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An Analysis of Discrepancies between Forward and Reverse ABO Blood Grouping.

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ABSTRACT

Analysis of discrepancies between forward and reverse ABO grouping and determining the etiology and root causes of discrepancies and resolving it for accurate and reliable ABO grouping. From June 2012 to February 2014, one hundred cases of discrepancy between forward and reverse methods were evaluated to determine the etiology and main cause of discrepancies. Eighty two (82) of the cases of discrepancy were due to age related weak or missing antibody, which had very low levels of antibody production or cannot produce the ABO antibodies. Sixteen (16) of the cases of discrepancies were due to Rouleaux formation and Cold agglutinins. Two cases of discrepancies were A_2 individuals which had Anti-A_1 in their serum. Accurate and reliable ABO grouping is the most important test in blood banking and transfusion medicine. For this reason, any discrepancy between forward and reverse methods should be resolved before transfusion of blood components.

Keywords: ABO blood group, Forward grouping, Reverse grouping, Discrepancy

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INTRODUCTION

ABO blood group system is the most important blood group in blood banking and transfusion medicine. This system consists of Three antigens—A, B and H ---, and Four phenotypes—A, B, AB, and O blood groups.

A feature of the ABO system is the regular occurrence of Anti-A and Anti-B in the absence of the corresponding red cell antigens [1]. Thus, the individuals with blood group A possesses A and H antigens on their red blood cells and demonstrate Anti-B in the serum, the individuals with blood group B possesses B and H antigens on red blood cells and demonstrate Anti-A in the serum, in blood group O there is only H antigen on red cells along with Anti-A and Anti-B in the serum, and finally in persons with blood group AB there are A, B and H antigens on red cells with no any antibody (Anti-A and Anti-B) in the serum.

Therefore, the ABO system is the only main system in which the reciprocal antibody is expected to be present in the serum of an individual whose red cells lack the corresponding ABO antigen.

An accurate ABO grouping is the most important test which is done in the blood bank. Mistyping either a donor or a recipient can lead to transfusion with ABO incompatible blood which results in severe intravascular hemolysis and may even result in the death of the recipient.

ABO grouping consists of testing the red cells with Anti-A and Anti-B reagents for determining the antigens on red cells (Forward or cell type) and testing the serum for expected antibodies by using A_1 and B red cells (Reverse or Backtype). ABO grouping should include both forward and reverse procedures, and the results of the two methods should agree with each other [2].

An ABO discrepancy implies that the forward, or red cell, ABO grouping does not agree with the reverse, or serum, ABO grouping. In patients, an ABO discrepancy must be resolved before transfusion of any blood components, and in donors, the discrepancy must be resolved before any blood is labeled with a blood type [3].

The most common cause of a discrepancy is a technical error. After this possibility has been ruled out, ABO discrepancies fall into four general categories:

1. Weak- reacting or missing antigens in the forward grouping
2. Unexpected or additional antigen reactions in the forward grouping
3. Weak- reacting or missing antibodies in the reverse grouping
4. Unexpected or additional antibody reactions in the reverse grouping

The aim of our study was to evaluate One Hundred cases of discrepancy between forward and reverse ABO blood grouping methods.

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MATERIALS AND METHODS

An analysis of ABO discrepancies was performed on patients during the period from June 2012 to February 2014 in our center. ABO blood groups of patients were determined by forward and reverse methods and the cases of discrepancies were recorded. The root causes and etiologies of discrepancies were analyzed with clinical details to classify the discrepancies and resolve them with suitable steps.

Patients and specimens:

All patients referred to our center from June 2012 to February 2014 were included in study. Two samples were obtained from every patients; one EDTA sample for forward grouping and a serum sample for reverse or back type method. The only exclusion criterion was hemolysed samples, and resampling was performed for patients with hemolysed samples. All specimens analyzed as soon as possible, or stored at 1-10 $^{\circ}C$ to reduce deterioration of weak antibodies or false reaction due to contamination of the specimen.

Procedures:

Forward grouping was performed by using standard anti-A and anti-B reagents to demonstrate the presence or absence of the A and B antigens on the patients' red cells. Reverse grouping was done by using A_1 and B red blood cells to demonstrate the presence or absence of anti-A and anti-B antibodies in the patients serum or plasma. In the case of occurring discrepancy between forward and reverse blood grouping, the etiology of discrepancy was analyzed with clinical details to classify the discrepancy and resolve it with suitable steps.

RESULTS

All the patients referred to our center from June 2012 to February 2014 are included in study. ABO blood groups of patients were determined by forward and backtype methods, and the cases of discrepancies were recorded. One hundred cases of discrepancy were selected and investigated for determining root causes and etiologies of discrepancies.

From one hundred cases of discrepancies, 71 of them were men, and 29 of them were female. The age range of discrepancy cases were from 3 months to 113 years.

Eighty two (82) of the cases of discrepancy were elderly and newborn patients (70 Elderly and 12 newborn and infants). At these patients, there were no reaction between patients' serum with A_1 and B red cells in backtype blood grouping and no agglutination was occurred in backtype method (age related weak or missing antibodies). The age range of elderly patients was from 88 to 113 years (mean: 93.5), and the age range of newborns and infants was from 3 to 8 months (mean: 6).

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The reason for the missing or weak antibodies is that the patients have depressed antibody production or cannot produce the ABO antibodies. At these patients, the ABO blood group was reported according to the results of front type. For resolving the discrepancy, the reverse grouping reactions was enhanced by incubation of the patients' serum with the reagent cells at room temperature for approximately 30 minutes.The basic information and main results of this group are summarized in Table 1.

Table 1: Age related weak or missing antibodies

	Elderly	Newborns & infants
Age	88-113 years (mean: 93.5)	3-8 months (mean: 6)
A	22	4
B	20	2
O	28	6

Sixteen (16) of the cases of discrepancy were due to Rouleaux formation and cold agglutinins. At these patients, the agglutination was occurred between red blood cells with Anti-A and Anti-B reagents in forward grouping, and the result of forward grouping seems to be AB; whereas, in reverse grouping, the patients' serum agglutinate the A_1 , B and O cells and the result of inverse grouping seems to be O. The pattern caused by Rouleaux formation and cold agglutinins is illustrated in Table 2.

Table 2: Rouleaux and Cold agglutinins

Forward grouping		Reverse grouping		
Anti-A	Anti-B	A_1 cells	B cells	O cells
2+	2+	2+	2+	2+

Table 3: Discrepancy cases caused by Rouleaux formation and cold agglutinins

	Rouleaux formation	Cold agglutinins
Age range	62-78 years (mean: 74.5)	56-67 years (mean: 63.2)
A	2	1
B	2	2
AB	1	1
O	5	2
Total	10	6

In the cases of Rouleaux formation (10 cases), the discrepancy was resolved by washing of red blood cells used in forward grouping with saline, and saline replacement techniques was used to obtain a valid reverse grouping.

In the cases of cold agglutinins (6 cases), the discrepancy was resolved by washing of red blood cells used in forward grouping with warm (37°^{c}) saline or a 45°^{c} elusion technique followed by warm washing to obtaining immunoglobulin-free red cells.The basic information and main results of discrepancy cases caused by Rouleaux formation and cold agglutinins is shown in Table 3.

In two cases of discrepancy, the result of forward grouping was A and the result of reverse grouping was O. Testing with Anti-A_1 lectin (Dolichos biflorus lectin) established that these patients have A_2 blood group. Approximately 1-8% of A_2 individuals demonstrate anti-A_1 along with anti-B in their serum, which causes discrepancy between forward and reverse methods. The pattern of forward and reverse grouping in A_2 individuals with anti-A_1 is shown in Table 4.

Table 4: A_2 individuals with anti-A_1

Forward grouping		Reverse grouping		
Anti-A	Anti-B	A_1 cells	B cells	O cells
4+	-	2+	4+	-

DISCUSSION

The ABO system is the most important blood group system. ABO grouping should include both forward (cell type) and reverse (serum or plasma typing) procedures. The results of forward and reverse methods must agree each other. An ABO discrepancy implies that the forward typing does not agree with the reverse method. It is important to identify an ABO discrepancy and resolve that before transfusion of any blood component.

In our study, age related weak or missing antibody was the most common cause of discrepancy. (Elderly and newborn patients) Infants normally begin to produce anti-A / or anti-B between 3 to 6 months of age, therefore, in newborns and infants younger than four months, only the result of forward grouping is reliable and grouping with anti-A and anti-B reagents is required to establish the ABO group.

Elderly patients, especially patients older than 65 years of age, have low titers of anti-A or anti-B and may have weak-reacting or missing expected antibodies. Therefore, the result of backtype or reverse grouping may not be reliable in elderly patients, and forward grouping method is recommended for determining ABO blood group in elderly patients.

The best way to resolve the discrepancy in age related weak or missing antibody group, is enhancing the reaction in reverse method by incubation the patients' serum with the reagent cells at room temperature for approximately 15- 30 minutes.

The Rouleaux formation was the second most common cause of discrepancy in our study. Rouleaux formation may occur in patients with elevated levels of globulins. To obtain a reliable and accurate result, the red blood cells must be washed with saline before using in forward grouping, and saline replacement technique is necessary for obtaining valid and reliable result in reverse grouping.

Six cases of discrepancy were as a result of cold agglutinins and antibody-coated red cells. The result of direct antiglobulin test (direct coombs test) was positive at these patients. For determining the valid and accurate ABO blood group of these patients, the red blood cells must be washed with warm ($37^{\circ C}$) saline at least six times to yield immunoglobulin-free red cells. Other methods which can be used for obtaining immunoglobulin-free red cells is $45^{\circ C}$ elution technique followed by warm washing of RBC's, and treatment of IgM-coated red cells with Dithiothreitol or 2-Mercaptoethanol.

Two of our discrepancy cases demonstrated blood group A in forward method, and Blood group O in reverse grouping. Testing the red cells with Anti-A_1 lectin established blood group A_2, and testing the serum with A_1 red cells demonstrated the presence of anti-A_1 in serum. (Blood group A_2 with anti-A_1) Anti-A_1 is found in 1-8% of A_2 and 22-35% of A_2B individuals. Anti-A_1 produced by an A_2 individual is not usually reactive at $37^{\circ C}$. Therefore, it is not considered a clinically significant antibody.

Various studies were performed by researchers on analysis of ABO discrepancies. In reports from Department of laboratory medicine in Korea, chimerism and mosaicism are found to be important causes of ABO discrepancy by studying the short tandem repeats loci with DNA-based techniques [4]. Another study on analysis of ABO discrepancies in 35 French hospitals suggests that incidence of ABO discrepancy was 1 per 3400 [5].

In an analysis of ABO discrepancies performed by M.H. Kim et al, an 8 year study revealed an incidence of 82 cases of discrepancy out of 93800 (0.08%) [6]. A study by B. Thakral on importance of weak ABO subgroups, showed 17 weak ABO subgroups in 86687 donors (0.02%) which causes discrepancy in ABO grouping [7].

In a case discussion by In Bum et al, two cases with hepatocellular and gall bladder carcinoma, revealed a discrepancy with red cells typed as O group and reverse typing showing anti-A only. They showed hypogammaglobulinemia which can be the reason for missing antibody in serum [8]. In another case discussion by Picker et al in 49 women with blood group A and AML, after the presentation of disease it was found that A antigen was undetectable even by tube-spin method [9].

Finally, as stated before, for prevention of incompatible blood transfusion, any discrepancy between forward and reverse blood grouping should be resolved before transfusion of blood components.

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CONCLUSION

ABO blood group system is the most important system in transfusion medicine and blood banking. ABO grouping should include both forward (cell type) and reverse (backtype) methods, and the results of two methods should match and agree with each other. Mistyping either a donor or a recipient can lead to transfusion with ABO-incompatible blood, which can result in severe hemolysis and may even result in the death of the recipient. Any discrepancy between forward and reverse blood grouping methods should be resolved before transfusion of blood components.

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