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ORIGINAL PAPER

Prevalence and specificities of red blood cell alloantibodies in transfused Ugandans with different diseases

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Background and Objectives Alloantibody formation against red blood cell (RBC) antigens is a common complication of transfusion therapy. However, the prevalence of RBC alloimmunization is hardly known in Black Africans. In Uganda, the practice is to transfuse ABO/D compatible blood without screening for immune antibodies. The aim of this study was to determine the prevalence and specificities of RBC alloantibodies in transfused Ugandans.

Materials and Methods Using a cross-sectional design, transfused patients at Mulago Hospital in Kampala, Uganda were investigated. Demographic characteristics and transfusion histories were recorded. EDTA blood samples were obtained from consenting patients and RBC alloimmunization was demonstrated using immunohaematological tests.

Results A total of 214 transfused patients (mean age, 30·3 years; F/M ratio, 1·0) were investigated. Thirteen patients (6·1%) possessed RBC alloantibodies whose specificities were six anti-E; three anti-S; one each of anti-D, -K and -Le^a; and two samples were pan-reactive. Eleven (84·6%) of the alloimmunized patients had experienced up to 10 transfusion episodes. The number of units of blood transfused and the transfusion episodes were significantly associated with the RBC alloimmunization rate (P = 0·01).

Conclusions The prevalence of RBC alloimmunization in transfused Ugandans was 6·1% and was associated with the number of donor exposures. This immunization rate is similar to that observed in transfused Caucasians despite differences in RBC antigen distributions. Patients with malaria were less likely to develop RBC alloantibodies. Alloantibodies were mainly against E and S antigens. We recommend the introduction of pretransfusion antibody tests in Uganda depending on the recipient's diagnosis.

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Introduction

Red blood cell (RBC) alloimmunization is an important adverse effect that follows repeated transfusions with allogeneic blood [1]. It results from an immune response due to the genetic differences between blood donors and recipients. Immune anti-RBC antibodies are generally formed early in the course of multiple transfusions, usually before the 10th transfusion [2,3]. In patients with disorders that often require multiple blood transfusions the rate of RBC alloimmunization has been reported in the range of 5–30% [2–10]. This range is even wider (3–76%) in patients with haemoglobinopathies [11–15]. Risk factors for RBC alloimmunization include female sex, a history of pregnancy, recipient clinical diagnosis and treatment, and racial

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differences between transfusion recipients and blood donors [16–18].

Patients receiving chemotherapy for myeloproliferative disorders do not seem to be suppressed in terms of their ability to produce blood group alloantibodies. However, patients with lymphoproliferative disorders are generally characterized by an impaired immunological response and therefore alloimmunization to RBC antigens following multiple transfusions is less common [2,3,19]. Absence or low rates of alloimmunization have been reported in some D negative AIDS patients receiving D positive RBC transfusions and in multiply transfused AIDS patients with AZT-associated anaemia [20,21]. Some reports indicate that multitransfused females register higher rates of alloantibody formation than males [18]. However, studies by Blumberg *et al.* [19] and Redman *et al.* [6] showed no difference in gender regarding alloimmunization rates.

Red blood cells alloimmunization is generally detected when patients need a blood transfusion. During pretransfusion testing, the patient's blood sample is usually screened for unexpected alloantibodies [22]. Antibodies against antigens of the Rh and Kell blood group systems are the specificities most frequently found in alloimmunized patients in Western Europe and the United States [3,5,6,9]. In Uganda, no such pretransfusion screening for immune antibodies is carried out on blood transfusion recipients. The aim of this study was to determine the prevalence and specificities of RBC alloantibodies in transfused Ugandan patients with different diseases.

Materials and methods

Patients

Using a cross-sectional design, transfused patients with different diseases admitted at Mulago National Referral Hospital in Kampala, Uganda were investigated. The initial part of the study involved patient recruitment and blood sample collection and took place between 1st February and 31st July, 2008. Eligibility criteria included inpatients that were at least 2 years of age and had received at least two previous allogeneic blood transfusions – the second transfusion episode being more than 2 weeks before enrolment into the study. This was intended to enrol a group of participants that were likely to have become alloimmunized at an appropriate age and time after exposures to blood transfusions. Patients with haemoglobinopathies were being investigated in a parallel study and were excluded. Obstetric patients were also excluded from this study. In general, patients received ABO/D compatible and non-leucocyte depleted whole blood or packed RBC transfusions.

Data collection

Patients' charts were reviewed for demographic characteristics and the transfusion history. In cases of incomplete or missing records, patients or their attendants were asked for additional information on the above history. The number of transfusion episodes, number of units of blood transfused, date of transfusion, indication for transfusion, age of first transfusion and for oncology patients whether they had received anti-cancer chemotherapy; were recorded in a data collection form. The study was approved by the research and ethical committees at Mbarara University of Science and Technology, Mbarara, Uganda, and Makerere University Medical School, Kampala, Uganda.

Laboratory investigations

After consent, blood was drawn into ethylenediaminetetraacetic acid (EDTA) tubes for laboratory investigations. Plasma and buffy coat samples were removed and stored frozen at the Joint Clinical Research Center (JCRC) in Kampala, Uganda, until they were shipped to the Sanquin Blood Supply in Leiden, The Netherlands, for immunohaematological studies.

Plasma samples were screened for the presence of RBC alloantibodies by use of a standard 3-cell panel of reagent group O RBCs. In the indirect antiglobulin test (IAT), a LISS-enhanced gel centrifugation technique (DiaMed ID, Micro Typing System, Cressier sur Morat, Switzerland) with polyspecific anti-human globulin (rabbit anti-IgG and monoclonal anti-C3d) was used. When the antibody screening was positive, antibody identification was performed by testing the plasma samples with commercial panels of reagent RBCs, of selected phenotypes, by similar or additional methods whenever needed. Patients were considered to be alloimmunized if antibodies to one or more RBC antigens could be identified. For one patient who possessed anti-D alloantibodies, an RHD multiplex PCR was carried out to determine the genotype and hence the molecular basis of this observation.

Statistical methods

Statistical software packages Excel 5·0 (Microsoft, Redmond, CA) and Statistical Package for the Social Sciences 12·0 (SPSS Inc., Chicago, IL) were used for data management and analysis respectively. For univariate analysis of possible associations between RBC alloimmunization and gender, history of pregnancy, whole blood transfusion, diagnosis of malignancy, anti-cancer therapy and HIV positivity, the Chi-squared test or Fisher's exact test were used. Logistic regression analysis was used for continuous variables of a non-Gaussian distribution i.e. the age at the time

of enrolment, the number of units of blood transfused, the number of transfusion episodes and the number of years since the last transfusion. Groups were assumed to differ significantly when the probability level was less than 0·05.

Results

Patient data

We recruited 214 transfused inpatients at Mulago National Referral Hospital during the study period. Of these, 113 (52·8%) were females and among them, 77 (68·1%) had a history of pregnancy. The mean age at the time of blood collection was 30·3 (median, 29·5; range, 2–80) years. The patients had been transfused with a total of 1 869 (mean, 8·7; median, 5·0 and range, 2–65) units of blood in 1 285 (mean, 6·0; median, 4·0 and range, 2–57) transfusion episodes. Half of the patients studied (108; 50·6%) had malignant disorders while 62 (29%) had infectious diseases (Table 1).

Thirteen patients (6·1%; 95% CI: 3·0–10·0%) were found to be alloimmunized to RBC antigens; 11 (84·6%) of them having experienced up to a maximum of 10 transfusion episodes. The number of units of blood transfused and the number of transfusion episodes were significantly associated with the rate of alloimmunization ($P = 0·01$). Other demographic and transfusion characteristics of alloimmunized patients were not significantly different to those in the non-immunized group (Table 2).

Table 1 Disease groups of the 214 Ugandan transfused patients and proportions of those who were alloimmunized in each group

Disease group	Patients (*n*; %)	RBC Alloimmunized (*n*; %)
Malignancies	108 (50·6)	9 (8·3)
Haematologic	64	4
Solid tumors	44	5
Infectious diseases	62 (29·0)	2 (3·2)
Malaria	34	1
AIDS	24	1
Bacterial infections	4	0
Others	44 (20·4)	2 (4·5)
Gastrointestinal disease	12	1
Renal disease	10	0
Trauma	10	0
Heart disease	7	1
Diabetes mellitus	3	0
Burns	2	0

RBC antibodies

Eleven of the alloimmunized patients produced a total of 12 RBC alloantibodies of known specificity. The remaining two patients possessed pan-reactive antibodies. The specificities of the alloantibodies identified were: anti-E, 6; anti-S, 3; and 1 each of anti-D, -K and -Le^a. In one patient (9·1%), two alloantibodies, anti-E plus anti-K, presented as a combination; the rest of the alloantibodies were as single specificities. D genotyping on DNA isolated from the buffy coat of the patient whose plasma contained alloanti-D revealed that this patient had partial D of the R_o^{Har} category.

Discussion

This cross-sectional study was undertaken to determine the prevalence and specificities of RBC alloantibodies in Ugandan patients who received blood transfusions. Out of 214 patients studied, 13 (6·1%; 95% CI: 3·0–10·0) possessed RBC alloantibodies. This overall RBC alloimmunization

Table 2 Demographic variables and transfusion characteristics of alloimmunized and non-immunized Ugandan patients who received blood transfusions

	Alloimmunized (*n* = 13)	Non-immunized (*n* = 201)	*P*-value
Mean age in years (median; range)	34·8 (35·0; 2–75)	30·0 (28·0; 2–80)	NS
Sex ratio (F/M)	1·6	1·0	NS
History of pregnancy (%)	62·5	67·6	NS
Mean transfusion episodes (median; range)	11·8 (4·0; 2–57)	5·6 (4·0; 2–50)	0·01
≤10 Transfusion episodes (%)	84·6	90·5	NS
Mean units of blood transfused (median; range)	16·2 (8·0; 2–65)	8·3 (5·0; 2–60)	0·01
Transfusion with whole blood (%)	92·3	88·1	NS
Patients with malignancy (%)	69·2	46·2	NS
Patients received anti-cancer chemotherapy (%)	55·6	50·5	NS
HIV positive patients (%)	23·1	30·3	NS
Mean years since the last transfusion (median; range)	2·54 (0; 0–29)	1·36 (0; 0–49)	NS

NS = *P*-value > 0·05

prevalence is comparable with rates previously reported on patients receiving transfusion support for chronic renal failure and haematological disorders in developed countries [2,3,5–10]. The antibody specificities (anti-E, 6; anti-S, 3; anti-D, 1; anti-K, 1; anti-Le^a, 1; and 2 pan-reactive) are also similar to the ones commonly detected and reported [3,6,9]. The only difference is the finding of a slight increase in the rate of alloimmunization against the S antigen. Also, we recently found an increased number of alloantibodies (13·3% of all antibody specificities identified) with anti-S specificity in a parallel study on RBC alloimmunization in Sickle Cell Disease (SCD) patients in Uganda (B. Natakunda, H. Schonewille, C. Ndugwa and A. Brand, unpublished data). There was a mixture of antibodies (anti-E and -K) in one of the alloimmunized patients which is comparable to the frequency of multiply alloimmunized patients in other studies [2,9]. The patient who was alloimmunized to the D antigen – a 50-year-old female with no history of pregnancy – had apparently been typed as D positive by serology. Using an *RHD* multiplex PCR [23], D genotyping was carried out on this patient and a partial D of the R_o^{Har} category was found.

Analysis of the transfusion characteristics revealed that the rate of RBC alloimmunization was significantly associated with the number of units of blood received and the number of transfusion episodes ($P = 0{\cdot}01$ in both cases) i.e. the alloimmunization risk increased with the number of donor exposures. This observation is in agreement with retrospective analyses by Fluit *et al.* [3] and Schonewille *et al.* [9]. According to Blumberg *et al.* [2], although the formation of RBC alloantibodies is influenced by the number of transfusions, most of the alloimmunization occurs early in the course of blood transfusion. This was also the case in 84·6% of alloimmunized patients in the present study. Female sex and a history of pregnancy were not associated with RBC alloimmunization. Our data indicate that 9 (8·3%) of 108 patients with malignant disorders became immunized to RBC antigens following blood transfusion and this prevalence is comparable with what has been observed in other studies on RBC alloimmunization in oncologic disorders [9]. Malaria is a serious disease and patients usually present with haemolytic anaemia that may be severe. The effect of malaria infection on the pathogenesis of RBC alloimmunization remains to be elucidated. Recent reports by Hendrickson *et al.* demonstrated suppression of RBC alloimmunization by a bacterial endotoxin lipopolysaccharide (LPS) and an enhanced magnitude of RBC alloimmunization in a setting of viral-like inflammation, in murine models [24,25]. In Uganda, severe malaria is a common indication for blood transfusion therapy and the occurrence of RBC alloantibodies in malaria patients in the present study was anticipated. However, only one patient (2·9%) was immunized in 34 transfused patients with severe malaria infection. These data suggest that there seems to be no enhanced RBC alloimmunization in patients with malaria. Absence of alloimmunization as a result of immunosuppressive effects of AIDS or anti-cancer chemotherapy has been reported in previous studies [20,26]. However, in this study 5 (55·6%) of the 9 alloimmunized patients with cancer had been receiving anti-cancer chemotherapy. Also, one patient with AIDS – a 45-year-old female who had received 11 units of whole blood in three transfusion episodes – was alloimmunized to the E antigen. However, the fact that anti-E may sometimes be a naturally occurring antibody [27] cannot be excluded in this case.

Thus, alloantibody formation remains a notable adverse effect of blood transfusion in the provision of clinical care even in a setting of immunesuppression, especially for cancer patients. This emphasizes the important role played by eliciting a good clinical history of previous blood transfusion(s) and routine laboratory screening for the presence of unexpected antibodies before transfusion. However, the implementation of routine antibody screening for all transfusion recipients in Uganda and in most of Sub-Saharan Africa is not currently feasible. This is because of the extra costs involved in the monthly procurement of reagent cell panels; the increased work load and hence additional staffing requirements for hospitals and transfusion services; the apparently low RBC alloimmune response rates in most of the transfusion recipients; the lack of organized administrative systems for laboratory record keeping and retrieval; and the lack of adequately qualified personnel in the fields of Immunohaematology and Transfusion Medicine. In a parallel study on RBC alloimmunization in transfused patients with sickle cell disease in Uganda, we observed a similar alloimmune response rate of 6·1% (unpublished data). Therefore, in case of patients with malignant diseases, haemoglobinopathies or other disorders likely to require repeated blood transfusions there should be special considerations. For these patient categories that are at higher risk of RBC alloimmunization, we recommend the introduction of a *pretransfusion typing and screening strategy* [22] comprised of the following three tests: ABO/D grouping of the donor and recipient; IAT screening for irregular RBC antibodies in the recipient's serum (a 3-cell panel); and a saline cross-match between the patient's serum and the donor RBCs at room temperature. Any detected antibodies in the patients can be identified accordingly so that transfusion with antigen-negative blood is given. Regarding other transfusion recipients, we recommend the use of a *complete cross-match* [28] before blood transfusion i.e. incubation of the patient's serum with the donor RBCs and addition of antihuman globulin serum. In so doing, we shall go a long way towards the prevention of morbidity related to immunological complications of blood transfusion in Ugandan patients with different diseases.

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