ABSTRACT

Background: Screening of alloantibodies in patients is not yet done in district hospitals of Rwanda. The practice is to transfuse ABO/D compatible blood following an immediate spin crossmatch (IS-XM) or indirect antiglobulin test crossmatch (IAT-XM).

Objectives: To assess the risk of red blood cell (RBC) alloimmunisation associated with the use of IS-XM compared to the IAT-XM in patients receiving blood transfusions in district hospitals in Rwanda.

Design: A cross-sectional comparative descriptive study.

Setting: Four Rwandan district hospitals. Kirehe and Nyanza hospitals used IS-XM while Muhima and Ruhengeri hospitals used IAT-XM.

Subjects: Blood samples were obtained from 187 patients (101 with IS-XM and 86 with IAT-XM) transfused in January, February, October, and November of 2012.

Results: The median age of blood recipients was 31 years (7 - 80) and 36% of them were male. Sixteen specific antibodies were identified in 12 patients: anti-RH1/D (2), anti-RH2/C (2), anti-RH3/E (2), anti-RH4/c (1), anti-RH5/e (2), anti-LE1/Lea (2), anti-JK1/Jka (1), anti-JK2/Jkb (1), anti-KEL1/K (1), anti-MNS1/M (1), and autoantibody (1). The global prevalence of red blood cell (RBC) alloimmunisation was 6.4% (12/187). That prevalence was significantly higher in the IS-XM group (10.4%) than in the IAT-XM group (2.3%) with an odds ratio of 4.8; [95% CI=1.2-19.8]; and a p-value of 0.031.

Conclusion: The prevalence of red blood cell (RBC) alloimmunisation in 187 patients receiving blood transfusions was 6.4% and was higher in recipients from hospitals using IS-XM, with Rhesus (RH) system antibodies widely predominant (56.2%). We recommend that IAT-XM be used in all district hospitals in Rwanda to minimise this risk.

INTRODUCTION

Red blood cell (RBC) alloimmunisation is the formation of single or multiple antibodies to red blood cell antigens following transfusion, transplantation or pregnancy. Clinically significant antibodies are those known to cause haemolytic disease of the newborn (HDN), haemolytic transfusion reaction, or shortened survival of transfused red blood cells. To prevent alloimmunisation, sensitive pretransfusion techniques, such as blood group typing combined with screening of alloantibodies (“type and screen”) or compatibility testing shall be those that detect clinically significant antibodies. They shall include incubation at 37°C prior to antiglobulin test using reagent red blood cells that are not pooled (1).

Compatibility “tube” testing is carried out in three main phases (D. Joe Chaffin, Pretransfusion testing?: Basic Immunohematology part 2). The first phase, called “immediate spin” (IS), consists in combining two drops of the recipient serum and 1 drop of a 2 to 5% saline suspension of the donor RBCs
with centrifugation for 15 to 30 seconds. This phase enables detection of ABO incompatibility and cold-reactive antibodies; the latter are IgM antibodies that are not clinically significant. In the second phase, a potentiator medium (low ionic strength saline [LISS], polyethylene glycol [PEG], or bovine albumin) is added and the solution is incubated at 37°C. The incubation period differs depending on the medium: 10-15 min for LISS, 15 min for PEG, and 15-30 min for bovine albumin. The third phase consists in adding antihuman globulin (AHG), an antibody against human immunoglobulins and/or complement to the mixture; this phase is also called indirect antiglobulin test (IAT) (2,3). The final phase is crucial for detecting weak anti-A or anti-B and warm-reacting IgG antibodies, which are clinically significant and not detected by IS-XM (4). Other methods of compatibility testing, such as column agglutination ("gel") testing and solid phase adherence assay (SPAA), are used in different countries (2,3). Each hospital must determine its preferred testing method and ensure adequate lab capacity to perform the selected procedure. IS-XM is a safe method only in centres where sera of donor and patient are screened for clinically significant antibodies and the screening is negative (1, 5, 6).

In district hospitals of Rwanda, screening of alloantibodies in patients is not performed. The practice is to transfuse ABO/D compatible blood following an IS-XM in most cases or IAT-XM in few hospitals. Despite this variability in practice, there are no studies to our knowledge that assess alloimmunisation outcome of blood transfusion in Rwandan patients.

The aim of this study was to measure the risk of RBC alloimmunisation in Rwandan patients receiving RBC transfusion where IS-XM testing is used versus IAT-XM testing in pretransfusion crossmatch. We determined the prevalence and specificities of RBC alloantibodies in transfused patients from four different district hospitals employing these two crossmatch testing techniques.

**MATERIALS AND METHODS**

**Study design:** Using cross-sectional design and systematic sampling, we selected a total of 187 transfused patients with different diseases in four district hospitals of different provinces of Rwanda. Study sites included four hospitals: Muhima, Nyanza, Ruhengeri and Kirhe District Hospitals. Kirhe and Nyanza Hospitals were using IS-XM and Muhima and Ruhengeri were using IAT-XM. A nurse at each hospital recruited patients for the study and a doctor at each hospital facilitated data collection and notified patients of results; both received basic information and training regarding the study.

Patients receiving transfusions were recruited in four months, February, March, October and November of 2012. Eligibility criteria included inpatients that were at least seven years of age and had received at least one transfusion. Children below seven years were excluded to ensure adequate volume of blood samples for immunohaematology analyses. Prior to discharge, enrolled patients were given information about the study and were provided with a follow-up appointment for repeat blood testing. Follow-up appointments were scheduled at least four weeks after transfusion, allowing sufficient time for minimal reactive antibody formation (7). Depending on the date of transfusion, the follow-up period was between four and 12 weeks. Data collection and blood samples were taken in the last week of April 2012 for those transfused in February and March and in the last week of December 2012 for those transfused in October and November.

**Data collection:** We used a structured questionnaire to record demographic and transfusion history characteristics, including: age, sex, parity, number of transfusion episodes, number of transfused units of packed red blood cells, clinical indication for the transfusion, type of transfused blood product, and technique used in pretransfusion crossmatch testing (IS-XM or IAT-XM). Prior to patient discharge, information about the study was given to patients and the questionnaire was completed by a doctor. Patients returned 4 to 12 weeks later for blood sample collection and the questionnaire was updated with the time period between transfusion and blood sample collection. After informed consent (assent for patients below 17 years) was obtained, six to nine milliliters of blood was collected in EDTA (ethylenediaminetetraacetic acid) vacutainer test tubes. Within 12 hours, samples underwent centrifugation at 3000 rpm lasting three minutes. Plasma samples were extracted and kept at -30°C and red blood cell samples at 2 to 6°C in the Regional Blood Centre of Rwamagana in Eastern Province (Rwanda) until they were transported to the Blood Bank of Liège in Belgium where laboratory analyses were performed.

**Laboratory investigations:** Immunohaematology analyses of blood samples were performed by the blood bank of the University Hospital of Liège. Plasma samples were screened for the presence of red blood cell alloantibodies (detection of irregular antibodies) using an automated technique, Immucor Capture-R Ready-Screen (4) (Immucor® Neo®) (sensitivity 88.3%, specificity 94.3%) (8). This technique uses microplate wells coated with a standard 4-cell panel of reagent group-O RBCs. When the antibody screening was positive, by similar technique, antibody identification was performed by testing the plasma samples with commercial panels of 14 reagent RBCs of selected phenotypes with one
positive and one negative control-cell. While some alloantibodies could be identified by this automated technique, others required further analysis using gel centrifugation technique (Diamed-ID®, Alba Bioscience®, Edinburgh) with a standard 3-cell panel of reagent group-O RBCs for screening and 11 phenotyped group-O RBCs for identification. In this gel technique, enhancers were “LISS/Coombs” and “NaCl/papain” (overall sensitivity 79%; 57% in Coombs and 59.3% in papain with overall specificity 98.6%) (Jérôme de MARCHIN and Christiane GERARD, Comparison of Immuno-Capture (Immucor® Galileo®) assay to the Gel (Diamed®) in pre-transfusion testing in a Blood Bank, CHU of Liège). The algorithm combining these two techniques allows the identification of more specific antibodies (9).

Patients were considered to be alloimmunised if antibodies to one or more RBC antigens could be identified. One patient with panreactive panels in both indirect antiglobulin test and papain, followed by a positive direct Coombs test was also considered alloimmunised.

Statistical analysis: Data entry and statistical analysis was performed using STATA/IC 12.1 for Windows. Descriptive analysis included frequency tables and mean or median calculation. Odds ratios for associations (with exact or test–based confidence interval), Pearson Chi-squared (chi-2) or Fisher’s exact test (when assumptions were not met for chi-2) and Mantel-Haenszel chi-2 for stratification were used to measure the relationship between red blood cell (RBC) alloimmunisation and related exposure factors. Pretransfusion crossmatch technique (IS-XM or IAT-XM) was the main factor to analyze. Other factors known to be associated with RBC alloimmunisation in other studies were also analysed; these include gender, number of parity, and number of transfusion episodes. Furthermore, we analysed possible confounders in the relationship between RBC alloimmunisation and pre-transfusion crossmatch technique by adjusting for gender, number of parity and number of transfusion episodes. Groups were assumed to differ significantly when the p-value was lower than 0.05.

Ethical consideration: This research has been approved by the Rwanda National Ethical Committee and was reviewed by the Rwanda Biomedical Centre/National Health Research Committee, where we obtained two different ethical and scientific approvals, respectively. Amendments to the study protocol during our study process were sent to both these institutions.

RESULTS

Demographic and transfusion related results: Of the 187 transfused patients who were recruited, 86 (46.0%) were from the two hospitals using IAT-XM and 101 (54.0%) were from the two hospitals using IS-XM. Females were predominant (64%) with sex ratio of 1.75. Age ranged from 7 to 80 years; the median age was 31 years. At the time of blood sample collection, 74.3% of patients reported one lifetime blood transfusion, 16.6% reported two while 9.1% were polytransfused. Packed RBCs were transfused in 93.0% of the cases, whole blood in 3.2%, platelets in 3.2% and plasma in 0.5%. Mean number of units by transfusion was 2 ± 0.65 (standard deviation). Mean time between last transfusion and blood sampling was 7 ± 3.6 weeks (standard deviation).

Antibodies detection and identification: Laboratory investigations showed 19 of 187 patients (10.2%) with reactive irregular antibodies. Panels for antibody identification specified 16 antibodies in 12 patients (6.4%). We were not able to determine specific antibodies in the remaining seven patients (3.7%), and so these samples were considered as missing for antibodies during statistical analysis. Hence, RBC alloimmunisation in our studied population is 6.4%. Among the 12 alloimmunised patients, eight patients had single specificity antibodies and 4 had mixed specificities. Table 1 shows the specificities of identified antibodies, with 9 (56.2%) belonging to the Rhesus blood group system, 2 (12.5%) to the Lewis system, 2 (12.5%) to the Kidd system, 1 (6.3%) to the Kell system, 1 (6.3%) to the MNS system, and 1 (6.3%) showed panreactive panel with positive direct Coombs test (auto-antibody).
**Table 1**
Specificities of 16 RBC alloantibodies identified in 12 (6.4%) transfused patients in Rwanda

<table>
<thead>
<tr>
<th>Blood group system</th>
<th>RBC alloantibody specificity</th>
<th>Number of antibodies (respectively)</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus</td>
<td>RH1/D , RH2/C, RH3/E, RH4/c, RH5/e</td>
<td>2,2,2,1,2</td>
<td>56.2</td>
</tr>
<tr>
<td>Lewis</td>
<td>LE1/Lea</td>
<td>2</td>
<td>12.5</td>
</tr>
<tr>
<td>Kidd</td>
<td>JK1/Jka, JK2/Jkb</td>
<td>1, 1</td>
<td>12.5</td>
</tr>
<tr>
<td>Kell</td>
<td>KEL1/K</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>MNSs</td>
<td>MNS1/M</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>Panreactive*</td>
<td>Auto</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>16</td>
<td>100%</td>
</tr>
</tbody>
</table>

*RBC with positive auto-antiglobulin test

**Risk factors of red blood cell (RBC) alloimmunisation:**
Excluding the 7 patients (5 in IS-XM test and 2 in IAT-XM test) with indeterminate profiles, 10 patients among 96 (10.4%) in the IS-XM group were alloimmunized versus 2 patients (2.3%) among 84 in the IAT-XM group, with odds ratio (OR) of 4.8, [95% CI = 1.2 -19.8] and a p-value = 0.031. Female patients, multiparous women and polytransfused patients were respectively 1.2; 1.9 and 2.2 times more likely to develop alloantibodies than other patients in their corresponding groups but these associations were not statistically significant. To check confounding factors, the Mantel-Haenszel (M-H) chi-squared (chi2) test of the relationship between RBC alloimmunisation and pretransfusion crossmatch technique adjusted for number of transfusion episodes remained significant with a p-value = 0.040. The M-H chi2 after stratification by gender and number of parity was not found due to small number of cases (antibodies) observed in respective strata. Table 2 summarises our findings related to risk factor analysis.

**Table 2**
Risk factors in 12 (6.4%) alloimmunised patients in Rwanda

<table>
<thead>
<tr>
<th>Variable in categories</th>
<th>Number of patients*</th>
<th>Number (with %) of Alloimmunised patients</th>
<th>Odds ratio</th>
<th>95%Confidence interval(CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crossmatch test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IS-XM</td>
<td>96</td>
<td>10 (10.4)</td>
<td>4.8</td>
<td>1.2 -19.8(tb)</td>
<td>0.031†</td>
</tr>
<tr>
<td>IAT-XM</td>
<td>84</td>
<td>2 (2.3)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>113</td>
<td>8 (7.0)</td>
<td>1.2</td>
<td>0.3 -5.7(exc)</td>
<td>0.773†</td>
</tr>
<tr>
<td>Males</td>
<td>67</td>
<td>4 (5.9)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>42</td>
<td>2 (4.8)</td>
<td>1</td>
<td></td>
<td>0.708‡‡</td>
</tr>
<tr>
<td>At least one pregnancy</td>
<td>71</td>
<td>6 (8.5)</td>
<td>1.9</td>
<td>0.3- 19.5(exc)</td>
<td></td>
</tr>
<tr>
<td>Number of transfusion episodes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.187‡‡</td>
</tr>
<tr>
<td>One lifetime transfusion</td>
<td>134</td>
<td>7 (5.2)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than one transfusion</td>
<td>46</td>
<td>5 (10.9)</td>
<td>2.2</td>
<td>0.5-8.6 (exc)</td>
<td></td>
</tr>
</tbody>
</table>

*The 7 patients with indeterminate profiles are excluded; **only in females; (tb) test based confidence interval; (exc) exact confidence interval; †Pearson Chi2; ‡‡Fisher’s exact.
DISCUSSION

This is the first ever study carried out to compare the frequency of red blood cell (RBC) alloimmunisation with specific antibodies in different pretransfusion crossmatch techniques after blood transfusion in Rwanda. The high proportion of women in this study was due to the inclusion of Muhima hospital, which only provides maternity services. Additionally, obstetric hemorrhage in women is common in Africa, especially in rural areas; leading to higher rates of transfusion in females (10). The high prevalence of packed RBC transfusion (93.0% of the cases) showed a low utilisation of other blood products. While RBC transfusion is more common in the context of emergency transfusions, as it is the case in district hospitals of Rwanda, this may also indicate a lack of clinician and blood bank staff awareness about the indications of blood product use, blood ordering practices and storage, as was shown in two studies published in 2010 assessing clinical transfusion practice and interface between blood preparation and use in Uganda (11, 12).

The prevalence of RBC alloimmunisation, excluding seven indeterminate profiles, was 6.4%. This prevalence may be an underestimate compared to the total reactive irregular antibody detection rate of 10.2%. The 6.4% rate of alloimmunisation in our hospitals is comparable to that seen in two studies carried out in patients receiving transfusions in Ugandans for different diseases and for sickle cell anemia (6.1% in both studies) (13, 14). Rates of alloimmunisation in other studies show a wider range: a study in polytransfused patients in Mali revealed 10.3% alloimmunisation whereas a study in Malawi showed 1.1% alloimmunisation (but included patients who had not received blood transfusion) (15, 16). The predominance of alloantibodies (56.2%) belonging to the Rhesus blood system in this study (Table 1) is compared to the results of the two studies conducted in Uganda (53.8% and 76.9% of alloantibodies belonging to RH blood system) (13, 14). That predominance is also explained by a high proportion of variant antigens (mainly D and C) within the RH blood system found in Afro-Caribbean population (17, 18). In the same context of alloimmunisation due to RH system antigens, Norol F et al. showed that RBC alloimmunisation can be reduced by 75% if matched RBCs for RH and Kell antigens are transfused instead of standard matched red blood cells (ABO/D only) (19).

In our study, pretransfusion crossmatch by IS-XM was significantly associated with a higher risk of RBC alloimmunisation (4.8 times more likely to cause RBC alloantibodies than IAT-XM). This was also demonstrated in a study conducted by T. Oduola et al., in which incompatible residual reactions (1.2%) were observed by both LISS-AHG (IAT) and full AHG crossmatches when ABO/D compatible blood issued by IS-XM was examined (4, 5). In our study, there was no significant relationship between RBC alloimmunisation with sex, parity, and number of transfusion episodes, as was seen in other studies (13, 20, 21, 22). Furthermore, these variables did not confer a confounding effect on the relationship between RBC alloimmunisation and XM tests.

In conclusion, the risk of developing RBCs alloantibodies is higher in patients where IS-XM pretransfusion crossmatch is used, compared to IAT-XM testing with predominance of RH system antibodies. We recommend using a standard operating procedure with IAT-XM in blood banks of district hospitals of Rwanda to minimize the risk of alloimmunisation. While the roll out of IAT-XM can be easily implemented, surveillance procedures must be put in place to assure the sustainability and quality of the technique in the whole country.

STUDY LIMITATIONS

Our sample size was limited by financial constraints. However, potential sources of bias were controlled by a good communication with participants to limit non-respondents, by using controlled procedures for blood sample storage, transport and laboratory analyses.

ACKNOWLEDGMENTS

To all on-site facilitators (hospital directors, medical doctors and nurses) who helped in recruiting patients and obtaining blood samples. We are grateful to the members of the Rwanda National Ethical Committee and Rwanda National Health Research Committee for their scientific input. We are indebted to Professor Filomena Valente, the Coordinator of the Master Complémentaire in Public Health Methodology at School of Public Health of the “Université Libre de Bruxelles”, who provided valuable input on data analysis. We would like to thank Prof. A. Gothot and Dr. Chr. Gérard and members of BTC study scholarships and training unit for financial support.

FUNDING

Funding for travel from Belgium to Rwanda was provided by the Belgium Technical Cooperation Unit of Scholarships for Studies and Training. All costs related to laboratory investigations were covered by the blood bank of Liège under the authorisation of Professor André Gothot and Dr. Sc. Christiane Gérard. There were no special funds for data collection. The author(s) received no financial support for the research, authorship and/or publication.
CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES


