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ORIGINAL PAPER

The role of improved pre-transfusion testing in the prevention of delayed serologic transfusion reactions among blood recipients in Uganda: a Randomized Controlled Trial (IPAT Study)

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Background and objectives The goal of pre-transfusion testing (PTT) is to provide patients with beneficial and safe transfusions. In Uganda, PTT includes ABO/RhD typing plus room temperature (RT) saline cross-matches without red-blood-cell (RBC) alloantibody screening. The aim of the IPAT study was to assess the role of improved PTT in the prevention of delayed serologic transfusion reactions (DSTRs).

Materials and methods In this randomized controlled trial, patients at Mbarara Hospital in Uganda, with a history of RBC exposure, were randomized 1:1 to have either RBC alloantibody screening (SCREEN group) or room temperature saline cross-matches (CONTROL group) during PTT. 'Home-made' reagent RBCs from group O RhD-positive volunteers were used for antibody screening in the indirect antiglobulin test. Participants were evaluated for RBC alloantibody production 7–14 days after transfusion. Post-transfusion haemoglobin estimation and direct antiglobulin tests (DATs) were also performed.

Results We randomized 220 patients to either the SCREEN or CONTROL group. Both study arms had similar demographic and transfusion characteristics at baseline. There were 19 (17·3%) individuals in the CONTROL group with DSTRs compared to 8 (7·3%) in the SCREEN group at the time of follow-up ($P = 0·02$). Overall, post-transfusion DATs were positive in 7 (3·5%) patients but there was no associated decrease in haemoglobin levels.

Conclusion Red-blood-cell alloantibody screening is associated with occurrence of significantly fewer DSTRs. The use of 'home-made' reagent cells during PTT in Uganda is feasible. We recommend a change in the local PTT policy to consider the introduction of RBC alloantibody screening.

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Introduction

The term pre-transfusion testing (PTT) refers to the serologic tests as well as clerical and history checks performed to determine compatibility between a blood

recipient and the donor. The aim of PTT is to provide patients with safe transfusions without accelerated destruction of red-blood-cells (RBCs). Elements of PTT include obtaining a well-labelled blood sample; comparing the information on the request form with that on the sample tube for identification; checking the patient's transfusion records and history; testing the patient sample for ABO/RhD groups; screening the patient sample for unexpected RBC alloantibodies; identifying the detected alloantibodies and performing a cross-match [1,2]. If performed properly, PTT detects most of the clinically significant RBC alloantibodies and ensures that patients are issued the designated blood components which are ABO/RhD compatible. An antibody is considered to be clinically significant if it is reactive at 37°C and examples of that specificity are known to have caused haemolytic transfusion reactions (HTRs), haemolytic disease of the foetus and newborn (HDFN) or unacceptably short survival of the transfused RBCs [3].

Despite the substantial progress made in the area of blood safety in Uganda, less attention has been paid to overall transfusion safety. *Transfusion safety* must be distinguished from *blood safety*: blood safety concerns the safety of the component itself and is largely the responsibility of blood suppliers. In contrast, transfusion safety focuses on the overall process which results in the delivery of transfusion therapies to patients [4]. Currently, PTT practices in Uganda are limited to ABO/RhD typing plus room temperature (RT) saline cross-matches – usually performed on a tile. These procedures only test for naturally occurring IgM antibodies and do not typically detect clinically significant immune alloantibodies which are of the IgG isotype and reactive at body temperature [3,5]. In sub-Saharan Africa, 6·7 per 100 transfused individuals (95% CI: 5·7–7·8) have clinically significant anti-RBC alloantibodies [6]. Research studies in Uganda reported the prevalence of RBC alloimmunization among sickle cell disease (SCD) and other multiply transfused (OMT) patients to be 6·1% with anti-E, -D, -C and -S being the common specificities [7,8]. In these two reports, some RBC alloantibodies might have been missed because cross-sectional study designs were employed and longitudinal studies indicate that up to 25% of the alloantibodies formed disappear within a median of 10 months [9,10]. Because RBC antibody screening procedures and 37°C IAT cross-matches are not performed on pre-transfusion samples in Uganda, previously exposed and alloimmunized patients are at increased risk of serologic complications of blood transfusion following additional exposures to RBCs. Delayed serologic transfusion reactions (DSTRs) and delayed haemolytic transfusion reactions (DHTRs) occur when a patient previously sensitized by pregnancy or blood transfusion receives 'incompatible RBCs' because the low titres of circulating alloantibodies escape detection by PTT. However, a rapid anamnestic antibody response occurs after transfusion with antigen-positive RBCs. These delayed reactions occur between 24 h and 28 days (usually within 5–14 days) after transfusion. Unlike DHTRs, DSTRs are identified serologically but not clinically. According to the USA National Healthcare Safety Network (NHSN) Hemovigilance protocol, DSTRs are defined as the absence of clinical signs of haemolysis and demonstration of new, clinically significant antibodies against RBCs by either positive direct antiglobulin test or positive antibody screen with newly identified RBC alloantibody [11]. DSTRs and DHTRs occur in approximately 1 in 1500 transfusions, with DSTRs being detected at rates two to fourfold higher than DHTRs [12–14]. Following an anamnestic antibody response there may be difficulties in getting compatible blood for future transfusions and an increased risk of haemolysis of the donor RBCs in the current or subsequent transfusion episodes [15,16].

It was recommended by the above authors in Uganda that there was a need to introduce RBC alloantibody screening in local blood banks to prevent post-transfusion serologic complications and hence improve transfusion safety. Therefore, we conducted a randomized controlled trial to assess whether improved PTT (pre-transfusion antibody screening using 'home-made' reagent cells followed by 37°C IAT cross-matches) had a role in the prevention of DSTRs among blood recipients in the Ugandan setting compared with the current standard of practice (performing room temperature saline cross-matches with no antibody screening). The research was code-named as the 'IPAT study' [IPAT ≡ Improved Pre-transfusion testing to prevent Anamnestic serologic reactions following Transfusions].

Materials and methods

Trial design

The IPAT study was a prospective randomized controlled trial in which eligible participants were randomized 1:1 to have either RBC alloantibody screening performed on their pre-transfusion blood samples [SCREEN group] or room temperature saline cross-matches carried out before transfusion as occurs in current practice [CONTROL group]. The PTT method remained the same for each participant throughout all the units of blood transfused.

Participants

Individuals with a history of previous RBC exposure—those who had received at least one blood transfusion and/or had had at least one pregnancy—were asked to

consent to participate in the study. Patients with immunosuppressive conditions such as HIV/AIDS, diabetes mellitus and concurrent immunosuppressive therapy were not excluded. However, children <1 year of age; patients in a moribund status; those pregnant or in the puerperium and those with lymphoid malignancies were not enrolled. Individuals randomized to the SCREEN group and found to have coexisting RBC alloimmunization at the time of PTT were not excluded.

The study was conducted at Mbarara Regional Referral Hospital (MRRH) in Mbarara, Uganda. MRRH has a capacity of approximately 400 beds and is the teaching hospital for Mbarara University of Science and Technology (MUST). Transfusion recipients admitted at MRRH in the Pediatric, Obstetric & Gynecological, Surgical and Medical wards were included in the study. Information regarding their demographic and transfusion characteristics was recorded on data collection forms.

Interventions

Approval to conduct the study was obtained from the Research and Ethics Committees at MUST and clearance was got from the Uganda National Council for Science and Technology (UNCST). Participants in the CONTROL group had PTT that involved room temperature saline cross-matches on a tile (as occurs in the current practice) while those in the SCREEN group had *improved PTT that is* RBC antibody screening followed by 37°C IAT cross-matches before transfusion. All patients were transfused with non-leucocyte reduced whole blood or packed RBCs.

Follow-up

Participants were asked to return for review after 1 week following discharge from hospital (approximately 7–14 days after blood transfusion). At follow-up, tests included Hb, DAT and RBC alloantibody screening using the IAT technique (tube method). For purposes of the IPAT study, participants were considered to have a DSTR if clinically significant antibodies to any of the RBC antigens expressed on the screening cells were detected in their plasma at the time of follow-up but with no clinical signs (e.g. pallor and jaundice) or laboratory evidence (e.g. reduced haemoglobin) of RBC destruction [12].

Outcomes

The primary outcome was the occurrence of a DSTR while secondary outcomes included the demonstration of a reduced Hb concentration and a positive DAT 7–14 days after transfusion.

Sample size

A sample size of 220 participants was calculated with an allocation ratio of 1:1 (110 patients in each study group) at 5% level of significance and a power of 80%; assuming a 10% loss to follow-up.

Randomization

The participants were randomized (using consecutively numbered, otherwise identical, opaque envelopes with the PTT method sealed therein) into either the SCREEN or CONTROL group before transfusion. There were equal numbers of envelopes for each PTT method. The envelopes were prepared in two separate batches of 110 each, thoroughly mixed and then stored in a locked cabinet. Only one envelope was opened after each enrolment, in consecutive order.

Measurements

Immunohematologic procedures were performed at the MRRH Transfusion Laboratory in Mbarara, Uganda. Four millilitres (4 ml) of venous blood samples were collected from all participants and put in ethylenediaminetetraacetic acid (EDTA) vacutainers. In all cases, a *Tabletop* centrifuge (Hettich); a water bath (Memmert); and 12 × 75 Kahn tubes were used. 'Home-made' reagent RBCs were prepared from group O RhD-positive volunteer donors (who were students or staff at MUST) and phenotyped for expression of D, C, c, E, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, S and s antigens. The screening cells were labelled accordingly as 3-cell panels, preserved in Alsever's solution (Sigma-Aldrich) and kept refrigerated at 4°C for 3–4 weeks. Antihuman globulin reagent (Immucor Inc.) and IgG check cells were used in the IAT technique. Plasma samples were kept frozen at −80°C and later shipped to the United States where confirmatory antibody screening of all positive samples alongside a corresponding number of negative patient samples as controls was done at the Immunohematology Reference Laboratory of Bloodworks Northwest in Seattle, WA, using the tube method.

Statistical methods

All analyses of primary and secondary outcomes were on an intention-to-treat (ITT) basis. Statistical software packages Excel version 5.0 (Microsoft Corporation, Redmond, CA) and STATA version 12.0 (StataCorp., College Station, TX) were used for data management and analysis, respectively. For comparisons between the two groups regarding recipient age at the time of enrolment, gender, pregnancy

history, components transfused, number of transfusion episodes, number of units received and the indication for transfusion, the chi-squared test was used for discrete variables. Student's *t*-test was used for comparing means of continuous variables of normal distribution. Groups were assumed to differ significantly when the probability level was <0·05.

Results

Blood recipients

From May through August 2017, a total of 426 patients at Mbarara Regional Referral Hospital were assessed for eligibility for inclusion in the study. Of these, 185 participants (43·4%) met at least one exclusion criterion; and the patient or a surrogate decision-maker declined consent in 21 instances. Therefore, 220 patients underwent randomization, received blood transfusion and were considered in the intention-to-treat analysis. Nineteen patients (8·6%) were unable to return for follow-up 7–14 days (11 days on average) after blood transfusion, leaving 201 patients (98 in the CONTROL group and 103 in the SCREEN group) who were assessed for the occurrence of DSTRs (Figure 1).

Transfusion characteristics

Of all participants, 102 (46·4%) were females and among them, 81 (79·4%) had a history of pregnancy. The mean

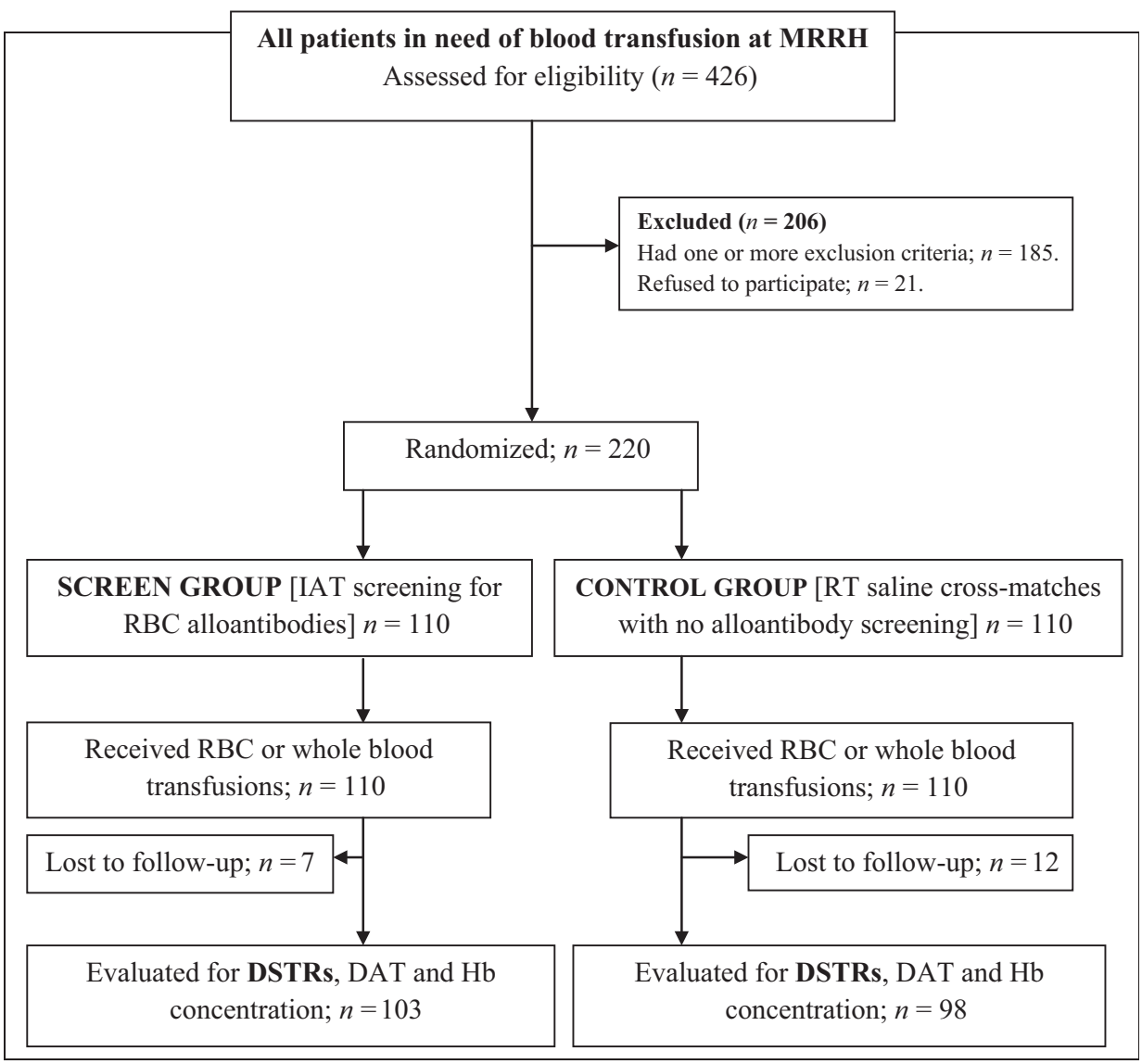

Fig. 1 Flow diagram showing the study course and interventions for participants randomized into the SCREEN and CONTROL arms of the IPAT study.

Table 1 Baseline demographic and transfusion characteristics for participants in the SCREEN and CONTROL groups of the IPAT study[a]

	SCREEN group (n = 110)	CONTROL group (n = 110)	*P*-value
Mean age of recipients (standard deviation)	33·5 (17·1)	31·1 (18·8)	0·32
Female participants	54 (49·1)	58 (52·7)	0·59
Adult females (≥18 years)	43 (79·6)	42 (72·4)	0·37
History of pregnancy	42 (77·8)	39 (72·4)	0·98
Whole blood transfusions given	87 (79·1)	82 (74·5)	0·64
>10 transfusion episodes	6 (5·5)	8 (7·3)	0·58
Mean units of blood received (median; range)	6·7 (5·0; 2-28)	6·4 (5·0; 2-50)	0·71
Mean transfusion episodes (median; range)	4·2 (3·0; 2-16)	4·3 (3·0; 2-20)	0·93
Recipients with malignancy	15 (13·6)	9 (8·2)	0·19
Recipients with HIV infection	18 (16·4)	15 (13·6)	0·57
Recipients with inflammatory diseases (malaria & bacterial infections)	47 (44·5)	46(41·8)	0·89

[a]Data are reported as number (%) or mean (median; range) unless otherwise specified.

age (standard deviation) was 31·1 (18·8) and 33·5 (17·1) years among individuals in the CONTROL and SCREEN groups, respectively. The patients were transfused with a total of 1434 (median, 5; range, 2–50) units of blood in 922 (median, 3; range, 2–20) transfusion episodes. The two study groups had similar characteristics at baseline (Table 1). For all transfused patients, the ABO blood group distribution was as follows: A = 29%; B = 20%; AB = 7% and O = 44%. For the RhD type, 6·4% of the patients were RhD negative and they all consistently received RhD negative blood.

Primary and secondary outcomes

Overall, there were 27 patients (12·3%) found to possess RBC alloantibodies at the time of follow-up; with 19 (17·3%) in the CONTROL group and 8 (7·3%) being in the SCREEN group. There was a significant difference in the two study arms regarding the rate of post-transfusion RBC alloantibody formation and hence the occurrence of DSTRs ($P = 0·02$). There were five patients randomized to the SCREEN group found to have RBC alloantibodies at the time of PTT. The number of individuals in the CONTROL group with pre-existing RBC antibodies at the time of randomization was not known. Post-transfusion DATs were positive in four (4·08%) individuals in the CONTROL group and in three individuals (2·91%) in the SCREEN group, suggesting the possibility of DHTRs. There was an incremental change in the mean Hb post-transfusion that is 1·72 g/dl in the CONTROL group and 1·69 g/dl for the SCREEN group; however, the difference between groups was not statistically significant (Table 2). Confirmatory studies performed in the United States were in agreement with the above findings.

Discussion

We describe a prospective randomized controlled trial that assessed the role of RBC antibody detection tests in the prevention of DSTRs after blood transfusion in Uganda. It presented a unique opportunity to answer a fundamental question regarding the need to improve clinical transfusion practice locally. Throughout Uganda, transfused patients are tested for their ABO/RhD blood groups and RT cross-matches are performed instead of the internationally recommended 37°C IAT cross-matches [17] or alloantibody detection tests using the IAT technique. Thus, sensitized blood recipients in Uganda might experience anamnestic antibody formation and immunohemolytic sequelae following further RBC exposures.

In this trial, participants were randomized to two PTT strategies: the SCREEN group in which RBC alloantibody screening followed by 37°C IAT cross-matches was carried out and the CONTROL group where the current PTT protocol (RT saline cross-matches with no RBC antibody screening) was used. After 7–14 days following blood transfusion, 17·3% of the patients in the CONTROL group were found to have RBC alloantibodies compared to 7·3% in the SCREEN group. Therefore, a significant reduction in the occurrence of DSTRs was observed when RBC antibody screening tests were performed and 37°C IAT cross-match compatible blood transfused. These findings have important implications for clinical transfusion practice and health policy formulation in Uganda. They underscore the urgent need to introduce improved immunohematologic testing procedures throughout the country. This will prevent complications associated with anamnestic anti-RBC alloantibody production and hence improve the safety as well as the efficacy of blood transfusions.

Table 2 Primary and secondary outcomes for participants in the SCREEN and CONTROL arms of the IPAT study[a]

Variable	SCREEN group (n = 103)	CONTROL group (n = 98)	*P*-value
Positive post-transfusion IAT screening	8 (7·3)	19 (17·3)	0·02
Positive post-transfusion DAT screening	3 (2·91)	4 (4·08)	0·65
Pre-transfusion haemoglobin concentration (g/dl)	4·64 (0·97)	5·07 (1·27)	0·03
Post-transfusion haemoglobin concentration (g/dl)	6·31 (1·02)	6·80 (1·49)	0·02
Increment in haemoglobin concentration (g/dl)	1·69 (0·55)	1·72 (0·53)	0·79

[a]Data are reported as number (%) or mean (standard deviation) unless otherwise specified.

The rate of RBC alloantibody formation in the SCREEN group was 7·3% and this was comparable to findings from other longitudinal studies among polytransfused patients in sub-Saharan Africa [18,19] and the rest of the world [20–22] where pre-transfusion screening for RBC alloantibodies is routinely practised. At the time of PTT, there was a 4·9% prevalence of RBC alloantibodies in the SCREEN group which was within the range for alloimmunization previously reported among multiply transfused Ugandans [8]. At baseline, patients in both the CONTROL and SCREEN arms had similar demographic characteristics. However, the proportion of CONTROL group patients with pre-existing RBC alloantibodies at the time of PTT was unknown, participants in both study arms did not differ significantly regarding the known risk factors for RBC alloantibody formation (Table 1). Therefore, the observed difference in the two groups concerning the occurrence of DSTRs (the primary outcome) appears to have been due to the study intervention that is PTT alloantibody screening followed by 37°C IAT cross-matches (Table 2). The improved PTT performed in the SCREEN group possibly reduced the number of 'incompatible transfusions' leading to fewer anamnestic antibody reactions. However, this was not the case in the CONTROL group (the current practice of RT saline cross-matches with no screening) where more DSTRs were observed presumably as part of a secondary immune response.

The IPAT study was designed to reflect the current situation in clinical transfusion laboratories in Uganda and demonstrate the PTT method that would be practically feasible to implement in the country. There have been concerns regarding the cost and cost-effectiveness of the technology given the poor laboratory infrastructure within hospitals and the need for monthly procurement of reagent RBC panels from manufacturers overseas. We have previously reported that the tube IAT method for RBC alloantibody screening in Uganda was cost-effective [23]. In this study, PTT using the tube method was employed as opposed to gel or microplate techniques which are generally more expensive. Furthermore, group O RhD-positive volunteers at MUST were mobilized, serologically typed and utilized as donors of reagent RBCs instead of using commercial cell panels. Five participants in the SCREEN group had positive IAT screening results before transfusion. In this scenario, 37°C IAT cross-matches were performed and compatible units of blood were transfused. Fortunately, none of the patients with pre-existing RBC alloantibodies at the time of PTT experienced any clinical or laboratory signs of haemolysis. While this approach was not devoid of compatibility risks, it was superior to the current practice where no 37°C IAT cross-matches are performed.

There are some limitations to the study. The research design was such that it was not possible to establish the number of CONTROL group participants with pre-existing antibodies at the time of PTT and hence the total number of DSTRs in this cohort could have been overestimated. Antibody identification was beyond the scope of this trial and was thus not done for both study arms. Therefore, one could not clearly distinguish between pre-existing and anamnestic antibodies at the time of follow-up. Using lessons learnt, we will endeavour to design future projects using similarly produced reagent RBCs, provide data on the RBC alloantibody specificities among transfused Ugandans and establish a local pool of phenotyped reagent cell donors. Another limitation is the timing for post-transfusion follow-up of 7–14 days (11 days on average) from the date of transfusion which was by no means adequate. While RBC alloantibodies may appear as early as 7–10 days after transfusion in primary immunization and within 2–7 days in a secondary response [24], RBC alloantibodies have been detected with increasing frequency at later follow-up time-points of 6 weeks up to 6 months [20,25–27]. In our case, there were financial and logistical challenges that could not allow the research team to arrange additional follow-up visits for participants. Also, the study lacks conclusive data on the occurrence of DHTRs. A DHTR may be defined as a significant drop in the Hb level (of ≥25%) between 24 h and 21 days after transfusion; with or without a newly detected alloantibody in the eluate and/or post-transfusion serum studies after a negative antibody screen in the pre-transfusion serum and haemoglobinuria [28,29]. Hb

concentrations as well as DATs were performed at the time of follow-up as surrogate indicators of possible delayed haemolysis. Regarding these secondary outcomes, the rate of DAT positivity was 3·5% overall and was similar in both study arms. However, the positive DAT tests were not associated with a drop in the Hb concentration. Although there were significant differences between the pre- and post-transfusion Hb levels for the two groups, the overall increment in Hb concentrations was not significantly different between the two study arms. Moreover, the post-transfusion Hb values in both groups did not show a negative trend that would be suggestive of a DHTR (Table 2). Other indicators for DHTRs such as post-transfusion bilirubin, reticulocyte counts and lactate dehydrogenase levels were not assessed in this trial. Therefore, the frequency and nature of DHTRs among patients in Uganda remains a subject for further investigations and research.

Conclusion and recommendations

Our study demonstrated the role of RBC alloantibody screening in the prevention of DSTRs and the feasibility of using 'home-made' reagent cells during PTT in Uganda. These are significant findings that should form evidence for the introduction of pre-transfusion RBC antibody screening in Uganda and other jurisdictions with restricted healthcare budgets. We strongly advocate for a change in the Ugandan Ministry of Health policy on PTT in order to prevent the consequences of anamnestic RBC antibody formation among blood recipients. The PTT programme could be rolled out countrywide using the available equipment at district hospital laboratories such as microscopes, centrifuges, water baths, Kahn tubes and refrigerators.

All paediatric and adult blood recipients should have 37°C IAT cross-matches before transfusion. Patients with a history of previous exposure to RBCs through transfusion and/or pregnancy should undergo a pre-transfusion RBC alloantibody detection test (using the tube method). Repeatedly transfused patients for example those with cancer, sickle cell anaemia or other haemoglobinopathies might have multiple antibodies and are usually 'difficult to transfuse'. The latter should be managed at regional referral hospitals where laboratory infrastructure for alloantibody identification and DHTR investigation will be put in place. The Uganda Blood Transfusion Service (UBTS) could mobilize group O RhD-positive volunteers so that 'home-made' reagent cells are routinely produced at regional blood centres and are supplied to nearby hospital blood banks. The National Medical Stores (NMS) could supply Coombs' serum to hospitals alongside other reagents, drugs and sundries. An immunohematology reference laboratory (IRL) could be set up at Mbarara University of Science and Technology (or elsewhere in Uganda) to handle issues of Transfusion Medicine training, complex diagnostics and applied research.

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Conflict of interests

The authors certify that they have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in this manuscript.

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