Prevalence, specificity and risk of red blood cell alloantibodies among hospitalised Hubei Han Chinese patients

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Background. The prevalence, specificity and risk of red blood cell alloantibodies vary widely among different geographic areas, races, and diseases and according to different methods of study, but no data are available on the Chinese Han population, who were investigated in the present study.

Materials and methods. Antibody screening was conducted among 42,517 hospitalised Hubei Han Chinese individuals using column agglutination technology. Samples that were positive in antibody screening were subjected to antibody identification by the tube test. Clinical data, including gender, age, race, transfusion history and records of alloantibody detection, transfusion reactions or haemolytic disease of the newborn, were collected to analyse the prevalence and specificity of alloantibodies and complications associated with them.

Results. A total of 212 patients with alloantibodies were identified among 42,517 patients, yielding a prevalence of 0.50% in this study. Significantly different prevalence rates were observed according to age and sex. The most frequently identified alloantibodies were anti-E (87/212, 41.0%), anti-D (45/212, 21.2%), anti-M (41/212, 19.3%) and a combination of anti-E and anti-c (13/212, 6.1%). Haemolytic disease was observed in 13 infants with anti-D, three infants with anti-E and one infant with anti-Fy^a alloantibodies. Delayed haemolytic transfusion reactions occurred in four patients with alloantibodies.

Discussion. In hospitalised Hubei Han Chinese individuals**,** the overall prevalence of alloantibodies was 0.50%, with anti-E, anti-D and anti-M being the most frequently identified alloantibodies. These results indicate that anti-D and anti-E alloantibodies were major risk factors for haemolytic disease of the newborn or delayed haemolytic transfusion reactions in this study population.

Keywords: RBC alloantibodies, antibody prevalence, antibody specificity, Han Chinese.

Introduction

Red blood cell (RBC) alloantibodies are directed against antigens expressed on the erythrocytes of patients. RBC alloimmunisation[1] follows transfusion, pregnancy and transplantation[2-4]. The alloantibodies aimed at RBC can be responsible for both immediate and delayed haemolytic transfusion reactions. Such complications, related to the omission of pre-transfusion tests or to their inability to recognise antibodies, can be very serious to the point of endangering the patient's life[5]. At the same time, maternal RBC alloantibodies can be harmful during pregnancy, potentially leading to severe haemolytic disease of the foetus or newborn (HDFN)[6]. In transplantation, RBC alloantibodies may raise the risk of haemolytic reactions, delayed engraftment and pure RBC aplasia[4]. Knowing the prevalence and specificity of RBC alloantibodies for a specific geographic area, race or disease will, therefore, assist with the management of blood transfusions and transplants and with the prevention of HDFN.

The published prevalence of RBC alloantibodies varies widely between different study populations, being up to 0.8% in blood donors, approximately 1% to 2% in hospital-based patients and higher in patients receiving chronic transfusion therapy among non-Chinese subjects[7-10]; lower prevalence rates were found in Chinese blood donors and patients, ranging from 0.10% to 0.27%[11-14]. Several inherent factors, including different blood group antigen frequencies in different races, diversity in immunogenicity, number of RH variants, prevalence of underlying diseases, the frequency of patients receiving chronic transfusions and age of the patients, may account for these differences[15-18]. However, some organisational or technological factors, including different transfusion management procedures and different detection techniques, may also account for these differences[19,20].

Previous studies on the specificity of RBC alloantibodies showed that the most frequently identified alloantibodies, as a percentage of total antibodies, were

E, D, M and Mi^a in Chinese individuals[12], Le^a, E, Mi^a and Le^b in southeast Asians[20], Mi^a and E in an eastern Taiwanese population[21], and E, Le^a, K, D, Le^b, M, P_1, Fy^a, C and c in Americans[8].

China is a multi-ethnic country, with the Han population being the most common ethnicity. Although there have been many reports in the medical literature on Chinese RBC alloantibody data[12,13], the prevalence, specificity and risk of RBC alloantibodies in the Hubei Han population remain unclear. This study therefore investigated these issues among a hospitalised Hubei Han Chinese population in Renmin Hospital of Wuhan University, an important hospital in the Hubei province of China.

Materials and methods

Patients

After approval by the Ethics Committee of Renmin Hospital of Wuhan University, 42,517 hospitalised patients (22,739 males and 19,778 females) who underwent blood typing and antibody screening in the hospital between February 2010 and June 2012 were enrolled in the study. All of the patients were from the Hubei province of China, one of the regions containing the largest density of Han people. Those patients of non-Han ethnicity and patients with autoantibodies, antibodies against low or high-incidence antigens, or false positive results caused by bubbles or fibrinogen were excluded from the study. Because record culling was performed regardless of the alloantibody status, there was no known selection bias.

To ensure that the same patient was not repeatedly included in the study, if a patient had multiple negative antibody screening results during the same period, only the first record was used in this study, and if any antibody screen was positive, the first positive record with information on all antibodies throughout the period was included.

The following data were collected when available: demographic information, including gender, race, transfusion history, antibody screening, identifying records and the number and specificity of the RBC alloantibodies, haemolytic disease in the newborn, and whether a transfusion reaction occurred in an individual with a positive antibody result.

Serological testing

The samples for alloantibody screening were subjected to a three-cell screening panel procedure with an AutoVue immunohaematology analyser using a panel (Shanghai Blood Medical Biotechnology Inc., Shanghai, PRC), which included antigens frequently found in Chinese individuals, such as Di^a and Mur. Low ionic strength saline (Ortho Clinical Diagnostics, Raritan, NJ, USA) was used to enhance the antigen-antibody interactions.

Samples found to be reactive to an antibody screening test were subjected to antibody identification procedures utilising, in every case, two antibody identification cell panels with the tube test. The alloantibody identification was first conducted with an 11-cell panel (Immucor Inc., Norcross, GA, USA) covering blood type antigens D, C, c, E, e, V, C^w, K, k, Kp^a, Kp^b, Js^a, Js^b, Fy^a, Fy^b, Jk^a, Jk^b, Le^a, Le^b, P_1, M, N, S, s, Lu^a, Lu^b and Xg^{*a} using indirect antiglobulin (Shanghai Blood Medical Biotechnology Inc.) and polybrene (Baso Biotechnology Inc., Zhuhai, PRC) tests. Then, a 10-cell panel (Shanghai Blood Medical Biotechnology Inc.) covering the characteristic Chinese blood type antigens of D, C, c, E, e, Jk^a, Jk^b, M, N, S, s, Mur, Fy^a, Fy^b, Di^a, Di^b, K, k, Le^a, Le^b, P_1, Do^a, Do^b, Yt^a and Yt^b was performed. Specialised technicians familiar with immunohaematology testing performed all the procedures.

Statistical analysis

The data were analysed using the statistical software SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). The statistical evaluation of the data was performed using the chi-square test. P values less than 0.05 were considered statistically significant.

Results

Overall, 212 patients with alloantibodies were identified among 42,517 hospitalised patients, yielding a prevalence of 0.50% in this study. Among these patients with alloantibodies, 184/212 (86.8%) had a history of transfusion and/or pregnancy. In the remaining 28/212 (13.2%) patients with no history of transfusion or pregnancy, 13 infants with anti-D, three infants with anti-E and one infant with anti-Fy^a were identified, and these alloantibodies were identical to those present in their mothers. In addition, one male adult with anti-E and ten male adults with anti-M were found: in these 11 subjects, no risk factor was identified for the development of the red cell antibodies. Of the 212 patients with alloantibodies, 192 (90.6%) had a single RBC antibody, 16 (7.5%) had a mixture of two RBC antibodies, and 4 (1.9%) had three antibodies. Among the 192 patients in whom a single RBC antibody was identified, most had anti-E (87/192, 45.3%), followed by anti-D (45/192, 23.4%), anti-M (41/192, 21.4%), and others (19/192, 9.9%). Among the patients in whom multiple antibodies were detected, patients with the combination of anti-E and anti-c were the most common (13/20, 65.0%). Overall, the most frequently identified alloantibodies, as a percentage of the total patients with antibodies, were anti-E (87/212, 41.0%), anti-D (45/212, 21.2%), anti-M (41/212, 19.3%) and a combination of anti-E and anti-c (13/212, 6.1%). Among the anti-D cases detected, one patient was identified with IgM and IgG anti-D (Table I).

A total of 236 RBC alloantibodies were identified among the 212 hospitalised patients with alloantibodies. The most frequently identified alloantibodies were of the Rhesus (RH) system (169/236, 71.6%), followed by the MNS system (54/236, 22.9%). Antibodies directed against other blood group systems were rare. Within the RH system, the identified alloantibodies included anti-E (100/169, 59.2%), anti-D (45/169, 26.6%), anti-C (5/169, 3.0%), anti-c (13/169, 7.7%) and anti-e (6/169, 3.5%). Within the MNS system, the identified alloantibodies included anti-M (44/54, 81.5%), anti-N (5/54, 9.2%), anti-S (2/54, 3.7%) and anti-Mur (3/54, 5.6%). Compared with males, females had a significantly higher prevalence of detected alloantibodies, particularly anti-D, anti-E and anti-M (Table II). The frequency of alloantibody detection was significantly different between age groups in females, with rates noticeably lower in patients aged 20 years of age or younger and higher in the 31- to 40-year old age group (Figure 1).

Table I - Results of antibody identification.

Alloantibodies	Total patients (n)	Male (n)	Female (n)	History of transfusion and/or pregnancy			
				Transfusion (n)	Pregnancy (n)	Transfusion + Pregnancy (n)	Other (n)
Anti-D	45	7	38	1	27	4	13
Anti-E	87	28	59	29	28	26	4
Anti-Jk^b	2	0	2	0	2	0	0
Anti-Le^a	5	1	4	3	2	0	0
Anti-M	41	14	27	12	13	6	10
Anti-N	5	2	3	3	2	0	0
Anti-S	2	1	1	1	1	0	0
Anti-Fy^a	2	1	1	0	1	0	1
Anti-Mur	3	1	2	1	1	1	0
Anti-C+e	2	1	1	1	1	0	0
Anti-E+c	13	5	8	6	5	2	0
Anti-e+Jk^b	1	1	0	1	0	0	0
Anti-C+e+Jk^b	1	0	1	0	0	1	0
Anti-M+C+e	2	0	2	1	1	0	0
Anti-M+Le^a+Jk^b	1	1	0	1	0	0	0
Total	212	58	154	60	84	40	28

n: number.

Table II - RBC alloantibodies according to sex.

	Male	Female	P-value
Anti-D	**7 (0.16)**	**38 (0.84)**	**0.000**
Anti-E	**33 (0.33)**	**67 (0.67)**	**0.000**
Anti-C	1 (0.20)	4 (0.80)	0.132
Anti-c	5 (0.38)	8 (0.62)	0.273
Anti-e	2 (0.33)	4 (0.67)	0.319
Anti-Jk^b	2 (0.40)	3 (0.60)	0.542
Anti-Le^a	2 (0.33)	4 (0.67)	0.319
Anti-M	**15 (0.34)**	**29 (0.66)**	**0.009**
Anti-N	2 (0.40)	3 (0.60)	0.542
Anti-S	1 (0.50)	1 (0.50)	0.918
Anti-Mur	1 (0.33)	2 (0.67)	0.481
Anti-Fy^a	1 (0.50)	1 (0.50)	0. 918
No antibody	22,652 (0.54)	19,601 (0.46)	

No antibody: the antibody screening result was negative.
Numbers in parentheses indicate percentage of the total number of antibodies.
Significant difference between males and females are shown in bold.

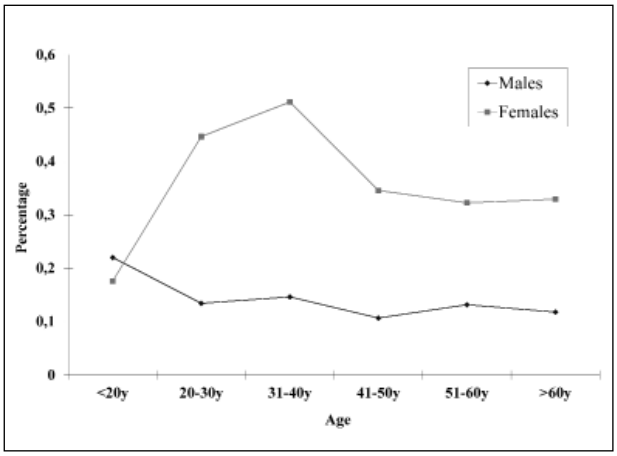

Figure 1 - Distribution of alloantibodies among different age groups.

During the period of hospitalisation in patients with alloantibodies, 13 infants with anti-D, three with anti-E and one with anti-Fy^a developed HDFN, and two patients with anti-E, one with anti-e+Jk^b and one with anti-M+Le^a+Jk^b developed delayed haemolytic transfusion reactions (DHTR). HDFN was diagnosed from a few hours to 3 days after birth. DHTR was detected from 1 day to 1 week after the first transfusion in patients whose antibody screening had been omitted or if antibodies were undetected before the first transfusion in our hospital. In these patients, DHTR manifested with the clinical features of acute haemolysis (haemoglobinuria, jaundice and pallor), and responsible alloantibodies were subsequently detected and identified, enabling these patients to receive compatible blood units negative for corresponding antigens (Table III).

Table III - Clinical consequences of RBC alloantibodies in patients.

	N. of patients	
	DHTR	**HDN**
Anti-D		13
Anti-E	2	3
Anti-Fy^a		1
Anti-e+Jk^b	1	
Anti-M+Le^a+Jk^b	1	

DHTR: delayed haemolytic transfusion reaction; HDN: haemolytic disease of newborn.
During the hospitalised period in patients with alloantibodies, 13 infants with anti-D, three with anti-E and one with anti-Fy^a developed HDN, and two patients with anti-E, one with anti-e+Jk^b and one with anti-M+Le^a+Jk^b developed DHTR.

Discussion

Screening for and identifying alloantibodies are important components of pre-transfusion testing. This study was an effort to characterise RBC alloantibodies among the hospitalised Chinese Hubei Han population. Our results revealed that the alloantibody prevalence in this area was 0.50%, which is slightly higher than that in other Han populations studied, such as the 0.17% of hospitalised Chinese patients at the Zhongshan Hospital of Fudan University[12], 0.21% of surgical Chinese patients at the Shengjing Hospital of China Medical University[13], and 0.27% of Chinese pregnant women at the Queen Mary Hospital and Tsan Yuk Hospital in Hong Kong[14]. All of these alloantibody prevalences were lower than those in eastern Taiwanese individuals (3.39%)[21], southeast Asians (0.66%-1.16%)[20] and Americans (2.5%)[8].

Although the Mongoloid-exclusive Di^a antigen and its antibody were found in some Chinese populations[12,22], no anti-Di^a was detected in our population, in accordance with the results of other studies[13,14,23]. Antibodies to the variant M antigen were more frequent in southeast Asians[20,21] but less frequent in our study, with these results being in accordance with those of previous reports[13,23]. The characteristic antibodies found in white populations, anti-K and anti-Fy^a[8,24], were not detected or were rare in our study, as in other Chinese studies[12,23]. The most frequently identified alloantibodies in our study, as percentages of the total antibodies, were anti-E (41.0%), anti-D (21.2%), anti-M (19.3%) and anti-c + anti-E (6.1%). Similarly, in hospitalised Chinese individuals from other areas, the most frequently identified alloantibodies were anti-E (53.1%), anti-D (10.9%), anti-M (14.1%) and and-Mi^a (10.9%)[12]. These results are remarkably different from those in Americans, among whom the most frequently identified alloantibodies were anti-E (20.8%), anti-Le^a (18.6%), anti-K (14.7%) and anti-D (12.9%)[8].

The heterogeneity of the populations involved, the different screening protocols, variations in the definition of clinically significant antibodies and different antibody identification techniques might account for the above differences.

However, a history of transfusion and/or pregnancy was significantly correlated with alloantibody formation. Pahuja[25] observed that pregnancy status in women is significantly and positively correlated with alloantibody formation, which may account for females having a significantly higher prevalence of alloantibodies than the males in our study. Interestingly, age was also significantly associated with the frequency of alloantibody detection, in accordance with a report by Jan *et al*.[21.] However, in our study the highest frequency of alloantibody detection was in female patients aged 31-40 years, whereas in the study by Jan *et al*. it was in patients aged 61-80 years. The heterogeneity of the populations involved may account for this difference. This finding in our study may be related to a greater exposure and immunisation with age.

Although their incidence is debated, RBC alloantibodies can pose serious problems, such as DHTR and HDFN. As far as concerns DHTR, such reactions have been found to occur in 4-11% of patients with sickle cell disease given blood transfusions[26-29]. With regards to HDFN, many studies[30,31] have suggested that this is caused by maternal antibodies that recognise paternally inherited antigens on foetal RBC during pregnancy, resulting in substantial pathology and/or death. In our present study, HDFN was diagnosed in 17 infants, and DHTR was detected in four patients. The infants' alloantibodies were transmitted from their mothers. DHTR occurred because all the affected patients had previous transfusions, and their antibody screenings were omitted or the antibodies could not be detected before the first transfusion in our hospital.

Anti-E, anti-D and anti-M were the most common alloantibodies in this study population. RhD-negative patients are rare in the Chinese Han population[32], and the prophylactic use of RhD-negative blood units is rigorous, which may explain the absence of DHTR induced by anti-D and the few anti-D produced by transfusions

among this study population. However, HDFN induced by anti-D and anti-E was notable, and its clinical significance among infants and foetuses should be emphasised in this area. Most anti-M antibodies are not active and have no clinical significance at 37 °C[33]; therefore, anti-E should be regarded as the most important alloantibody with clinical consequences. If the E antigen in the Rhesus blood group is determined as often as the D antigen in transfusion practice, high-risk groups, such as those with the anti-E antibody and those undergoing chronic transfusion therapy or transplantation, will benefit.

In conclusion, we showed that the overall prevalence of alloantibodies was 0.50%, with anti-E, anti-D and anti-M being the most frequently identified alloantibodies in the hospitalised Chinese Hubei Han population. These results indicate that anti-D and anti-E were the major risk factors inducing HDFN or DHTR in this study population and that these two antibodies should be carefully detected. Finally, further studies should be performed to collect sufficient evidence for anti-D prophylaxis in obstetric practice.

The Authors declare no conflicts of interest.

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