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Situation analysis and blood transfusion strategy of Lewis antibodies in Hunan Province

Xiaoxiang Wei[a,b], Fengxia Liu[c], Dong Ran[d], Ping Yin[c], Lin Qu[a]*

[a]*Department of Medicine Clinical Laboratory, Xiangya Third Hospital, Central South University, Changsha, China*
[b]*Department of Blood Transfusion, The First People's Hospital, Nanning, China*
[c]*Department of Blood Transfusion, Xiangya Third Hospital, Central South University, Changsha, China*
[d]*Department of Medicine Clinical Laboratory, The Eighth People's Hospital, Nanning, China*

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Abstract
Background: Detection rate, serological characteristics, and clinical data of patients with Lewis blood group antibodies in Hunan Province were analyzed through retrospective analysis. This was undertaken in order to optimize the detection methods and blood transfusion strategies of these patients.
Methods: Blood typing, antibody screening, and cross-matching were performed by microcolumn gel, and Lewis antigen was detected by immediate spin test, antibody identification of positive and negative ABO samples, positive antibody screening, and cross-blood mismatch samples. Antibodies were identified by immediate spin test and microcolumn gel antiglobulin method, and the clinical data of the patients with Lewis antibody characteristics were analyzed.
Results: A total of 74 samples (15.91%) with Lewis antibodies were detected from 465 positive samples; cases were distributed in different cities of Hunan Province, with Changsha city being the most frequent (28%) one, with mostly non-O (66), anti-Le^a (31; 41.89%), anti-Le^a+anti-Le^b (23; 31.08%), anti-Le^b (5; 6.76%), anti-Le^{bH} and anti-Le^a+anti-Le^{bH} (1+4; 6.76%), and antibody types immunoglobulin M (IgM) (51; 68.92%), immunoglobulin G (8; 10.81%), and IgG+IgM (4; 5.41%) cases. Patients included more females (67.57%) than males. The detection rate of gynecological diseases and patients with solid tumors was highest (44.59%). In all cases, the Lewis blood group was Le (a-b-); none of the 15 transfusion patients had hemolytic transfusion reaction.
Conclusion: A variety of experimental methods must be adopted simultaneously to determine specificity and prevent the leakage of Lewis antibodies. The infusion of red blood cells matching with antiglobulin media at 37°C was recommended to ensure safe transfusion for recipients with Lewis antibodies.

***Corresponding author:** Lin Qu, Department of Medicine Clinical Laboratory, Xiangya Third Hospital, Central South University, No.138, Tongzipo Road, Yuelu District, Changsha, Hunan 410013, China. *Email address:* xy3yyqulin@csu.edu.cn

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Introduction

Lewis blood group antibodies, first discovered in 1946, are reactive below 22°C, while rendered inactive or become very weak at 37°C, hence considered of little clinical significance. However, the high titer of Lewis antibodies in the Southeast Asian population may cause serious hemolytic transfusion reactions. Moreover, Xiao-Fei et al. found that the positive rate of Lewis antibodies in the Chinese population with red blood cell (RBC) blood group antibodies is only lower than that of Rhesus factor (Rh) and MNS blood group antibodies.[1-3] In recent years, many domestic literature reports have established that Lewis antibodies are active at 37°C, which causes the occurrence of hemolytic blood transfusion reaction.[4-6] Global literature reports have demonstrated that anti-Le^a and anti-Le^b antibodies can cause acute hemolytic transfusion reactions under 37°C. The common feature of these meaningful Lewis antibodies is that they are active at 37°C and have strong reaction in the direct agglutination test. Some were not found during the pre-transfusion antibody detection test but were found during acute hemolytic transfusion reaction after blood transfusion.[7-9]

Thus, the clinical significance of Lewis antibodies must be valued and deeply explored. In this study, we conducted a retrospective analysis of the Lewis blood group system antibodies of patients detected at the Third Xiangya Hospital of Central South University from 2019 to 2021.

Materials and Methods

Specimen origin

A total of 465 cases of ABO positive definite incompatibility, positive antibody screening, and cross-blood incompatibility were selected from the patients examined during 2019–2021. Lewis blood group system antibodies were detected in 74 patients, including 24 males and 50 females aged 2–97 years, with a median age of 50 years.

Main reagents and instruments

Red blood cell accidental antibody screening kit (Lot.: 202107005) and reverse setting reagent red blood kit (Lot.: 202107016) were purchased from Jiangsu Libo Pharmaceutical Biotechnology Co. Ltd. Jiangyin, China. Microcolumn antiglobulin card (IgG+C3d; Lot.: 20210306), ABO, and rhesus D (RhD) blood type detection card (Lot.: 20210602) were purchased from Changchun Boxun Biotechnology Co. Ltd. Changchun, China; 16 spectrum kit (Lot.: 8000454924) was purchased from Sanquin, Beijing, China. Anti-Le^a (Lot.: 991021) and anti-Le^b (Lot.: 992020) were obtained from Immucor, Norcross, GA, USA. Anti-A and anti-B reagents (Lot.: 20201000704) were purchased from Beijing Jinhao Pharmaceutical Co. Ltd., Beijing, China. Dithiothreitol (DTT; Lot.: N1804230075) was purchased from Dulai, Nanjing, China, and the salivary reagent was self-made. Constant 37°C incubator and card centrifuge were obtained from Changchun Boyan Scientific Instrument Co. Ltd., Changchun, China. The automatic blood type/antibody screening analyzer (Johnson & Johnson, Shanghai, China) and the Kubota, Shanghai, China, centrifuge KA-2200 were used. All test cards and reagents were used within the validity period according to manufacturers' instructions.

Detection method

ABO blood group identification

The ABO and RhD blood groups of the patients were identified by fully automatic microcolumn gel method. The immediate spin test was used to identify antibody groups according to reference instructions for operation method and interpretation standards.

Accidental antibody screening test

Automatic microcolumn gel antiglobulin method was used for accidental antibody screening of patient serum. The agglutating RBCs were more than the volume of the pore allowed to enter the gel mesh and could not pass through the gel layer. These were left on top of or dispersed in gel as positive antibodies; unagglutated free RBCs, which were able to pass through the gel layer because of their small size, were negative and precipitated at the bottom of microtubules. Positive specimens were retested by immediate spin test according to reference instructions for operation method and interpretation standard.

Accidental antibody specificity identification

After performing RBC antibody screening of positive samples, patients' serum samples were confirmed by immediate spin test and microcolumn gel antiglobulin method; if necessary, blood type substance (saliva) neutralization tests were used to identify the specificity of accidental antibodies. The interpretation principle was similar to the ABO blood group identification and the accidental antibody screening test.

Identification of antibody type

After mixing patients' serum samples with an equal amount of 0.01 mol/L DTT, the samples were placed in 37°C constant temperature incubator for 30 min, followed by reacting with spectrum cells in saline (4°C; room temperature 37°C) and microcolumn gel antiglobulin medium.

Identification of Lewis blood group antigen

Immediate spin test was used to identify RBCs of the patients with Lewis antibodies following reagent instructions.

Cross-blood matching test

Patients with positive antibodies were used for cross-blood matching test using immediate spin test and microcolumn gel antiglobulin method. Three samples of main side,

secondary side, and self-control were designed. When all samples were negative using the microcolumn gel antiglobulin method at the condition of beginning and room temperature of 37°C, it was accepted as matching of the blood.

Statistical methods

Descriptive statistics were used for data counting using the SPSS22.0 statistical software. Differences of Lewis antibodies in ABO blood type, antibody type, and disease distribution were analyzed using Chi-square test; $P < 0.05$ was statistically significant, statistics has the significance difference ($P < 0.01$).

Statement of ethics

This study was approved by the Third Xiangya Hospital of Central South University (No. 2021-S307). Written informed consent was obtained from patients to participate in the study.

Results

Detection rate of Lewis antibodies

Among the 465 positive specimens, the detection rate of Lewis antibodies was second only to Rh and MNS system antibodies, with 74 cases (16%) detected (Figure 1).

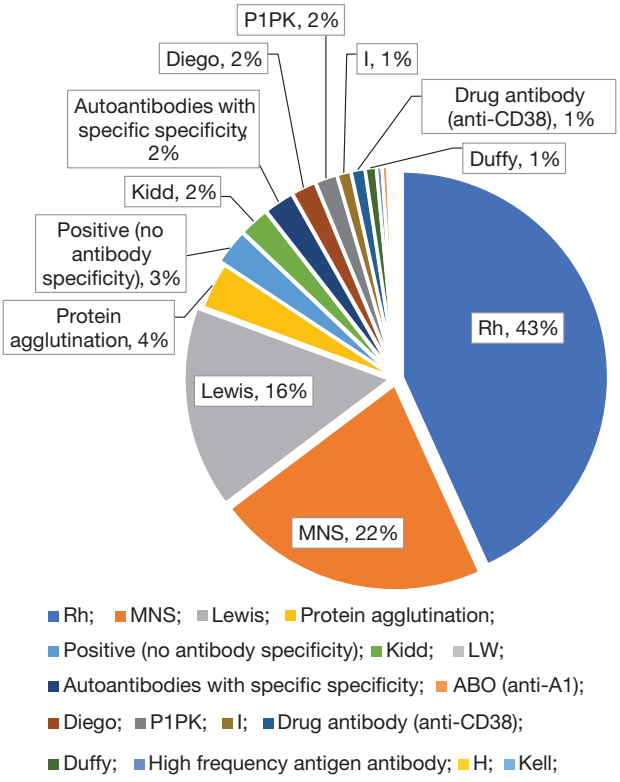

Figure 1 Detection rate of Lewis antibodies in 465 positive specimens.

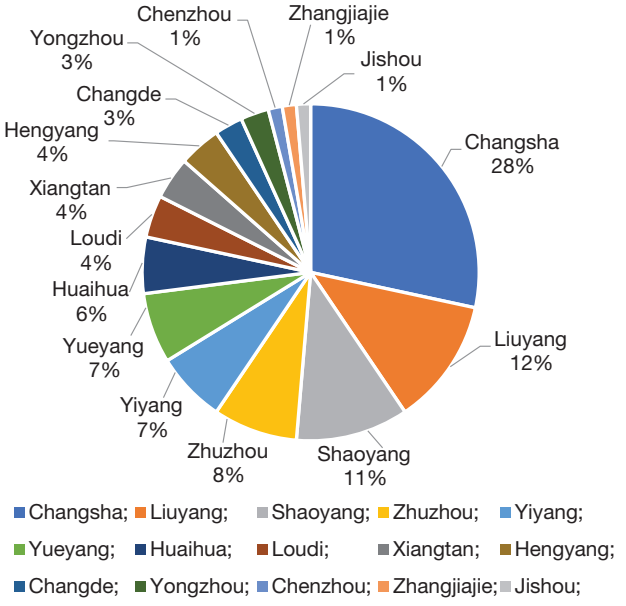

Figure 2 Distribution diagram of 74 positive cases of Lewis antibodies in Hunan Province.

Table 1 Sex comparisons of 74 positive patients of Lewis blood group system antibodies.

	Male	Female
Lewis blood group system antibodies were detected	24 (32.43%)	50 (67.57%)

Distribution of positive Lewis antibodies

The 74 positive cases of Lewis antibodies were distributed in all the cities of Hunan Province, with maximum cases found in Changsha city (28%), followed by Liuyang city. The proportion of cases in each city is shown in Figure 2.

Sex comparison of positive cases of Lewis antibodies

Sex comparison of 74 positive cases of Lewis antibodies is shown in Table 1.

Identification of the type of Lewis antibodies and their distribution

Identification of Lewis antibodies and their distribution in 74 positive patients with ABO blood group demonstrated no difference for the types of Lewis antibodies with common immunoglobulin M (IgM) ($P > 0.05$) and ABO types with common non-O type distribution ($P > 0.05$; Table 2).

Blood group substance (saliva) neutralization test

Blood group substance (saliva) neutralization test for 52 patients suspected to be Lewis antibodies positive

combined with other blood type system antibodies or Lewis antibody pattern was unclear. The result of no reaction or reaction significantly weakened by using blood type substance (saliva) neutralization of its plasma, and identifying with antibody cell reaction (Table 2).

Le (a-b-) antibodies were found in 74 patients of positive Lewis blood group. Not difference ($P > 0.05$) was found for the distribution of Lewis antibodies in the ABO blood type individuals and in patients with different diseases, although Lewis antibodies were commonly found in type A individuals (52.7%; Table 3).

Of the 74 patients with Lewis blood group system antibodies, 15 patients were treated for blood transfusion, of which 11 patients were transfused with a RBC suspension cross-matching by microcolumn gel antiglobulin method at 37°C. Hemoglobin level in nine patients increased after transfusion, but no significant change was found in bilirubin. In addition, the unsatisfied increase in hemoglobin level was found in two patients with active bleeding and liver injury after blood transfusion accompanied by continuous increase in bilirubin, which could be due to original disease (Table 4 and Figure 3). All patients with effective blood transfusion showed no acute and delayed hemolysis.

Discussion

The Lewis blood group system consists of two major antigens, namely, Le^a and Le^b, and four other Lewis antigens represented as Le^{ab}, Le^{bH}, ALe^b, and BLe^b. Three common

Table 2 Identification results of Lewis antibodies and ABO distribution in 74 positive cases.

		Blood group substance (saliva) neutralization test		ABO blood group					Antibody type				Methods of antibody identification			
													Saline medium			
	N	Tested	Not Tested	A	B	O	AB	Not quite clear	IgG	IgM	IgG+IgM	Undistinguished	RT	4°C	37°C	IAT
anti-Lea	31	23	8	17	7	4	2	1	3	24	1	3	21	28	22	25
anti-Leb	5	3	2	2	1	0	2	0	2	2	0	1	2	3	2	2
anti-LebH	1	1	0	1	0	0	0	0	0	1	0	0	1	1	1	1
anti-Lea+anti-Leb	23	15	8	12	3	2	6	0	1	15	2	5	18	22	14	14
anti-Lea+anti-LebH	4	2	2	1	1	0	2	0	0	4	0	0	3	4	4	1
Lewis combined with other blood group system antibodies (e.g., anti-E, anti-cE, anti-M, anti-Mur, anti-Wra, anti-IH, anti-P1)	10	8	2	6	1	0	2	1	2	5	1	2	8	8	7	8
Total (N)	74	52	22	39	13	6	14	2	8	51	4	11	53	66	50	51
(%)	100	70.27	29.73	52.70	17.57	8.11	18.92	2.70	10.81	68.92	5.41	14.86	71.62	89.19	67.57	68.92

Comparison of blood group distribution of Lewis antibodies in ABO: $\chi^2 = 18.749$, $P = 0.613 > 0.05$. Comparison of antibody types of various Lewis antibodies: $\chi^2 = 14.036$, and $P = 0.503 > 0.05$. IAT: indirect antiglobulin test; RT: room temperature.

Table 3 The ABO blood group and disease distribution in 74 positive cases of Lewis blood group system.

	N (%)	A	B	O	AB	Not quite clear
Gynecological diseases (including cervical cancer, uterine fibroids, polycystic ovary, ovarian cyst, bilateral adnexal cyst, etc.)	16 (21.62)	7	3	3	3	0
Pregnant women (late pregnancy, placenta residue, inevitable abortion, premature rupture of membranes)	10 (13.51)	3	2	1	3	1
Kidney diseases (kidney stones, kidney cysts)	11 (14.86)	5	2	1	2	1
Solid tumors (gastrointestinal benign, malignant tumor, renal, adenocarcinoma, auditory nerve tumor, orbital tumor, renal cancer, etc.)	17 (22.97)	11	3	0	3	0
Hyperlipoidemia	3 (4.05)	3	0	0	0	0
Liver diseases (alcoholic liver cirrhosis, liver cyst)	3 (4.05)	2	1	0	0	0
Other diseases	14 (18.92)	8	2	1	3	0
Total(*N*)	74	39	13	6	14	2
(%)	100	52.70	17.57	8.11	18.92	2.70

$\chi^2 = 16.300$, $P = 0.942 > 0.05$.

Table 4 Relevant information of 11 patients with RBCs.

Case number	ABO blood group	Lewis antibodies	Antibody type
1	A	anti-Le^a + anti-E	IgG
2	A	anti-Le^a	IgG
3	AB	anti-Le^a	IgG
4	B	anti-Le^a	IgM
5	AB	anti-Le^a + anti-Le^b	IgM
6	AB	anti-Le^a + anti-Le^b	IgM
7	A	anti-Le^a + anti-M	IgM
8	A	anti-Le^a + anti-Le^{bH}	IgM
9	A	anti-Le^a	IgM
10	A	anti-Le^a + anti-Le^b	IgM
11	B	anti-Le^a + anti-Le^{bH}	IgM

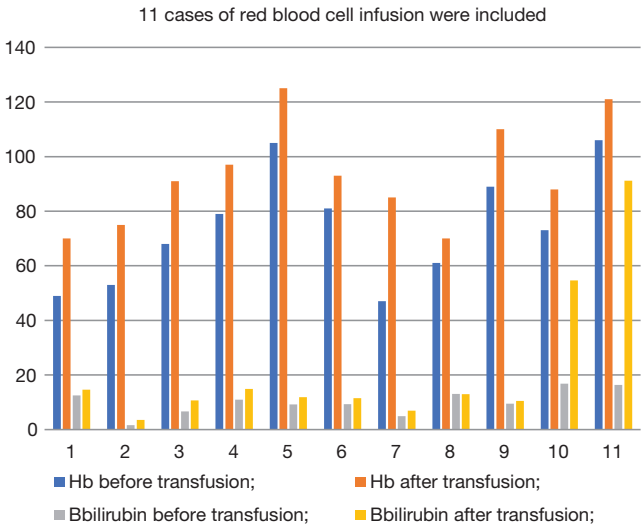

Figure 3 Comparison of hemoglobin and bilirubin levels before and after transfusion in 11 patients.

phenotypes, including Le (a+b-), Le (a-b+), Le (a-b-), and rare Le (a+b+), were found in some Asian populations. This types of antigens are widely expressed in platelets, endothelium cells, kidney tissues, genitourinary system, gastrointestinal epithelial cells, and saliva but not synthesized by RBCs.[10]

The expression of Lewis blood group antigens is regulated by the secretively type *SE* gene (*FUT2*) and *LE* gene (*FUT3*), both of which belong to the *FUT* family of cachalots glycosyltransferases (GTFs). In the case of inactivation of *LE* gene, the Lewis blood group antigen was shown as Le (a-b-), regardless of the *SE* gene status. In addition, the expression levels of Lewis antigens on RBC membranes are also affected by patient's age, ABO blood group, tumor, and sample storage time.[11]

Lewis blood group system antibodies are most frequently detected in patients with Le (a-b-) phenotype. In this study, the Lewis blood group of 74 Lewis antibody positive patients was Le (a-b-), and the anti-Le^a antibody was high, while anti-Le^b antibodies were relatively rare, often produced simultaneously with anti-Le^a antibodies, and a few anti-Lewis antibodies combined with other blood group system antibodies. Anti-Le^a antibodies can be measured at both room temperature and low temperature, some by indirect antiglobulin test (IAT) and enzyme, often combining complement, and showing a positive *in vitro* hemolysis (Figure 4). Anti-Le^a+anti-Le^b antibodies can be detected at different temperatures. However, anti-Le^a pattern appears only at low or room temperature, while anti-Le^b pattern appears only using anti-human sphere medium or enzymatic methods. This survey demonstrated that anti-Lewis antibodies were mostly produced in non-O blood group individuals, which was significantly higher than that of O blood group individuals (66 cases vs. 6 cases). This result was slightly different from the previous report, which indicated that a high frequency of anti-Le^a antibodies occurred in O blood group individuals, and anti-Le^b antibodies were found in non-O type individuals. However, this is still not clear whether this was related to the nature of H antigen in type O individuals and the nature of the Le antigen, both being trehalose (mycose or tremalose).[12] Lewis antibodies are more common in non-O blood group individuals because the gene products of *ABH* and *Le* come from the same precursor, the synthesis process of Lewis antigen and *ABH* antigen is a crossover, and A and B antigens have competitive inhibition for Lewis synthesis, that is, A1 can reduce the expression of Le^a and Le^b.

Since all the patients with Lewis antibodies in this study were Chinese, and the *SE* gene was rare in the Chinese population, and the Le (a+b-) phenotype was almost absent, so Chinese anti-Le^b or anti-Le^{bH} antibodies are only found in individuals with Le (a-b-) phenotype. Besides, correlation

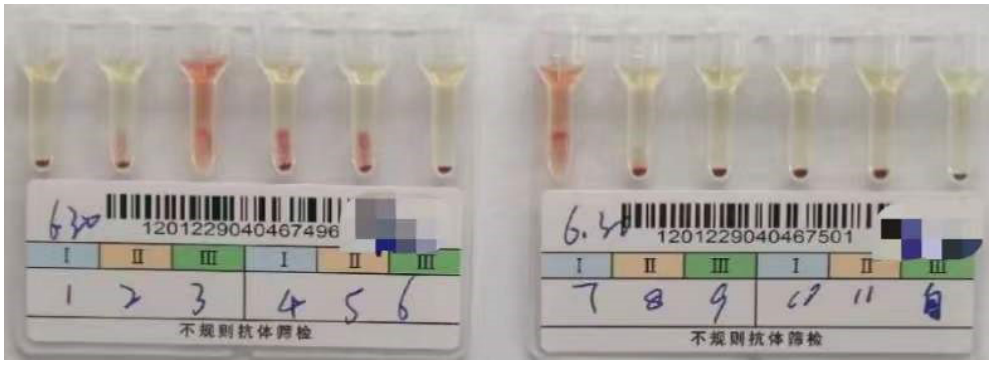

Figure 4 Results of anti-human globulin cards with anti-Le^a+anti-Le^b antibodies.

exists between the precursor material of the Lewis blood group system and the ABO. The anti-Le^b produced by non-O group individuals of the Chinese population is most likely anti-Le^{bH}, and it is only specific for the Le^b antigen of type O blood group (in fact, it should be Le^{bH} antigen). Therefore, in ABO, the cross-type of Le^b positive RBCs, the main side is weakly agglutinated or not agglutinated; this is significantly different from the reaction intensity of antibody-identified cells (O cells).[13] Type O RBCs are not recommended for such patients, and an ABO isotype RBC infusion should be selected. Therefore, when the detected anti-Le^b antibodies are non-O group individuals, it is necessary to distinguish between anti-Le^b and anti-Le^{bH} to ensure safe transfusion.

Lewis antibodies can be produced both naturally and by immune stimulation. A total of 74 cases of Lewis antibodies were detected in this study, accounting for 15.91% (74/465), and the detection rate was higher than that in Nanning area studied by Liu et al. (4.78%).[14] In all, 47 patients had a history of blood transfusion and (or) pregnancy, which was higher than that found among patients with no history of blood transfusion or pregnancy (27 patients). This was consistent with the higher positive rate of Lewis antibodies with a history of pregnancy or blood transfusion, such as immunization, reported by Zhang et al.,[15] but the type of antibody detected was different. This survey identified IgM 51 (68.92%), IgG 8 (10.81%), IgG + IgM 4 (5.41%), and 11 unclassified (14.86%) immunoglobulins. Most Lewis antibodies are reactive in saline, the reactivity temperature domain is wide, and can be reactive at room temperature, 4°C, and 37°C, but the agglutination intensity is weak and easy to disperse, requiring centrifugation and gentle resuspension. The agglutination is also observed after incubation at 37°C, but is usually weaker than that at room temperature, reported in 50 cases (67.57%).

This survey indicated the highest detection rate (52.70%) of Lewis antibodies in type A individuals. The incidence of gynecological diseases and other solid tumors was higher, followed by patients with kidney diseases, maternal, hyperlipidemia, liver diseases, etc. Simon et al. reported that high incidence of Lewis antibodies was found in pregnant women and women with gynecological diseases.[16] The difference between the two studies could be due to differences in patients admitted to hospitals. Studies have shown that ABO (H) and Lewis blood group antibodies are associated with viral infection and occurrence of cancer.[17] The absence of Lewis antibodies on the surface of RBCs can lead to different tumors (e.g., pancreas, stomach, colorectal, and gallbladder tumors), alcoholic cirrhosis, alcoholic pancreatitis, and severe nephropathy; another phenomenon associated with malignancy is incompatible A antigen, which is occasionally expressed in type A or B tumors. The high incidence of gastrointestinal and ovarian adenocarcinoma in type A individuals could result from the natural presence of anti-A in type O and B type individuals, thus inhibiting the development of an antigen carried by tumor molecules. During pregnancy, most of the Lewis antigen determination clusters were adsorbed on plasma lipoproteins with high abundance rather than on the surface of RBCs. Thus, pregnant women may produce Lewis antibodies because of the appearance of transient Le (a-b-) phenotype. In abdominal diseases, less absorption of Le^a active molecules by the gut may cause less Le^a antigen in plasma, resulting in less antigen or even no expression, possibly producing Lewis blood group antibodies.[1]

Patients containing Lewis antibodies often indicated negative phenotypes because of low titer, low affinity of antibodies or IgM or IgG anti-Le^a alone.[18-20] In addition, a variety of accidental antibodies presenting at the same time caused difficulty to identify blood types. Moreover, in the antibody screening and spectrum cell test, the agglutination of different antibodies may cause mutual interference, making it difficult to identify antibodies and cross-blood matching.[21,22] In order to improve the detection rate, the following details must be considered: (1) increase incubation time at room temperature and perform repeat centrifugation; (2) detect Lewis antibodies of samples as early as possible to avoid false negativity resulting from shedding during storage; (3) wash identified cells with normal saline before antibody identification; (4) use enzymatic treatment profile to enhance reaction sensitivity; (5) combination of Lewis blood group system antibodies with other blood group system antibodies can result in the neutralization of Lewis antibodies by the corresponding phenotype of blood type substance (saliva) before the identification of other antibodies; and (6) saliva neutralization tests must be followed to detect Lewis antibodies at the condition of low liter and incomplete pattern of cells for antibody identification because of activity difference and shedding of surface antigens.

Transfusion strategy: Most antigen-negative RBCs are unessential because Lewis antigen is an exogenous glycolipid antigen and is prone to elution and shedding within several days; abundance of Lewis blood group antibodies in the plasma of a donor can neutralize antibodies in the plasma of a blood recipient. Therefore, hemolysis is rare after infusion of Le (a+) or Le (b+) erythrocytes.[8] In this investigation, 11 patients of RBC transfusion were non-O blood group individuals, 8 patients of Lewis antibodies had IgM type antibodies, and 3 patients were of IgG type. We did not screen blood for negativity of Lewis antigens, but used microcolumn gel anti-globulin method at 37°C for cross-matched blood transfusion. This transfusion strategy was chosen due to two main reasons: first, most Lewis antibodies were IgM types and inactive without clinical significance at 37°C; second, about 93% of Chinese have Le (a+) blood type substances in their plasma, and about 99% of people have Le (b+) blood type substances in their plasma; so, the Lewis antibodies produced by patients are neutralized by the blood type substances in imported blood types. The above-mentioned 11 patients with blood transfusion had no acute or delayed hemolysis transfusion reaction, and the blood transfusion was effective. In fact, Lewis antibodies rarely caused neonatal hemolysis, as most Lewisantibodies are of IgM type or IgM + IgG type. Meanwhile, common Lewis antigens of Le (a-b-) type were weakly expressed on neonatal RBCs.[23]

Conclusion

The combined identification of Lewis blood group antibodies is crucial for the rapid and correct classification

of antibody type, and the accurate judgment of antibody activity. Therefore, the infusion of RBC with antiglobulin medium at 37°C is recommended for recipients with Lewis antibodies *in vivo*. Optimization of experimental method can shorten the time for difficult blood type identification and cross-blood matching, which accordingly save time for the treatment of patients.

Author Contributions

Xiaoxiang Wei, Fengxia Liu, Dong Ran, Ping Yin and Lin Qu conceived the study; Xiaoxiang Wei and Lin Qu participated in the study design, performance, coordination, and manuscript writing; Fengxia Liu, Dong Ran and Ping Yin performed the research. All authors have read and approved the final manuscript.

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