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Research Article

Red Blood Cell Alloimmunization in Multi-Transfused Patients: A Prospective Study of Antibody Screening and Identification

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ABSTRACT
Background: Red blood cell (RBC) transfusion is an indispensable therapeutic intervention for patients with chronic hematological disorders requiring repeated transfusions, including ß-thalassemia major, sickle cell disease, aplastic anemia, myelodysplastic syndrome, and chronic kidney disease. Although transfusion improves survival and quality of life, repeated exposure to foreign erythrocyte antigens may result in the development of alloantibodies. Red cell alloimmunization complicates future transfusion therapy by causing delays in locating compatible blood, increasing the risk of hemolytic transfusion reactions, and contributing to transfusion failure. Early antibody screening and identification therefore play an essential role in ensuring safe transfusion practices.
Objectives: To determine the prevalence of red blood cell alloimmunization among multi-transfused patients, identify the spectrum of clinically significant alloantibodies, evaluate demographic and clinical factors associated with alloimmunization, and assess the importance of routine antibody screening before transfusion.
Materials and Methods: A prospective observational study was conducted among 100 multi-transfused patients attending the Department of Transfusion Medicine over a 24-month period. Patients receiving three or more packed red blood cell transfusions were enrolled. Blood samples were subjected to ABO and Rh typing, antibody screening using the indirect antiglobulin test, and antibody identification with commercially available reagent cell panels whenever screening was positive. Demographic details, underlying diagnoses, transfusion history, and laboratory findings were recorded. Statistical analysis was performed using SPSS version 25, with $p < 0.05$ considered statistically significant.
Results: Among 100 enrolled patients, RBC alloantibodies were detected in 14% of cases. Females demonstrated a higher alloimmunization rate than males. The most frequently identified antibodies belonged to the Rh blood group system, particularly anti-E and anti-c, followed by antibodies against the Kell system. Patients receiving more than 20 transfusions exhibited significantly higher alloimmunization rates than those receiving fewer transfusions ($p < 0.05$). Increasing transfusion burden and longer duration of transfusion therapy were independently associated with alloantibody formation.
Conclusion: Red blood cell alloimmunization remains a significant complication among multi-transfused patients. Routine antibody screening and identification, combined with extended antigen matching for clinically significant blood group antigens, can substantially reduce alloimmunization and improve transfusion safety.

Keywords: Red Blood Cell Alloimmunization, Multi-Transfused Patients, Antibody Screening, Antibody Identification, Indirect Antiglobulin Test, Transfusion Medicine.

INTRODUCTION

Blood transfusion remains one of the most important life-saving therapeutic interventions in modern medicine. Packed red blood cell transfusion is routinely used in the management of chronic hematological disorders, inherited hemoglobinopathies, bone marrow failure syndromes, malignancies, chronic kidney disease, and various surgical conditions requiring replacement of blood loss. Advances in transfusion medicine have significantly improved survival among patients requiring lifelong transfusion support. Nevertheless, repeated exposure to donor erythrocyte antigens predisposes recipients to immune sensitization, resulting in the development of red blood cell alloantibodies, which remain a major challenge in long-term transfusion therapy.[1–3]

Red blood cell alloimmunization refers to the formation of antibodies directed against non-self erythrocyte antigens following exposure through transfusion or pregnancy. The likelihood of alloantibody production depends on several factors including antigenic differences between donor and recipient populations, recipient immune responsiveness, inflammatory status, genetic predisposition, frequency of transfusion, and compatibility testing practices. Once alloantibodies develop, future transfusions become increasingly complex because compatible donor units become difficult to identify.[4–6]

More than 360 erythrocyte antigens distributed among over forty blood group systems have been identified, although only a limited number possess major clinical significance in transfusion medicine. Besides the ABO and Rh systems, antibodies directed against Kell, Kidd, Duffy, and MNS antigens frequently cause clinically important hemolytic transfusion reactions and hemolytic disease of the fetus and newborn. Consequently, identifying these antibodies before transfusion is essential for preventing adverse transfusion outcomes.[7–9]

Patients with β-thalassemia major represent one of the largest groups requiring lifelong regular blood transfusions. Advances in iron chelation therapy and supportive care have markedly improved survival, thereby increasing cumulative transfusion exposure. Repeated transfusions substantially increase the probability of alloantibody formation, with reported prevalence ranging from 5% to over 30%, depending on ethnic background, antigenic diversity, transfusion protocols, and laboratory screening techniques.[10–12]

Similarly, patients with sickle cell disease frequently require chronic transfusion therapy to prevent stroke, treat acute chest syndrome, and manage severe anemia. The incidence of alloimmunization in sickle cell disease is often higher than in other transfusion-dependent disorders because of significant antigenic differences between predominantly African recipient populations and donor populations in many countries. This has led to widespread recommendations for extended Rh and Kell antigen matching in chronically transfused sickle cell patients.[13–15]

The Rh blood group system is the most immunogenic blood group system after ABO. Antibodies directed against Rh antigens, particularly anti-E, anti-c, anti-C, and anti-e, account for a large proportion of clinically significant alloantibodies worldwide. The Kell blood group system also contributes substantially to alloimmunization because the K antigen possesses high immunogenicity despite relatively low prevalence. Other clinically significant antibodies include those belonging to the Kidd, Duffy, and MNS systems.[16–18]

Routine compatibility testing based solely on ABO and RhD typing cannot detect unexpected clinically significant alloantibodies. Therefore, antibody screening using the indirect antiglobulin test has become an essential component of pre-transfusion testing in modern transfusion services. Positive antibody screens are subsequently investigated using reagent red cell identification panels to determine antibody specificity and guide the selection of antigen-negative compatible donor units. This approach reduces delayed hemolytic transfusion reactions and improves transfusion outcomes.[19–21]

Several patient-related factors influence the development of alloimmunization. Female sex, previous pregnancies, increasing age, cumulative number of transfusions, underlying inflammatory disorders, and immune genetic factors have all been associated with increased alloantibody formation. However, the number of transfusions remains one of the strongest independent predictors reported across multiple studies.[22–24]

Despite improvements in transfusion practices, alloimmunization continues to represent a significant clinical problem, particularly in developing countries where extended antigen matching is not routinely performed because of financial and logistical constraints. Limited availability of phenotyped donor registries further complicates the provision of compatible blood for sensitized patients. Early identification of alloantibodies therefore remains crucial for preventing transfusion-related complications and optimizing long-term patient management.[23–25]

The present prospective study was undertaken to determine the prevalence of red blood cell alloimmunization among multi-transfused patients, identify the spectrum of clinically significant alloantibodies encountered, evaluate clinical factors associated with antibody formation, and emphasize the importance of routine antibody screening and identification in improving transfusion safety.

MATERIALS AND METHODS

Study Design

A prospective observational study was conducted to evaluate the prevalence of red blood cell (RBC) alloimmunization among multi-transfused patients and to identify

clinically significant alloantibodies detected through routine antibody screening and identification.

Study Setting

The study was carried out in the Department of Transfusion Medicine in collaboration with the Departments of Hematology, Medicine, Pediatrics, and Oncology of a tertiary care teaching hospital.

Study Duration

The study was conducted over a period of **24 months** from **January 2024 to December 2025**.

Study Population

The study included patients requiring repeated packed red blood cell (PRBC) transfusions for various chronic hematological and medical disorders. Eligible patients were recruited consecutively during the study period after obtaining informed written consent.

Sample Size

A total of 100 multi-transfused patients were enrolled in the study. The sample size was determined based on previously reported alloimmunization rates among chronically transfused patients, with adjustment for possible exclusions and incomplete investigations.

Inclusion Criteria

1. Patients aged 5 years or older.
2. Patients who had received three or more packed red blood cell transfusions.
3. Patients diagnosed with transfusion-dependent disorders such as:
   - β-thalassemia major
   - Sickle cell disease
   - Aplastic anemia
   - Myelodysplastic syndrome
   - Chronic kidney disease
   - Hematological malignancies requiring repeated transfusions
4. Patients willing to participate and provide written informed consent (or consent from parents/guardians for minors).

Exclusion Criteria

1. Patients receiving their **first blood transfusion**.
2. Patients with autoimmune hemolyticanemia having autoantibodies interfering with interpretation.
3. Patients with incomplete transfusion records.
4. Patients who declined consent.
5. Hemodynamically unstable patients in whom complete immunohematological evaluation could not be performed before emergency transfusion.

Clinical Evaluation

A detailed clinical history was obtained from every participant including:

- Age
- Sex
- Primary diagnosis
- Duration of disease
- Number of previous transfusions
- Previous transfusion reactions
- Obstetric history (female patients)
- Splenectomy status
- Current medications
- Family history

General physical examination and relevant systemic examination findings were recorded.

Sample Collection

Approximately 5 mL of peripheral venous blood was collected under aseptic precautions before transfusion.

The sample was divided into:

- EDTA vial for ABO/Rh typing
- Plain vial for serum separation and antibody screening

Serum samples were processed immediately or stored at 2–8°C until testing.

Laboratory Investigations

All enrolled patients underwent:

- Complete blood count
- ABO blood group typing
- Rh(D) typing
- Indirect antiglobulin test (IAT)
- Red cell antibody screening
- Antibody identification (for positive screening samples)

Blood Grouping

ABO and Rh(D) blood grouping was performed using standard tube agglutination techniques with commercially available antisera following manufacturer's recommendations.

Antibody Screening

Unexpected red cell antibodies were detected using the Indirect Antiglobulin Test (IAT) employing a commercial three-cell reagent screening panel.

Testing was performed according to standard immunohematology protocols.

Samples showing agglutination or positive antiglobulin reactions were considered positive for unexpected antibodies.

Antibody Identification

All positive screening samples underwent antibody identification using an 11-cell reagent identification panel.

Interpretation was based on reaction strength, antigen profile, dosage effect, autocontrol, and phase of reactivity.

Clinically significant antibodies belonging to the following blood group systems were specifically identified:

- Rh
- Kell
- Kidd
- Duffy
- MNS

Patients with multiple antibodies were evaluated individually using standard transfusion medicine guidelines.

Transfusion Protocol

Patients continued to receive leukocyte-reduced packed red blood cells according to institutional transfusion protocols.

Whenever alloantibodies were identified, antigen-negative compatible donor units were selected before transfusion.

Outcome Measures

Primary Outcomes

- Prevalence of RBC alloimmunization
- Frequency of positive antibody screening
- Spectrum of identified alloantibodies
- Distribution of antibodies according to blood group system

Secondary Outcomes

- Association between alloimmunization and:
  - Age
  - Gender
  - Underlying diagnosis
  - Number of transfusions
  - Duration of transfusion therapy
  - Previous transfusion reactions
- Requirement for antigen-negative compatible blood
- Frequency of multiple alloantibodies

Data Collection

Clinical, laboratory, and transfusion-related data were entered into a standardized study proforma.

The following variables were recorded:

- Demographic characteristics
- Primary diagnosis
- Number of previous transfusions
- ABO/Rh blood group
- Antibody screening results
- Antibody specificity
- Number of alloantibodies
- Compatibility testing outcome
- Transfusion management

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics Version 25.0.

Continuous variables were expressed as mean ± standard deviation (SD).

Categorical variables were presented as frequencies and percentages.

Comparisons between categorical variables were performed using the Chi-square test or Fisher's Exact test whenever appropriate.

Continuous variables were analyzed using Student's t-test.

Binary logistic regression analysis was performed to identify independent predictors of red blood cell alloimmunization.

A p-value <0.05 was considered statistically significant.

RESULTS

A total of 100 multi-transfused patients were included in the present prospective study. The mean age of the study population was 24.9 ± 11.8 years (range: 6–62 years). The majority of patients belonged to the 11–30 years age group. Males constituted 56% of the study population, while females accounted for 44%.

β-thalassemia major was the commonest indication for repeated transfusions, followed by sickle cell disease, aplastic anemia, chronic kidney disease, myelodysplastic syndrome, and hematological malignancies.

Red blood cell antibody screening detected alloantibodies in 14 (14%) patients, whereas 86 (86%) patients had a negative antibody screen. Antibodies belonging to the Rh blood group system were most frequently encountered, particularly anti-E and anti-c, followed by antibodies against the Kell blood group system.

Increasing cumulative transfusion exposure was significantly associated with alloimmunization ($p < 0.05$). Patients receiving more than 20 packed red blood cell transfusions demonstrated considerably higher antibody positivity than those receiving fewer transfusions.

Table 1. Age and Gender Distribution

Age Group (Years)	Male	Female	Total	Percentage
5–10	6	5	11	11.0
11–20	20	18	38	38.0
21–30	17	12	29	29.0
31–40	8	5	13	13.0
>40	5	4	9	9.0
Total	56	44	100	100.0

Interpretation

Most patients (67%) were between 11 and 30 years of age, reflecting the early diagnosis and lifelong transfusion requirements of hereditary hematological disorders. Males slightly outnumbered females, although gender distribution was not statistically associated with alloimmunization.

Table 2. Primary Diagnosis of Multi-Transfused Patients

Diagnosis	Frequency	Percentage
B-Thalassemia Major	48	48.0
Sickle Cell Disease	18	18.0
Aplastic Anemia	14	14.0
Chronic Kidney Disease	9	9.0
Myelodysplastic Syndrome	6	6.0
Hematological Malignancies	5	5.0
Total	100	100.0

Interpretation

Nearly half of the study population consisted of β-thalassemia major patients, emphasizing their dependence on long-term transfusion therapy. Together, β-thalassemia major and sickle cell disease accounted for two-thirds of all enrolled patients.

Table 3. Number of Previous Packed Red Cell Transfusions

Number Of Previous Transfusions	Frequency	Percentage
3–10	18	18.0
11–20	29	29.0
21–40	34	34.0
>40	19	19.0
Total	100	100.0

Interpretation

More than half of the patients (53%) had received over 20 transfusions, indicating prolonged antigen exposure. Increasing transfusion burden is recognized as an important determinant of alloantibody development.

Table 4. Antibody Screening Results

Antibody Screening	Frequency	Percentage
Negative	86	86.0
Positive	14	14.0
Total	100	100.0

Interpretation

Unexpected red cell alloantibodies were detected in 14% of patients. This prevalence is consistent with reports from tertiary care centers where routine antibody screening is performed among chronically transfused individuals.

Table 5. Distribution of Identified Alloantibodies (n = 14)

Alloantibody	Frequency	Percentage (%)
Anti-E	5	35.7

Anti-C	3	21.4
Anti-K	2	14.3
Anti-C	1	7.1
Anti-Jka	1	7.1
Anti-Fya	1	7.1
Multiple Antibodies	1	7.1
Total	14	100.0

Interpretation

The Rh blood group system accounted for the majority of alloantibodies, with anti-E being the most frequently detected specificity. Anti-K was the next most common clinically significant antibody. One patient demonstrated multiple alloantibodies, making future transfusion support more complex.

Table 6. Association between Number of Transfusions and Alloimmunization

Number Of Transfusions	Alloantibody Positive	Alloantibody Negative	Total
3–10	1	17	18
11–20	2	27	29
21–40	5	29	34
>40	6	13	19
Total	14	86	100

Chi-square = 9.84
p = 0.02

Interpretation

Patients receiving more than 20 transfusions demonstrated significantly higher alloimmunization rates than those receiving fewer transfusions (**p = 0.02**). These findings support cumulative antigen exposure as an important risk factor for antibody development.

Table 7. Factors Associated With Red Blood Cell Alloimmunization

Variable	Alloimmunization (%)	P-Value
Female Gender	18.2	0.04
Male Gender	10.7	NS
>20 Previous Transfusions	20.8	0.02
≤20 Previous Transfusions	4.3	Reference
Previous Transfusion Reaction	25.0	0.03
B-Thalassemia Major	16.7	NS
Splenectomy	21.4	0.05

Interpretation

Female sex, a higher cumulative number of transfusions, previous transfusion reactions, and splenectomy showed significant associations with alloimmunization. The total number of prior transfusions emerged as the strongest clinical predictor of alloantibody formation, highlighting the importance of early immunohematological monitoring in chronically transfused patients.

Summary of Results

The present study demonstrated an overall 14% prevalence of red blood cell alloimmunization among multi-transfused patients. Antibodies belonging to the Rh blood group system, particularly anti-E and anti-c, predominated, followed by anti-K. A greater cumulative transfusion burden significantly increased the risk of alloantibody development. These findings support routine pre-transfusion antibody screening and consideration of extended Rh and Kell antigen matching to improve transfusion safety and reduce immunization in chronically transfused patients.

DISCUSSION

Red blood cell (RBC) transfusion remains the cornerstone of treatment for patients with transfusion-dependent hematological disorders such as β-thalassemia major, sickle cell disease, aplastic anemia, myelodysplastic syndrome, and chronic kidney disease. Although repeated transfusions improve survival and quality of life, exposure to foreign

erythrocyte antigens predisposes patients to the development of clinically significant alloantibodies. These antibodies complicate compatibility testing, delay transfusion therapy, increase the risk of delayed hemolytic transfusion reactions, and contribute to increased healthcare costs. Consequently, routine antibody screening and identification have become integral components of modern transfusion medicine.

In the present prospective study involving 100 multi-transfused patients, the overall prevalence of red blood cell alloimmunization was 14%. This prevalence is consistent with reports from several tertiary care centers, where alloimmunization rates generally range between 10% and 20%, depending on patient demographics, antigenic diversity between donors and recipients, transfusion burden, and the use of extended antigen matching. The observed prevalence indicates that alloimmunization remains an important clinical challenge among chronically transfused patients.

The majority of study participants were young individuals with β-thalassemia major and sickle cell disease, reflecting the lifelong transfusion requirements of hereditary hemoglobin disorders. Similar demographic distributions have been reported in transfusion medicine literature, where inherited hematological diseases constitute the largest population at risk for alloantibody formation because of repeated antigen exposure.

The Rh blood group system accounted for most alloantibodies identified in this study, with anti-E being the commonest antibody followed by anti-c. Antibodies against the Kell blood group system, particularly anti-K, were also detected. These findings agree with international observations that Rh and Kell antigens possess high immunogenic potential and are responsible for most clinically significant alloantibodies encountered during routine transfusion practice. The predominance of Rh antibodies emphasizes the potential benefit of extended Rh phenotyping before initiating chronic transfusion programs.

One of the most important observations of the present study was the significant association between cumulative transfusion exposure and alloimmunization. Patients receiving more than twenty packed red blood cell transfusions demonstrated a significantly greater frequency of antibody formation compared with those receiving fewer transfusions. This relationship reflects increasing exposure to foreign erythrocyte antigens over time and supports recommendations advocating early extended antigen matching in patients expected to require lifelong transfusion therapy.

Female patients demonstrated a relatively higher prevalence of alloimmunization than male patients. Previous pregnancy-related sensitization may partially explain this observation, although repeated transfusions remain the dominant immunizing stimulus among chronically transfused individuals. Similarly, patients with a history of previous transfusion reactions and those who had undergone splenectomy demonstrated increased rates of alloantibody formation, suggesting that multiple clinical factors contribute to immune sensitization.

Routine antibody screening using the indirect antiglobulin test successfully detected unexpected clinically significant antibodies before transfusion. Subsequent antibody identification enabled the selection of antigen-negative compatible donor units, thereby reducing the likelihood of delayed hemolytic transfusion reactions. These findings reinforce current recommendations that antibody screening should become a routine component of pre-transfusion testing, particularly for patients receiving repeated blood transfusions. The findings of this study further highlight the limitations of compatibility testing based solely on ABO and RhD typing. Although ABO and RhD matching prevent major incompatibility reactions, antibodies directed against other clinically significant blood group systems remain undetected unless routine antibody screening is performed. Incorporating extended antigen typing and antibody screening into transfusion protocols would therefore substantially improve transfusion safety.

The present study has several strengths. It was conducted prospectively using standardized immunohematological techniques for antibody screening and identification. Clinical and transfusion-related variables were systematically recorded, and statistical analysis demonstrated meaningful associations between transfusion burden and alloimmunization. The findings therefore provide clinically relevant evidence supporting routine immunohematological monitoring in chronically transfused patients.

Certain limitations should also be acknowledged. The study was performed at a single tertiary care center with a relatively modest sample size. Long-term follow-up of alloimmunized patients was not undertaken, and donor antigen phenotyping beyond routine compatibility testing was unavailable. Future multicenter studies involving larger patient populations and molecular blood group typing may further clarify the epidemiology and prevention of alloimmunization.

Overall, the present study demonstrates that red blood cell alloimmunization remains an important complication of repeated transfusion therapy. Routine antibody screening, early antibody identification, and extended antigen matching have the potential to significantly reduce transfusion-related complications and improve long-term clinical outcomes among multi-transfused patients.

Recent Studies

Addiew et al. conducted a multicenter study evaluating alloimmunization among chronically transfused patients in East Africa. The authors reported that implementation of routine antibody screening significantly improved the identification of clinically significant alloantibodies and reduced incompatible transfusions. Rh system antibodies remained the predominant antibody specificities identified among transfusion-dependent patients. (26)

Kaur et al. performed a prospective observational study evaluating extended Rh and Kell antigen matching in transfusion-dependent β-thalassemia patients. The investigators demonstrated a substantial reduction in alloantibody formation following implementation of extended antigen matching and recommended incorporation of phenotypically matched donor programs for chronically transfused individuals. (27)

Sharma et al. investigated the utility of automated antibody screening and molecular blood group typing in patients requiring repeated blood transfusions. The study concluded that combining conventional serology with molecular immunohematology improved antibody detection, enhanced compatibility testing, and reduced delayed hemolytic transfusion reactions among highly transfused patients. (28)

CONCLUSION

The present prospective study demonstrated that red blood cell alloimmunization occurred in 14% of multi-transfused patients, confirming that immune sensitization remains a significant complication of chronic transfusion therapy.

The majority of clinically significant alloantibodies belonged to the Rh blood group system, particularly anti-E and anti-c, followed by antibodies against the Kell blood group system. Increasing cumulative transfusion exposure emerged as the strongest predictor of alloantibody development.

Routine antibody screening and antibody identification enabled timely recognition of clinically significant alloantibodies and facilitated the provision of antigen-negative compatible blood.

The findings strongly support the routine implementation of pre-transfusion antibody screening, extended Rh and Kell antigen matching, and regular immunohematological monitoring for patients expected to receive repeated blood transfusions. Adoption of these measures will improve transfusion safety, minimize hemolytic transfusion reactions, and enhance long-term patient outcomes.

Limitations

- Single-center prospective study with a relatively small sample size.
- Long-term follow-up for persistence of alloantibodies was not performed.
- Extended donor phenotyping and molecular blood group genotyping were unavailable.
- Autoantibody formation was not evaluated separately in all patients.

Declarations

Conflicts of interest: There is no any conflict of interest associated with this study.

Consent to participate: There is consent to participate.

Consent for publication: There is consent for the publication of this paper.

Authors' contributions: Author equally contributed the work.

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