Original Article

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Red Blood Cell Alloantibodies among Multi-Transfused Patients in a Single Tertiary Center

Hannah Ismail, M.Med[1], Sabariah Mohd Noor, M.Med[1], Rohayu Hami, DrPH[2], Siti Salmah Noordin, M.Med[3]

[1]Department of Transfusion Medicine, Hospital Raja Permaisuri Bainun, Jalan Raja Ashman Shah, 30450 Ipoh, Perak Darul Ridzuan, Malaysia.

[2]Department of Community Health, Pusat Kanser Tun Abdullah Ahmad Badawi, Universiti Sains Malaysia, 13200 Kepala Batas, Pulau Pinang, Malaysia.

[3]Department of Clinical Medicine, Pusat Kanser Tun Abdullah Ahmad Badawi, Universiti Sains Malaysia, 13200 Kepala Batas, Pulau Pinang, Malaysia.

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Abstract:

Objective: This study investigated the prevalence and specificity of red blood cell (RBC) alloantibodies and assessed the factors associated with their development in multi-transfused patients at a single tertiary center.

Material and Methods: This cross-sectional study retrospectively analyzed data from 384 multi-transfused patients who underwent pre-transfusion or immunohematology (IH) testing at Hospital Raja Permaisuri Bainun, Malaysia. Patients were selected using systematic random sampling, and data were extracted from electronic hospital systems and IH test reports. Variables included demographics, clinical diagnosis, transfusion history, and transplant status. Data were analyzed using descriptive statistics and logistic regression.

Results: A total of 5,675 multi-transfused patients were identified during the study period. Of the 384 patients selected for analysis, 155 (44.5%) exhibited RBC alloantibodies**.** Anti-E was the most frequently identified alloantibody (39.6%), followed by anti-Mia (26.2%). Patients with chronic kidney disease and thalassemia had a significantly higher risk of alloantibody development (p-value<0.001 and p-value=0.032, respectively).

Conclusion: The high rate of RBC alloimmunization in multi-transfused patients highlights the importance of extended RBC antigen matching, not only for thalassemia patients but also for those with chronic kidney disease, to reduce the risk of alloantibody formation.

Contact: Siti Salmah Noordin, M.Med.
Department of Clinical Medicine, Pusat Kanser Tun Abdullah Ahmad Badawi , Universiti Sains Malaysia, 13200 Kepala Batas, Pulau Pinang, Malaysia.
E-mail: ssalmah@usm.my

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Introduction

Red blood cell (RBC) alloimmunization is one of the most significant adverse effects of transfusion, with clinical consequences that vary depending on the specificity of the antibody involved[1]. The development of RBC alloantibodies is influenced by several factors, including genetic disparities between donor and recipient, exposure to foreign antigens, and the immunogenicity of those antigens. Additional contributing factors include the recipient's age, sex, underlying clinical condition, and immune status[2]. As a result, multi-transfused and transfusion-dependent patients are at an increased risk for RBC alloimmunization. Reported incidences vary widely across different patient populations, ranging from 8% to 76%[3-5].

Globally, ABO and RhD typing and crossmatching are routinely performed for all patients requiring RBC transfusions. In addition, extended antigen matching for blood group systems such as Rh (C/c/E/e), Kell (K/k), Kidd (Jk^a/Jk^b), Duffy (Fy^a/Fy^b), and MNS (M/N/S/s) is recommended for transfusion-dependent patients, including those with thalassemia and sickle cell disease[6]. However, this practice is not consistently implemented in some laboratories, except for Rh and Kell blood group systems, primarily due to limitations in reagent availability, blood inventory, equipment, and trained staff, thereby increasing the risk of RBC alloimmunization[7].

The frequency of RBC alloantibodies varies among different patient groups, largely due to genetic heterogeneity across populations. This study aimed to determine the prevalence and specificity of RBC alloantibodies and to assess the factors associated with their development in multi-transfused patients. Understanding these factors will facilitate resource allocation and subsequently enhance the transfusion practices.

Material and Methods

This cross-sectional study involved retrospective data collection from multi-transfused patients who underwent pre-transfusion testing or immunohematology (IH) tests from 1st January 2020 until 31st December 2021 at the Department of Transfusion Medicine at Hospital Raja Permaisuri Bainun (HRPB), Ipoh, Perak, Malaysia. HRPB is a tertiary care hospital offering specialized medical services across all major disciplines and subspecialties. It ranks fourth among the ten hospitals in Malaysia with the highest number of thalassemia patients[8].

The inclusion criteria for the study were multi-transfused patients who required pre-transfusion or IH tests during the study period. According to a study by Obi et al., a multi-transfused patient is defined as an individual who has received more than one unit of packed red blood cell (PRBC) within a one-month timeframe, regardless of the number of transfusion events, or a cumulative total of at least ten units of PRBCs within three months[9].

Clinical diagnoses were categorized into four groups: thalassemia, hematological disorders, chronic kidney disease (CKD), or 'others'. Patients with missing data, concomitant autoantibodies, or a history of receiving non-identical ABO or RhD PRBC transfusions were excluded to minimize potential interference from such antibodies, which could affect the interpretation of antibody identification tests.

The sample size was calculated using a double proportion approach, based on an estimated prevalence of RBC alloantibodies being higher in CKD patients (23.8%) than in those with hematological diseases (11.76%)[10]. With a significance level of 0.05 and a study power of 80%, the minimum required sample size, including a 10% dropout rate, was 384. A systematic random sampling was employed to select subjects based on the inclusion and exclusion

criteria; every 17th multi-transfused patient on the transfusion list was selected.

All transfusion records and clinical information were retrieved from integrated electronic medical record applications: the Blood Bank Information System version 2.0 (BBISv2), the Hospital Information System (HIS@KKM), and IH test reports. Data collected included demographic and clinical characteristics of the patients, such as age, sex, ethnicity, ABO blood group, clinical diagnosis, PRBC transfusion frequency, and transplant history.

Data entry and analysis were conducted using IBM SPSS Statistics for Macintosh, version 26.0 (IBM Corp., Armonk, New York, USA). Data were analyzed using descriptive and inferential statistics. Numerical data were presented as mean±standard deviation (S.D.) or median (interquartile range, IQR), depending on normality, while categorical data were presented as frequencies and percentages. Simple and multiple logistic regression analyses were performed to identify the factors associated with RBC alloantibody development. A p-value of <0.05 was considered statistically significant.

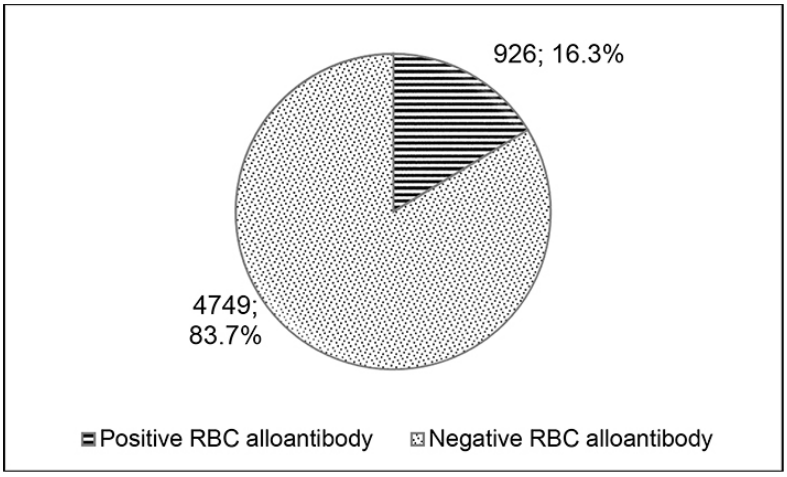
Ethical approval (USM/JEPeM/21010108) was obtained from the Human Research Ethics Committee (HREC), Universiti Sains Malaysia. Additional approval was also obtained from the Medical Research and Ethics Committee (MREC), Ministry of Health, Malaysia, under protocol number NMRR-21-45-58083 (IIR).

Results

Prevalence of RBC alloantibodies and characteristics of multi-transfused patients

During the study period, 5,675 multi-transfused patients were identified, of whom 926 (16.3%) were recorded as having RBC alloantibodies (Figure 1). For this study, a total of 348 patients who met the inclusion and exclusion criteria were selected for detailed analysis. Among these, 155 patients (44.5%) tested positive for RBC alloantibodies, while 193 (55.5%) tested negative.

Table 1 summarizes the demographic and clinical characteristics of the study population. The patients' ages ranged from 1 to 97 years, with a mean of 49.4±19.7 years. Among the clinical categories, patients with CKD



RBC=red blood cells

Figure 1 Overall prevalence of red blood cell alloantibodies in multi-transfused patients during the study period

Table 1 Demographic and clinical characteristics of multi-transfused patients (n=348)

Variables	Red blood cell alloantibodies, n (%)		Total n (%)
	Absence (n=193)	Presence (n=155)	
Age (years)			
Mean (±standard deviation)	49.03 (±19.8)	49.69 (±19.9)	49.4 (±19.7)
0–20	32 (16.6)	18 (11.6)	50 (14.4)
21–40	28 (14.5)	30 (19.4)	58 (16.7)
41–60	54 (28.0)	51 (32.9)	105 (30.1)
>60	79 (40.9)	56 (36.1)	135 (38.8)
Sex			
Male	105 (54.4)	78 (50.3)	183 (52.6)
Female	88 (45.6)	77 (49.7)	165 (47.4)
Ethnicity			
Malay	81 (42.0)	80 (51.6)	161 (46.3)
Chinese	45 (23.3)	47 (30.3)	92 (26.4)
Indian	56 (29.0)	26 (16.8)	82 (23.6)
Others	11 (5.7)	2 (1.3)	13 (3.7)
Blood group			
O	78 (40.4)	43 (27.7)	121 (34.7)
A	48 (24.9)	42 (27.1)	90 (25.9)
B	56 (29.0)	54 (34.8)	110 (31.6)
AB	11 (5.7)	16 (10.3)	27 (7.8)
Transfusion frequency			
>1 unit/month	184 (95.3)	130 (83.9)	314 (90.2)
>10 units/3 months	9 (4.7)	25 (16.1)	34 (9.8)
Clinical diagnosis			
Thalassemia	2 (1.0)	10 (6.5)	12 (3.4)
Chronic kidney disease	11 (5.7)	50 (32.3)	61 (17.6)
Hematological disorders (n=84)			
Aplastic anemia	5 (2.6)	0 (0)	5 (1.4)
Chronic and symptomatic anemia	2 (1.0)	11 (7.1)	13 (3.7)
Autoimmune hemolytic anemia	8 (4.1)	3 (1.9)	11 (3.2)
Acute leukemia	6 (3.1)	2 (1.3)	8 (2.3)
Chronic leukemia	7 (3.6)	2 (1.3)	9 (2.6)
Lymphoma	5 (2.6)	1 (0.6)	6 (1.7)
Multiple myeloma	9 (4.7)	0 (0)	9 (2.6)
Myelodysplastic syndrome	10 (5.2)	2 (1.3)	12 (3.4)
Immune thrombocytopenic purpura with anemia	4 (2.1)	3 (1.9)	7 (2.0)
Others	3 (1.6)	1 (0.6)	4 (1.1)
Others (n=191)			
Gastrointestinal bleeding	32 (16.6)	5 (3.2)	37 (10.6)
Intra-abdominal injuries	7 (3.6)	5 (3.2)	12 (3.4)
Intracranial bleeding	10 (5.2)	17 (11.0)	27 (7.8)
Polytrauma	31 (16.1)	18 (11.6)	49 (14.1)
Solid organ malignancies	11 (5.7)	14 (9.0)	25 (7.2)
Surgical bleeding	10 (5.2)	2 (1.3)	12 (3.4)
Obstetric bleeding	7 (3.6)	2 (1.3)	9 (2.6)
Miscellaneous	13 (6.7)	7 (4.5)	20 (5.7)

had the highest prevalence of RBC alloantibodies (n=50; 32.3%). More than half of the patients (n=191; 54.9%) were classified under the 'others' category. The 'others' category included patients with gastrointestinal bleeding (n=37; 10.6%), traumatic injuries such as polytrauma (n=49; 14.1%), intra-abdominal injuries (n=12; 3.4%), intracranial bleeding (n=27; 7.8%), and solid organ malignancies (n=25; 7.2%); notably pancreatic, colon, cervical, and ovarian cancers.

None of the patients with hematological disorders had undergone hematopoietic stem cell transplantation. However, among hematological disorder patients, 56 (66.7%) were receiving chemotherapy and/or immunosuppressive therapy, while 28 (33.3%) were not undergoing any treatment (Supplementary Table 1).

Frequency and specificity of RBC alloantibodies among multi-transfused patients

The majority of multi-transfused patients developed a single alloantibody (n=111; 71.6%), while 32 patients (20.6%) developed two alloantibodies, and 12 patients (7.8%) developed multiple alloantibodies (Table 2). Anti-E was the most frequently identified single alloantibody (n=44; 39.6%), followed by anti-Mia (n=29; 26.2%), and anti-M and anti-Le^a (6.3% each). The most common combination of double alloantibodies was anti-Le^a and anti-Le^b (n=11; 34.4%), followed by anti-E and anti-c (n=10; 31.3%). All patients with alloantibodies were serologically negative for the corresponding RBC antigens, consistent with alloimmunization.

Among 12 patients with thalassemia, two had no detectable alloantibodies, four had either single or multiple RBC antibodies, and two had double RBC alloantibodies (Table 3). Anti-E was the most frequently identified alloantibody in thalassemia patients (n=7), followed by anti-Mia (n=5).

A total of 214 RBC alloantibodies were identified among the 155 multi-transfused patients. The most common specificities of these alloantibodies were directed against the Rh blood group system (n=97; 45.3%), followed by MNS (n=49; 23%), Lewis (n=40; 18.7%), Kidd (n=12; 5.6%), and Duffy (n=9; 4.2%) (Table 4).

Table 2 Specificities of the red blood cell alloantibodies among multi-transfused patients (n=155)

Single antibody (n=111)		Double antibodies (n=32)		Multiple antibodies (n=12)	
Antibody specificities	**Number of patients, n (%)**	**Antibody specificities**	**Number of patients, n (%)**	**Antibody specificities**	**Number of patients, n (%)**
Anti-E	44 (39.6)	Anti-Le^a + Le^b	11 (34.4)	Anti-E + c + Jk^b	2 (17.0)
Anti-Mia	29 (26.2)	Anti-E + c	10 (31.3)	Anti-E + c + Jk^a	1 (8.3)
Anti-M	7 (6.3)	Anti-E + Mia	4 (12.5)	Anti-E + C + D	1 (8.3)
Anti-Le^a	7 (6.3)	Anti-c + Mia	1 (3.1)	Anti-E + Jk^a + S	1 (8.3)
Anti-Jk^a	5 (4.5)	Anti-C + e	1 (3.1)	Anti-E + Le^a + Le^b	1 (8.3)
Anti-P1	5 (4.5)	Anti-E + C	1 (3.1)	Anti-Fy^b + Le^a + Le^b	1 (8.3)
Anti-Le^b	4 (3.6)	Anti-E + Jk^b	1 (3.1)	Anti-Fy^b + Le^a + Mia	1 (8.3)
Anti-Fy^b	3 (2.7)	Anti-E + Le^a	1 (3.1)	Anti-Fy^b + S + Ch/Rg	1 (8.3)
Anti-S	2 (1.8)	Anti-E + Le^b	1 (3.1)	Anti-E + c + S + Fy^b	1 (8.3)
Anti-c	2 (1.8)	Anti-S + Fy^b	1 (3.1)	Anti-E + c + Jk^a + Mia	1 (8.3)
Anti-C	1 (0.9)			Anti-E + c + Jk^a + C^w	1 (8.3)
Anti-Fy^a	1 (0.9)				
Anti-Ch/Rg	1 (0.9)				

[a] and [b]=reflect antithetical antigens (allelic variants),[w]=weak antigen expression, Mean number of alloantibodies per patient is 1.38

Table 3 Clinical characteristics of thalassemia patients (n=12)

No.	Age (years)	Sex	Ethnicity	Diagnosis	ABO RhD blood group	Red blood cell (RBC) phenotype	Types of RBC antibodies	Transfusion frequency
1	3	Female	Malay	Beta Thalassemia Intermedia	O RhD positive	CDe/CDe (R1R1), Jk^a+Jk^b+kk, Fy^a+Fy^b-Ss	Anti-E	>10 units/ 3 months
2	9	Female	Malay	Beta Thalassemia Major	B RhD positive	CDe/CDe (R1R1), Jk^a+Jk^b- kk Fy^a+Fy^b+Ss	Anti-E, Anti-c, Anti-Jkb	>10 units/ 3 months
3	11	Male	Chinese	Beta Thalassemia Intermedia	O RhD positive	CDe/cDE (R1R2), Jk^a+Jk^b-Kk Fy^a+Fy^b+Ss	Probably Anti-Mia	>10 units/ 3 months
4	11	Female	Malay	Beta Thalassemia Major	O RhD positive	CDe/CDe (R1R1), Jk^a+Jk^b+ kk Fy^a+Fy^b+ss	-	>10 units/ 3 months
5	13	Female	Malay	HbE Beta Thalassemia	A RhD positive	cDe/cde (Ror), Jk^a+Jk^b- kk Fy^a+Fy^b+ss	Anti-E	>10 units/ 3 months
6	19	Female	Chinese	Beta Thalassemia Major	B RhD positive	cDE/cde (R2r), Jk^a+Jk^b+ kk Fy^a+Fy^b- ss	Anti-E, Anti-Jka, Anti-S	>10 units/ 3 months
7	38	Female	Malay	Beta Thalassemia Major	O RhD positive	CDe/cde (R1r), Jk^a+Jk^b- kk Fy^a+Fy^b- ss	Anti-S, Anti-Fyb Anti-Ch/Rg	>10 units/ 3 months
8	23	Male	Chinese	Beta Thalassemia Intermedia	A RhD positive	CDe/CDe (R1R1)*	Anti-E, Probably Anti-Mia	>10 units/ 3 months
9	29	Male	Malay	HbE Beta Thalassemia	B RhD positive	CDe/cDE (R1R2)*	Probably Anti-Mia	>10 units/ 3 months
10	35	Male	Malay	Beta Thalassemia Major	O RhD positive	CDe/CDe (R1R1)*	-	>10 units/ 3 months
11	61	Female	Chinese	HbH disease	B RhD positive	CDe/cde (R1r)*	Anti-E, Probably Anti-Mia	>10 units/ 3 months
12	68	Male	Malay	HbE Beta Thalassemia	B RhD positive	CDe/CDe (R1R1), Jk^a+Jk^b+ kk, Fy^a+Fy^b Ss	Anti-E, Anti-c, Anti-Jka, Probably Anti-Mia	>10 units/ 3 months

*Missing data for additional extended RBC phenotype

Factors associated with RBC alloantibody development among multi-transfused patients

Univariate analysis using simple logistic regression showed significant associations between RBC alloantibody development and variables such as ethnicity, ABO blood group, frequency of PRBC transfusions, and clinical diagnosis (Table 5). In the final multivariate logistic regression model, clinical diagnosis remained significantly associated with alloantibody development, particularly among patients with CKD and thalassemia (Table 6).

Patients with CKD had significantly higher odds of developing RBC alloantibodies compared to those with other clinical diagnoses (adjusted odds ratio [aOR]=5.623, 95% confidence interval [CI]: 3.461–6.327; p-value<0.001). Similarly, thalassemia patients had increased odds of alloantibody formation (aOR=2.127; 95% CI: 1.221–10.309; p-value=0.032).

Discussion

Providing safe and adequate blood is a fundamental component of blood transfusion services. The development of RBC alloantibodies against one or more RBC antigens can significantly complicate transfusion therapy, particularly among multi-transfused patients, by limiting compatible blood availability and increasing the risk of hemolytic transfusion reactions.

In this study, the overall prevalence of RBC alloantibodies among multi-transfused patients was 16.3%, comparable to findings by Valle Neto et al., who reported a prevalence of 15.7%[10]. However, among the selected cohort

Table 4 Red blood cell alloantibodies distribution according to blood group systems

Blood group system	Number of antibodies	Percentage (%)
Rh	97	45.3
Anti-E	71	33
Anti-c	19	8.9
Anti-C	4	1.9
Anti-C^{w}	1	0.5
Anti-D	1	0.5
Anti-e	1	0.5
MNS	49	23
Anti-Mia	36	16.9
Anti-M	7	3.3
Anti-S	6	2.8
Lewis	40	18.7
Anti-Le^{a}	22	10.3
Anti-Le^{b}	18	8.4
Kidd	12	5.6
Anti-Jk^{a}	9	4.2
Anti-Jk^{b}	3	1.4
Duffy	9	4.2
Anti-Fy^{a}	1	0.5
Anti-Fy^{b}	8	3.7
P1PK		
Anti-P1	5	2.3
Chido/Rodgers		
Anti-Ch/Rg	2	0.9
Total	214	100

a and b=antithetical antigens (allelic variants),^{w}=weak antigen expression, Mean alloantibodies per blood group system is 30.6

of 348 patients, the prevalence was substantially higher at 44.5%, with CKD patients demonstrating the highest frequency (50/348; 14.4%). This finding aligns with a local study conducted at Hospital Universiti Sains Malaysia, which reported a 12.4% alloimmunization rate among transfused CKD patients[11]. In contrast, the alloantibody rate among thalassemia patients in this study was lower than that reported in another local study (10.59%)[12]. Previous literature has shown considerable variation in alloimmunization rates among thalassemia patients (ranging from 2.9% to 37%) due to clinical, methodological, and statistical heterogeneity across studies[13].

In addition to recipient-related factors such as demographic characteristics and immune status, ethnicity and antigenic disparities between donor and recipient populations have been shown to influence alloimmunization rates. Lower rates are typically observed in populations with a high degree of donor-recipient phenotypic homogeneity, while higher rates are reported in settings with ethnic or racial diversity[14,15]. Interestingly, in Malaysia, despite its multi-ethnic population, the most common RBC phenotypes across ethnic groups are DCe/DCe (R1R1), Jk (a+b+), Fy (a+b-), MNss, and kk. However, the second most common Rh phenotype differs by ethnicity; for instance, DCe/dce (R1r) is more frequent among Malays and Indians, while DCe/DcE (R1R2) is more common among Chinese and Kadazan individuals[16].

Consistent with previous studies in Kelantan, Malaysia, and India[5,11], most multi-transfused patients in our cohort developed a single alloantibody rather than multiple. Among all RBC alloantibodies identified, 45.3% were directed against antigens in the Rh blood group system, a trend also observed in many other studies[17-19]. Anti-E was the most frequently detected single alloantibody, consistent with the predominance of the R1R1 phenotype in the Malaysian population. Previous studies have reported an anti-E prevalence ranging from 36.4% to 42.9%[12,20,21], supporting the high immunogenicity of the E antigen in this population.

Anti-Mia was the second most frequently detected single alloantibody in this study. This finding is consistent with previous reports involving Chinese populations in Southeast and East Asian countries, including Malaysia, where the frequency of anti-Mia has been reported to range between 26.4% and 55.02%[20,22]. The Mia antigen is associated with six glycophorin variants: GP. Mur, GP. Bun, GP. HF, GP. Hop, GP. Hut, and GP. Vw. A study conducted in Taiwan reported that, among 78,327 blood donors, 4.71% were GP.Mur, 0.025% were GP.Hut, and 0.022% were GP.Vw. Overall, 4.66% (67,348/1,444,541) of donors were found to be Mi(a+)[23]. Similarly, a study

Table 5 Association between patients' demographic and clinical characteristics and the development of red blood cell alloantibodies by simple logistic regression (n=348)

Variables	Regression Coefficient (b)	Crude OR (95% CI)	Wald statistics	p-value
Age (years)				
0–20	0	1		
21–40	0.314	1.324 (0.625, 2.691)	1.237	0.287
41–60	0.487	1.087 (0.351, 3.105)	1.199	0.438
>60	0.633	0.861 (0.843, 1.297)	0.474	0.502
Sex				
Male	0	1		
Female	0.794	1.659 (0.593, 4.828)	1.341	0.396
Ethnicity				
Malay	0	1		
Chinese	1.812	2.554 (1.015, 14.436)	3.558	0.042**
Indian	0.943	1.229 (0.816, 13.164)	3.062	0.059*
Others	0.227	1.113 (0.432, 4.791)	1.186	0.243*
Blood group				
O	0	1		
A	0.462	1.587 (0.909, 2.770)	2.644	0.104*
B	0.559	1.749 (1.032, 2.964)	4.315	0.038**
AB	0.970	2.638 (1.124, 6.193)	4.968	0.026**
Transfusion frequency				
>1 unit/month	0	1		
>10 units/3 months	0.967	1.812 (0.892, 1.938)	1.727	0.057*
Clinical diagnosis				
Others	0	1		
Thalassemia	1.174	3.832 (1.633, 12.514)	4.426	0.024**
Hematological disorders	−0.435	0.547 (0.361, 1.627)	1.318	0.119
Chronic kidney disease	2.826	6.129 (3.405, 8.078)	11.825	<0.001**

CI=confidence intervals, OR=odds ratio, *p-value<0.250, **significant at p-value<0.05

from a university hospital in Malaysia reported a low overall prevalence of the Mia antigen (2.6%; 18/694), with the highest frequency among Chinese donors (4.7%; 11/233), followed by Malays (1.7%; 6/347) and Indians (0.9%; 1/114)[22]. Although relatively rare, anti-Mia has been implicated in severe hemolytic disease of the fetus and newborn (HDFN), requiring exchange transfusion and fatal intravascular hemolytic transfusion reaction[24,25].

In our study, the frequency of RBC alloantibodies was similar between male and female patients, with no significant association between sex and RBC alloantibody development. This finding aligns with some studies[10,15,17]. On the contrary, earlier studies described female sex as an independent risk factor for RBC alloimmunization. For example, women over 45 years of age have been shown to have an 80% higher risk of developing post-transfusion RBC alloantibodies, possibly due to increased antigenic exposure through pregnancy and transfusion[26].

Regarding transfusion frequency, previous studies have reported mixed findings. Some demonstrated a significantly increased risk of alloimmunization in patients receiving ten or more units of PRBC[27]; conversely, others found no significant association between alloantibody development and the number of units transfused[28]. Our study did not demonstrate a significant association between transfusion frequency and alloantibody development. This result may be attributable to the heterogeneity of our patient cohort, which included individuals with trauma and

Table 6 Association between patients' demographic and clinical characteristics and the development of red blood cell alloantibodies by multiple logistic regression (n=348)

Variables	Regression Coefficient (b)	Adjusted OR (95% CI)	Wald statistics	p-value
Ethnicity				
Malay	0	1		
Chinese	1.897	5.793 (0.813, 8.270)	3.054	0.087
Indian	1.126	2.561 (0.134, 6.583)	1.406	0.264
Others	0.731	1.086 (0.491, 5.472)	1.285	0.402
Blood Group				
O	0	1		
A	0.381	1.154 (0.405, 1.147)	1.302	0.468
B	0.670	1.827 (0.729, 2.453)	1.659	0.153
AB	0.892	2.358 (0.831, 3.505)	2.806	0.091
Transfusion frequency				
>1 unit/month	0	1		
>10 units/3 months	0.316	0.495 (0.713, 1.928)	2.792	0.119
Clinical diagnosis				
Others	0	1		
Thalassemia	1.131	2.127 (1.221, 10.309)	2.358	0.032*
Hematological disorders	−0.315	0.538 (0.543, 1.396)	0.862	0.421
Chronic kidney disease	2.416	5.623 (3.461, 6.327)	9.735	<0.001*

*significant at p-value<0.05, The multicollinearity test was checked and not found, The Hosmer-Lemeshow test (p-value=0.516) and classification table (correctly classified percentage=88.1%) were applied to check model fitness, CI=confidence intervals, OR=odds ratio

active bleeding who were more likely to receive multiple units of PRBCs within a one-month period. Prior studies have suggested that systemic inflammatory states, such as polytrauma or major surgical interventions, may enhance immune recognition of foreign RBC antigens, thereby increasing the risk of alloimmunization[29].

Our study indicated that patients with CKD had a 5.6-fold increased risk of developing RBC alloantibodies compared to patients with other clinical diagnoses. This finding may be attributed to the transfusion policy at our center, where PRBC units for CKD patients are matched only for ABO and RhD antigens. Previous studies have established that limiting antigen matching to only ABO and RhD significantly increases the risk of RBC alloimmunization[30].

We also observed a significant association between thalassemia and RBC alloantibody development. At our center, thalassemia patients routinely receive extended RBC antigen matching for DCcEe, Kidd, Duffy, and Kell antigens, which likely reduces the risk of alloimmunization. When donor and recipient RBC antigen profiles are closely matched, particularly for non-ABO systems, alloantibody development is significantly reduced[31].

Preventing RBC alloantibody formation remains a critical goal in transfusion-dependent populations. The most effective preventive strategy is antigen avoidance, ideally achieved through phenotypic or genotypic matching of donor and recipient RBC antigens. However, even comprehensive matching may not completely eliminate alloimmunization risk[32,33]. Moreover, implementing such strategies poses substantial financial and logistical challenges, particularly in resource-limited blood bank settings. Thus, transfusion policies must strike a balance between clinical benefit and cost-effectiveness, using risk stratification to guide extended antigen matching. For instance, maintaining a hemoglobin level above 9 g/dL in thalassemia patients helps suppress

ineffective erythropoiesis, prevent hepatosplenomegaly, and reduce marrow expansion[34]. In other multi-transfused populations, patient blood management (PBM) strategies are key to minimizing allogeneic transfusion and, consequently, the risk of alloimmunization[35].

Despite the strengths of this study, several limitations must be acknowledged. First, this study was retrospective in nature, relying on a single time-point assessment of RBC alloantibody prevalence. Second, it was conducted in a single center, which may limit the generalizability of the findings. Additionally, although systematic random sampling was used to reduce selection bias, the sample size was smaller than that of several comparable studies. Moreover, the use of systematic random sampling in this study may have contributed to an uneven distribution of multi-transfused patients with and without RBC alloantibodies. Furthermore, the high variability of diagnoses among the included patients, such as trauma and bleeding cases, might have introduced residual confounding despite the use of systematic random sampling, thereby potentially attenuating or obscuring associations between transfusion-related factors and outcomes. Therefore, future research should include larger, prospective, multicenter studies to better understand the dynamics of alloimmunization in diverse patient populations.

Conclusion

Our study highlights a high prevalence of RBC alloantibodies among multi-transfused patients, particularly those with CKD and thalassemia. These findings emphasize the importance of implementing extended RBC antigen matching, especially for antigens within the Rh system (DCcEe), alongside judicious blood utilization and patient blood management (PBM) strategies. Such measures are critical to reducing the risk of alloimmunization and improving transfusion outcomes in high-risk patient populations.

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Conflict of interest

The authors declare no conflict of interest.

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Supplementary Table 1 Types of treatment in hematological disorders patients (n=84)

Types of treatment	RBC alloantibodies, n (%)		Total (n=84)
	Absence (n=59)	Presence (n=25)	
Chemotherapy/immunosuppressant	47 (79.7)	9 (36.0)	56 (66.7)
Not on chemotherapy/immunosuppressant	12 (20.3)	16 (64.0)	28 (33.3)
Transplantation	0 (0)	0 (0)	0 (0)

RBC=red blood cell