences and have been updated with the experience gained in validating automated systems in blood establishments.

The benefits of validation are that it:

- improves the use of technology;
- increases the business benefits of computerized systems;

- improves the relationship between stakeholders (users, suppliers, authorities, etc.);
- improves operational efficiency;
- reduces the risk of failure and
- improves compliance with regulations.

These guidelines address the validation needs for automated systems, that is, those that have some degree of computer control. The use of a project process methodology facilitates the achievement of validation requirements and provides the necessary level of control.

Testing of software is not in itself 'validation'; it is a verification activity. It should not be separated from the overall validation of a process/system.

Validation is more than simply testing an application, process or system. Its objectives are:

- to demonstrate control;
- to ensure compliance;
- to generate knowledge and
- to establish future requirements (e.g., training and maintenance).

Validation requires a structured approach. The approach normally used for automated systems makes use of the concept of a computer system life cycle. Approaches may include methodologies developed initially to manage software development and medical device verification. Approaches such as agile validation may be used as long as there is adequate documentation of work performed.

A computerized system life cycle includes phases from the initial concept of the system, through project and operation phases, and through the retirement of the system. The activities of these phases are systematically defined when adopting a life cycle approach within the QMS. The life cycle activities should be scaled depending on the outcome of a risk assessment, systems components, supplier capability and business impact. The life cycle approach enables management control and a consistent approach across systems. It ensures compliance with regulatory requirements and assurance of quality and fitness for the intended use.

Determining what to validate

Blood establishments have to validate all automated systems and computer systems that are considered critical. The system is considered critical if:

- The automated system is directly linked to the decision-making process for blood or blood product manufacturing, testing (donor/patient), labelling and release for transfusion and/or it is used to manipulate the related information.
- The computer system is critical to product and quality, information management, record storage, and tools for operational decision-making and control.

The objective is to produce documented evidence that provides a high level of assurance that all parts related to the use of an automated system will work correctly and consistently.

Deciding how much validation is enough

A question often posed by blood establishments is: How much validation do we need to perform?

Validation is essentially a component of the organization's quality management system, so this question could be rephrased as 'How much quality do we need?' The product quality and cost benefits to be achieved by an organization through adopting the Total Quality Management principles of customer satisfaction, employee involvement and continuous improvement are well-established and are equally applicable to validation.

The answer to the question, therefore, is that the blood establishment needs to ensure that enough of the right work is done by the right people to achieve system acceptance in a way that satisfies the Quality System.

With this in mind, it is worth considering what makes validation projects successful, namely:

- senior management commitment;
- sufficient resources;
- competent project management;
- collaborative team approach, that is, users/technical representatives/validation/quality assurance (QA)/Information Technology (IT) professionals;
- risk assessment and
- cost efficiency.

Validation is a complex process. The skill sets and experience of the team are very important in ascertaining the scope of work to be carried out and that not too much or unnecessary work is performed. There may be a temptation to disregard particular elements to reduce workload. This approach is not recommended and should be avoided.

Determining the resources needed

The process is easier to perform with qualified staff and where validation processes are already established and embedded into the organization. For those organizations that are about to adopt validation practices and that may be lacking validation resources, it is important to consider the following:

- It takes time for validation processes to be developed and become embedded in the organization. In the meantime, the blood establishment wants to continue with its activities.
- It is essential that validation and acceptance of systems are performed before systems are used operationally.
- Use should be made, where possible, of the supplier's system and test documentation to reduce the blood establishment's qualification effort.

PURPOSE

These guidelines were first developed and have been updated by the Validation Task Force of the International Society of Blood Transfusion Working Party on Information Technology (ISBT WPIT).

The aim of these guidelines is to provide guidance on the validation of automated systems in blood establishments which may affect the safety and quality of blood components and services provided by organizations involved in blood collection, testing, processing, distribution and/or transfusion.

Technology is rapidly advancing. These guidelines should serve as a basis for validating emerging as well as existing automated systems.

SCOPE

This document addresses related activities such as application validation, supplier qualification, risk assessment, data migration, disaster recovery planning and IT-specific topics.

- This document does not cover steps in the validation of interfaced automated equipment.
- This document does not cover national regulations.

RESPONSIBILITY

The overall responsibility for ensuring that all critical automated systems are validated lies with senior management.

The validation team may include validation specialists, quality assurance staff, operational users, information technology staff, engineering staff, suppliers, purchasing staff and consultants. The minimum membership of a validation team should be representatives of the process owner, IT and quality assurance. The actual membership will be determined by the scope of the validation. Within certain constraints (e.g., personnel reviewing the validation should not have executed the tests they review), individuals on the validation team may have multiple responsibilities. All validation activities must be communicated to or even involve the top management of the blood establishment.

The following are examples of responsibilities that may need to be assigned to members of the validation team:

- management of the validation process;
- quality assessments of third-party suppliers;
- preparation, execution, review and approval of validation plan and protocols;
- problem resolution;
- identification of required materials and support;
- filing and maintenance of all completed validation documentation;
- verification of data migration;
- development of documents, including Standard Operating Procedures (SOPs) and
- preparation, execution, review and approval of training plans.

CHANGE CONTROL

Any change occurring during a project (before releasing an automated system) or to an operational automated system should be documented in order to ensure that the system is maintained in a state of control.

Change may be initiated by the process owner or others, but it should be controlled by the process owner.

Project change control

During the validation process, before releasing an automated system for operational use, modifications to the configuration of the automated system may be made to comply with specifications and/or end user expectations.

Any change occurring during the validation process must be documented and controlled.

All deliverables in the context of the project or system should be identified, so the items subject to change control may be defined. These include:

- IT Infrastructure;
- hardware;
- software: including application software, operating systems, Database Management Systems (DBMS); firmware, library files, configurable packages, drivers and compilers;
- configuration files/reference tables;
- data migration files and programmes;
- manuals (user manuals, system manuals);
- development documentation;
- validation documentation;
- training materials and
- Standard Operating Procedures (SOPs).

Modifications to system configuration and/or validation deliverables resulting from test deviations encountered during the qualification phases are subject to project change control.

Operational change control

Changes to a live, automated system are managed through the facility's change management procedure. Some changes may require notification to or license amendment from regulatory agencies. Since this varies among countries, users must consult local requirements. Operational change management should continue until system retirement.

All proposed modifications, enhancements or additions should be reviewed and assessed to determine the effect each change would have on the system. This operation should determine the degree of required validation. When changes are made to an automated system, sufficient validation should be conducted to demonstrate that portions of the IT infrastructure and software not involved in the change were not adversely impacted. This is in addition to testing that evaluates the correctness of the implemented change(s). Where required, SOPs and Configuration Management Database (CMDB) should be updated and user training updated and delivered before implementing the changes. All other relevant documentation should also be updated.

Operational change control SOPs should allow for specific variation for certain types of changes such as system administration

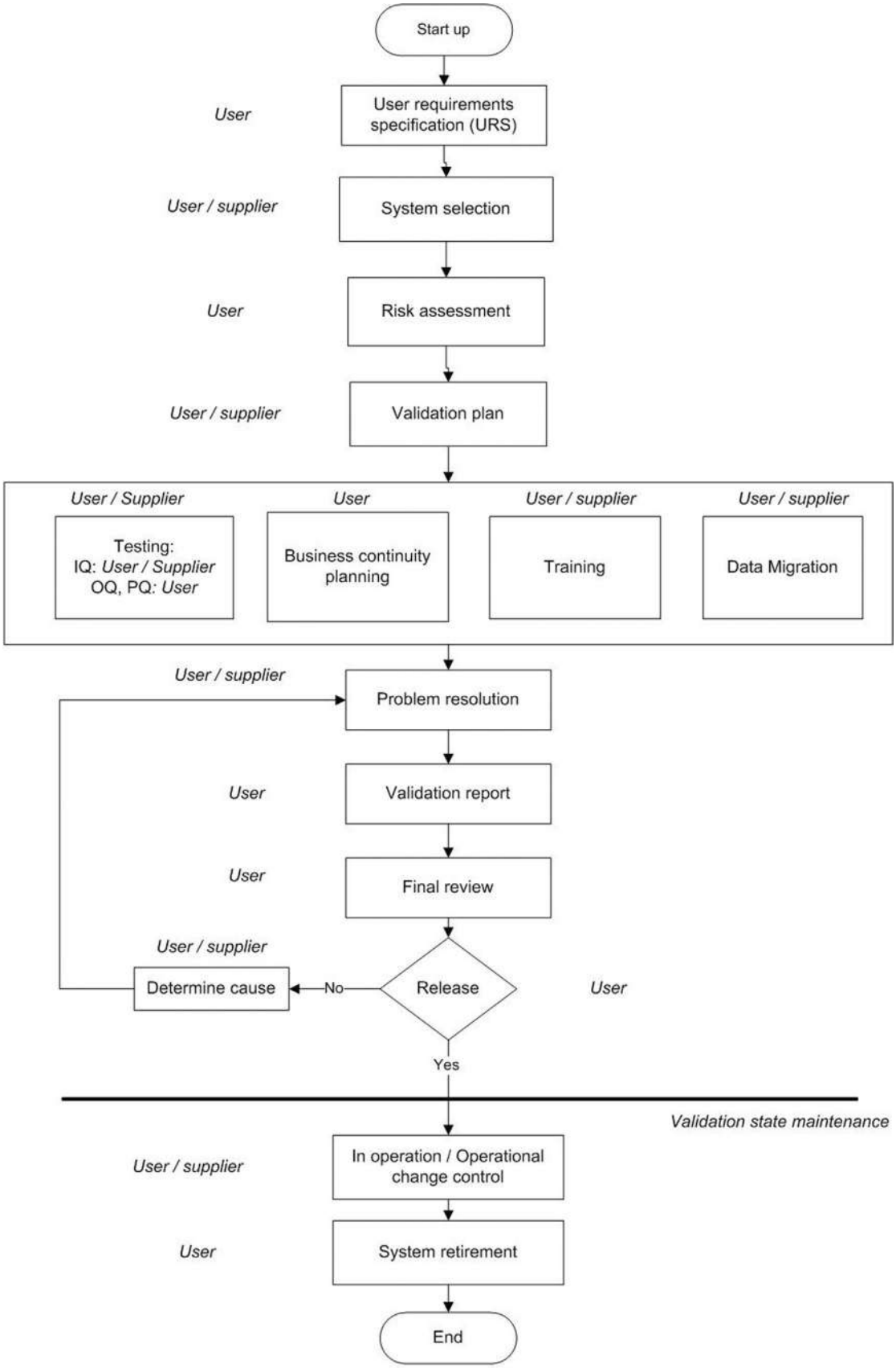

FIGURE 1 Validation process throughout automated system life cycle

Responsibility of the user and supplier is attributed for each step of the validation process

modifications, emergency or repair changes or for workarounds provided by the software vendor.

It is the responsibility of

- the system process owner to ensure that a change control process and associated procedures are in place to support changes to the system;
- the management team to ensure SOPs are followed and
- each member of the change team to execute the assigned activities accurately and completely.

Just as in the guideline sections above, operational changes require a well-thought-out, defined and documented change management plan and process – whether for planned or unplanned changes. This process assures that the system remains in a state of control or can be returned to the previous state of control and is authorized by the appropriate personnel as defined in the facilities plan.

At a minimum, an infrastructure change management plan should consider the complexity and impact of the change and include the following:

- defined change control team
  - quality staff;
  - IT personnel appropriate to the change;
  - review/advisory panel knowledgeable in the system;
  - stakeholders (others impacted by the change) and
  - final authority;
- request – a defined and controlled method to request a change to the infrastructure, including a description of the change, scope and proposed timeline;
- risk and impact analysis;
- consideration of human and financial resources;
- justification – specific outcomes and impact on business, technical and other systems/services, including a risk analysis of these systems;
- test and validation plan – defined testing requirements, at what stage(s), validation requirements, who is responsible and who signs off – the amount of detail will depend on the complexity and the impact of the change;
- backout plan – how to return the system to a state of control if there is a problem either during the change or after the change is implemented;
- implementation plan – including a timeline, resources required and any training/competency required in a manner that will minimize disruption to end users;
- post-implementation review/lessons learned – to assure that the change is working as planned and has not created problems and
- final change review and documented acceptance – a review of all aspects of the change and sign-off by the appropriate individuals as identified in the change management policy.

In an electronically connected world, there are attacks on entities through the Internet. Therefore, it is critical to accept some changes to a system, such as a security patch after a breach. Sometimes a change to the system is less risky than not making the change or waiting until after the completion of testing before making the change. There should be documentation which details why the critical safety patch was needed and that it was installed. It is necessary to test the system to ensure critical functionalities of the system are not affected. In these cases, it is also important to monitor the system closely after installing a critical safety patch to ensure there is no unintentional negative impact of the patch.

Because critical safety patches may be installed without verification, suppliers that provide these services should also follow good IT practices, including quality planning which defines the activities, procedures, deliverables and responsibilities for delivering and monitoring their services.

System inventory

An up-to-date listing (inventory) of all relevant systems and their GMP functionality should be available.

It should consist of:

- name of the system;
- version or model number of the system;
- the owner of the system and
- its validation status.

For critical systems, an up-to-date system description detailing the physical and logical arrangements, data flows and interfaces with other systems or processes, any hardware and software prerequisites and security measures should be available. For this purpose, a CMDB can be used.

VALIDATION PROCESS THROUGHOUT THE AUTOMATED SYSTEM LIFECYCLE

Figure 1 outlines the validation process.

Start-up of validation

Validation should start when the decision is made to acquire a new automated system (including a new information system or new equipment) or to implement a new process. Change to an existing process should also initiate validation as part of the change control procedure. This first step requires the identification of the stakeholders involved.

User requirements specification

An automated system is validated against its User Requirements Specification (URS). The URS is a key document that describes what the process owner wants or expects from the system. It is required for a new automated system or significant change to an existing system (minor changes

should be captured by the change control process), whereas it does not include any 'how', it should clearly state what is required. The URS should form the basis of the contract with the supplier providing additional documentation and system definitions to support the procurement process.

The development of a URS is not an easy task and requires both expert knowledge of the business and analytical skills. It is the user's responsibility but often may only be completed following consultation with potential suppliers or independent experts. The approval of the URS should be documented in accordance with the facility's QMS followed.

In the case of custom-developed software, the URS will form the basis for a Functional Specification (FS), which describes each of the system functions necessary to meet the user requirements. Within the URS, use cases can be used to provide more detail.

GAMP® recommends the following during the production of the specification:

- each requirement statement should be uniquely referenced and be no longer than 250 words;
- requirement statements should not be duplicated or contradicted;
- the URS should express requirements and not design solutions;
- each requirement should be testable and traceable;
- both user and supplier need to understand the URS; ambiguity and jargon should be avoided;
- wherever possible, the URS should distinguish between mandatory/regulatory requirements and desirable features;
- GMP requirements regarding the supplier's quality system should be included;
- in the case of cloud service providers, the supplier will support the facility during the validation process by leveraging validation documents, and
- information security requirements should be included.

System selection

System selection is based on the following considerations covering the entire life-cycle of the system.

URS review

The URS is sent to potential suppliers as a request for a proposal. A supplier's response should be based on the functionality of their system (functional and technical specification) and how well they meet the user requirements. During the URS review, the responses of the supplier candidates and/or their system FSs are compared with the URS in order to identify suppliers and systems that may qualify.

Supplier qualification

An important part of the validation, which is not a testing activity but is vital to the quality of blood and blood components, is assessing a supplier's ability to provide critical components of computerized systems [including a Blood Establishment Computer System (BECS)], whether hardware, infrastructure, cloud services or software.

Purchasers must ensure that suppliers have implemented QMS. Suppliers of BECS must follow the Quality System Requirements in the country where the BECS will be installed. Since suppliers of BECS may install their systems in multiple countries, harmonization of their Quality Systems used across the world has become best practice. There should be a methodology in place to verify the suitability of suppliers of BECS, critical IT equipment and services prior to selection.

A Computerized System has been defined in GAMP 5 [3] as: "Any automated or digital system used in the business. The Computerized System consists of the hardware, software and network components, together with the controlled functions and associated documentation."

The supplier of critical IT products and services should be assessed using a questionnaire/survey, an on-site audit or a combination of both. A qualified auditor or a third party can also perform the audit of the supplier if needed.

The assessment should evaluate the status of the supplier's quality system. Attention should be given to the supplier's procedures for the development, support, maintenance and distribution of updates. If the supplier is an existing supplier, the results of previous assessments should be reviewed and taken into account when evaluating the supplier. Arrangements for the supplier assessment should be formally agreed upon by the user and supplier and documented.

Supplier qualifications such as ISO 9001 and 27001 can be taken into consideration when qualifying a supplier.

For less critical services and products and for suppliers of infrastructure and professional services, assessment by questionnaire is sufficient.

The use of referrals and information found on the Internet about the service-oriented company may be as useful as an audit to verify the company's adherence to their QMS and how successful it is at adhering to requirements and delivering its services to clients. However, it is recommended that a combination of both methods (audit plus Internet search) be used. If possible, find another blood establishment that used the service company for a similar service and ask probing questions to uncover any non-adherence to cGMPs.

Supplier qualification may vary depending on the type of service provided. For example,

- For an infrastructure or platform supplier, infrastructure life cycle, security control/measure implementation, continuity of infrastructure and availability of installation documentation should be assessed.
- For a software provider, the existence of a software life cycle, change control policies, data integrity, user documentation, control of user access and availability of validation plans for software should be assessed.

Each potential BECs supplier's QMS should include a documented policy to ensure that the automated systems introduced to blood establishments are compliant with GMPs and adequate for the systems' intended use.

The supplier's QMS should:

- integrate life cycle activities for the process being followed to deliver and support the product, application or service;
- specify responsibilities, including making it clear there should be a separation of authority between quality assurance and other groups, such as product development, product support, finance or marketing;
- determine the appropriate deliverables, documentation and planned periodic reviews of supplier's adherence to the QMS;
- follow a validation framework, including the use of validation plans and validation reports as necessary and
- maintain compliance throughout the life of a system and have an approach to continuous improvement of the QMS and its use.

Some potential questions that can be used to ascertain a supplier's suitability for activities, services or supplies as part of a medical device may be found in Appendix A.

System evaluation

System evaluation consists of assessing:

- the system against regulations and standards, including GxP;
- the system against established user requirements;
- the needs of the system and environment configuration;
- the requirements for installation;
- the training requirements;
- the technological standard of the system, that is, future proofing and the road-map of the future development and
- the supplier to ensure it uses a recognized development methodology.

Evaluation will be against criteria specified by the user in the URS. Results of the system evaluation should be presented in a report.

From the user perspective, system evaluation should be performed on critical automated systems that are configurable, off-the-shelf packages or bespoke developments.

Financial considerations

Depending on national policies, financial consideration is an important element in the selection of a new automated system. The user should consider costs of the entire life-cycle of the system including:

- one-time implementation costs such as:
  - software licensing;
  - hardware, interfacing and peripheral costs;
  - data migration, installation and training costs;
  - validation effort needed and
  - travel and lodging expenses.
- on-going costs, such as:
  - software support;
  - hardware and network maintenance;
  - yearly fees for infrastructure and software licenses and/or service;
- archiving of historical data and records;
  - frequency of anticipated software updates and system upgrades and amount of revalidating involved;
  - additional staffing in technical, quality, end user areas, etc. and
  - retirement costs.

Risk assessment

Risk assessment is required when an automated system is new and to be implemented, changed, upgraded or its retirement planned. It must be performed to identify critical control points, determine the degree of testing required and define risk mitigation plans. This requires considerations of the impact, probability and detectability of a potential hazard or harm to a computerized system.

Risk assessment also looks at the critical control points in the software and can identify those areas where, if there is a failure or malfunction, harm to the patient, donor or business may occur. Risk assessment should at least consider the following elements: patient risk, product quality and data integrity.

A risk assessment has an important place in the validation process as it can maximize testing resources. Since it is impossible to test everything, it is best to identify the higher risk functionalities and spend proportionally more time and effort on validating these processes.

Many automated systems used in blood banking are considered to be configurable software packages. A typical feature of these systems is that they permit each institution to develop its own applications by customizing/configuring the system. Each application then becomes specific to this institution, and maintenance of the system becomes an important process, especially when new updates to the system are installed. Often, the configurable system is part of a much bigger network, which, in turn, becomes the entire system. This makes it impossible for the vendor to validate each different type of final system. The amount of testing and how many times the same process is tested is dependent on the amount of risk the functionality may present. This should provide the user with a higher degree of assurance that the system will consistently produce a quality product.

A systematic approach is needed to perform a thorough risk assessment. First, each potential risk of a system or subsystem is identified and traced to a trigger, event or cause. Information regarding each potential risk is collected, analysed and a GAMP [3] category assigned (see Appendix B). For an example of risk categorization see Table 1.

TABLE 1 Risk categorization

High	Risks are considered to be intolerable
Medium	Risks are undesirable
Low	Risks are so low as to be negligible

TABLE 2 Documentation important to the validation process

Steps of the validation process	Type of documents	Supplied by
User requirements specification	User requirements specification (URS)	User
System selection	Installation requirements *Functional specifications *Hardware design specifications *Software design specifications *Engineering diagrams Manual/user guides Supplier questionnaire/survey Supplier audit report system evaluation report change management policy	Supplier
Risk assessment	Risk analysis	User
Throughout	SOPs Use of the automated system Support activities Backup and recovery Archiving and record retention Change control Security management Periodic review Business continuity/disaster planning System retirement Training Maintenance and monitoring	User
Validation plan	Validation plan Validation protocol	User
Training	Training plan Documentation of training Training material	User and supplier
Testing (IQ)	Test results	User and supplier
Testing (OQ, PQ)	Test results	User and supplier
Disaster recovery plan	Countermeasures plans Disaster recovery plans Continuity action plans	User
Problem resolution	Problem resolution records	User
Validation report	Validation report	User
In operation	Maintenance and monitoring plans and records Periodic audit/review plans and reports Change control and incident records Data migration plan	User
	Change notification	Supplier
System retirement	System retirement plan and report	User

Note: *User may have to assume that supplier has these documents.

Next, options should be provided for risk reduction to either mitigate and/or eliminate the risk. It may be decided that the risks in the system are so high that it should not be implemented. If it is decided to go forward with implementation, controls, either process or product, need to be used to mitigate and/or eliminate the identified potential risks. Mitigation generally involves testing or creating workarounds, either with independent software or written SOPs that prevent the end user from replicating the risk identified in the system process. Documentation of the entire process must be produced, approved, controlled and retained for the period required by national regulations. Table 2 includes documents that are typically required to provide an audit trail and assure the quality of the validation process, including the maintenance of the validation state.

Validation plan

A validation plan should be prepared after a decision is made to implement a new or change an existing, system. It is recommended that the validation plan be prepared as a cooperative effort by subject matter experts, IT, quality and production management staff. The level of risk is a major factor in determining the level of effort to be applied in testing and other verification and validation tasks. It may be revised,

under change control, during the life of the validation process. Once the validation is performed, the plan becomes a historical record.

The validation plan should provide a description of:

- the automated system;
- the validation activities;
- responsibilities;
- the procedures used during the validation;
- operating procedures post-implementation;
- expected outcomes and
- acceptance requirements.

User and supplier roles and responsibilities for validation activities must be defined. The identity of authors, reviewers and approvers of the deliverables must be identified in the plan. Procedures for documenting, reporting, evaluating and resolving incidents and deviations discovered during the validation process should be included, as well as a mechanism for documenting and justifying exceptions to these procedures and the validation plan.

The completed validation plan must be reviewed and approved according to the facility's quality system policies. The validation protocols are used to produce documented evidence that the system performs as intended.

Content of the validation plan

The validation plan should cover the following topics:

Scope of the validation. The scope of validation should specify the automated system's identification, the context of the use of the automated system, the automated system's definition, the automated system's boundaries, that is, what is in and out of scope for this validation project, the processes to be employed and the aim of the validation.

Risk management. Risk management should involve an initial assessment, including a decision on whether the system or its part(s) is GxP regulated or not.

Validation strategy. The strategy to follow for validation will depend on the type and complexity of the automated system and the degree of risks of its use. It is mainly based on the different elements identified in the risk assessment and documents provided by the supplier concerning the supplier testing performed, use and administration of the automated system. The amount, type and results of supplier testing may be used to focus on and determine the amount of testing needed during the validation efforts.

Validation strategy should define which activities may be performed *prospectively*, *retrospectively or concurrently* (see Glossary for definitions of validation, prospective; validation, retrospective and validation, concurrent). The strategy must define the system platform(s) and controlled environment upon which the qualification processes are to be performed. Qualification of complex blood management systems would ideally take place upon a frozen test system, which is identical to and separate from the live environment. Less complicated equipment should be isolated from the operational environment during the validation testing. Installation Qualification (IQ), Operational Qualification (OQ) and Performance Qualification (PQ) classify the different validation tasks and testing that have to be performed to ensure the quality of the use of an automated system.

Installation qualification (IQ). IQ shows that the system has been installed correctly. Once IQ has commenced, the system and infrastructure should be under formal change control. Support from the supplier is required during IQ testing. Important IQ considerations are:

- hardware and software installation;
- installation conditions [wiring, utilities, uninterrupted power source (UPS) etc.];
- interface connections exist;
- preventative maintenance;
- safety features;
- supplier documentation, prints, drawings and manuals;
- software and hardware documentation;
- spare parts list;
- software backup;
- security aspects and
- environmental conditions (such as temperature and humidity).

Operational qualification (OQ). In this phase, the automated system and process operating parameters should be challenged to ensure that they will result in a product that meets all defined user requirements under all anticipated conditions of manufacturing, including worst-case testing.

OQ considerations include:

- functionality of the automated system;
- alarms and limits;
- configuration;
- process control limits monitored by the automated system;
- software operational parameters (ideally linked to the functional and design specifications as provided by supplier);
- automated system operational specifications;
- interface testing;
- process operating procedures;
- process change control;
- training;
- preventive maintenance and monitoring;
- evaluations for potential failure modes and worst-case conditions (risk analysis and critical control points, failure mode and effect analysis, fault tree analysis);
- backup and recovery and
- system access and security.

Performance qualification (PQ). The objective is to demonstrate that the computerized process will consistently produce acceptable product/output under normal operating conditions. Due to practical reasons, part of the limiting and boundary conditions testing is often performed at this stage. The demonstration is achieved by using the appropriate methods and tools for process validation.

PQ considerations include:

- use of actual computerized parameters and procedures established in OQ and used during the operation;
- reconfirmation of acceptability of the computerized processes as established in OQ;
- reconfirmation of process repeatability and assurance of process stability when used in the field with trained operators;
- data migration to the new platform and
- stress or load testing (data to prove stability and capability of the automated system).

Challenges to the process should simulate conditions that will be encountered during the operation. Challenges should include the ranges of conditions covered by the SOPs and should be repeated enough times to assure that the results are meaningful and consistent. Challenges will need to include forcing the process to operate at its allowed upper and lower limits.

Reports of qualification activities should be written and adherence to the requirements documented. The qualified infrastructure should be under change control.

Supplementary qualification(s). For more complex systems, it is often necessary to expand the qualification exercise to include functionally specific testing, which does not readily conform to the criteria for IQ/OQ/PQ defined above. For example, a separate Interface Qualification may be required when validating interconnected systems or a Cutover Qualification may be required to verify system security or operational features following the installation of the system in the live environment.

Formation of the validation team. The use of a team ensures that the validation processes are well analysed that the protocols are comprehensive and that the final validation package is well documented and easy to follow. The team should advise about 'worst-case' scenarios, communicate with key functional areas about new and changed products and foster cross-functional cooperation. Members of the validation team include end users, quality assurance staff, IT staff and others (facilities engineering, manufacturing, laboratory, technical services, research and development, regulatory affairs, purchasing and top management) depending on the subject.

Timeline. Depending on the complexity of the validation process, a timeline should be established in order to:

- evaluate the time and resources needed for the validation;
- define the period over which the validation should be performed and
- define the time when the automated system should be operational.

Validation deliverables. Relevant documents that must be obtained during the testing process should be specified (screen prints, installation reports, SOPs that have to be produced, graphical displays, electronic data, etc.). These documents will be used to evaluate whether the automated system can or cannot be released.

Acceptance criteria. The general acceptance criteria for validation testing and the acceptable overall outcome of the validation process should be defined in the validation plan.

Responsibilities and approvals. The plan should identify roles and responsibilities, as well as the individual(s) responsible for approval for release into production.

Validation protocols

Validation testing is performed using detailed validation protocols, which are developed as required from the validation plan and the risk assessment. For IQ, OQ and PQ, validation protocols should contain:

- the scope covered;
- the test instructions;
- the expected results;
- the acceptance/rejection criteria;
- spaces for capturing results of the tests, including a pass or fail statement that confirms the outcome of the test and
- a section for the tester and the reviewer to sign and date.

Validation protocols should be independently reviewed upon completion.

Data migration

Data migration is the process of transferring existing data, either manually or electronically, from a source system to a target system (usually from an old system to a new system). The source, as well as the target, can be single or multiple systems. Data migration may vary in scope, complexity and risk. The data migration process should be managed according to a specific plan and requirements described in a data migration plan. The goal of a data migration validation is to ensure data integrity in the new system(s).

The content of the data migration plan may vary depending on the complexity of the data migration processes. It must set forward sufficient elements to guide the data conversion team to a successful data migration. The plan should cover:

- migration scope;
- roles and responsibilities;
- requirements and deliverables;
- risk analysis;
- configuration management strategy;
- software tools and strategies for ensuring compliance and fitness for the intended use;
- data quality assessment;
- data mapping;
- data cleansing rationale;
- data transformation rules;
- migration steps;

- data verification strategy and acceptance criteria;
- system transition plan and
- rollback or workaround strategy if the migration fails.

Plan

In the planning stage, the first step is to perform a general assessment of the requirements. Based on a risk assessment approach it is essential to identify and develop key elements of a data migration plan. Although data migration may vary in complexity, the objective is that the integrity of the data is not compromised and that its context value remains.

For a successful migration, it is important that there is a good understanding of the data that exists in the current system. All possible data sources for the migration should be identified, and extractions and queries should be used to assess the 'cleanliness' of the data. The rationale for cleansing the data should be documented.

User requirements are formulated for the desired functionality of the data on the target system. If the target system is already in use in the production environment, care should be taken to ensure that there is no discrepancy between the user requirements and the existing functionality. A plan to deal with discrepant data (e.g., different blood groups on a given patient) should be documented and followed.

A migration specification document must be created describing the mapping of the fields from the old system to the new system. The document should also contain all necessary translations and/or modifications of database fields during the migration process.

All migration steps, as well as actions between the extraction and the import, must be documented in the data migration plan. If it is necessary to perform additional actions on the target system (i.e., on the imported data or the system as such), these actions should also be included in the document. Data migration requires several steps and should include verification of the data to ensure that the data being migrated is correct and in the proper format for use in the target system. There may be considerable differences between the database structure of the source system and the target system. The format and the functional usage of data in the receiving system can be significantly different; for example, limitations in the field length can create severe data integrity errors.

Execute and report

Once the data migration plan is written and approved, migration test runs should be performed in a test environment. For achieving an effective data migration procedure, data on the old system is mapped to the new system providing a design for data extraction and data loading. The design relates old data formats to the new system formats and requirements. The migration may involve many phases, but it minimally includes data extraction, where data is read from the old system and data loading, where data is written to the new system.

Iterations are part of the execution of the migration process. Prior to any iteration, parameters, translation tables, and code should be frozen to provide a stable platform for the iteration. Once the data is transferred, it must be verified. If corrupted data is identified, scripts must be corrected and data migration testing repeated.

Each iteration of the process should at least include these control check points:

- collation of migration process timings (extraction, transmission, transformation and load);
- continual identification of data cleanse issues;
- confirmation of parameter settings and parameter translations;
- identification of any migration merge issues;
- reconciliation and
- deviations.

The execution of a data migration process should be consistently repeatable and accurate. The data migration process should be repeated until it reaches consistent results and meets the requirements set in the data migration plan. Once the migration test runs are completed and the data accurately and completely translated, the integral end-to-end data migration process, as described in the data migration plan, can be performed in the production environment.

Perform migration verification in the production system

After loading into the new system, results are subjected to data verification to determine whether data was accurately translated, is complete and supports processes in the new system. During verification, there may be a need to run both systems in parallel to identify areas of disparity and prevent erroneous or lost data.

Points for consideration are:

- Is all user data correctly converted to the new format?
- Are there any missing records or fields?
- Are new fields initialized to correct values?

One of the methods for testing and verifying results is sampling. In addition, there are manual inspections that examine the results of a migration and process checking, which, unlike sampling and inspections, focuses on verifying that the tool or script used to move the data works as intended.

The migration plan is executed, and the process and migrated data are validated. Ideally, validation should be performed on the production system. In some cases, this is not a possibility. This situation can arise when the production system is in use or because validation requires manipulation of the imported data that cannot be reversed. It may then be necessary to perform the validation on a copy of the production system. In this case, the validation report should contain a precise description of the differences between the validation and the production environments and the impact the differences may have on the validation result.

When validation has been performed on a copied system, the actual migration can subsequently be performed with minimal testing on the production system.

It is important after successful data migration that the access to data in the old system is locked. This does not prevent access to the data by authorized, knowledgeable staff; it prevents modification of the data.

Data integrity

Data Integrity is the accuracy and consistency of data stored in a database, data warehouse or other similar data storing constructs. Data Validation comprises the tests and evaluations used to determine the correctness and reasonableness of data. It is not feasible to check all data during a validation. A sampling of data, the size of which is determined by risk analysis, should be performed.

General expectations

Data types. In a computer application, the data types are classifications of data such as Character, Number (integer or real numbers), Boolean and Date (date or date and time). Validation test cases must ensure that data types stored will be data types retrieved.

Data elements. Data elements are basic units of information built from standard structures having a unique meaning and distinct units or values. Examples of data elements are customer name, address and date of birth. Validation test cases must ensure that data elements stored will be data elements retrieved.

A record. A record is data associated with a given item (e.g., product, donor, patient, instrument). It consists of a group of data elements assembled in a particular order and with the same data types. Validation test cases must ensure that records stored in their entirety will be retrieved without compromising data elements in the record and their associated data types.

Count. The number of records stored in the database. Validation test cases must ensure that the number of records stored equals the number of records retrieved.

Record updates. An update to a record is changing the record to reflect new information. Validation test cases must ensure that similar data types are updated with compatible data types and that the key data fields are not modified. For example, the date of birth cannot be updated by an integer or character; similarly, a number cannot be updated to a character data type.

Deletes. A deleted record is when information is removed from the record. When computer application requirements allow deletion, the validation test cases must prove that deleted records are not retrieved.

Related records. A primary record is a unique identifier. Information associated with a primary record is captured in the related record(s). For example, a donor or patient identifier is a primary record, but there may be multiple addresses (related records) associated with the identifier. The validation test cases must prove that primary records and related records are retrieved as expected.

Dangling records. A dangling record is a record that has become disassociated with the primary record. When a primary record is deleted or deactivated, the related records must be deleted or deactivated accordingly. If a related record is not deleted or deactivated, then a dangling record exists in the database. The validation test cases must prove that there are no dangling records in the database.

Truncation. Truncation is when a data element is abbreviated. When the stored data in a data element is larger than the field can hold, information may be lost. Validation test cases must prove that truncation is accepted or rejected depending on the requirements.

Calculations. Calculations are when an input to a data element is converted before storing it in the database. The validation test cases must ensure that the retrieved value is equivalent to the entered value. For example, an employee's weekly work hours may be stored as seconds in the database, but when retrieved from the database, the data element is accurately displayed as hours.

Algorithms. An algorithm is a sequence of instructions where the main goal is to solve a problem. For example, calculating donor eligibility based on the last donation date or deferral information. The validation must ensure that the algorithm works as expected.

Encryption and decryption. These terms express the process of converting data to prevent unauthorized access to digital information. When the input to a data element or the entire record is encrypted before storing in the database, it must be accurately decrypted back to its original value.

Zero-size database

When a computer application is launched with a zero-size database—meaning there is no history or any existing data in the database—validation test cases must prove that:

- new data captured in the database is mapped properly to data types; for example, characters to character, date to date, number to number, etc.;
- the number of records entered is equal to the number of records stored;
- the number of records stored is equal to the number of records retrieved;
- updates applied to data elements are reflected when the record is retrieved and
- retrieved records exactly match the stored data elements in each record.

Converting the existing database to the new database

It is common practice to convert an existing database to a new database when:

- a new computer application is launched;
- the existing computer system is modified;
- the database provider has a major upgrade;
- there is an upgrade of the hardware and

- there is an upgrade of the operating system.

When converting (or migrating) to a new database, the validation test cases must prove data integrity by verifying the following:

- the number of records for each component (often known as tables) in the old database is equal to that in the converted (new) database;
- the required data elements have been migrated properly to the new database;
- data types are mapped properly in the new database;
- no truncation or loss of data has occurred in the new database;
- no loss of precision has occurred in the new database (e.g., 3.44445 is converted as 3.44445) and
- no loss of referential integrity such that there are no dangling records (for example, all donated units must be associated with a donor).

Additional data integrity checks may include the following match between the old and new databases:

- Number of donors;
- Number of blood donations for each donor;
- Number of donors by gender;
- Number of donors by postal code, city and state;
- Number of donors by each blood group and Rh type and
- Number of donors in each age group (e.g., 18–25, 26–30, 31–40, 41–50, etc.).

Additional record sampling validation

Automated reports. Test cases must include automated reports that ensure data integrity.

Reports and queries: when reports and/or queries are available in the computer application that is designed to extract data from the database, these reports or queries must be tested to verify each data element.

Manual verification. Test cases must include manual verification when automated reports are not possible (e.g., counting the number of Group O units in inventory manually to verify the number of Group O units in the electronic inventory is correct).

Manual spot checks of data elements. It is important to consider including validation test cases for manually spot-checking data elements. For example, it is reasonable to use the following guidelines for sample size as appropriate based on risk analysis.

- If the database size is in thousands, consider 1% to 2% of records for spot checks.
- If the database size is in millions, consider numbers like 500–1000 records selected randomly or by an algorithm (e.g., one out of every 100 donors in an alphabetical list) to perform spot checks.
- Special cases: If there are known special cases, run reports or queries against select special cases and perform spot checks.

Infrastructure qualification

IT infrastructure refers to the composite hardware, software, network resources and services required for the existence, operation and management of an enterprise IT environment.

A separate Performance Qualification is not expected for infrastructure as the PQ of the infrastructure is included in the OQ and PQ of the application(s) using the infrastructure.

The following hardware components (physical or virtual) are part of the IT infrastructure:

- connectivity elements [Local Area Network (LAN), Wireless Fidelity Network (WIFI) and Wide Area Network (WAN)];
- connectivity infrastructure that includes active and passive components. (Examples of active components are repeaters, switches and routers. Passive components include cables, connections and outlets.);
- servers enabling office automation that manages business applications, databases and data storage;
- workspace clients, such as thin or fat clients, personal computers, handhelds and
- peripherals (for example, scanners, printers and label printers).

In addition to visible physical components, IT infrastructure includes software. Software may:

- be central office automated services, like mail, file and web services. These services are not directly related to business applications;
- control the hardware (operating systems, firmware);
- be used for processing, storage and transport of data (databases, interfaces);
- manage the communication with the users (user interfaces, web servers);
- control the security of the system or
- manage virtual platforms.

When qualifying cloud services [particularly infrastructure as a service (IaaS) and platform as a service (PaaS)], the importance of qualifying the supplier becomes evident; in many situations, it is not possible for the organization to actually perform the qualification actions. Suppliers may not be keen on sharing their inside information (necessary for IQ/OQ activities) with customers for security reasons.

When qualifying the supplier, the emphasis is on reports from third parties regarding General IT controls (for instance, ISO 27001 certification and/or ISAE 3402 reports).

A prerequisite to ensuring a controlled and validated automated system is a qualified infrastructure including servers, networks and clients and other devices that are part of the network. This provides the foundation upon which the automated system, that is, the GxP application runs in an environment that is continuously maintained and in control.

Normally the infrastructure is qualified with an IQ and sometimes OQ. The PQ of the infrastructure is performed during the validation of the application(s).

According to GAMP®, recording version numbers and verification of correct installations are sufficient for qualifying infrastructure software, which is composed of established and commercially available layered infrastructure software (upon which the automated system application is built). The documentation and tests described below can be included in the validation of the application. At least minimal testing is needed for infrastructure qualification since proving the application runs correctly using the established infrastructure is what is important. A risk-based assessment approach should be used.

Servers

Qualification deliverables for servers should be limited to the equipment and its associated operating system and utilities. System upgrades require updated documentation and possible retesting.

- Requirements Specification should specify the functional requirements for servers and their operating systems and utilities.
- Design Specification should specify the actual configuration and setup of the equipment, operating system and utilities of the servers.
- Installation Qualification should:
  - capture the installation of the server. Serial numbers and models should be included. Any additional components not installed by the manufacturer should be documented
  - include the operating system, patches and upgrades, additional utilities and toolkits
  - Include start-up and shutdown procedures.
- Operational Qualification should include, at a minimum: the process of backup and recovery, data archival and retrieval (if applicable), critical aspects of security, functionality of the uninterruptible power supply, communications between servers and interfaced equipment, existence according to design of any system redundancy (such as mirrored drives) and secondary/failover systems.

Network infrastructure

- The network infrastructure can be defined as transportation and communication systems. Testing of the Wide Area Network (WAN) and Local Area Network (LAN) should be limited to the major components of the WAN/LAN. The network infrastructure is a dynamic environment; therefore, it is necessary to establish and follow good engineering, documentation and quality assurance practices. Network upgrades require updated documentation and possible retesting.
- Requirements Specification should (1) specify the functional requirements for the major components of the WAN/LAN infrastructure and (2) specify the required redundancy of the infrastructure.
- Design Specification should specify the actual equipment for the major parts of the WAN/LAN infrastructure. It is a description of the physical hardware components, such as hubs, switches, routers, patch panels and of the software components, such as transport protocols and network operating systems. WAN/LAN interfaces are included; other components, such as cabling, power supplies and interface cards, should also be captured.
- Installation Qualification should (1) capture the physical installation of the major components. Serial number and model should be included. (2) include the documentation of software on standalone switches and routers.
- Operational Qualification should use automated test equipment to verify that the appropriate security levels and filters are operating correctly and that the cabling works according to the requirements.

Clients

Where possible, organizations should be in control of the clients by disabling administrator rights for non-IT department users. The clients should be controlled via policies, procedures, CD images and audits. System upgrades require updated documentation and possible retesting.

- User Requirements Specification should (1) specify the functional requirements for the type of client workstations and laptops and (2) document the organization's standard type of clients and the minimum hardware requirements, as well as the current operating system including its patches, upgrades and software to be used.
- Installation Qualification should record system information (type of hardware, serial number of image/build in accordance with the established procedure).
- Operational Qualification should be performed by testing the applications running on the client in order to ensure that the applications operate according to their intended use in the client/server environment.

Qualification of virtual computerized systems

A virtual machine (VM) is an image file that exhibits the behaviour of a separate computer, capable of performing tasks such as running applications and programmes like a separate computer.

Qualification of virtual machines can be conducted as for non-virtualized systems while effectively mitigating the specific risks.

While considering risks associated with a virtualized environment, some of these risks are the same as for all other items of infrastructure; however, the complexity of the virtualized environment means that additional failure modes must be considered and that risk likelihood and detectability must be reconsidered.

Specific risks:

- Because of complicated data backup/restore procedures, there is a risk of corrupt, incorrect or lost data in certain parts of the virtual environment (e.g., wrong Storage Area Network, restoration to a wrong storage location, misconfigured failover resources);

- Suppliers may not support applications which are not designed for a virtual environment;
- There may be a dependency on a supplier to manage access to the platform and servers and
- Since physical network connections are replaced by virtual networks (VLAN), security risks to consider are (1) incorrect configuration of VLANs and (2) faulty interconnections between virtualized systems.

Complete virtual machines (including virtual hardware, operating systems, prerequisite software and configuration) can be qualified once and deployed many times in a consistent and repeatable manner.

Training

All personnel developing, maintaining or participating in the qualification process must be trained before beginning any validation activity in accordance with the facility training policies.

A plan must be developed to ensure staff are trained on the various functions they will be performing and that they are declared to be competent. It should be specified who requires training, at which level they have to be trained, and the documents on which the training is based. The choice of the appropriate training methods will be determined based on system complexity, the tasks to be performed and the background of the trainees. Suppliers may provide training support.

Once training documentation and SOPs are written and the automated system installed, training can be performed with or without instructors. It must be supported with clear training instructions and concurrent documentation of the training. The competency of the trained staff should be evaluated and documented. By the completion of training, operators should be able to perform the intended functions and respond in an appropriate and timely manner to all alarms, warnings and error messages.

Testing

Prior to testing, the system must be configured and frozen, and a change control mechanism must be established. All documents required for the qualification phase as defined in the validation plan must be available.

The results from testing should be documented on the validation protocol or an annex document against predefined acceptance criteria stated in the test instructions. Test anomalies should be captured and reviewed with the outcome documented (see Problem resolution).

The following rules for testing must be applied:

- All test results should be recorded indelibly.
- Any corrections should be performed according to the rules specified in the QMS of the institution, and a reason for the change should be specified if not obvious to an auditor of the information.
- Shorthand notations such as ticks should be avoided.
- Test results should be directly documented as testing occurs and should be retained (e.g., screen prints, reports, queries). Suppliers may provide documentation of validation testing electronically by means of grids with each step, acceptance criteria, interpretation and documentation of results using a screen print.
- Problem logs and resolution should be maintained.
- Testing summaries should be established.
- Test results should be reviewed and approved by a competent, independent person(s).

Problem resolution

All problems encountered during testing should be documented. Problems will fall into two categories: validation test case failures (for example, the system does not perform as expected, operator input errors that cause a test failure, errors due to configuration settings, outcomes that are not as expected but are acceptable) and test cases are inappropriately written.

Validation test case failures. The following tasks must be performed:

- documentation of all incidences of test case failure;
- investigation of all incidents to determine if:
  - the test case was properly written;
  - there was user error in executing the test case;
  - there was a specification error or
  - there was a system limitation.
- reporting of software programming problems to the vendor;
- identification of a solution (e.g., workaround, reconfiguration);
- documentation of resolution and
- depending on the change required to fix the problem, determine if only the test case should be re-executed or if regression testing of several functions is required. A risk assessment-based approach should be used to determine the amount of additional testing to be performed.

Validation report and final review

The validation report presents the results of the validation activities, including data migration, interpretation of the validation outcome and the conclusions drawn. If unexpected outcomes are obtained, they should be summarized. The summary should define what changes and/or 'workarounds' will be needed to mitigate the risk.

The final review is performed by staff identified in the validation plan upon completion of the validation process and consists of reviewing documents as specified in the plan. The review should confirm that:

- the documentation is complete;
- the testing proves, with a high degree of assurance that the system will consistently meet its acceptance criteria;
- data migration is complete and accurate;

- any non-conformance was addressed through problem-solving;
- training requirements have been met and
- a disaster recovery plan is in place.

The possible outcomes from this review are:

- release (go-live),
- conditional release (go-live with issues that do not impact patient safety, product quality and data integrity) or
- do not release.

The system can only be released by qualified personnel. If the system cannot be released or can only be conditionally released, the reason for the decision must be documented. In all instances, the decisions made must focus upon the importance of patient and product safety and data integrity.

After release, the facility is responsible for maintaining the validated state of the automated system according to pre-established plans.

Go-live process

Go-live is the process of going from project to operational status. This involves a transfer of responsibility from the project team to operational staff. The transfer process scope, acceptance criteria and transfer checklist should be established beforehand. Transfer activities performed should be described and approved paying special attention to the communication of open issues and incomplete activities or documentation. A period of monitoring the system after go-live is needed, and a rollback strategy is defined for serious problems emerging. The formal acceptance of the automated system and controlled transfer into the live operational environment should be documented.

ON-GOING ACTIVITIES

Disaster recovery plan (DRP)

A Disaster Recovery Plan (DRP), part of a business continuity plan, is required and consists of a number of elements designed to minimize disruption to the business in case of system failure/unavailability. An approach based on risk assessment is recommended. The following is recommended:

- Prepare a countermeasure plan to first identify risks and then mitigate those risks. This can include hardware redundancy, maintenance, system monitoring and data backup procedures, training and security arrangements.
- Prepare a DRP detailing how the system will be recovered and brought back into operation.
- Define the responsibilities of business, IT and IT suppliers.
- Periodically test the DRP.
- Identify individuals within a command centre for managing the disaster process. There must always be a team of experts (DR Team) in control of the DRP. The leader of this team must have enough authority for decision-making.

A **DRP** should consist of the following phases:

1. *Activation and notification phase*: Activation of the DRP may occur during planned events or after a disruption or outage that may extend beyond the Recovery Time Objective (RTO). The RTO is defined by a Service Level Agreement (SLA) for a system.
   The DRP team will notify application owners and process owners of the situation and (if applicable) about a possible long-term outage.
2. *Assessment phase*: Once the DRP is activated, perform an outage assessment and impact analysis for the system. Present findings from the outage assessment to a central disaster management team.
3. *Determining appropriate steps*: Based on the impact analysis, determine which disaster recovery steps will be invoked. Where the plan does not cover the situation, define appropriate measures.
4. *Recovery phase*: Implement the activities and procedures for recovery of the affected environment. Notify and escalate procedures for communication of recovery status to application owners and process owners as needed. Verify that alternate computerized systems used during recovery are working as intended.
5. *Reconciliation phase*: Reconciliation begins when operations return to their normal status. Perform actions to verify system capability and functionality has been restored at the original or new permanent location. Verification procedures may include functionality or regression testing, operational testing and/or data verification. At a minimum, the primary system's capability and functionality are verified. The system is declared recovered and operational upon successful completion of verification testing.
6. *Deactivation phase*: The deactivation phase includes activities to notify application owners and process owners. This phase also addresses recovery effort documentation, activity log finalization, incorporation of lessons learned into plan updates, readying resources for any future events and discharging the Disaster Management Team.

Validation state maintenance

Maintaining the validated state is one of the most difficult activities in guaranteeing the regulatory compliance and fitness of the use of an automated system. The maintenance phase spans the time between the automated system's start-up and the retirement of the system. The following items, which are essential to maintaining the validated state, may already be covered within the facility's quality system:

- preventive maintenance;
- incident management;
- software patches/service packs installation;
- training and competency;
- supplier requalification;

- periodic review;
- performance monitoring and
- system retirement.

Operational change control, document control and quality control procedures support the maintenance of the validated state.

IT equipment preventative maintenance

All critical equipment should have regular, planned maintenance to detect or prevent avoidable errors. This Planned Preventative Maintenance (PPM) should include routine day-to-day and periodic maintenance. PPM will ensure that the equipment required for any process remains in its optimum functional state.

- All equipment that requires PPM should be identified.
- Maintenance intervals should be determined for each item of equipment.
- The maintenance status of all equipment that requires PPM should be readily available.

Software patches/service packs installation

Transfusion services and donor centres work with software that may be regulated by the national competent authority, as well as software resources that are not regulated. The unregulated software and hardware provide the infrastructure for data transfer and connectivity between systems. Making changes to the software to update new functionality, installing security patches to minimize an identified vulnerability or adding new software to fix a software bug all require standardized processes and procedures. Facility change control policies and procedures apply to these changes. These requirements include the need to:

- assess the risks of making or not making the changes;
- document the decision-making and testing performed and
- monitor the system after the change is made.

This evaluation helps to determine the need for IQ, OQ and/or PQ. What differs between installing a new system and installing a patch to a system is the scope of the validation necessary to maintain a safe system. These requirements do not change regardless of the perceived urgency of implementing the update or software patch.

Timing of implementation. The urgency of implementing software is related to the level of need for the security of the system. Table 3 outlines urgency levels and appropriate actions.

Blood establishment software. It is unlikely that changes to the blood establishment software are critical for IT security. The standard process for making changes should be followed. There is time to assess the risks and perform testing before implementation in a process that meets all regulatory requirements for pre-implementation validation.

Security patches to the infrastructure. Security breaches are common, and software developers frequently issue updates and patches as frequently as weekly or when defects are found and a countermeasure created. Infrastructure changes can be planned or may come as an urgent patch to prevent a security breach. The institution should have a policy that outlines the risk assessment and approval processes for implementing a change at any level of risk or urgency. Regardless of the extent and urgency of the requested change, the risks need to be assessed and the decision documented.

TABLE 3 Actions indicated based on urgency levels for software implementation

Urgency level	Action
Patch or fix is optional	Assess the need for the fix and plan routine validation if required
Routine patch	Routinely scheduled, includes a review manufacturer's information, risk assessment and testing before implementation
Urgent security or operational fix to ensure data integrity	Review manufacturer's information, assess risk, notify users, implement, test critical functions
Mission critical security issue	Review manufacturer's information, implement, notify users, assess for negative impact, test critical functions

Blood establishment software is connected to other systems and is supported by the information technology infrastructure. This makes the system vulnerable to outside disruption and security breaches. The failure to handle the patch in a timely manner creates its own risks, and compliance with regulatory requirements is compromised.

The amount of validation testing performed is based on the risk of not making the change versus the risk of negative outcomes if the change is made. Routine and urgent implementation of patches and fixes should be:

1. scheduled;
2. placed into a test environment and
3. tested before implementation.

Only mission-critical software may be deployed without notification to the blood establishment. Any incident where software was deployed to end users without notification should be reviewed to ensure there was adequate documentation of the event and justification for implementation without notification and testing.

Review of manufacturer's release notes. The manufacturer's information about the changes provides details on how the changes affect the operation of the software and hardware. Some changes may provide the facility with a choice of options. The facility needs to determine if and how these changes impact their operating procedures and determine which, if any, option will be deployed. Based on this information, the risks of making the changes can be assessed, and a validation plan could be developed.

The goal of the patch may be to reduce a security risk or allow a mission critical task to continue. The risk analysis is still performed and documented as it is an important part of the decision-making process.

In some instances, the manufacturer may have limited information on the change or the downstream impact on the current system configuration. The extent of post-implementation testing and monitoring may increase when the deployment is done quickly and or with minimal or no information from the vendor.

Responsibilities. The facility's policies and procedures should define the groups or individuals responsible for the implementation. The facilities infrastructure team may be responsible for assessing the risks, documenting the decisions made and implementing the changes needed. Communication of the changes is a critical part of the process, even if making the changes is urgently required. The systems connected by the infrastructure may be adversely affected by the change. Communication is critically important when third parties have been contracted to manage the infrastructure or components of the system.

Versioning

In order to provide tracing and tracking computer applications it is necessary to uniquely identify the version of the application and module or programme units within the application. Methods of numbering versions may be found in Appendix C.

Training and competency

The ability of staff to use and support an automated system correctly should be maintained. The training programme should be reassessed for any critical change in environment, process or to the automated system.

The training programme should be adapted for each significant staff reassignment or newly assigned task related to the automated system.

Training records, including plans and protocols of the training status, ensure that training needs are properly identified, planned, delivered and documented for the entire validation process.

Suppliers of IT services may need to be trained in GMP requirements.

Supplier requalification

The frequency and the detail of the requalification process depends on the level of risk from using the automated system. Requalification should be planned for every supplier concerned.

This process can be performed through an audit similar to the one used for system selection. An internal procedure should be written to describe the level of auditing required for re-qualifying suppliers based on the purpose of the audit.

Supplier's requalification is not limited to the audit; it also concerns the follow-up of audit findings.

The decision to continue with a supplier will depend on the criteria established by the blood establishment and the level of compliance to the regulatory requirements applicable in the country concerned.

Periodic review

The aim of the periodic review of computerized systems is to establish that procedures continue to meet requirements and are approved. The review also confirms that qualification documents are complete, current and accurate.

Elements of the system should be verified before implementation and reverified at regular intervals as defined by the facility. The frequency of verification intervals for elements can be determined based on risk assessment.

A periodic review should be planned and scheduled to comply with the guidelines of the competent authority. The facility should define the scope, depth and frequency of the periodic review assessment.

It should consider:

- specification and design of components (Are specifications in place?);
- asset list (Is there a configuration item list, and are physical components in place and updated?);
- qualification documents (Was the OQ in accordance with qualification plans? Was the qualification testing based on risk assessments?);
- change management (Are procedures in place including consideration of the need for testing?);
- security management (Are virus protection and firewalls in place and maintained? Are there reports of security issues? Are physical and logical controls in place?);
- incident and problem management (Is there a process in place for reporting, assessing and documenting? Is there an overview of incidents and problems?);
- backup and recovery (Are procedures in place, tested and assessed against requirements?);
- disaster recovery (Is the process defined, tested and documented?);
- supplier qualification (Are suppliers assessed against quality requirements? Have there been any changes in procedures with suppliers?);
- changes in the environment, process, business requirement, legislation or accepted best practices;
- personnel qualification, training and competences;
- documentation for using and supporting the system (Are there policies, processes, procedures, operational plans and related records) and
- audit reports.

A report of the review process should be prepared and should include:

- relevant results obtained;
- deviations or problems found;
- required corrective actions and
- the ratification of the continued acceptability for the system use.

Identified actions should be prioritized and planned. A risk assessment-based approach should be used.

A date when the computerized system is due for periodic review/re-validation should be established.

Performance monitoring

To ensure the proper operation of an automated system consisiting of computers, networks and applications, a monitoring plan should be developed and implemented to ensure the proper operation of an automated system consisting of computers, networks and applications. The plan should take into account the criticality of the system being monitored and outline monitoring, user notification and problem-solving mechanisms.

Critical system conditions should be monitored with suitable monitoring tools at appropriate time intervals. The monitoring plan should state acceptable and unacceptable system parameters, the monitoring tool to be used and the frequency of observation.

If an unusual event is observed, personnel should follow the standard response outlined in the monitoring plan. The standard response will likely involve notifying affected personnel and initiating a resolution to the problem. Depending on the severity of the problem and the criticality of the system, backup and restoration plans may need to be implemented. See Disaster Recovery Plan (DRP).

System retirement

At the end of the operation, the automated system should be decommissioned. The following rules should be applied:

- If the retirement of the automated system involves a replacement, it should be planned.
- Consideration should be given to archiving system software.
- If the data is archived, it should be done in such a way that it can be retrieved and read during the required time frame unless the data is migrated to a validated replacement system.
- An archive report should be generated describing the archive approach and listing the documents, raw data and electronic records archived.
- It may be necessary to retrieve the data independently of the original system.
- The data should be retained as required by the regulations and company policy.

SECURITY

Security policies should be developed for defining the rules and guidance regarding the use and access to critical information. It could be performed through the Guidelines on Information Security from ISBT.

User access policies

User access policies should be developed requiring unique identification codes for each user, periodic password changes, prohibition of sharing passwords and mechanisms to ensure users are added to and deleted from the system as appropriate and when authorized. Users should have access only to the information they need to perform their job duties. Appropriate measures should be taken against unauthorized input, deletion or modification of critical data. Any deviations and/or modifications to these access policies will be documented and approved.

System access policies

System access policies should be developed in order to protect the system from unauthorized access. They should include:

- physical security;
- system access security, including user access;
- e-mail systems;
- shared network resources;
- internet access and use;
- system network connection security;
- software licenses and
- external automated systems.

Procedures should describe how the policies are implemented.

BACKUP AND RECOVERY

To ensure the availability and reliability of stored electronic data, backups should be

- made to reconstruct GxP relevant records;
- routinely performed as defined by the Quality Management System and any existing Service Agreements (e.g., the backup process, the number of backup copies, the frequency of backup, the backup verification process and the restore process) and
- performed before any significant software changes.

This applies to any system, including software, environment configuration and operating system.

- The backup process should ensure data integrity; each backup should be verified that it is complete and error-free.
- Physical backup copies should be stored in a secure place and in an appropriate environment (protected from fire, water and other hazards) that guarantees the quality of the storage medium and complies with confidentiality and privacy regulations and should be stored in an offsite location.
- Each backup medium should be clearly identified, for example, CD, tape, cloud.
- A log of backups should be maintained.
- The method of restoring and establishing control should be specified in the event recovery is required.
- The recovery process should be validated and routinely tested.

ARCHIVE AND RECORD RETENTION

All information produced within a critical automated system should be managed according to defined processes and with appropriate support.

A records retention policy and its use should be established. The type of records should be documented as well as the defined period of retention for each.

Archiving of electronic records involves the use of offline electronic storage. The archive process to follow should be documented, and consideration should be given to the following:

- documentary evidence to be taken when records are archived;
- indexing facilities;
- data should be secured by physical and electronic means against willful or accidental damage;
- storage facilities and environmental conditions should minimize the degradation of record storage media that could result in the loss of data;
- archived data should be secured in a manner that satisfies confidentiality and privacy regulations;
- electronically stored records should be periodically regenerated, based on the specification of the technology used;
- retained or archived records should be readily retrievable for business or regulatory purposes and
- access to the hardware needed to read these media needs to be maintained.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ACRONYMS

BECS:	Blood Establishment Computer System
cGMP:	Current GMP
CMDB:	Configuration Management Database
DBMS:	Database Management System
DHF:	Design History File
DRP:	Disaster Recovery Plan
DR:	Disaster Recovery
DHR:	Device History Record
DMR:	Device Master Record
DS:	Design Specification
FDA:	Food and Drug Administration
FS:	Functional Specification
GAMP®:	Good Automated Manufacturing Practice
GLP:	Good Laboratory Practice
GMP:	Good Manufacturing Practice
GxP:	Good 'x' Practice, where 'x' represents

- Clinical
- Quality
- Distribution
- Laboratory
- Manufacturing

IaaS:	Infrastructure as a Service
ICH:	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ISO:	International Organisation for Standardization
IQ:	Installation Qualification
IT:	Information Technology
ISPE:	International Society for Pharmaceutical Engineering
LAN:	Local Area Network
OQ:	Operational Qualification
PaaS:	Platform as a Service
PPM:	Planned Preventive Maintenance
PQ:	Performance Qualification
QA:	Quality Assurance
QMS:	Quality Management System
RTO:	Recovery Time Objective

SLA: Service Level Agreement
SOP: Standard Operating Procedure
URS: User Requirement Specification
UPS: Uninterruptible Power Supply
VLAN: Virtual Local Area Network
VM: Virtual Machine
WAN: Wide Area Network
WPIT: Working Party on Information Technology

GLOSSARY

Automated system: Term used to cover a broad range of systems, including automated manufacturing equipment, control systems, automated laboratory systems, manufacturing execution systems and computers running on manufacturing database systems. The automated system consists of the hardware, software and network components, together with the controlled functions and associated documentation.

Client: An application or system that accesses a remote service on another computer system, known as a server, by way of a network. The term was first applied to devices that were not capable of running their own stand-alone programmes, but could interact with remote computers via a network. These dumb terminals were clients of the time-sharing mainframe computer. A fat client (also known as a thick client or rich client) is a client that performs the bulk of any data processing operations itself and does not necessarily rely on the server. A thin client is a minimal sort of client. Thin clients use the resources of the host computer. A thin client's job is generally just to graphically display pictures provided by an application server, which performs the bulk of any required data processing.

Computer system: A functional unit consisting of one or more computers, associated peripheral input and output devices and associated software that uses common storage for all or part of a programme and also for all or part of the data necessary for the execution of the programme; executes user-written or user-designated programmes; performs user-designated data manipulation, including arithmetic operations and logic operations and that can execute programmes that modify themselves during their execution. A computer system may be a stand-alone unit or may consist of several interconnected units.

Computerised system: Includes hardware, software, peripheral devices, personnel and documentation; for example, manuals and Standard Operating Procedures.

Critical safety patch: A software patch that is considered mandatory by the vendor. It typically improves security or mitigates a known threat.

Disaster recovery: A set of policies, tools and procedures to enable the recovery or continuation of vital technology infrastructure and systems following a natural or human-induced disaster. The disaster recovery plan includes policies and testing and may involve a separate physical site for restoring operations. This preparation needs to be taken very seriously and will involve a significant investment of time and money to ensure minimal losses in the event of a disaster.

Engineering diagrams: Description of the way a device is built. It could be electrical wiring schema, technical information, etc. Where information must be presented by means of a signal flow chart or circuit diagram, such visual aids shall be divided into discrete units, simplified and standardized.

Functional specification (FS): Description of the product to be supplied in terms of the functions it will perform and the facilities required to meet the user requirements. It covers mechanical, electrical layout, hardware and software elements. This kind of document is written in such a way that both supplier and user understand it.

Hardware design specifications: Description of the architecture and configuration of the hardware. It includes controllers, PCs, instrumentation and interfaces.

Installation requirements: Description of the environment into which the automated system should be installed.

Manuals/User guides: Documents describing the use of the system and the maintenance tasks that have to be performed by the user. It is a description of the product in terms of the functions it may perform and the facilities required to appropriately utilize the product.

Purchasing documentation: Document ordering any significant part of the automated system, including equipment, computer system or part of it and new development. It may be used for tracking the purchasing process.

Patches/Service packs: Code added to the software in order to fix a bug, especially as a temporary correction between two releases.

Process owner: The person ultimately responsible for the business process or processes being managed.

Qualification: The act of proving and documenting that equipment or ancillary systems are properly installed, work correctly and comply with specified requirements. Qualification is part of the validation, but the individual qualification steps alone do not constitute process validation (Qualification is an act or process to assure something complies with some condition, standard or specific requirements.)

Recovery time objective: The maximum tolerable time allowed for the recovery of a computer, system, network or application.

(IT) Services: The application of business and technical expertise to enable organizations in supporting their business processes

Software: Software is often divided into two categories: Systems software includes the operating system and all the utilities that enable the computer to function. Applications software includes programmes that do real work for users. For example, word processors, spreadsheets and database management systems fall under the category of applications software.

Software design specifications: Description of logical and physical structures of the programme, the standards to be used for file naming, label allocation and module naming. It defines how the software implements the requirements based on the functional specification.

Standard operating procedure (SOP): Written and approved description of essential steps, their sequence, responsibilities and

precautionary measures necessary to assure that operations can be accomplished routinely and in a uniform manner.

Supplier audit report: Presentation of the results of the investigation of the adequacy of the supplier to assure the quality and the reliability of the supplied automated system.

User requirements specification (URS): Clear and precise definition of what the user wants the system to do. It defines the functions to be carried out, the data on which the system will operate and the operating environment. The URS also defines any non-functional requirements, constraints such as time and costs and what deliverables are to be supplied. The emphasis should be on the required functions and not the method of implementing those functions.

Verification: The process of checking that the software meets specifications.

Validation: The process of checking whether the specification captures the customer's requirements.

Validation master plan: Describes the areas of the company within which validation is to take place and provides an overview of the status of planning. It lists the areas, systems and projects being managed, defines the status of validation for each and gives a broad indication of when validation is to be completed. It is a general plan and would normally cover all production areas and/or processes. It should include all systems for which validation is planned.

Validation plan: Description of the validation activities, responsibilities and procedures. It describes specifically how the validation is to be done.

Validation protocol: Prospective experimental (testing) plan that, when executed, is intended to produce documented evidence that the system performs as intended.

Validation report: Presentation of the results of validation activities, interpretation of the results and the conclusions drawn. If unexpected results are obtained during validation testing, it defines what changes will need to be made or what workarounds will be implemented to mitigate risk.

Validation, concurrent: Validation is conducted when there is no possibility of completing a validation programme before releasing a product or part of it. In this case, all validation concerns should be documented prior to the release of the product.

Validation, prospective: Validation is conducted prior to the distribution of either a new product or product made under a revised manufacturing process, where the revisions may affect the product's characteristics.

Validation, retrospective: Validation of a process for a product already in distribution based upon accumulated production, testing and control data. Test data is useful only if methods and results are adequately specific.

APPENDIX A: SAMPLE QUESTIONS FOR SUPPLIER QUALIFICATION

1. Are you a supplier of Infrastructure or a Service organization monitoring Infrastructure? If "yes", please describe the type of infrastructure you supply and the certification process you have been qualified by. If "no", please complete the following questions.
2. Do you have a Quality Policy? Do you have a Quality Manual?
3. Is there a Quality Management System in place? What is your familiarity with ICH Q10 Pharmaceutical Quality System?
4. Is there a security policy in place for the system?
5. Has there been a previous Quality Audit which is a systematic, independent examination of your adherence to the Quality System? What were the findings?
6. Is there an effective communication and escalation process in place in order to raise Quality issues to the appropriate levels of management?
7. What part does the Management Team play in the intentions and direction and application of the Quality System to your product or service?
8. Are there SOPs for all of the Development, Implementation and Maintenance phases?
9. What is the Change Control Process employed? Has it been documented? Do you use a Version Control system that allows the users to use, test and validate the system without interfering with LIVE use? Is Design Control part of the process?
10. How do you use a product and process understanding to enhance understanding throughout the lifecycle of your product or service?
11. Is the system configurable and if so, how is configuration management controlled?
12. Do you have a DHF (Design History File) for the current finished device as it is today? DHR (Device History Record)? DMR (Device Master Record)?
13. Are there Functional Requirements that have been developed for the System?
14. Do you have adequate resources for design issues such as: assessing new products; training and retraining of design managers and design staff; use of consultants, evaluation of the design process; product evaluation, including third-party product certification and approvals; patenting or other means of design protection?
15. How do you ensure that your Design Specifications have been implemented in the System? Is there a final validation before release of the system? Enhancements tested as part of a new/upgrade Version? What documentation do you have that describes your design and development planning?
16. Has there been validation of the system with respect to the Functional Requirements of the finished product? What kinds of documentation has been developed for the Testing and Validation of the system? Validation Master Plan? Test Plans? Matrix Document: Requirements versus Test Scripts; Test Scripts
17. Is there a document control system in place?
18. Are there any other activities that you feel help to improve the Quality Assurance activities that prove the development and implementation of the system are under control?
19. Is the customer informed of planned changes, and is there an opportunity for the customer to validate the changes?

APPENDIX B: CLASSIFICATION OF AUTOMATED SYSTEMS

GAMP® 5 A Risk-based Approach to Compliant GxP Computerised systems [3] and the PIC/S Good Practices for Computerised Systems in Regulated 'GxP' Environments [5] categorize automated systems and the applied tasks as follows.

Category 1: Infrastructure software

Established operating systems are not subject to specific validation. However, functions used by a critical software application should be validated. The name and version of the operating system should be documented and verified during Installation Qualification (IQ).

TABLE B1 Classification of automated systems used in blood banking

Automated system	Automated system categories
Air handling systems	4
Alarm system	4
Apheresis machines	4
Automated component processing system	4
Autonomous computer system with critical information (e.g., laptop)	5, 4, 3
Balance/mixer	4
Barcode reader	1
Blood product storage devices	4
Blood pressure automated system	1
Centrifuge	4
Computer system (including emulator)	5, 4, 3
DBMS (Database Management System)	1
ECG machine	1
Electronic archive system	5, 4, 3
Electricity backup system, UPS	4
Electronic balance	1
Electronic thermometer	4
Fast freezer	4
Hb meter	1
Heat sealer	1
Incubator	1
Irradiator	4, 3
Analytic automated system	4
LIMS (Laboratory Information Management System)	5, 4
Network	1
Network device	4
Printer	1
Operating system	1
Software application	5, 4, 3
Tube docking system	1

Note: Some automated systems are classified under more than one category since they may have different configurations.

Category 2: No longer used

Category 3: Non-configured software

These are commercially available standard software packages where configuration is limited to establishing its runtime environment (e.g., network and printer connections). The name, version and any configuration should be documented and verified during Installation Qualification (IQ). Functionality and user requirements (e.g., security, alarm and event handling, calculations and algorithms) should be tested within Operational Qualification (OQ).

Category 4: Configured products

Configured products provide standard interfaces and functions that enable the configuration of user-specific business or manufacturing processes. The development process should be assessed through a supplier audit. The audit should focus on the quality system and that application and support organizations are robust and competent.

The name, version and any configuration should be documented and verified during Installation Qualification (IQ). Functionality and user requirements (e.g., security, alarm and event handling, calculations and algorithms) should be tested within Operational Qualification (OQ) and the Performance Qualification (PQ).

Category 5: Custom applications

Custom applications are developed to meet the specific needs of the user company. It may be a complete system or extension to an existing system. The development process should be assessed through a supplier audit. The audit should focus on the quality system and that the application and support organizations are robust and competent.

The name, version and any configuration should be documented and verified during Installation Qualification (IQ). Functionality and user requirements (e.g., security, alarm and event handling, calculations and algorithms) should be tested within Operational Qualification (OQ) and Performance Qualification (PQ).

Table B1 lists classifications of automated systems used in blood banking based on the system described above.

APPENDIX C: VERSIONING METHOD

The versioning may use a simple numbering sequencing scheme. In general, for the software application versioning will be applied for two entities (i) at the application level and (ii) at the programme-unit level (or module, package, script, etc.). The following will describe the convention.

At the application level, the following may be applied:

v[major release].[minor release].[identity]

Where: v – indicates version (always lowercase)

[major release] – a major release relates to when significant changes are made to more than one of the following: the application including drastic changes to look and feel, extensive new features, major technology upgrade such as database and/or operating system impacting full re-compile and other high-impact events as determined by the product development and support team.

[minor release] – a minor release related to one of the above conditions as stated in the major release. Typically, it would be a new feature added to the application having significant impact to the process and users.

[identity] – an identity refers to application status whether it is in alpha, beta, limited release or final release.

0 is for alpha status
1 is for beta status
2 is for limited release
3 is for production status

Examples:

1. v3.0.0 – at the application level means the software application is running at major version 3, minor version 0 and is at the alpha testing
2. v3.1.1 – means the software application is running at major version 3, minor version 1 and is at the beta testing
3. v3.2.3 – means the software application is running at major version 3, minor version 2 and is at its final release

At each program (or the Form, Module, Package, Script) level following will be applied:

FFFFFFFF rel: nnn ("rel" always in lower case)

Where:

FFFFFFFF – is the upper-case Program-Unit Name (the first 8 significant characters)

rel – represent production release of the Form

nnn – indicates release number of the Form

Example:

MODPROG rel: 004

Means MODPROG is the program-unit name

rel: 004 is the release number of the form MODPROG

Both Application and Program Unit put together.

The following string will describe the combined Application and Program Unit together.

MYAPP v3.1.3MODPROG rel: 004

meaning – MYAPP is at major version 3, minor version 1, in production mode 3 and currently running the form MODPROG with release level 004.

Internal Mechanics:

a. Application version may be stored in a controlled text file
b. fter connecting to the application, the initiating program may read the text file for App version
c. The version for program unit may be hard-coded in each program or stored in controlled text-file
d. When the program unit is launched within the App, it can read the text-file, match the program unit name to display the program version
e. When Application is launched through Windows, the title MDI will be formatted as below:

MYAPP v3.1.3MODPROG rel: 004 Login User: UUUUUU on MM-DD-YYYY HH:24MI.SS

Where:

MYAPP is the application name
V3.1.3 is the application version
MODPROG is name of the program Unit
rel: 004 is modification level for the program MODPROG
UUUUUU is the user currently logged-in
MM-DD-YYYY HH24:MI.SS is the user login date and time with seconds

The program unit release history:

The program unit release history will display last 5 changes made to the program unit:

For example for the Form MODPROG, it will be: (CR may represent Change Request)

rel 001 CR17000021
rel 002 CR18000010
rel 003 CR18000012
rel 004 CR18000111

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DIARY OF EVENTS

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

