Frequency of unexpected red blood cell antibodies in Nanjing

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ABSTRACT

The development of unexpected red blood cell antibodies can significantly complicate transfusion therapy and result in more difficulties in cross−matching of blood. This study aimed to determine the occurrence rate of red blood cell alloimmunization in patients from Nanjing and the surrounding area. The antibody screening tests were carried out on 604 patients in Nanjing Red Cross Blood Center from January 2014 to December 2016, and the re−sults were compiled and statistically analyzed. In the 604 patients, 483 cases revealed autoantibodies with or with−out underlying alloantibodies, while 121 patients had only alloantibodies in their serum. The overall frequency of alloimmunization was 32.5%. The most frequent antibodies were what against the Rh systerm(72.39%), followed by MN system (25.71%).

Keywords: incidence, autoantibody, alloantibody, alloimmunization

INTRODUCTION

All redblood cell antibodies other than naturally occurred anti−A and anti−B antibodies are defined as "unexpected antibodies" including autoantibody and alloantibody[1]. An autoantibody is an antibody pro−duced by the immune system that is directed against one or more of the individual´s own proteins. Many autoimmune diseases (notably lupus erythematosus) are caused by such autoantibodies[2]. Production of al−loantibodies may result from exposure to foreign an−tigens such as pregnancy, transfusion, transplantation, or injections of immunogenic material[3]. These anti−bodies can cause acute and delayed hemolytic trans−fusion reactions and there are many reports on such cases[4]. Therefore, it is important to conduct testing to screen and identify the unexpected antibodies before transfusion.

The occurrence of red blood cell(RBC) alloanti−bodies has been reported in a variety of studies, and usually correlated with a previous history of transfu−sion or pregnancy, human leukocyte antigen (HLA)−DR typing, immune status and RBC density among people of different ethnical groups[5]. RBC alloan−tibodies have been detected in up to 0.8% of blood donors, and in 1%−2% of hospitalized patients. The occurrence of unexpected antibodies is approximately 2%−9% in patients with a history of blood transfu−sion and 9%−30% in patients with chronic transfusion therapy[6]. It is generally known that there are large differences in the frequency and type of unexpected antibodies found during screening according to the research subjects, blood type, genotypic frequency, screening method, and ability of the decoder[7]. The frequency of unexpected antibodies is reported to be 1.35% in Denmark[8], 0.78% in Germany[9], and 0.3%−2.0% in the USA[10]. The frequency and type of unex−pected antibodies in Nanjing has not been studied.

It is essential to know the frequency and distribu−tion of unexpected antibodies among China for prompt and safe blood transfusion.The purpose of this study was to examine the frequency and type of unexpected

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antibodies by antibody screening and identification in patients of Nanjing Red Cross Blood Center (NRCBC) from 2014 to 2016.

MATERIALS AND METHODS

Study population

The samples that showed positive results of anti–body screening test before blood transfusion or clini–cal difficulties in blood cross–matching were included in this study. All samples came from transfusion can–didates in hospital of Nanjing and surrounding area.

Antibody screening and identification

Antibody screening and identification were done using gel column assay (Boxun, Changchun, China) or tube test. A commercially available three cell panel (Shanghai Blood Biological Medical Co., LTD, Shanghai, China) was used for antibody screening procedure in which the subject´s plasma was reacted with a panel of red cells. The cards were incubated at 37℃ for 15 minutes and then centrifuged for 5 min–utes. The serum samples which were positive on the antibody screening were conducted with antibody identification. An extended 10–cell panel was used for antibody identification (Shanghai Blood Biological Medical Co., LTD, Shanghai, China). The serum of patients was reacted with the 10–cell panel of red cells and the cards were incubated at 37 ℃ for 15 minutes and then centrifuged for 5 minutes. The antigens on RBC were also determined by tube test to confirm the results of antibody identification.

RESULTS

Frequency of unexpected antibodies

The frequencies of unexpected antibodies in 604 cases delivered to NRCBC were identified. The number of patients with each type of antibody was shown in ***Table 1***. There were 408(67.5%) of them with autoantibodies, 121(20.0%) with alloantibodies, and 75(12.5%) cases had both autoantibodies and alloantibodies. The number of patients with autoantibodies was significantly higher than those with alloantibodies.

Type and distribution of unexpected antibodies

The identification tests for typing the unexpected

Table 1 **Autoantibody and alloantibody detected in patients**

Antibody	Case/*n*(%)
Autoantibody only	408(67.5)
Alloantibody only	121(20.0)
Autoantibody and alloantibody	75(12.5)

antibody were conducted in the 196 patients with al–loantibodies. The results showed that Rh antibody had the highest frequency (72.39%), followed by MN system antibody (25.71%). The frequencies of Rh an–tibodies were 38.10% for anti–cE, 21.90% for anti–E, 2.86% for anti–Ce, and 0.95% for anti–c. Anti–D and anti–C shared the same percentage which is 4.29%. Only anti–M antibodies (25.71%) were found in the MN system, however, it was the second most common antibody besides anti–cE***(Table 2)***.

Table 2 **The type and frequency of alloantibody**

System	Antibody specificity	Case/*n*(%)
Rh	Anti–cE	80(38.10)
	Anti–E	46(21.90)
	Anti–Ce	6(2.86)
	Anti–c	2(0.95)
	Anti–C	9(4.29)
	Anti–D	9(4.29)
MN	Anti–M	54(25.71)
Lewis	Anti–Le[a]	2(0.95)
Kidd	Anti–Jk[a]	1(0.48)
P1	Anti–P1	1(0.48)

Characteristics of patients with unexpected antibodies

In these 604 patients with positive unexpected an–tibodies, 318 (52.65%) were female and 286 (47.35%) were male. The distributions of both autoantibody and alloantibody between male and female were sig–nificantly different. As shown in ***Table 3***, the fre–quency of autoantibodies in the male group (86.62%) was significantly higher than that in the female group (61.64%). However, the distribution of alloantibodie spresented an opposite tendency, which was higher among the females. The percentage of female in 182 patients with positive alloantibodies was 76.92%.

Table 3 **Gender characteristics of patients with un–expected antibodies**

Gender	Case/*n*(%)	Autoantibody/*n*(%)	Alloantibody/*n*(%)
Male	286 (47.35)	272 (86.62)	42 (13.38)
Female	318 (52.65)	225 (61.64)	140 (38.36)

The age distribution in patients with unexpected antibodies was as follows: ≤ 30 yr, 114 (18.87%); 31–60 yr, 174 (28.81%); ≥ 61 yr, 316 (52.32%). As shown in ***Table 4***, the frequencies of autoantibody increased while alloantibody decreased with age gradually. The percentage of elderly people (≥ 61 yr) in patients with autoantibody was 61.12%.

As shown in ***Table 5***, the ABO blood group dis–tribution in patients with unexpected antibodies was as follows: A, 208 (34.44%); B, 164 (27.15%); AB, 77 (12.75%); O, 155 (25.66%). The frequencies of either autoantibody or alloantibody showed no differ–

ence among patients of A, B and O type. However, the frequencyof autoantibody in AB type was lower and alloantibody was higher than other blood types sig-nificantly (***Table 5***).

Table 4 **Age characteristics of patients with unex-pected antibodies**

Age(yr)	Case/*n*(%)	Autoantibody/*n*(%)	Alloantibody/*n*(%)
≤ 30	114 (18.87)	67 (51.94)	62 (48.06)
31–60	174 (28.81)	127 (64.80)	69 (35.20)
≥ 61	316 (52.32)	305 (86.16)	49 (13.84)

Table 5 **Blood group characteristics of patients with unexpected antibodies**

Blood Group	Case/*n*(%)	Autoantibody/*n*(%)	Alloantibody/*n*(%)
A	208 (34.44)	174 (76.32)	54 (23.68)
B	164 (27.15)	130 (75.14)	43 (24.86)
AB	77 (12.75)	57 (61.29)	36 (38.71)
O	155 (25.66)	138 (74.59)	47 (25.41)

DISCUSSION

Regular antibodies were usually referred to the isohemagglutinins and were directed against antigens of the ABO system[11]. It is not certain whether they are natural antibodies or caused by infection in early life. The irregular antibody is a different kind of blood group antibody from the anti–A, anti–B antibody[12]. In China, irregular antibody screening methods use a standard series of red blood cells[2,5]. With red blood cells containing the corresponding antigen and IgM antibody in a saline medium, visible condensation oc-curs (traditional ABO grouping); IgG antibodies with the corresponding cell reaction are analyzed by means of an enzymatic test or antiglobulin test to visible condensation[13].

Elevated frequencies of transfusion reaction have been reported, about one percent of all transfusions de-veloped antibodies that may eventually lead to the re-action[6,14]. Some of the reactions may occur as a result of irregular antibody production including a delayed hemolytic reaction, which happened when the antibod-ies attack antigens (other than ABO antigens) on the transfused blood cells slowly. The blood cells would break down by days or weeks after the transfusion[7,15].

In this study, we demonstrated that the frequency of autoantibody (80.0%) is higher than that of al-loantibody (32.5%), which is different from previous reports. This may be due to the high proportion of elderly patients in the study population. And our study also showed the frequency of autoantibody increased, while alloantibody decreased gradually with age.

The analysis of alloantibody showed that Rh an-tibody had the highest frequency (72.39%), followed by MNS antibody (25.71%); and the incidence rate of alloantibody in female (38.36%) was significantly higher than that in male (13.38%). Interestingly, we also found the frequency of alloantibody in AB blood type significantly different from other blood types.

Our study still has some limitations such as lacking clinical data, including ethnic group, pregnancy his-tory, transfusion history which may influence the oc-currence rate of unexpected antibodies.

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