The incidence and features of serological ABO subgroups among one million blood donors in Beijing

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ABSTRACT

This study aims to determine the incidence of serological ABO subgroups from a large-scale database, along with the features of blood samples with serological ABO discrepancies. The serological ABO results of one million individuals were randomly sampled from a blood donor database in Beijing between 2009 and 2010. All samples were diagnosed by serological reverse and forward ABO typing using an automatic analyzer. The proportions of the normal ABO types were 27.28%, 31.57%, 30.56%, and 10.16% for blood types A, B, O, and AB, respectively. In samples in which ABO discrepancies or obvious weak agglutinin were identified in the forward or reverse typing, further tests to analyze the ABO subgroup were conducted. The overall incidence of ABO subgroups was 0.047%, with 14 ABO subgroups observed: A2, A3, Ax, Am, Aint, Aend, B2, B3, Bx, Bm, Bel, B(A), cisAB, and AB^h. In conclusion, this study revealed the exact normal ABO and subgroup distributions in the general, healthy population of Beijing using samples from a blood donor database.

Keywords: ABO subtypes, ABO discrepancy, blood donor, large-scale study

INTRODUCTION

Accurate serological typing for ABO types is the primary and most important test in clinical transfusion medicine, because the development of ABO antigen-antibody complexes can induce severe acute or delayed hemolytic reactions^[1,2]. The distributions of the ABO blood types and predilections of ABO subgroups show various incidences among different ethnicities and even among people from the same country but in different areas. For instance, para-Bombay is the most common ABO subgroup in southwestern Asia, whereas it has a very low frequency in northern China^[3,4]. Therefore, exact data on the ABO distribution

and frequencies of the ABO subgroups are necessary for establishing national transfusion and transplant policies and for offering a reference to create strategies related to blood transfusion and serological typing in each country.

Several case reports focusing on ABO subgroups have been published, whereas large-scale studies on the incidence of ABO subgroups in the normal population are limited^[5,6]. However, there are certain factors that need to be considered, including unexpected antibody interference, and diseases causing weak serological ABO typing, which are two major factors responsible for ABO discrepancies in patients who require repeated transfusions. The ABO distribution among blood donors may well represent that in normal population in that area. Furthermore, another important factor responsible for the difficulty in performing ABO subgroup frequency studies is the very low frequency thereof; thus, extremely large sample sizes are needed.

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With this in mind, the aims of the present study were to determine the incidence of serological ABO subgroups from a large-scale database and describe the features of blood samples with serological ABO discrepancies.

MATERIALS AND METHODS

Sample collection

One million samples with available ABO results were randomly selected from the blood donor database from the Beijing Red Cross Blood Center between 2009 and 2010. Duplicate results were excluded. Furthermore, while the database included Rhesus D-negative individuals, specimens with blood transmitted diseases or that failed to meet the standards of blood donation were excluded.

Serological reverse and forward ABO typing

Anti-A, anti-B, and anti-A,B reagents (Beckman Coulter, USA) were used in the determination of the forward ABO blood group by determining the absence or presence of erythrocytic antigens A and/or B on the surface of human red blood cells. A cell, B cell, and O cell reagents (Beckman Coulter, USA) were used to type the reverse ABO blood groups. A control reagent was used in parallel testing with the blood grouping and phenotyping reagents to differentiate between specific and non-specific agglutination. For the serological typing, an automatic analyzer was used (Beckman Coulter PK7300 System; Beckman Coulter, USA).

ABO subgroup identification

Further serological tests, including the forward test with anti-A1, anti-H, and anti-A,B; direct agglutinin test; antibody screen test; antibody identification if the antibody screen test was positive; and absorption elution test if the direct agglutinin test was positive, were conducted in all ABO subgroup samples. All tests followed the standard operation procedures from the Blood Group Lab, Beijing Red Cross Blood Center.

Analysis of the ABO exon 6, 7 sequence and genotyping (Inno-Train Diagnostik GmbH, Germany) were used to confirm cases of B(A) and cisAB with other subgroups. DNA extraction(QIAamp DNA Blood Mini Kit; Qiagen GmbH, Germany) was conducted using the column extract method.

Statistical analysis

The frequencies and constituent ratios of the serological ABO normal types and subgroups were analyzed. The equilibrium between the corresponding normal ABO types and subgroups was used to confirm the incidence of the A and B subgroups. The odds ratio (OR) with 95% confidence interval (CI) was used to calculate the hazards for subgroups occurring among types A, B, and AB. The chi-square test was used to compare the characteristics between the study groups, with a 2-sided *P* value < 0.05 considered statistically significant.

RESULTS

ABO distributions for the normal type and subgroups

Normal ABO types accounted for 99.95% of the samples (**Table 1**). Among all normal ABO types, the frequency of the B type was the highest (31.57%; constituent ratio of the normal type, 31.68%), followed by O (30.56%; constituent ratio, 30.68%), A (27.28%; constituent ratio, 27.39%), and AB(10.16%; constituent ratio, 10.19%). The overall frequency of ABO subgroups was 0.047%, with AsubgroupB being the most frequently observed subgroup (0.0288%; constituent ratio of the subgroups, 57.53%), followed by the B subgroup (0.0073%, constituent ratio, 14.48%), AB subgroup (0.0058%; constituent ratio, 11.55%), A subgroup (0.0056%; constituent ratio, 11.15%), and others, including para-Bombay, B(A), and cisAB (0.0026%; constituent ratio, 5.29%).

Frequencies of serological ABO subgroups

Table 1 ABO distribution and incidence of subgroup in one million blood donors

ABO group	Normal ABO distribution (99.950%)		Subgroup	ABO subgroup (0.047%)	
	Frequency/%	Constituent rate/%		Frequency/%	Constituent rate/%
A	27.28	27.39	Asub	0.0056	11.15
B	31.57	31.68	Bsub	0.0073	14.48
O	30.56	30.68			
AB	10.16	10.19	AsubB	0.0288	57.53
			ABsub	0.0058	11.55
			Other*	0.0026*	5.29

*Including para-Bmbay, cisAB and B(A).

The constituent ratios of the serological ABO subgroups (0.047%) were as follows (**Table 2**): 7.05%, 47.36%, 10.96%, 9.00%, and 3.91% for A2, A2B, B3, AB3, and B(A), respectively. There were 6 types of the A subgroup (A2, A3, Ax, Am, Aint and Aend) and 6 types of A subgroup B (A2B, A3B, AxB, AintB, AendB and AelB) in the A subgroup, 5 types of the B subgroup (B2, B3, Bx, Bm and Bel) and 4 types of the AB subgroup (AB3, ABx, ABel, ABend) in the B subgroup, other subgroups including B(A), cisAB, and para-Bombay AB.

Table 2 Frequencies of serological ABO subgroup-typing in blood donors

Subgroup	A or B sub	Frequency/%	AsubB/ABsub	Frequency/%
Asub	A2	7.05	A2B	47.36
	A3	1.76	A3B	8.61
	Ax	1.37	AxB	0.20
	Am	0.59		
	Aint	0.20	AintB	0.98
	Aend	0.20	AendB	0.20
			AelB	0.20
Bsub	B2	1.57		
	B3	10.96	AB3	9.00
	Bx	0.98	ABx	1.76
	Bm	0.59		
	Bel	0.39	ABel	0.59
			ABend	0.20
Other	B(A)	3.91		
	cisAB	1.17		
	AB ^h	0.20		

Equilibrium of ABO distribution

We used the risk of the AB subgroup, including the A, B and AB subgroup, among the normal AB type as the reference for calculating the odds of the A subgroup among the normal A type and the B subgroup among the normal B type. Compared to the risk of the AB subtype, the ORs were 0.06(95%CI: 0.05–0.08) for the A subgroup and 0.07(95%CI: 0.05–0.08) for the B subgroup(**Table 3**), indicating that the risk of subgroups occurring in people with the AB type was 14–17-fold higher than that in those with ABO type A or B.

DISCUSSION

This paper reported the distributions of normal ABO blood types and 14 types of subgroups, along

Table 3 The equilibrium between normal types and subtypes

ABO group	Normal type/%	Subtype/%	OR	95%CI	P
AB	10.24	0.0346	–	–	–
A	27.38	0.0056	0.06	0.05–0.08	<0.0001
B	31.67	0.0073	0.07	0.05–0.08	<0.0001

with their respective constituent ratios, among one million blood donors in Beijing. These results may offer reference values for studies investigating the associations between the ABO types and various diseases as a control group from northeastern China, and the data collected may help in establishing strategies related to blood donation or transplantation. Many hospitals in China are currently planning to or have recently established molecular laboratories for transfusion medicine, and our subgroup data may thus also allow labs from different areas to choose the major examination items.

As mentioned above, several case reports focusing on patients with ABO subgroups have been published, whereas studies on the incidences of ABO subgroups in the normal population are scarce. Recent studies have demonstrated that the incidences of ABO subgroups were 0.014% among 190,960 blood donors in Fuzhou^[7], 0.015% among blood donors in Dongguan^[8], 0.016% among 39,106 blood donors in Pan-yu^[9], and 0.024% among blood donors in Nanning^[10], all of which are located in the southeast of China. Similar to our study, the A2 subgroup was the most prevalent ABO subgroup in these previous studies, indicating that this is the most common subgroup both in the north and south of China. On the other hand, para-Bombay showed an area-specific prevalence in the southeast of China, whereas B(A) was only identified in northern China.

Exact serological ABO typing is the most important examination in transfusion medicine, owing to the risk of severe hemolytic reactions induced by anti-A and anti-B^[11]. However, differences between the ABO genotyping and serotyping results may exist, because some subgroups present with the same serotype, such as Ael/Bel and O with the forward typing–negative serotype, and several cases of A205 have moreover been reported to present the same serotyping panel as A1 with anti-A4+^[12]. The present study showed that, among others, A205, A207, A208, and A209 reacted with anti-A1, although the guidelines from the State Food and Drug Administration mention that anti-A1 cannot react with A2 and that the agglutinin level of anti-A1 with A2 cells should be as strong as that of A cells^[6,12]. Due to the limited number of large-scale studies on ABO genotyping and ABO sequencing in China, further molecular studies to analyze the ABO genotype and compare its concordance to the results of serological ABO typing may result in the development of useful tools to more easily determine the presence of the ABO subtypes and ensure safe transfusions. Although the incidence is low in China, with only approximately 5/10,000 individuals having an

ABO subgroup in Beijing, the department of transfusion in each hospital should establish a standard procedure for these rare cases. Currently, in Beijing, the Beijing Red Cross Blood Center solves more than 400 cases of suspected ABO subgroups each year.

In conclusion, the present study analyzed a large-scale sample of serological ABO typing results in one million healthy blood donors in Beijing in order to demonstrate the incidence of ABO subgroups in this population (0.0047%). The ABO distribution showed that B was the most common normal type, accounting for 31.57% of cases, followed by O (30.56%), A (27.28%), and AB (10.16%). Among the different serological ABO subtypes, the A2 subtype showed the highest frequency.

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