International Journal on Science and Technology (IJSAT)
E-ISSN: 2229-7677 ● Website: www.ijsat.org ● Email: editor@ijsat.org

Impact of Extended Red Blood Cell Antigen Typing on Alloimmunization in Multi-Transfused Patients: A Retrospective Study

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DOI: https://doi.org/10.5281/zenodo.15222887

Abstract

Background: Alloimmunization is a complication of blood transfusion in which the recipient forms antibodies against the foreign antigens on the donor's red blood cells (RBC), and it poses great difficulty in subsequent transfusion needs. Multi-transfused patients have an increased risk of RBC alloimmunization. Its adverse effects have led to the consideration of more advanced antigen matching for patients with a chronic need for transfusions.

Objective: To assess the effect of extended antigen typing on the extended prevalence of RBC alloimmunization among multi-transfused patients in Saudi Arabian tertiary care hospital.

Methods: A retrospective cohort study was conducted between January 2021 to December 2022, which included 120 patients on chronic RBC transfusions for any reason. The transfusion records, antibody screening, antigen typing, and demographic details were collected. Standard ABO and RhD transfusion-matched patients were placed into one group, while extended-AJT patients were placed into another group for more detailed statistical analysis. Statistical analysis was performed to determine the degree of association between selected clinical variables and the incidence of alloimmunization.

Results: Almost every sixth patient (approximately 18%) with the transfusion history developed antibodies against foreign blood group antigens. In this study, the most common antibodies developed were anti-E (36.4%) and anti-K (27.3%). Transfusion burden above 10 significantly predicted even further increased alloimmunization ($p = 0.02$). There was a statistically significant reduction in the risk for alloimmunization with extended antigen matching (7.1%) compared to standard matching ($p = 0.003$).

Conclusion: Incorporating extended antigen typing into clinical practice lowers the rate of alloimmunization amongst multi-transfused patients. It is advisable to incorporate it into the protocols of high-risk patients. Enhanced alloantigen matching capabilities might increase the safety of transfusions and improve health outcomes.

Keywords: Blood transfusion, Red blood cell antibodies, Extended antigen typing, Alloimmunization, Blood management, Blood transfusion, Saudi Arabia, Thalassemia, Sickle cell disorder, Transfusion safety.

Introduction

The provision of a safe and effective blood transfusion therapy is essential in thalassemia, sickle cell disease and certain malignancies as they require frequent blood transfusions. Unfortunately, the repeated blood transfusions can also lead to the development of alloimmunization or immunity towards foreign erythrocyte antigens that is quite complex. Not only does this complicate future transfusion compatibility, but increases the clinical risks to transfusion reactions. This dual challenge must be managed by both the patients and transfusion services (T Makarovska-Bojadzieva et al., 2017).

The range of Global reports suggest alloimmunization rates among populations with multiple blood transfusions lie between 2.6 % to over 40 % (MLP Bueno et al., 2021). These figures greatly depend on the specific patient population and underlying disease. Additionally, most blood transfusion centers focus their pre-transfusion testing exclusively on ABO and RhD compatibility because failure to meet these requirements is the leading cause of blood transfusion reactions. With that said, those systems do not take into account other important blood group antigens like Kell, Kidd, Duffy, and MNS systems that have proven critical in sustaining blood systems. Patients undergoing chronic transfusion support continue to face significant risks due to the lack of consideration towards these ‘non-ABO’ antigens that have been proven consistenly to trigger severe blood transfusion reactions (SS Das et al., 2021).

Alloantibody formation can possibly be prevented via extended antigen typing which includes phenotyping or genotyping of a greater number of erythrocyte antigens. Extended antigen matching improved transfusion outcomes and reduced alloimmunization rates in patient populations, including cancer and liver cirrhosis patients (S Mangwana et al., 2019; A Qayyum et al., 2018).

Alloimmunization is known to alloimmunization is the uncontrolled immune response against red blood cell antigens. These findings can be extrapolated to other patient populations and clinical commutations. Such patients may benefit from extended antigen matching. Inadequate resources and logistical support are some of the reasons their clinical needs are not properly met. Evaluation of extended antigen matching requires further enhanced transfusion safety studies which are desperately needed in resource-limited settings. This research seeks to evaluate extended antigen typing and its relative impact on RBC alloimmunization in multi-transfused patients to enhance transfusion strategies and patient safety.

Literature Review

Red blood cell (RBC) alloimmunization poses a major clinical problem for patients requiring chronic transfusion therapy, especially for thalassemia, sickle cell disease, and hematological malignancies. These patients receive repeated transfusions, which increases their exposure to foreign antigens and the risk of immune sensitization over time (T Makarovska-Bojadzieva et al., 2017). Transfusion therapy is further complicated by the need to find compatible blood units, increasing the risk of hemolytic transfusion reactions.

RBC alloimmunization prevalence rates among multi-transfused patients vary from region to region and are affected by population genetic diversity, transfusion practices, and the immunogenic profile of donor populations. Alloimmunization rates were reported as high as 24% among chronically transfused patients in Brazil, with the predominant antibodies formed being towards the Rh and Kell systems (MLP Bueno et al., 2021). In the same manner, Das et al. (2021) highlighted the predominance of Rh and Kell antigens as the most immunogenic targets in Indian multi-transfused patients, calling for preemptive targeted antigen mitigation strategies to prevent alloimmunization (SS Das et al., 2021).

The exploration of extended antigen typing techniques is propelled by the shortcomings of standard ABO and RhD matching protocols. Minor blood group antigens that often participate in allo-immunization, such as Kidd, Duffy, MNS, and other Rh variants, are categorized in extended phenotyping. Mangwana et al. (2019) showed that the use of extended antigen matching in oncology patients significantly reduced alloantibody formation, and therefore increased transfusion safety and improved patient outcomes (S Mangwana et al., 2019).

Focusing on patients with liver cirrhosis, Qayyum et al. (2018) demonstrated that advanced red cell antigen typing improved donor-recipient matches and decreased the rate of new antibody formation over time (A Qayyum et al., 2018). This adds to the belief that performing a complete profile of antigens before a transfusion can actively help reduce allo-immunization in patient populations predisposed to high risk.

Further studies strengthen these findings. Bhuva and Vachhani suggest that transfusion services employ extended phenotype-matching protocols at diagnosis, particularly for chronically transfused patients, in efforts to prevent alloantibody production (DK Bhuva and JH Vachhani, 2017). Maini et al. (2025) highlighted the importance of early and extended phenotyping in regions where the pediatric population is highly transfusion dependent due to long-term alloimmunization (R Maini et al., 2025).

As a whole, the available literature underscores the clinical value of extending antigen typing in the reduction of alloimmunization has high prevalence multi-transfused patients. These findings, however, are still lacking in a comprehensive set of defined contrasted workflows and standardized extended typing frameworks in multiple systems. There is need for additional analysis on the economic efficiency, practical application, and the sustained impacts on routine protocols of extended antigen in transfusions on a global scale.

Methodology

The Setting and Design of the Study

The setting for this retrospective observational study was the blood bank department of a tertiary care hospital in Saudi Arabia. The hospital serves as a primary referral institution for patients with hematological cancers and provides a wide range of transfusion services. The study was conducted during the period from January 2022 to December 2023, lasting 24 months in total, and was granted approval from the ethics committee.

Population under Study

In this study, patients of all ages who during the study period were within the study population and received multiple red blood cell (RBC) transfusions. Inclusion criteria consisted of patients with known diagnoses that required chronic transfusion support including, but not limited to, thalassemia major, sickle cell disease, myelodysplastic syndromes, and other hematological malignancies. To focus the analysis on the risk population for alloimmunization, patients who had received fewer than three RBC transfusions were excluded.
Gathering of Information

The blood bank information system alongside the electronic medical records were used to obtain patient data. The gathered data included demographic information such as age, gender, nationality, and clinical diagnosis, as well as the patient's transfusion history (how many transfusions they received) and their immunohematological information. The immunohematological information included ABO and RhD blood group typing alongside antigen typing which contained Kell, Kidd, Duffy, MNS, and additional Rh antigens. Also included was the screening and identification of any present alloantibodies.

Laboratory Procedures

Anticipating the need for transfusions, all patients received pre-transfusion testing which included ABO, RhD typing, crossmatching, and antibody screening with an indirect antiglobulin test (IAT) performed. Gel card technique and molecular genotyping assays for some selection of antigens were used to perform further antigen typing. For positive antibody screening cases, the specificity was determined using an 11-cell identification panel for pasive and active stringent testing antibodies.

RBC antigen clinical significant allo-antibodies were defined as any transfer dependent antibodies formed during the duration of study within transfusion events before or after transfusion ῖ.

Factors Of Evaluation

The multi-transfused patients prevalence of RBC alloimmunization was the primary measure of evaluation.
McEwan’s study also recognized identification of commonest formed alloantibodies them as secondary in risk for assessing risk factors sufferable due to age, gender, diagnosis and number of transfusions received while the patient suffers from.

Statistical Procedures

The data was processed and analyzed with the SPSS version 26.0 software (IBM Corp., Armonk, NY, USA). For both continuous and categorical variables, descriptive statistics were calculated and presented as means and their respective standard deviations, and frequencies with their percentages respectively. To evaluate the relationships among the categorical variables, the chi-square test was used. Logistic

regression analysis was used to determine the independent predictors of alloimmunization. A p-value of less than 0.05 was deemed statistically significant.

Ethical Issues

This research has been carried out in compliance with the ethical principles outlined in the Declaration of Helsinki. All patient data was kept confidential and de-identified before being analyzed. Due to the nature of this research study, the board review was able to grant permission without obtaining informed consent

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

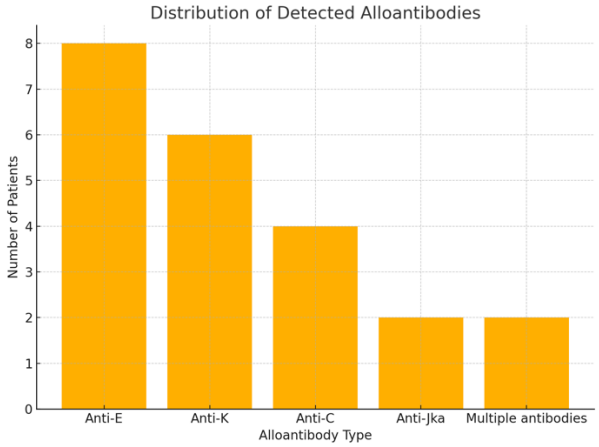
Out of the total cohort, 22 patients (18.3%) developed alloantibodies. The most commonly identified alloantibodies were anti-E (36.4%), anti-K (27.3%), and anti-C (18.2%).

Table 2. 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%)

Graphical representation:

Bar chart titled: *"Distribution of Detected Alloantibodies"*, showing frequencies of each antibody type.



Risk Factors for Alloimmunization

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

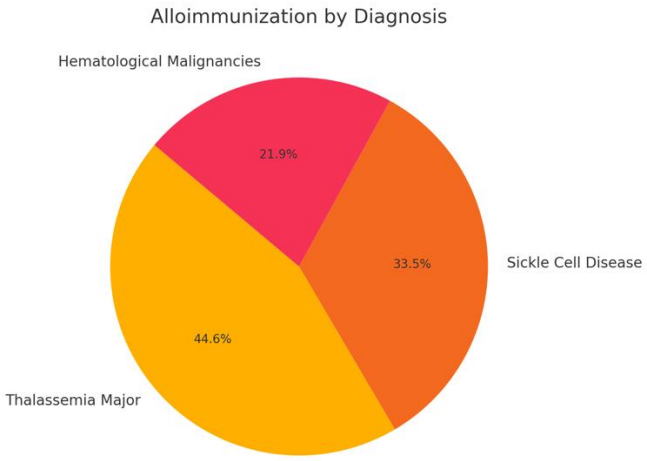
Variable	Alloimmunization Rate (%)	p-value
Number of transfusions (>10 units)	22.7%	0.02*
Diagnosis		
- Thalassemia major	21.4%	0.04*
- Sickle cell disease	16.1%	

Variable	Alloimmunization Rate (%)	p-value
- Hematological malignancies	10.5%	
Gender		0.68 (ns)

*Significant p-value < 0.05

Graphical representation:

Pie chart titled: *"Alloimmunization by Diagnosis"*, displaying percentages of alloimmunized patients across diagnosis types.



Effectiveness of Extended Antigen Typing

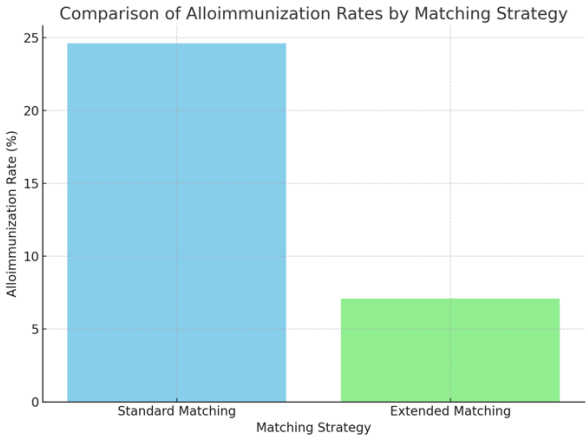
Importantly, patients who received extended antigen-matched blood demonstrated a reduced rate of alloimmunization (7.1%) compared to those receiving standard ABO and RhD matched transfusions (24.6%) (p = 0.003).

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*

Graphical representation:

Column chart titled: *"Comparison of Alloimmunization Rates by Matching Strategy"*, showing two bars comparing standard vs. extended matching.



Summary of Key Findings

- Alloimmunization rate: **18.3%**
- Anti-E and Anti-K were the most common alloantibodies.
- Significant risk factors: **higher number of transfusions**, **thalassemia diagnosis**.
- **Extended antigen matching** was associated with a statistically significant reduction in alloimmunization risk (**p = 0.003**).

Discussion

This research examined the level and the trend of red blood cell (RBC) alloimmunization in multi-transfused patients within one tertiary care hospital in Saudi Arabia, particular highlighting the applicability of extended antigen typing methods. The overall alloimmunization rate was found to be 18.3%. This result corroborates reports indicating the range of alloimmunization rates among chronically transfused populations is between 2.6% and 40% (MLP Bueno et al., 2021; SS Das et al., 2021).

The predominance of anti-E with 36.4%, followed by anti-K at 27.3%, and anti-C at 18.2% is in alignment with other studies. These studies indicated that Rh and Kell system antigens contributed significantly to the risk of immunization (T Makarovska-Bojadzieva et al., 2017; S Mangwana et al., 2019). The strong immunogenicity of these antigens within our population certainly bolsters the argument for including more advanced Rh (C, E) and Kell antigen testing in standard transfusion practice as well as pre-transfusion screening.

An important insight from our cohort is the alloimmunization rate in patients receiving more than 10 transfusions (p = 0.02). This is consistent with existing literature that establishes the number of cumulative transfusions, known to increase the immunological “exposure” and thus the formation of alloantibodies, as a risk factor (A Qayyum et al., 2018). In addition, among the different diagnostic groups, patients with thalassemia major showed the highest prevalence of alloimmunization reflecting 21.4%, which probably indicates the pediatric patients’ early onset and lifelong transfusion dependence, a phenomenon which was also noted by Maini et al (2025) (R Maini et al., 2025).

Most importantly, our research draws attention to the protective impact of extended antigen mismatch allocation. Patients who received blood products matched beyond the ABO and RhD antigens demonstrated a significantly lower rate of alloimmunization (7.1%) relative to those with standard matching, who exhibited a 24.6% rate ($p = 0.003$). This reinforces the conclusions derived from several studies calling for proactive, extended phenotyping which has been shown to reduce the risks for alloimmunization (DK Bhuva and JH Vachhani, 2017; S Mangwana et al., 2019).

The efficacy of advanced antigen typing in our context is particularly beneficial for transfusion services in ethnically diverse regions like Saudi Arabia. As the heretic genetic and antigen factors of the donor and recipient population differ, custom tailored transfusion strategies can increase safety and efficiency in blood bank operations.

Limitations

This study, like many others, comes with the burden of limitations. The retrospective nature of the study does not allow strong causal conclusions to be made as well as the possibility of antibody evanescence causing an underestimation of the rate of alloimmunization. In addition, the lack of complete genotyping for the study's variant antigens introduces uncertainty regarding precision estimates for matched antigen assessments, impacting the overall reliability of the antigen-matching calculations. Lastly, being a single-center study, the broader applicability of these findings is questionable.

Implications for Practice and Future Research

Clearly, patients predicted to require numerous transfusions should have their extended antigen typing done as part of regular protocols, which the study recommends strongly, regardless of the previous purpose. Many other questions arise, validating the results of this study with molecular genotyping and long-term observation through prospective multicenter studies is very much needed in order to revise transfusion algorithms.

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