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Red blood cell alloantibodies in transfused patients with haematological malignancies at Mbarara Regional Referral Hospital and the Uganda Cancer Institute: Prevalence, specificities and associated factors

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Abstract

Background and Objectives: Patients with haematological malignancies often require blood transfusion support. Multiple allogeneic blood transfusions may result in alloimmunization, complicating future transfusions. This study determined alloantibody prevalence, specificities and factors associated with the presence of red blood cell (RBC) alloantibodies among transfused patients with haematological malignancies at Mbarara Regional Referral Hospital (MRRH) and the Uganda Cancer Institute (UCI).

Materials and Methods: This was a cross-sectional study among patients with haematological malignancies who had been multiply transfused and were seeking cancer care at MRRH and the UCI, in Uganda. Patient plasma was screened for the presence of RBC alloantibodies using haemagglutination testing with a 3-cell commercial reagent RBC and antibody identification with 11-cell antibody panels.

Results: A total of 427 patients with a median age of 36 (inter-quartile range: 26–56 years) were investigated. Twenty-five participants (5.9%) possessed RBC alloantibodies whose specificities were as follows: anti-C, two; anti-D, four; anti-E, six; anti-K, four; and anti-c, anti-Fy^a, anti-Jk^a, anti-Le^a and anti-M, one each. Four patients possessed pan-reactive antibodies. Patients with chronic cancer (adjusted odds ratio [AOR] = 2.62, 95% confidence interval [CI]: 1.16–7.21), leukaemia (AOR = 2.71, 95% CI: 1.81–4.03), human immunodeficiency virus (HIV) infection

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(AOR = 4.34, 95% CI: 1.69–5.11), antibiotic use (AOR = 5.08, 95% CI: 2.11–7.41) and a history of ≥5 transfusions were significantly associated with RBC alloimmunization ($p \leq 0.05$).

Conclusion: RBC alloimmunization prevalence was 5.9% and associated with clinical and transfusion-related factors. Alloantibodies to Rh, Kell, MNS, Duffy, Kidd and Lewis blood group systems were detected, underscoring the need for improved pre-transfusion testing in Uganda.

Keywords

blood transfusion, chemotherapy naïve, haematological malignancies, RBC alloimmunization, Uganda

Highlights

- The study found a 5.9% prevalence of red blood cell (RBC) alloantibodies among patients with haematological malignancies who received repeated transfusions.
- The study identified alloantibodies against Rh, Kell, MNS, Duffy, Kidd and Lewis blood group system antigens, with four cases showing pan-reactivity. The diversity of alloantibodies increases the risk of haemolytic transfusion reactions and the risk of haemolytic disease of the foetus and newborn and complicates donor matching for transfusion.
- RBC alloimmunization was associated with chronic cancer, diagnosis of leukaemia, human immunodeficiency virus (HIV) infection, antibiotic use, ≥5 transfusion episodes and need for ≥5 donor units, emphasizing the need for improved strategies for preventing alloimmunization in multi-transfused patients.

INTRODUCTION

Patients with haematological malignancies often require blood transfusion support because of bone marrow infiltration, anaemia caused by inflammation and/or bone marrow suppression due to cancer chemotherapy [1]. It is important to note that, despite the current safety precautions, including ABO and D typing and compatibility testing, blood transfusions are not entirely risk-free [2]. The formation of red blood cell (RBC) antibodies against foreign RBC antigens remains a major concern in transfusion [3]. In fact, multiply transfused recipients, including patients with haematological malignancies, are at higher risk of RBC alloimmunization [4, 5], a concern heightened by evidence that alloimmunization can develop after only a few transfusions [4]. Studies on RBC alloimmunization in patients with haematological malignancies have shown varying prevalence rates, ranging from 3% to 11% [6–10]. Noteworthy, varied RBC alloantibodies against the antigens of different blood group systems have been reported [6–8]. RBC alloimmunization is associated with varied factors such as genetics, antigen disparity between the donor and recipient, the underlying disease, recipient sex, the use of multiple allogeneic blood units and a history of immune-sensitizing events [3, 4, 11–14].

RBC alloimmunization can lead to serious complications, such as delayed haemolytic transfusion reactions and, although rare, acute haemolytic transfusion reactions [2, 3]. Among women of childbearing age, alloimmunization carries the additional risk of haemolytic disease of the foetus and newborn (HDFN) [15]. Moreover, the need for extensive pre-transfusion testing and the potential requirement for antigen-negative donor units may lead to delays in blood transfusions [3]. Such consequences underscore the need for strategies to prevent alloimmunization, including improved pre-transfusion recipient testing [16, 17]. However, implementing comprehensive transfusion safety measures remains challenging in resource-limited settings [18]. In Uganda, pre-transfusion testing is currently limited to ABO and RhD typing and room-temperature saline cross-matching, all performed using the tile method [16, 18, 19]. Despite the frequent need for transfusions among patients with haematological malignancies, research data on RBC alloimmunization in Uganda remain scarce. This study determined alloantibody prevalence, specificities and factors associated with the presence of RBC alloantibodies among transfused patients with haematological malignancies at Mbarara Regional Referral Hospital (MRRH) and the Uganda Cancer Institute (UCI).

MATERIALS AND METHODS

Participants

A cross-sectional study was designed, involving transfused haematological cancer patients seeking cancer care at MRRH and UCI, in Uganda. Participants were consecutively recruited between 30 August 2024 and 4 January 2025. Eligibility criteria included ≥2 years of age with a confirmed diagnosis of haematological malignancy; chemotherapy-naïve; and a previous history of ≥2 transfusions of allogeneic blood, with the last transfusion episode being ≥2 weeks before

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enrolment in the study. These criteria were aimed at enrolling participants who were likely to have become alloimmunized after exposure to blood transfusion at the time of enrolment. Participants who were on chemotherapy and had been transfused within <2 weeks were investigated in a parallel study and thus excluded.

Data collection

Participants' charts were reviewed, and their demographic characteristics, cancer diagnosis, Eastern Cooperative Oncology Group (ECOG) performance score [20], other comorbidities, cancer treatment–related transfusion and other related factors (particularly, ABO/D group, obstetric history for female patients, vaccination history and autoimmune diseases) were recorded on a data capture form. In cases where the records were incomplete or missing, we sought additional information from participants or their caregivers. The study was approved by the Mbarara University of Science and Technology Research and Ethics Committee (MUST-2024-1505), the Uganda National Council for Science and Technology (HS4551ES) and the Health Research Ethics Board of Alberta-Cancer Committee (HREBA-CC-25-0052). Written informed consent was obtained from adult participants or their caregivers. Also, informed written assent was obtained from stable participants under 18 years.

Laboratory investigations

Following consent/assent, about 3 mL of venous blood was drawn into an ethylene di-aminetetraacetic acid vacutainer as per the phlebotomy procedure. Plasma and buffy coat samples were aliquoted into a cryogenic vial and stored at −80°C at the study sites until they were shipped to the Cumming School of Medicine, University of Calgary, for immunohaematological testing. Before testing, samples and reagents were allowed to attain room temperature (18–22°C).

Plasma samples were screened for the presence of RBC alloantibodies by use of a standard 3-cell panel of reagent group O RBCs (ID-DiaCell I-II-III, Bio-Rad Laboratories, DiaMed GmbH, Switzerland). An indirect anti-human globulin (AHG) test was performed using the tube agglutination method [21] in three phases (immediate spin [IS]; enhancement with low-ionic-strength saline at 37°C; AHG steps). During the IS, two drops (about 100 µL) of plasma were added to one drop (50 µL) of 5% reagent RBCs in a labelled glass tube. Agglutination in the IS phase was checked for cold agglutinating antibodies, which were interpreted as autoantibodies. Unagglutinated tubes proceeded to the enhancement and AHG steps. Agglutination was graded 0–4+, corresponding to the amount of red cell agglutinin [22]. Tubes that did not show agglutination during the AHG phase were confirmed microscopically and validated by adding one drop (50 µL) of immunoglobulin G-coated RBCs (Bio-Rad Laboratories). A positive reaction was considered to validate the result; otherwise, it was repeated. The presence of alloimmunization among participants was deemed positive only if antibodies to one or more RBC antigens were identified.

Samples that showed agglutination in the screening phase were further characterized using an 11-cell commercial panel of reagent group O RBCs of selected phenotypes (ID-DiaCell 1-11) (Bio-Rad Laboratories). The specificity of the alloantibodies was established through a systematic interpretation of the reaction patterns on the antigram, panel cell antigen profiles, hetero- and homozygosity of the identification panels, the manufacturers' reported cell panel specificities, exclusion criteria and the 'rule of three' (i.e., at least three positive reactions with antigen-positive cells and three negative reactions with antigen-negative cells), as previously described [23]. All laboratory testing followed the previously reported procedures and conformed to the manufacturer's guidelines [24–26].

Statistical methods

The data entered in the Epi Info software suite (Atlanta, Georgia) were exported and cleaned for analysis using R version 4.4.2. Descriptive statistics such as frequencies, percentages, mean and standard deviation (SD) were generated to describe the participants' characteristics. The prevalence of RBC alloimmunization was determined as a proportion at 95% confidence interval (CI). Univariate analysis was performed to determine the individual effect of each of the factors on RBC alloimmunization. At the bivariate level, variables with $p \leq 0.2$ and those supported by the literature as biologically plausible were considered for multivariate logistic regression while controlling for confounding factors (history of sickle cell disease, autoimmune disorders, splenectomy and status of the RhD blood group). The effect of each factor was determined by adjusted odds ratios (AORs), and statistical significance was set at $p < 0.05$.

RESULTS

Participant characteristics

The study enrolled 427 participants whose mean age was 38.9 (SD 19.2; range 2–87) years. The median age was 36 years (interquartile range: 26–56). Of these, 309 (72.4%) had acute haematological malignancies and 195 (45.7%) had a leukaemia diagnosis (Table 1).

Prevalence of RBC alloimmunization

Twenty-five participants (5.9%; 95% CI: 3.8%–7.9%) were found to be alloimmunized to RBC antigens. The prevalence of alloimmunization was 5.1% (7/136; 95% CI: 1.3–9.0) among participants enrolled at MRRH and 6.2% (18/291; 95% CI: 3.3–9.1) among those recruited at UCI. No significant differences in demographic or clinical characteristics were observed between the two sites. Of the 25 participants,

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TABLE 1 Participant characteristics and proportions of those alloimmunized.

Variable	Total participants ($N = 427$), *n* (%)	RBC alloimmunized ($N = 25$), *n* (%)
Age category (years)		
<5	23 (5.4)	1 (4.0)
5–12	15 (3.5)	1 (4.0)
13–19	31 (7.3)	2 (8.0)
20–30	77 (18.0)	4 (16.0)
31–65	239 (56.0)	14 (56.0)
>65	42 (9.8)	3 (12.0)
Sex		
Female	198 (46.4)	16 (64.0)
Male	229 (53.6)	9 (36.0)
Occupation		
Children/pupil/student	85 (19.9)	1 (4.0)
Formal employment	97 (22.7)	3 (12.0)
Informal employment	209 (48.9)	16 (64.0)
Unemployed	36 (8.4)	5 (20.0)
ABO blood group		
A	113 (26.5)	6 (24.0)
B	78 (18.3)	4 (16.0)
AB	19 (4.4)	1 (4.0)
O	217 (50.8)	14 (56.0)
Status of the RhD blood group		
Negative	9 (2.1)	1 (4.0)
Positive	418 (97.9)	24 (96.0)
Cancer status		
Acute	309 (72.4)	10 (40)
Chronic	118 (27.6)	15 (60.0)
Histological diagnosis		
Leukaemia	195 (45.7)	18 (72.0)
Lymphoma	126 (29.5)	4 (16.0)
Myeloma	69 (16.2)	1 (4.0)
Myelodysplastic syndromes	29 (6.8)	2 (8.0)
Myeloproliferative neoplasms	8 (1.9)	0 (0.0)
Eastern Cooperative Oncology Group score[a]		
0	91 (21.3)	4 (16.0)
1	129 (30.2)	10 (40.0)
2	130 (30.4)	7 (28.0)
3	47 (11.0)	3 (12.0)
4	30 (7.1)	1 (4.0)
Comorbidities		
Diabetes mellitus	129 (30.2)	5 (20.0)
Hypertension	78 (18.3)	3 (12.0)
HIV infection	56 (13.1)	8 (32.0)
Others (heart disease, hypersplenism)	18 (4.2)	0 (0.0)
Sickle cell disease	3 (0.7)	0 (0.0)
None	94 (22.0)	5 (20.0)
Unknown	49 (11.5)	4 (16.0)
Number of transfusion episodes		
<5	215 (50.4)	10 (40.0)
5–10	139 (32.6)	13 (52.0)
>10	73 (17.1)	2 (8.0)
Number of transfused units		
<5	78 (18.3)	9 (36.0)
5–10	172 (40.3)	15 (60.0)
>10	177 (41.5)	1 (4.0)
History of transfusion reactions		
Yes	4 (0.9)	1 (4.0)
No	356 (83.4)	20 (80.0)
Unknown	67 (15.7)	4 (16.0)
Indications for transfusion		
Anaemia	381 (89.2)	21 (84.0)
Bleeding disorders	29 (6.8)	2 (8.0)
Hypovolaemia	9 (2.1)	1 (4.0)
Others (surgery, chemotherapy)	8 (1.8)	1 (4.0)
Type of the transfusion product ($N = 402$)[b]		
Whole blood	165 (41.1)	17 (68.0)
RBC concentrates	237 (58.9)	8 (32.0)
History of pregnancy ($N = 198$; 9[c])		
Yes	132 (66.7)	4 (44.4)
No	66 (33.3)	5 (55.6)
History of abortion ($N = 132$; 7[c])		
Yes	23 (17.4)	1 (14.3)
No	109 (82.6)	6 (85.7)
Parity ($N = 132$; 9[c])		
0–2	38 (28.8)	2 (22.2)
≥3	94 (71.2)	7 (77.8)
History of autoimmune disease		
Yes	1 (0.2)	0 (0.0)
No	189 (44.3)	22 (88.0)
Unknown	237 (55.5)	3 (12.0)
History of splenectomy		
Yes	7 (1.60)	0 (0.0)
No	317 (74.2)	19 (76.0)
Unknown	103 (24.1)	6 (24.0)
Antibiotic use		

(Continues)

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TABLE 1 (Continued)

Variable	Total participants ($N = 427$), *n* (%)	RBC alloimmunized ($N = 25$), *n* (%)
Yes	79 (18.5)	13 (52.0)
No	348 (81.5)	12 (48.0)
Vaccination status[d]		
Yes	141 (33.0)	21 (84.0)
No	219 (51.3)	3 (12.0)
Unknown	67 (15.7)	1 (4.0)

Note: (i) Acute haematological malignancies are the rapidly progressing cancers of the blood and bone marrow, that is, acute lymphoblastic leukaemia and acute myeloid leukaemia. (ii) Chronic haematological malignancies are slowly progressing blood cancers that involve the accumulation of one or more mature or differentiated haematopoietic cells. These were: chronic lymphocytic leukaemia, chronic myeloid leukaemia, multiple myeloma, myelodysplastic syndromes and myeloproliferative neoplasms.

Abbreviations: HIV, human immunodeficiency virus; RBC, red blood cell.

[a]Eastern Cooperative Oncology Group (ECOG) score system for the participant's level of functioning [20].

[b]Type of the transfusion product. Of the 427 participants, 402 received either whole blood or red cell concentrates, while 25 participants received other transfusion products.

[c]Number of women of childbearing age who had a history of the respective variable and had RBC alloantibodies. Specifically, (i) Of the 198 females, 9 had RBC alloantibodies; of whom, 4 had a history of pregnancy. (ii) Of the 132 women of childbearing age, 7 had RBC alloantibodies; of whom, only 1 reported a history of abortion. (iii) Of the 132 women, 9 had RBC alloantibodies, of whom, 7 were multiparous (≥3).

[d]Vaccination status against the routine and other prescribed vaccines, including the coronavirus disease-2019, yellow fever and hepatitis vaccines.

14 (56.0%) were 31–65 years of age, 16 (64.0%) were females and 15 (60.0%) had chronic haematological malignancies (Table 1).

Frequencies of RBC alloantibodies

Of the 25 participants who were RBC alloimmunized, 21 had single alloantibodies of known specificities (Table 2).

Factors associated with RBC alloimmunization

To identify participant characteristics associated with red blood cell alloimmunization, bivariate analyses were first conducted (Table 3). At multivariate level, the variable of chronic cancer (AOR = 2.62, 95% CI: 1.16–7.21, $p = 0.02$), diagnosis of leukaemia (AOR = 2.71, 95% CI: 1.81–4.03, $p = 0.01$), human immunodeficiency virus (HIV) infection (AOR = 4.34, 95% CI: 1.69–5.11, $p = 0.03$), antibiotic use (AOR = 5.08, 95% CI: 2.11–7.41, $p = 0.02$) and ≥5 both transfusion episodes (AOR = 2.51, 95% CI: 2.28–3.81, $p = 0.02$) and donor units (AOR = 4.09, 95% CI: 2.73–6.94, $p = 0.01$) showed a significant statistical association. There was no association with age, gender or obstetric history (Table 4).

DISCUSSION

The prevalence of RBC alloimmunization was 5.9%. The identified RBC alloantibodies were anti-C, anti-c, anti-D, anti-E, anti-K, anti-M, anti-Fy[a], anti-Jk[a] and anti-Le[a]. Four participants had pan-reactive alloantibodies. RBC alloimmunization was associated with chronic cancer, diagnosis of leukaemia, HIV infection, antibiotic use and a history of ≥5 transfusions.

The 5.9% prevalence of RBC alloimmunization lies within the range of 3% to 11% as reported by various studies [6–10]. As well, it is comparable to the 6.25% previously reported in Uganda [9] and 6.5% in Egypt [8]. However, the reported prevalence is lower than 11% and 16% reported among patients diagnosed with leukaemia in Sudan [27, 28]. On the other hand, this study found a higher prevalence than the 4.2% reported in Kenya [29], and notably, it contradicts a previous finding of no RBC alloimmunization in Uganda [19]. The variability across different studies may be attributed, in part, to methodological differences. In this study, the inclusion of participants who had received ≥2 units of blood, with the most recent transfusion being at least 2 weeks before enrolment, may have influenced the observed prevalence of RBC alloimmunization. The 2-week interval could have affected the antibody detection, as some alloantibodies may not have developed or had waned by the time of testing. Additionally, a previous study has shown that the use of standard screening cells can impact antibody detection, as these cells often express antigen profiles representative of specific ethnic populations [30]. Repeated exposure to donor RBC antigen also increases the likelihood of alloantibody formation and may have contributed to the observed prevalence in this study. Consistent with previous studies [9, 31–33], blood transfusion remains a well-established risk factor for RBC alloimmunization, suggesting that our findings are likely the result of blood transfusion. Moreover, HIV infection and antibiotic use were associated with RBC alloimmunization, probably reflecting mechanistic immunological pathways in patients with haematological malignancies. Chronic HIV infection is associated with persistent systemic immune activation, systemic inflammation and dysregulated T- and B-cell function, leading to a hyperresponsive environment for alloantigen recognition and alloantibody formation [34]. On the other hand, antibiotic therapy, while essential for managing infections, exerts immunomodulatory effects on cytokine networks, immune cell activation and inflammatory signalling. In the context of systemic infection, these mechanisms enhance antigen presentation and B-cell activation at transfusion, lowering the threshold for alloantibody formation [35]. Collectively, HIV-induced immune dysregulation and antibiotic-mediated immune modulation likely synergize to amplify the likelihood of RBC alloimmunization.

As found in this study, alloantibodies against Rh, Kell, MNS, Lewis and additional blood group systems identified have been reported in other studies. For instance, anti-S, -K, Le[a], -Rh (D, E) and a combination of anti-E plus anti-K were previously reported in Uganda [9]. Also, anti-E, -D, -K and anti-C were reported as the dominant alloantibodies in sub-Saharan Africa [36]. Relatedly, anti-K, -Kp[a], -Fy[a], -Fy[b], -Jk[a], -Jk[b], -M, -N, -S, -Le[a] and -Rh (C, D, E, c) were reported from Egypt [8].

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TABLE 2 Frequency of different red blood cell alloantibodies.

Alloantibody	Anti-C	Anti-D	Anti-E	Anti-K	Anti-c	Anti-M	Anti-Fy^a	Anti-Jk^a	Anti-Le^a	Pan-reactive
Number ($N = 25$)	2	4	6	4	1	1	1	1	1	4
Percentage	8.0	16.0	24.0	16.0	4.0	4.0	4.0	4.0	4.0	16.0

In Sudan, four alloantibody specificities were reported, with anti-Kell being the most common, followed by anti-Le^a, P and M [28]. In Kenya, anti-K and anti-Le^a were the predominant antibodies reported [29]. These findings conform to studies that have consistently reported Rh and Kell system antibodies as the most prevalent [4, 5, 37]. Noteworthy, the identification of varied RBC alloantibodies highlights the burden and the risks posed by alloimmunization among patients with haematological malignancies who may require frequent transfusions. Anti-D alloantibodies were identified in four participants: two females and two males. Of these, two participants were group O, one was group A and the other was group AB; and all these participants were D-positive. The two female participants had histories of both pregnancy and transfusion. The RBC alloimmunization among these participants likely reflects the presence of D-variant alleles: individuals who are serologically D-positive but possess partial or weak D antigens, predisposing them to anti-D formation when transfused with standard D-positive units [38]. Notably, all four had >5 transfusions, which, in addition, is a recognized risk factor for RBC alloimmunization [33]. Of concern, the pan-reactive alloantibodies were identified in four participants: three males and a female. All of these participants had received >5 transfusions, and none reported a history of either a transfusion reaction or autoimmune disease. The specific cause of pan-reactivity in this study could not be determined; however, the screening cells used may have contributed to this finding. As previously reported, the antigenic profile of screening cells is often selected to represent a particular ethnic population, which may influence antibody detection [30]. The use of non-ethnically matched reagent cells could therefore limit the accurate characterization in our study population. Pan-reactivity may also result from the presence of autoantibodies, single or multiple alloantibodies as well as antibodies targeting high-incidence antigens. This complexity may lead to a masking effect and thus impede the detection of alloantibodies [39].

Some factors previously reported to be of significance did not show any statistical association in this study or appeared to differ from earlier findings. Notably, increasing age was associated with decreased likelihood of alloimmunization, in contrast to prior studies that reported a positive association between age and alloimmunization risk [40, 41]. The inverse association observed in this study may reflect immunosenescence, resulting in reduced antibody formation following transfusion [33]. In contrast, studies reporting higher alloimmunization risk with advancing age have attributed this to cumulative immune sensitization from prior transfusions or pregnancy [40, 41]. Also, the female sex has been reported as a risk of RBC alloimmunization [12, 32, 41]. In this study, most alloimmunized participants were women of childbearing age and multiparous, many of whom had received ≥5 units of donor blood. RBC alloimmunization among females may arise from foetal–maternal blood exposure during pregnancy, as well as transfusions associated with pregnancy-related complications, consistent with prior reports [40, 41]. Additionally, neither a history of pregnancy nor abortion showed a statistical association with RBC alloimmunization. This finding suggests that obstetric sensitizing events may have been obscured by the immunological impact of transfusion burden, underlying disease biology, and other immunomodulatory exposures. This observation is consistent with prior reporting that, while obstetric factors may elicit alloantibody formation, in populations with frequent transfusion exposure the transfusion stimulus may predominate as the primary driver of alloimmunization [42]. Furthermore, multiparity is associated with an increase in the transplacental foetal–maternal leaks, contributing to RBC immune sensitization [43]. The fact that we did not observe significance may be the result of our selection criteria, as participants had received ≥2 transfusions before enrolment. This would hide the particular sex-based effect, as both males and females would have received previous transfusions. Noteworthy, transfusion with whole blood was associated with a 4.71-fold increased odds of RBC alloimmunization compared with RBC concentrates, probably due to its higher antigenic load. Although not statistically significant, this finding is clinically relevant given the frequent transfusion requirements of patients with haematological malignancies. Because of limited hemovigilance in our setting, only four participants reported a history of transfusion reactions. Among them, only one—a male participant who had been transfused with 8 units of blood across three transfusion episodes—was alloimmunized. The reported transfusion reaction had occurred at another facility, and no further details were available.

This study investigated RBC alloantibodies by enrolling a large cohort of participants from two tertiary healthcare facilities in Uganda. However, the findings of this study ought to be interpreted in light of the following: first, the inclusion criteria involved participants likely to have been alloimmunized. Despite this, the study design was suboptimal, as antibodies may not have developed or may have waned at the time of testing. Also, because of logistical challenges in obtaining auto-control sera, the study did not investigate autoimmunity. However, three participants showed positive immediate-spin screen results, which were concluded as cold autoantibodies on prewarming. Further, the study utilized a double ratio of the participants' plasma during both the screening and antibody identification stages, an approach that resulted in a volume deficit and limited further testing of pan-reactive cases. Thus, we could not conclude whether the pan-reactive cases were true RBC alloimmunization. Also, the genotyping profiles of participants with anti-D alloantibodies were not determined. Moreover, the self-reported participant characteristics were dependent on the respondents' recall and providing accurate information. Although vaccination status showed a statistical association in the bivariate analysis, it was excluded from the multivariate model because of residual confounding, having small numbers, and a lack of biological plausibility.

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TABLE 3 Bivariate analysis of factors associated with red blood cell alloimmunization.

Variable	Alloimmunized, $n = 25$	Non-alloimmunized, $n = 402$	Percentage alloimmunized	Crude odds ratio (95% CI)	*p*-Value
Age category (years)					
<5 (reference)	1	22	4.3	1	
5–12	1	14	6.7	1.57 (0.09–27.2)	0.76
13–19	2	29	6.5	1.52 (0.13–17.8)	0.74
20–30	4	73	5.2	1.21 (0.13–11.4)	0.87
31–65	14	225	5.9	1.37 (0.18–10.9)	0.77
>65	3	39	7.7	1.69 (0.17–17.3)	0.66
Sex					
Male (reference)	9	220	3.9	1	
Female	16	182	8.1	2.15 (0.93–4.98)	0.07
ABO blood group					
O (reference)	14	203	6.5	1	
A	6	107	5.3	0.81 (0.30–2.18)	0.68
B	4	74	5.1	0.78 (0.25–2.46)	0.68
AB	1	18	5.6	0.81 (0.10–6.48)	0.84
Status of the RhD blood group					
Positive (reference)	24	394	5.7	1	
Negative	1	8	11.1	2.05 (0.25–17.1)	0.51
Cancer status					
Acute (reference)	10	299	3.2	1	
Chronic	15	103	12.7	4.35 (1.90–10.0)	0.0005
Histological diagnosis					
Lymphoma (reference)	4	122	3.2	1	
Leukaemia	18	177	9.2	3.10 (1.02–9.39)	0.045
Myeloma	1	68	1.4	0.45 (0.05–4.09)	0.48
Myelodysplastic syndromes	2	27	6.9	2.26 (0.39–13.0)	0.36
Myeloproliferative neoplasms	0	8	0.0	-	
Eastern Cooperative Oncology Group score					
0 (reference)	4	87	4.4	1	
1	10	119	7.8	1.83 (0.55–6.02)	0.32
2	7	123	5.4	1.24 (0.35–4.36)	0.74
3	3	44	6.4	1.48 (0.35–4.36)	0.62
4	1	29	3.3	0.75 (0.08–6.98)	0.80
History of sickle cell disease					
No (reference)	25	399			
Yes	0	3	0.0	-	-
History of autoimmune disease					
No (reference)	25	401			
Yes	0	1	0.0	-	-
History of diabetes mellitus ($N = 134$)					
No (reference)	20	278	6.7	1	
Yes	5	124	3.9	0.56 (0.21–1.53)	0.26
History of hypertension					
No (reference)	22	327	6.3	1	
Yes	3	75	3.8	0.59 (0.17–2.04)	0.41
History of HIV infection					
No (reference)	17	354	4.5	1	

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TABLE 3 (Continued)

Variable	Alloimmunized, $n = 25$	Non-alloimmunized, $n = 402$	Percentage alloimmunized	Crude odds ratio (95% CI)	p-Value
Yes	8	48	14.3	3.51 (1.44–8.57)	0.0058
Number of transfusion episodes					
<5 (reference)	10	205	4.7	1	
5–10	13	126	9.4	2.12 (0.90–4.97)	0.086
>10	2	71	2.7	0.58 (0.12–2.70)	0.49
Number of transfused units					
<5 (reference)	9	69	11.5	1	
5–10	15	157	8.7	0.73 (0.31–1.75)	0.48
>10	1	176	0.6	0.04 (0.005–0.35)	0.0032
History of transfusion reaction					
No (reference)	20	336	5.6	1	
Yes	1	3	25.0	5.60 (0.56–56.3)	0.14
Unknown	4	63	6.0	1.07 (0.35–3.23)	0.91
Indications for transfusion					
Anaemia (reference)	21	360	5.5	1	
Bleeding disorders	2	27	6.9	1.27 (0.28–5.70)	0.76
Hypovolaemia	1	8	11.1	2.14 (0.26–17.9)	0.48
Others (surgery, chemotherapy)	1	7	12.5	2.45 (0.29–20.8)	0.41
Type of the transfusion product ($N = 402$)[a]					
RBC concentrates (reference)	8	229	3.4	1	
Whole blood	17	148	10.3	3.28 (1.38–7.81)	0.0070
History of pregnancy ($N = 198$; 9[b])					
No (reference)	5	61	7.6	1	
Yes	4	128	3.0	0.38 (0.10–1.47)	0.16
History of abortion ($N = 132$; 7[b])					
No (reference)	6	103	5.5	1	
Yes	1	22	4.3	0.78 (0.09–6.81)	0.82
Parity ($N = 132$; 9[b])					
0–2 (reference)	2	36	5.3	1	
≥3	7	87	7.4	1.45 (0.29–7.31)	0.65
History of splenectomy					
No (reference)	25	395			
Yes	0	7	0.0	-	-
Antibiotic use					
No (reference)	12	336	3.4	1	
Yes	13	66	16.5	5.51 (2.41–12.6)	0.001
Vaccination status[c]					
No (reference)	3	216	1.4	1	
Unknown	1	66	1.5	1.07 (0.11–10.5)	0.96
Yes	21	120	14.9	12.6 (3.68–43.1)	0.0001

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; RBC, red blood cell.

[a]A total of 402 units of red cell concentrates and whole blood were transfused, of which 377 units were transfused to the non-alloimmunized participants.

[b]The number of non-alloimmunized participants varied across obstetric variables, with 189 (history of pregnancy), 125 (history of abortion) and 123 (parity).

[c]For myeloproliferative neoplasms ($n = 8$), sickle cell disease ($n = 3$), autoimmune disease ($n = 1$) and splenectomy ($n = 7$), bivariate analyses (crude odds ratio, 95% CI and p-value) were not calculated because of sparse data limiting inference.

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TABLE 4 Multivariate analysis of factors associated with red blood cell alloimmunization.

Variables	Adjusted odd ratio (95% CI)	*p*-Value
Age category (years)		
<5 (reference)		
5–12	1.48 (0.08–14.17)	0.61
13–19	2.19 (0.07–7.12)	0.69
20–30	1.68 (0.59–5.37)	0.78
31–65	0.81 (0.19–4.06)	0.47
>65	0.95 (0.11–8.41)	0.39
Sex		
Male (reference)		
Female	1.89 (0.71–2.18)	0.19
Cancer status		
Acute (reference)		
Chronic	2.62 (1.16–7.21)	0.02[a]
Histological diagnosis		
Lymphoma (reference)		
Leukaemia	2.71 (1.81–4.03)	0.01[a]
Myeloma	0.71 (0.32–1.09)	0.41
Myelodysplastic syndromes	1.16 (1.03–2.78)	0.33
Myeloproliferative neoplasms	-	-
No history of HIV infection		
History of HIV infection	4.34 (1.69–5.11)	0.03[a]
No history of transfusion reaction (reference)		
History of transfusion reaction	0.36 (0.38–1.94)	0.13
<5 transfusion episodes (reference)		
≥5 transfusion episodes	2.51 (2.28–3.81)	0.02[a]
<5 transfused units (reference)		
≥5 transfused units	4.09 (2.73–6.94)	0.01[a]
Type of transfusion product		
RBC concentrates (reference)		
Whole blood	4.71 (0.98–7.01)	0.19
No history of pregnancy (reference)		
History of pregnancy	0.88 (0.31–1.54)	0.10
Parity <3 (reference)		
Parity ≥3	0.60 (0.18–2.08)	0.26
No history of abortion (reference)		
History of abortion	1.49 (0.81–5.78)	0.08
Antibiotic use		
No (reference)		
Yes	5.08 (2.11–7.41)	0.02[a]

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus.
[a]Variables that showed a statistical association ($p < 0.05$).

In conclusion, this study found a 5.9% prevalence of RBC alloimmunization among transfused patients with haematological malignancy. Chronic cancer, the diagnosis of leukaemia, HIV infection, antibiotic use and previous transfusions were significantly associated with RBC alloimmunization. RBC alloantibodies belonging to the Rh, Kell, MNS, Duffy, Kidd and Lewis blood group systems were identified. The detection of clinically significant RBC alloantibodies indicates the likely adverse transfusion events, including transfusion reactions and challenges in future transfusions. As previously recommended [9, 16], these findings highlight the need for improved pre-transfusion testing. This study adds to the scarce data on RBC alloimmunization among patients with haematological malignancies and reinforces the need for further research.

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I.M.T., N.N., S.A., E.K., D.T., B.O., G.N., M.Y.C., C.N.J., G.v.M. and B.N. conceived the study idea, participated in study design as well as data acquisition, analysis and interpretation and manuscript drafting and revision. N.N., E.K., G.N., M.Y.C., C.N.J., G.v.M. and B.N. actively participated in the research design, selection of methods and writing of the manuscript. N.N., S.A., E.K., G.N., D.T., M.Y.C., C.N.J., G.v.M. and B.N. critically reviewed the manuscript. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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