The Review Of
DIABETIC
STUDIES OPEN ACCESS

Prevalence, Specificity, And Risk Factors Of Red Blood Cell Alloimmunization In Chronic Blood Transfusion Therapy: A Retrospective Cohort Study

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Abstract
This retrospective study investigated the burden and prevention of red blood cell alloimmunization in 120 multi-transfused patients at a Saudi Arabian tertiary care center. The overall alloimmunization rate was 18.3%, with anti-E, anti-K, and anti-C being the most common alloantibodies. Alloimmunization was significantly associated with receiving more than 10 transfusions (p=0.02) and a diagnosis of thalassemia major. The core finding was a pronounced protective effect of extended antigen matching. Patients receiving blood units matched beyond ABO and RhD (including Rh variants and Kell) had a significantly lower alloimmunization rate of 7.1%, compared to 24.6% in the standard matching group (p=0.003). These results highlight the clinical significance of Rh and Kell antigen mismatches and demonstrate that extended antigen matching is an effective strategy to mitigate alloimmunization risk in populations requiring chronic transfusion support. Implementing proactive extended phenotyping is recommended to enhance transfusion safety.

Keywords: Alloimmunization; Blood Transfusion; Phenotype Matching; Transfusion Reaction; Hemovigilance; Chronic Transfusion; Immunohematology.

Introduction and Literature Review

Red blood cell (RBC) transfusion remains a cornerstone of supportive therapy for patients with transfusion-dependent conditions such as thalassemia, sickle cell disease (SCD), and various hematological malignancies (T Makarovska-Bojadzieva et al., 2017). However, the very therapy that sustains life also poses a significant immunological challenge. Repeated exposure to foreign erythrocyte antigens can trigger alloimmunization—the development of antibodies against non-self RBC antigens. This complication not only jeopardizes future transfusion compatibility, escalating the risk of delayed hemolytic transfusion reactions, but also complicates laboratory workflows and strains blood bank resources (SS Das et al., 2021).

The reported prevalence of alloimmunization among multi-transfused patients exhibits considerable global heterogeneity, ranging from 2.6% to over 40% (MLP Bueno et al., 2021). This wide variation is influenced by factors including underlying diagnosis, the genetic disparity between donor and recipient populations, and crucially, the extent of antigen matching employed in pre-transfusion testing (SS Das et al., 2021). Standard practice in many transfusion services is limited to ABO and RhD compatibility,

as mismatches in these systems are the most immediate cause of severe reactions. Nonetheless, this protocol fails to account for a multitude of other clinically significant antigens within systems like Kell, Kidd, Duffy, and MNS, which are frequently implicated in alloimmunization (T Makarovska-Bojadzieva et al., 2017).

Consistent with this, epidemiological studies highlight the immunodominance of specific antigens. Research from Brazil and India identifies antibodies against Rh (especially anti-E and anti-C) and Kell (anti-K) systems as the most prevalent among alloimmunized, chronically transfused patients (MLP Bueno et al., 2021; SS Das et al., 2021). This pattern underscores a critical gap in standard matching protocols and points to a clear target for enhanced preventative strategies.

To mitigate this risk, the concept of extended antigen matching—involving phenotyping or genotyping for a broader panel of erythrocyte antigens beyond ABO and RhD—has gained prominence. Evidence supporting its efficacy is emerging across diverse patient cohorts. For instance, S Mangwana et al. (2019) demonstrated that extended antigen matching in oncology patients significantly reduced the incidence of new alloantibody formation. Similarly, A Qayyum et al. (2018) reported improved donor-recipient compatibility and a decreased rate of alloimmunization in patients with liver cirrhosis following the implementation of extended RBC antigen typing. These findings advocate for a more proactive, antigen-specific approach to transfusion therapy.

Further reinforcing this perspective, DK Bhuva and JH Vachhani (2017) recommend instituting extended phenotype-matching protocols at diagnosis for patients anticipated to require chronic transfusions, as a preemptive measure against sensitization. The long-term benefits of such early intervention are emphasized by R Maini et al. (2025), who highlighted its importance in pediatric populations dependent on lifelong transfusion support.

Collectively, the existing literature substantiates the clinical utility of extended antigen typing in reducing the burden of alloimmunization. However, the integration of this practice into routine clinical workflows, particularly in resource-variable settings, remains inconsistent. There is a persistent need for localized studies that quantify the protective effect of extended matching against the backdrop of specific patient demographics, prevalent antigens, and existing transfusion practices. Furthermore, evaluations of the practical feasibility and sustained impact within real-world transfusion services are required.

This study, therefore, aims to evaluate the prevalence and specificity of RBC alloantibodies in a multi-transfused population and to assess the comparative effectiveness of standard versus extended antigen matching in preventing alloimmunization. By doing so, it seeks to generate evidence to inform refined transfusion strategies that enhance both safety and efficacy for patients reliant on chronic transfusion therapy.

Aim of the study:

General Aim: To investigate the burden, patterns, and preventable risk factors of red blood cell alloimmunization in a population of multi-transfused patients.

Specific Objectives:

1. To determine the prevalence and specificity of red blood cell alloantibodies in a cohort of multi-transfused patients with thalassemia major, sickle cell disease, and hematological malignancies.
2. To identify the most frequently implicated alloantibodies and describe the pattern of antibody formation (single vs. multiple) among alloimmunized patients.
3. To evaluate the association between clinical risk factors (diagnosis, number of transfusions, gender) and the development of alloimmunization.
4. To assess the comparative effectiveness of standard (ABO/RhD) versus extended antigen matching (e.g., including Rh subgroups like C/c/E/e and Kell) in reducing the incidence of new alloantibody formation.
5. To provide evidence-based recommendations for targeted antigen typing and matching protocols for specific patient subgroups (e.g., thalassemia major patients) to minimize alloimmunization risk.

Methodology

Study Design and Setting

This retrospective observational study was conducted in the blood bank department of a tertiary care referral hospital in Saudi Arabia. The study period spanned 24 months, from January 2022 to December 2023. The study protocol received approval from the Institutional Ethics Committee.

Study Population

The study cohort comprised patients of all ages who received multiple red blood cell (RBC) transfusions during the study period. Inclusion criteria were: 1) a diagnosis necessitating chronic transfusion support (e.g., thalassemia major, sickle cell disease, myelodysplastic syndromes, or other hematological malignancies), and 2) a history of three or more RBC transfusion episodes. Patients who had received fewer than three transfusions were excluded to focus on a population at substantial risk for alloimmunization.

Data Collection

Data were extracted retrospectively from two primary sources: the hospital's Blood Bank Information System and the Electronic Medical Records. Collected variables included demographics (age, gender, and nationality), clinical data (primary diagnosis), transfusion history (total number of RBC transfusion episodes and units received), and immunohematological data (ABO and RhD type, extended RBC antigen phenotyping, including Kell, Kidd, Duffy, MNS, and Rh variants, as well as results of alloantibody screening and identification).

Laboratory Procedures

Pre-transfusion testing for all patients followed standard protocols, including ABO/RhD typing, antibody screening via the indirect antiglobulin test (IAT), and crossmatching. Antibody screening and identification were performed using the column agglutination (gel card) technique. For patients with a positive antibody screen, specificity was determined using an 11-cell identification panel. Extended antigen typing was performed using serological methods (gel cards) for phenotyping; molecular genotyping was utilized in select cases for antigen prediction.

For this study, a clinically significant alloantibody was defined as any RBC antibody identified during the study period that is known to cause hemolytic transfusion reactions or complicate crossmatching.

Study Outcomes and Variables

The primary outcome was the prevalence of RBC alloimmunization within the multi-transfused cohort. Secondary outcomes included:

- The frequency and specificity of identified alloantibodies.
- The association between alloimmunization and potential risk factors: age, gender, primary diagnosis, and total transfusion burden.
- The comparative analysis of alloimmunization rates between patients who received standard ABO/RhD-matched blood versus those who received RBC units matched for extended antigens.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics software (Version 26.0, Armonk, NY, USA). Descriptive statistics were presented as mean ± standard deviation (SD) for continuous variables and as frequency (percentage) for categorical variables. Associations between categorical variables (e.g., diagnosis and alloimmunization status) were assessed using the Chi-square test or Fisher's exact test, as appropriate. To identify independent predictors of alloimmunization, multivariate binary logistic regression analysis was employed. A two-tailed *p*-value of < 0.05 was considered statistically significant.

Ethical Considerations

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. The Institutional Ethics Committee granted a waiver for informed consent due to the retrospective, anonymized nature of the data analysis. All patient data were de-identified prior to analysis to ensure confidentiality.

regression analysis was used to determine the independent predictors of alloimmunization. A p-value

of less than 0.05 was deemed statistically significant.

Results

Study Population

A total of 120 multi-transfused patients were included in the analysis. The mean age of the study population was 28.4 ± 12.6 years, with a slight male predominance (54.2% male, 45.8% female). The majority of patients were diagnosed with thalassemia major (58.3%), followed by sickle cell disease (25.8%), and hematological malignancies (15.9%).

Table 1. Demographic and Clinical Characteristics of Study Population (n = 120)

Variable	Frequency (%)
Age (mean ± SD)	28.4 ± 12.6 years
Gender	
- Male	65 (54.2%)
- Female	55 (45.8%)
Diagnosis	
- Thalassemia major	70 (58.3%)
- Sickle cell disease	31 (25.8%)
- Hematological malignancies	19 (15.9%)
Mean number of transfusions per patient	12.7 ± 4.3 units

Prevalence of Alloimmunization

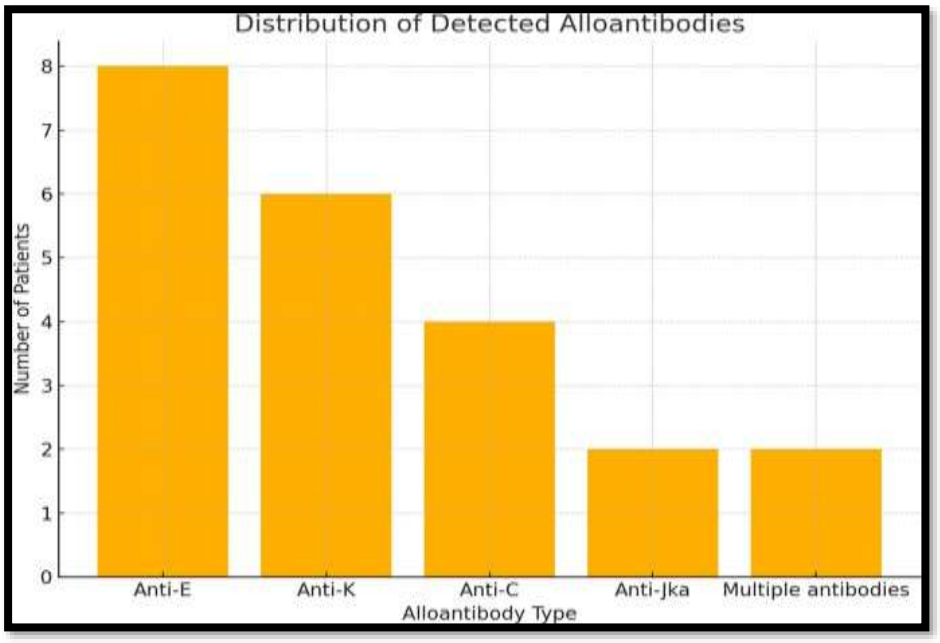
Out of the total cohort, 22 patients (18.3%) developed alloantibodies. The most commonly identified alloantibodies were anti-E (36.4%), anti-K (27.3%), and anti-C (18.2%).Table 2 and Figure 1

Table 2. Alloantibody Specificities in Alloimmunized Patients (n = 22)

Alloantibody Detected	Frequency (%)
Anti-E	8 (36.4%)
Anti-K	6 (27.3%)
Anti-C	4 (18.2%)
Anti-Jka	2 (9.1%)
Multiple antibodies	2 (9.1%)

Risk Factors for Alloimmunization

Figure 1:Distribution of Detected Alloantibodies", showing frequencies of each antibody type



Statistical analysis revealed a significant association between number of transfusions and alloimmunization. Patients who received more than 10 transfusions had a higher risk of developing alloantibodies ($p = 0.02$). Additionally, diagnosis type was associated with alloimmunization rates, with thalassemia major patients exhibiting the highest prevalence (21.4%), followed by sickle cell disease (16.1%) and hematological malignancies (10.5%).

Table 3. Association between Clinical Variables and Alloimmunization

Variable	Alloimmunization Rate (%)	p-value
Number of transfusions (>10 units)	22.7%	**0.02***
Diagnosis		
- Thalassemia major	21.4%	**0.04***
- Sickle cell disease	16.1%	
- Hematological malignancies	10.5%	
Gender		**0.68 (ns)**

*Significant p-value < 0.05

Effectiveness of Extended Antigen Typing

Importantly, patients who received extended antigen-matched blood demonstrated a reduced rate of alloimmunization (7.1%) compared to those receiving standard ABO and RhD matched transfusions (24.6%) ($p = 0.003$).

Figure 2:Alloimmunization by Diagnosis", displaying percentages of alloimmunized patients across diagnosis types

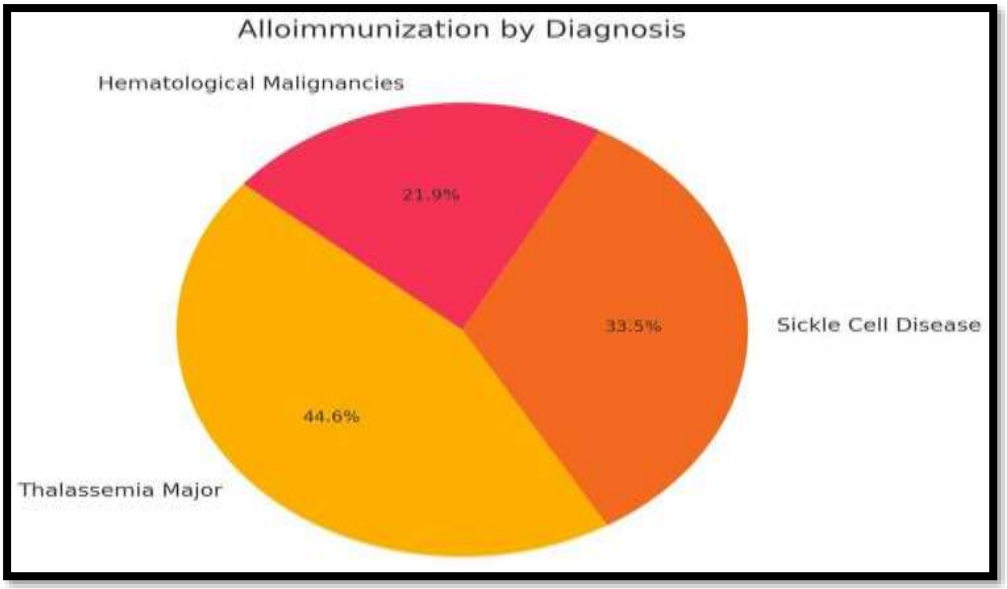
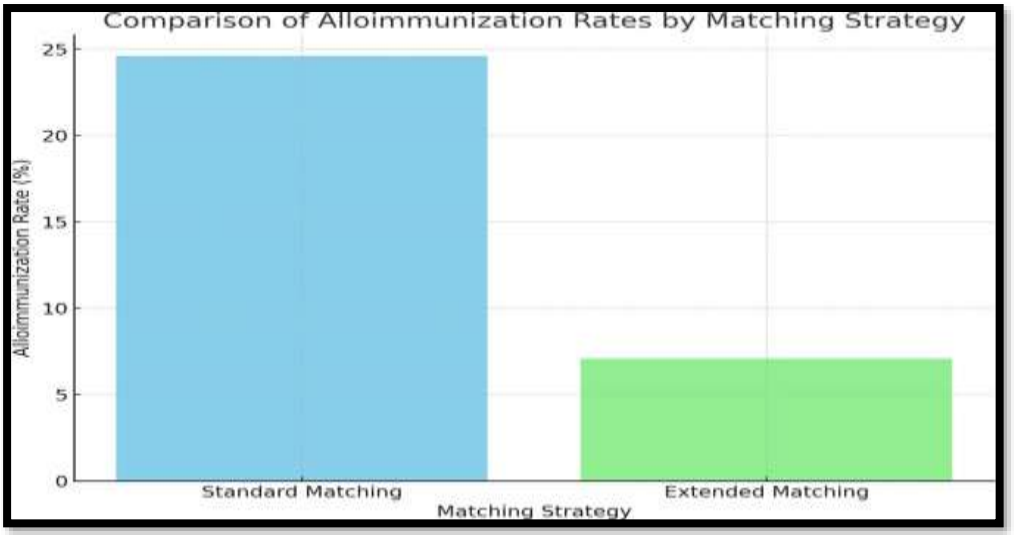


Table 4. Impact of Extended Antigen Matching on Alloimmunization Rates

Matching Strategy	Alloimmunization Rate (%)	p-value
Standard ABO and RhD matching	24.6%	
Extended antigen matching (including Kell, etc.)	7.1%	0.003*

Figure 3:Comparison of Alloimmunization Rates by Matching Strategy", showing two bars comparing standard vs. extended matching.



Discussion

This study assessed the prevalence, specificity, and risk factors for red blood cell (RBC) alloimmunization in a multi-transfused population at a tertiary care center in Saudi Arabia, with a specific focus on evaluating the efficacy of extended antigen matching.

The overall alloimmunization rate of 18.3% aligns with the broad range (2.6%–40%) reported in global literature for chronically transfused patients, reflecting the influence of local patient demographics and transfusion practices (MLP Bueno et al., 2021; SS Das et al., 2021). The pattern of antibody specificities observed—with anti-E (36.4%), anti-K (27.3%), and anti-C (18.2%) being most prevalent—is consistent with established data identifying Rh and Kell system antigens as highly immunogenic (T Makarovska-Bojadzieva et al., 2017; S Mangwana et al., 2019). This finding reinforces the clinical necessity of proactively matching for these antigens beyond standard ABO/RhD compatibility.

Consistent with the principle of cumulative antigenic exposure, a transfusion burden of >10 units was a significant risk factor for alloimmunization (p=0.02), as documented elsewhere (A Qayyum et al., 2018). Furthermore, the diagnosis-specific analysis revealed thalassemia major patients to have the highest alloimmunization prevalence (21.4%). This likely reflects their early initiation of and lifelong dependence on transfusion therapy, a vulnerability highlighted in similar populations (R Maini et al., 2025).

The most significant finding of this study is the pronounced protective effect of extended antigen matching. Patients receiving blood matched for antigens such as Kell and Rh variants (C, c, E, e) demonstrated a substantially lower alloimmunization rate (7.1%) compared to those receiving only ABO/RhD-matched units (24.6%) (p=0.003). This provides strong, localized evidence supporting the recommendations of prior research advocating for proactive, extended phenotyping to mitigate alloimmunization risk (DK Bhuva and JH Vachhani, 2017; S Mangwana et al., 2019).

Implementing such a strategy in genetically diverse regions like Saudi Arabia could significantly enhance transfusion safety and streamline blood bank operations by preventing complex antibody workups.

Limitations:

This study has several limitations inherent to its design. Its retrospective nature limits the ability to establish causal relationships and may lead to an underestimation of the true alloimmunization rate due to potential antibody evanescence. The reliance primarily on serological phenotyping, rather than comprehensive molecular genotyping, may introduce imprecision in antigen matching assessments and fail to detect some variant antigens. Finally, as a single-center study, the generalizability of our findings to other populations with different genetic backgrounds and transfusion protocols may be limited.

Conclusion

This study confirms a clinically significant burden of RBC alloimmunization among multi-transfused patients in our setting, primarily driven by antibodies against Rh and Kell system antigens. It identifies a high transfusion burden and a diagnosis of thalassemia major as key risk factors. Most importantly, it provides compelling evidence that extended antigen matching is a highly effective strategy for reducing alloimmunization risk. We therefore recommend the integration of extended RBC phenotyping—at a minimum for Rh (C, c, E, e) and Kell—into the standard pre-transfusion workup for all patients diagnosed with conditions requiring chronic transfusion therapy. Future prospective, multicenter studies incorporating molecular genotyping are warranted to refine optimal matching protocols and evaluate the long-term clinical and economic benefits of this strategy.

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