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The Analysis of Antibody Distribution and Factors Influencing the Efficacy of Blood Transfusion in Patients with Unexpected Red Cell Blood Antibodies

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Abstract: *Objective:* To analyze the distribution of unexpected red blood cell (RBC) antibodies and explore the factors that influence transfusion efficacy in patients with positive unexpected antibodies. *Methods:* A retrospective review of 161 cases of positive unexpected antibodies recorded in our hospital from January 2022 to December 2025 was performed. Clinical data were collected. We first analyzed the proportion of various types of unexpected RBC antibodies. Patients were divided into two groups, including an ineffective group and an effective group (partially effective, effective), according to transfusion efficacy, and the influencing factors that led to the ineffectiveness of RBC transfusion were explored. *Results:* Unexpected antibodies accounted for 77.02%, with Rh blood group system antibodies (34.78%) being the most common, followed by MNS blood group system antibodies (28.57%); anti-E (31.06%) accounted for the highest proportion of monospecific antibodies, followed by anti-M (18.63%). Ineffective RBC transfusion accounted for 12.10%. The average transfusion of 1 U RBCs per kilogram of body weight increased the median hemoglobin to 5.65 g/L. The therapeutic effect of RBC transfusion was related to the patient's gender, presence of Rh blood group system antibodies, autoimmune history, pregnancy history, immune/targeted drug use history, and tumor history ($p < 0.05$). Rh blood group system antibodies and pre-transfusion hemoglobin levels are independent risk factors for ineffective RBC transfusion. The risk of ineffective RBC transfusion in patients with Rh blood group system antibodies is 7.68 times that of patients without (OR: 7.68, 95% CI: 1.54–38.31); the risk of patients with pre-transfusion hemoglobin between 40 g/L and 70 g/L (excluding 40 g/L) causing ineffective transfusion is 0.03 times that of ≤ 40 g/L (OR: 0.03, 95% CI: 0.00–0.33) (OR: 0.03, 95% CI: 0.01–0.15), the risk of pre-transfusion hemoglobin > 70 g/L causing ineffective transfusion is 0.16 times that of ≤ 40 g/L (OR: 0.16, 95% CI: 0.03–0.98). *Conclusions:* Rh blood group system antibodies are the most common, and anti-E is the most common monospecific antibody. Unexpected antibodies affect the efficacy of RBC transfusion, and Rh blood group system antibodies are an important risk factor for ineffective RBC transfusion. The hemoglobin level before transfusion is also a factor that affects the efficacy of RBC transfusion.

Keywords: Unexpected antibodies; Antibody identification; Red blood cell transfusion; Blood transfusion efficacy

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1. Introduction

Unexpected antibodies, also known as irregular antibodies, refer to antibodies against blood group systems other than the ABO system (antiA and antiB) [1]. Because unexpected antibodies can lead to ineffective transfusion of red blood cells and even cause acute or delayed hemolytic transfusion reactions and hemolytic disease of the newborn, it is clinically necessary to further identify the antibody type in patients with positive antibody screening results and to precisely match antigennegative red blood cells for transfusion based on the detected monospecific antibodies [2–4]. The distribution of unexpected antibodies exhibits regional and population differences [5]. In practice, there are frequently cases of emergency blood transfusion where antibody screening is positive, but there is insufficient time to send for monospecific antibody identification, or cases where the specificity of the unexpected antibody is unclear or difficult to identify; in such situations, transfusion can only be performed via crossmatchcompatible blind matching. Therefore, clarifying the local distribution of unexpected antibodies is helpful in improving the success rate of blind crossmatch compatibility. Moreover, whether the presence of unexpected antibodies affects the transfusion efficacy of red blood cells in matched individuals remains an issue that warrants further investigation. This retrospective study analyzed the distribution of unexpected blood group antibodies in our hospital and explored other factors influencing transfusion efficacy in patients with positive unexpected antibodies.

2. Materials and methods

2.1. Study subjects

Patients who applied for surgical blood preparation and therapeutic transfusion in our hospital from January 2022 to December 2025 were enrolled. A 5mL sample of EDTAK2 anticoagulated whole blood was collected from each patient for unexpected antibody screening. Patients with positive screening results underwent monospecific antibody identification and red blood cell crossmatching.

2.2. Reagents and instruments

The following equipment and reagents were used: automated blood group analyzer (Blozer 120), cardtype medical centrifuge (LB-3000), reagent card incubator (LB-CO2-1), antihuman globulin (anti-IgG + C3d) test cards (all from Jiangsu Libo), and antibody screening cell kits (I, II, III) (from Shanghai Blood Biological Medicine). All reagents met quality control and internal quality control requirements and were used within their expiration dates.

2.3. Methods

Unexpected antibody screening was performed for each patient specimen using the automated blood group analyzer combined with manual methods. Specimens with positive screening results were sent to the Guangzhou Blood Center for further antibody identification to determine the type and specificity of the antibodies, and compatible red blood cell components were then transfused. Clinical data of patients with positive antibody screening were collected, including sex, age, blood type, antibody type, diagnosis, transfusion history, pregnancy history, history of autoimmune diseases, history of immune/targeted drug use, height, weight, transfusion volume, and hemoglobin (Hb) levels before and after transfusion (within 24 hours posttransfusion).

2.3.1. Evaluation of posttransfusion efficacy

The efficacy of red blood cell transfusion was objectively assessed by the increase in Hb level within 24

hours after transfusion. The specific method was based on the calculation of Hb increment per unit of red blood cells transfused (g/L) × 60 / patient body weight (kg) [1]. An increase of ≥ 5 g/L per unit was defined as effective transfusion, an increase of 0–5 g/L as partially effective, and an increase of ≤ 0 g/L as ineffective.

2.4. Statistical analysis

Data were processed using SPSS 26.0 software. Normally distributed continuous variables were expressed as mean ± SD; categorical variables were expressed as n (%) and compared using the chisquare test; nonnormally distributed continuous variables were expressed as median. Logistic regression analysis was used to identify factors influencing ineffective red blood cell transfusion in patients with positive unexpected antibodies. A *p-value* < 0.05 was considered statistically significant.

3. Results

3.1. Basic characteristics of patients with positive unexpected antibodies

A total of 75,307 patients in our hospital underwent unexpected antibody screening, and only 238 were ultimately confirmed to have specific antibodies, yielding a positivity rate of 0.32% (238/75,307).

Among the 161 patients with positive antibody screening (after excluding 77 duplicate cases), the mean age was 53.06 ± 15.40 years; females (63.35%) outnumbered males; blood type distribution was O (40.99%) > B (26.71%) > A (24.84%) > AB; 34.16% had a history of transfusion, 56.52% had a history of pregnancy, 50.31% had a history of immune/targeted drug use, and 57.76% had a history of malignancy.

3.2. Distribution of unexpected antibodies

A single antibody was detected in 77.02% (124/161) of cases, while two or more antibodies were concurrently present in 22.98% (37/161). Antibodies of the Rh blood group system were the most common, accounting for 34.78% (56/161), followed by MNS (28.57%), Lewis (9.32%), I (4.35%), Kidd and Diego (1.86% each), and Cromer, H, and P (0.62% each). Among nonspecific antibodies, autoantibodies accounted for 23.60% and druginduced antibodies for 5.59%. See **Figure 1**.

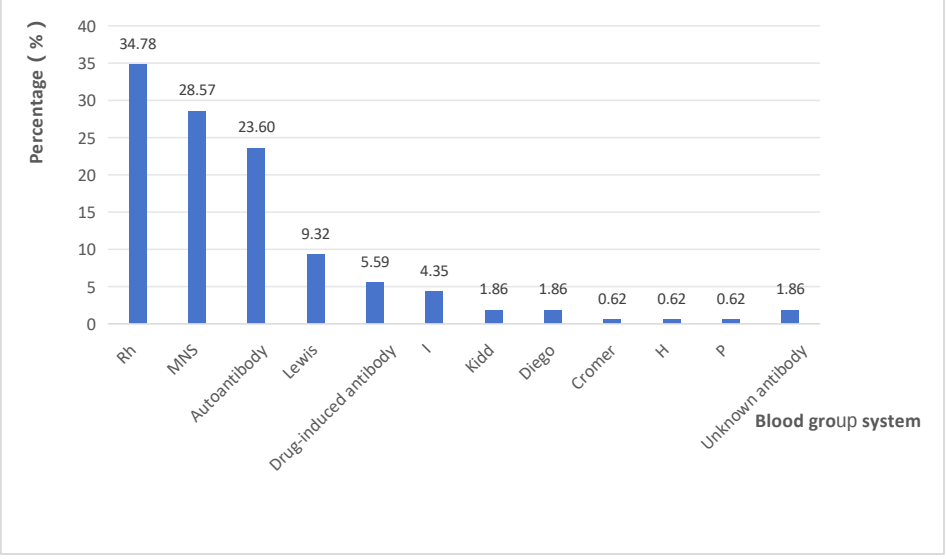

Figure 1. Distribution of unexpected antibody blood group systems.

3.2.1. Monospecific antibody distribution

Among monospecific antibodies, anti-E accounted for the highest proportion (31.06%), followed by anti-M (18.63%), anti-c, anti-Mia and anti-Lea (9.32% each), anti-I (4.35%), anti-D (2.48%), anti-C and anti-e (1.86% each), anti-Jkb and anti-Dia (1.24% each), and anti-S, anti-Leb, anti-Jka, anti-Dib, anti-Cromer, anti -H, and anti-P1 (0.62% each). See **Figure 2**.

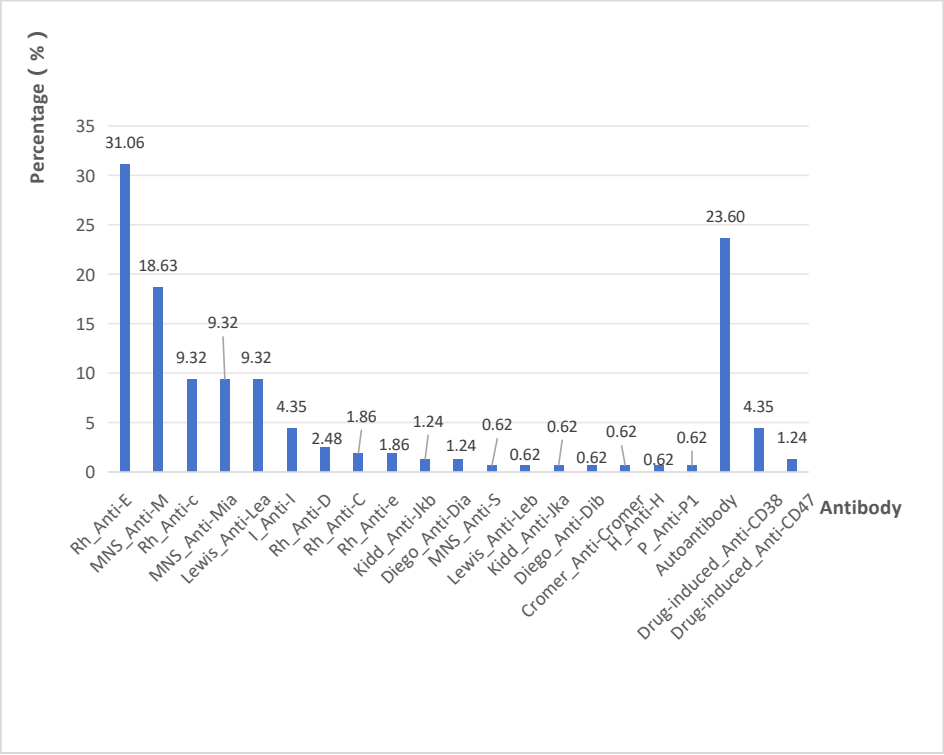

Figure 2. Distribution of unexpected antibodies.

3.3. Erythrocyte transfusion efficacy in patients with unexpected antibody positivity

Among the 140 transfusion cases with unexpected antibody positivity, 14 patients were not reexamined within 24 h after transfusion; therefore, only 124 cases (after excluding 2 patients with unknown antibody status) were included in the posttransfusion efficacy analysis. The transfusion treatment was effective in 54.03% (67/124), partially effective in 33.87% (42/124), and ineffective in 12.10% (15/124). The median hemoglobin increment per 1 unit of red blood cells transfused per kilogram of body weight was 5.65 g/L.

The efficacy of transfusion treatment was significantly associated with patient sex, presence of Rh blood group antibodies, history of autoimmune disease, history of pregnancy, history of immunotherapy/targeted drug use, and history of malignancy (all $p < 0.05$). No significant correlations were found with patient age, blood type, antibody type (single vs. multiple), MNS blood group antibodies, transfusion history, history of metastasis, or concurrent infection (all $p > 0.05$). See **Table 1**.

Table 1. Analysis of factors influencing the efficacy of red blood cell transfusion in patients with unexpected antibody

		Number of cases with therapeutic effect of red blood cell transfusion (n)/composition ratio (%)			Total (n)	χ^2	*p*
		Effective (n/%)	Partially effective (n/%)	Ineffective (n/%)			
Gender	Male	26 (41.94)	31 (50.00)	5 (8.06)	62	14.55	0.001 < 0.05*
	Female	41 (66.13)	11 (17.74)	10 (16.13)	62		
Age	≤ 30 years	9 (75.00)	3 (25.00)	0 (0.00)	12	0.26	0.611 > 0.05
	31–60 years	29 (46.03)	26 (41.27)	8 (12.70)	63		
	≥ 61 years	29 (59.18)	13 (26.53)	7 (14.29)	49		
Blood type	A	23 (76.67)	3 (10.00)	4 (13.33)	30	0.97	0.324 > 0.05
	B	13 (44.83)	10 (34.48)	6 (20.69)	29		
	O	25 (43.9)	29 (50.9)	3 (5.3)	57		
	AB	6 (75.0)	0 (0.0)	2 (25.0)	8		
Antibody type	Single type	49 (57.65)	26 (30.59)	10 (11.76)	85	0.86	0.354 > 0.05
	Multiple types	18 (46.15)	16 (41.03)	5 (12.82)	39		
Rh blood group antibody	Absent	43 (63.24)	13 (19.12)	12 (17.65)	68	15.87	0.000 < 0.05*
	Present	24 (42.86)	29 (51.79)	3 (5.36)	56		
MNS blood group antibody	Absent	58 (53.70)	40 (37.04)	10 (9.26)	108	1.08	0.299 > 0.05
	Present	9 (56.25)	2 (12.50)	5 (31.25)	16		
History of autoimmune disease	Present	24 (75.00)	5 (15.63)	3 (9.38)	32	4.95	0.026 < 0.05*
	Absent	43 (46.74)	37 (40.22)	12 (13.04)	92		
History of transfusion	Present	50 (53.19)	36 (38.30)	8 (8.51)	94	1.30	0.255 > 0.05
	Absent	14 (53.85)	5 (19.23)	7 (26.92)	26		
History of pregnancy	Present	37 (62.71)	11 (18.64)	11 (18.64)	59	13.26	0.001 < 0.05*
	Absent	30 (46.15)	31 (47.69)	4 (6.15)	65		
History of immunotherapy/ targeted drug use	Present	45 (63.38)	15 (21.13)	11 (15.49)	71	12.24	0.002 < 0.05*
	Absent	22 (41.51)	27 (50.94)	4 (7.55)	53		
History of tumor	Present	29 (63.04)	7 (15.22)	10 (21.74)	46	14.23	0.001 < 0.05*
	Absent	38 (48.72)	35 (44.87)	5 (6.41)	78		
Tumor with metastasis	Present	13 (61.90)	2 (9.52)	6 (28.57)	21	0.38	0.536 > 0.05
	Absent	54 (52.43)	40 (38.83)	9 (8.74)	103		
Comorbid infection	Present	28 (48.28)	25 (43.10)	5 (8.62)	58	4.50	0.105 > 0.05
	Absent	39 (59.09)	17 (25.76)	10 (15.15)	66		

Note: * indicates $p < 0.05$, indicating a statistically significant difference.

3.4. Logistic regression analysis of factors influencing ineffective red blood cell transfusion in patients with unexpected positive antibodies

3.4.1. Univariate analysis

Univariate logistic regression analysis was performed to evaluate the effects of sex, age, blood type, antibody blood group type (including type, Rh blood group antibodies, MNS blood group antibodies, Lewis blood group antibodies, and autoantibodies), history of autoimmune disease, transfusion history, pregnancy history,

history of immunotherapy/targeted drug use, disease status (tumor history, tumor with metastasis, and concurrent infection), and pretransfusion hemoglobin level on ineffective red blood cell transfusion.

The results showed that blood type (type O), antibody blood group system (Rh blood group antibodies, MNS blood group antibodies, and Lewis blood group antibodies), pregnancy history, tumor history, and pretransfusion hemoglobin level all had statistically significant effects on ineffective red blood cell transfusion ($p < 0.05$), as presented in **Table 2**.

Table 2. Univariate analysis of ineffective red blood cell transfusion in patients with unexpected positive antibodies

		Therapeutic effect after blood transfusion			chi-square test		Univariate logistic regression	
		Total (n)	Ineffective (n)	Ineffective proportion(%)	χ^2	*p*	OR (95% CI)	*p*
Blood type	Non-O	67	12	17.91	4.63	0.031 < 0.05*	Ref.	
	O	57	3	5.26			3.93 (1.05–14.70)	0.042 < 0.05*
Blood group antibody	Non-Rh	68	12	17.65	4.36	0.037 < 0.05*	Ref.	
	Rh	56	3	5.36			3.79 (1.01–14.17)	0.048 < 0.05*
	Non-MNS	108	10	9.26%	6.29	0.012 < 0.05*	Ref	
	MNS	16	5	31.25%			0.224 (0.07–0.78)	0.018 < 0.05*
	Non-Lewis	121	13	10.74	8.54	0.003 < 0.05*	Ref.	
	Lewis	3	2	66.67			0.06 (0.01–0.71)	0.026 < 0.05*
Pregnancy history	No	65	4	6.15	4.54	0.033 < 0.05*	Ref.	
	Yes	59	11	18.64			0.29 (0.09–0.96)	0.042 < 0.05*
Disease status	No tumor	78	5	6.41	6.39	0.011 < 0.05*	Ref.	
	Tumor	46	10	21.74			0.25 (0.08–0.78)	0.017 < 0.05*
	No metastasis	103	9	8.74	6.40	0.011 < 0.05*	Ref.	
	With metastasis	21	6	28.57			0.24 (0.07–0.77)	0.016 < 0.05*
Pretransfusion Hb (g/L)	≤ 40	20	1	5.00	20.79	0.000 < 0.05*	Ref.	
	> 40 and ≤ 60	71	3	4.23			0.05 (0.01–0.49)	0.01 < 0.05*
	> 60 and ≤ 70	17	3	17.65			0.04 (0.01–0.20)	0.000 < 0.05*
	> 70	16	8	50.00			0.21 (0.04–1.05)	0.057 > 0.05

Note: * indicates $p < 0.05$, and the difference is statistically significant.

3.4.2. Multivariable analysis

The statistically significant variables from the above univariate logistic regression analysis (blood type O, Rh blood group antibodies, MNS blood group antibodies, Lewis blood group antibodies, history of

pregnancy, history of tumor, history of tumor metastasis, and pretransfusion hemoglobin [Hb]) were included in a multivariable logistic regression model. The results showed that only Rh blood group antibodies and pretransfusion Hb level were independent risk factors for ineffective red blood cell transfusion. Patients with Rh blood group system antibodies had a 7.68fold higher risk of ineffective transfusion compared with those without Rh antibodies (OR: 7.68, 95% CI: 1.54–38.31). Compared with patients with pretransfusion Hb ≤ 40 g/L, the risk of ineffective transfusion was 0.03fold for those with Hb > 40 g/L and ≤ 60 g/L (OR: 0.03, 95% CI: 0.00–0.33), 0.03fold for those with Hb > 60 g/L and ≤ 70 g/L (OR: 0.03, 95% CI: 0.01–0.15), and 0.16fold for those with Hb > 70 g/L (OR: 0.16, 95% CI: 0.03–0.98). See **Table 3**.

Table 3. Multivariable logistic regression analysis of ineffective red blood cell transfusion in patients with unexpected antibodies

		Therapeutic effect after blood transfusion			Multivariate Logistic Regression	
		Total (n)	**Ineffective (n)**	**Ineffective proportion (%)**	**OR (95% CI)**	***p***
Blood group antibody	NonRh	68	12	17.65	Ref.	
	Rh	56	3	5.36	7.68 (1.54–38.31)	0.013 < 0.05*
Pretransfusion Hb (g/L)	≤ 40	20	1	5.00	Ref.	
	> 40, ≤ 60	71	3	4.23	0.03 (0.00–0.33)	0.004 < 0.05*
	> 60, ≤ 70	17	3	17.65	0.03 (0.01–0.15)	0.000 < 0.05*
	> 70	16	8	50.00	0.16 (0.03–0.98)	0.047 < 0.05*

Note: * indicates $p < 0.05$, and the difference is statistically significant.

4. Discussion

The production of unexpected antibodies refers to antibodies stimulated by transfusion, pregnancy, drugs, and other factors, mainly including specific antibodies, nonspecific antibodies (autoantibodies and druginduced antibodies), and antibodies of undetermined specificity (unknown antibodies) [6]. Studies have shown that the distribution of unexpected antibodies is related to population and regional differences [5]. The positive rate of unexpected antibody screening in our hospital was 0.32%, which lies between the 0.20% reported by Guo Weijie et al. and the 0.47% reported by Song Aowei et al., possibly due to the specific patient population and disease spectrum in our hospital, with high proportions of females (63.35%), patients with tumors (57.76%), and those receiving immunotherapy/targeted therapy (50.31%) [5,7].

In this study, 77.02% of patients had only a single type of unexpected antibody, while approximately 22.98% had two or more types simultaneously. Specific antibodies accounted for 82.60%, with antibodies of the Rh blood group system (34.78%) being the most common, and antiE was the most prevalent antibody among all blood group system antibodies (31.06%), which is consistent with most studies [6,8]. The ranking of specific antibody blood group systems in our hospitalized patients was Rh > MNS > Lewis > I > Kidd, Diego > Cromer, H, P, involving a total of 9 blood group systems, with the top three systems accounting for 72.67%. The ranking of singlespecificity antibodies was anti-E > anti-M > anti-c, anti-Lea, anti-Mia > anti-I > anti-D > anti-C, anti-e > anti-Jkb, anti-Dia > anti-S, anti-Leb, anti-Jka, anti-Dib, anti-Cromer, anti-H, anti-P1, and the top three antibodies accounted for 77.65%. Nonspecific antibodies accounted for 29.19 %, among which druginduced antibodies accounted for 5.59%, specifically anti-CD38 and anti-CD47, mainly because 57.76% of patients with unexpected antibody positivity in our hospital had a history of tumors, and up to 50.31% had received immunotherapy/targeted therapy. Nonspecific autoantibodies for 23.

60%; autoantibodies are antibodies produced against selfantigens, or antibodies generated after foreign antigens combine with certain components in the body, which can cause autoimmune diseases or even destroy the patient's own and/or transfused red blood cells, leading to ineffective transfusion [1,9].

In this study, among the 124 patients with unexpected antibody positivity who received transfusions, 54.03% showed effective transfusion therapy, 33.87% showed partial effectiveness, and 12.10% showed ineffectiveness. The median increase in hemoglobin per 1 unit of red blood cells transfused per kilogram of body weight was 5.65 g/L, which met the expected therapeutic efficacy [1], and no transfusionrelated adverse reactions occurred.

All patients with unexpected antibody positivity in our hospital underwent single-specificity antibody identification first, and red blood cell units were matched according to the antibody results for transfusion. Nevertheless, 12.10% of patients still had ineffective transfusions, suggesting that other factors might influence the transfusion efficacy in patients with unexpected antibodies. Our results showed that the transfusion efficacy (effective, partially effective, ineffective) in patients with unexpected antibodies was associated with sex, presence of Rh blood group antibodies, history of autoimmune disease, history of pregnancy, history of immunotherapy/targeted therapy use, and history of tumors. Previous studies have indicated that transfusion efficacy is related to sex, pregnancy history, and tumor history, possibly because females, patients with pregnancy history, autoimmune diseases, and those on long-term immunotherapy/targeted agents can cause sensitization of their own red blood cells or produce other antibodies, more readily triggering immune responses that affect transfusion outcomes [10–12]. To further explore the risk factors for ineffective red blood cell transfusion, we first performed univariate logistic regression analysis, which showed that blood type, type of blood group antibodies, pregnancy history, tumor history, and pre-transfusion hemoglobin level were statistically significant factors affecting transfusion ineffectiveness. Subsequent multivariable logistic regression analysis revealed that only Rh blood group antibodies and pre-transfusion Hb level were independent risk factors for ineffective red blood cell transfusion. In clinical practice, according to relevant technical specifications in China, before red blood cell transfusion, only ABO blood grouping (forward and reverse typing) and Rh(D) typing of both recipient and donor samples are routinely rechecked, and cross-matching is performed for compatible transfusion [13]. Therefore, there is a possibility that individuals negative for other blood group antigens (e.g., Rh, MNS, Lewis) may, after blind-matched transfusion of antigen-positive red blood cells, produce corresponding antibodies. Even if subsequent transfusions are matched according to single-specificity antibody identification results, the blood group antibodies already present in the recipient can still affect transfusion efficacy through a series of immune reactions [14]. In this study, Rh blood group system antibodies were the most common, and patients with Rh blood group system antibodies had a 7.68-fold higher risk of ineffective transfusion than those without Rh blood group system antibodies (OR: 7.68, 95% CI: 1.54–38.31). Therefore, well-equipped medical institutions should routinely identify other Rh antigens (E, e, C, c) in both recipients and donors to avoid the production of Rh blood group system antibodies and reduce ineffective red blood cell transfusions.

The AABB guidelines recommend a restrictive red blood cell transfusion threshold (Hb 70 g/L) for hemodynamically stable adults [15]. Domestic guidelines recommend that for internal medicine patients with 60 g/L ≤ Hb < 100 g/L, or surgical patients with 70 g/L ≤ Hb < 100 g/L, transfusion should be considered based on clinical symptoms and signs, with avoidance of transfusion whenever possible; for internal

medicine patients with Hb < 60 g/L or surgical patients with Hb < 70 g/L accompanied by obvious anemia symptoms, red blood cell transfusion should be given[16]. Our study showed that in patients with unexpected antibodies, pretransfusion Hb > 40 g/L and ≤ 70 g/L was associated with a 0.03fold risk of ineffective transfusion compared with Hb ≤ 40 g/L (OR: 0.03, 95% CI: 0.00–0.33; OR: 0.03, 95% CI: 0.01–0.15), and pretransfusion Hb > 70 g/L was associated with a 0.16fold risk (OR: 0.16, 95% CI: 0.03–0.98). Therefore, transfusion at Hb > 70 g/L in patients with unexpected antibodies significantly increases the risk of ineffective red blood cell transfusion .

5. Conclusion

In summary, Rh blood group system antibodies are the most common unexpected antibodies, with anti -E being the highest proportion among singlespecificity antibodies. Factors affecting the transfusion efficacy in patients with unexpected antibodies include sex, presence of Rh antibodies, history of autoimmune disease, pregnancy history, history of immunotherapy/targeted therapy use, and tumor history, among which Rh blood group system antibodies are also an important risk factor for transfusion ineffectiveness. Therefore, it is recommended that wellequipped medical institutions include all D, E, e, C, and c antigens of the Rh blood group system in both recipients and donors for routine testing, so as to avoid transfusing antigenpositive red blood cells to antigennegative individuals as much as possible, thereby reducing the production of Rh blood group system antibodies and the resulting ineffective red blood cell transfusions. For patients with unexpected antibodies and Hb > 70 g/L, transfusion indications should be strictly assessed, and red blood cell transfusions should be avoided whenever possible.

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Disclosure statement

The authors declare no conflict of interest.

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