Case report

ABO Blood Group Discrepancy Due to An Unusual Naturally Occurring, Clinically Significant Anti-M antibody: A Case Report

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Abstract

ABO blood group is the most clinically significant blood group determined by forward and reverse grouping, which should match with each other. Any discrepancy between the forward and reverse grouping should be resolved to avoid incompatible transfusion reactions. Anti-M is a naturally occurring antibody that can lead to ABO discrepancy triggering a challenge for the blood bank. The objective of this case report was to present a rare case of anti-M leading to ABO discrepancy. A 2-year-old girl, diagnosed with right canine space cellulitis, was admitted for incision and drainage and tooth extraction under general anaesthesia. Her ABO blood group showed discrepancy where forward grouping was A, but reverse grouping was O with 4+ reaction in both A-cell and B-cell. Repeat testing with a new sample showed similar results. The patient's probable Rh genotype was CDe/Cde (R1R1). The direct Coombs test and autocontrol were negative. She had no prior sensitization events. Antibody screening was positive and antibody identification revealed anti-M with a wide thermal range from 4°C to 37°C. MN phenotype was NN. Subsequent testing showed that the reverse grouping reagent A-cell possessed M-antigen and the positive reaction in reverse grouping was due to anti-M rather than anti-A. Repeat reverse grouping using M-antigen negative A-cells was negative. Crossmatch with M-antigen negative blood was compatible. Any ABO discrepancies should be resolved before transfusion. Anti-M reactive at 37°C is a clinically significant antibody. Therefore, careful interpretation of the results and a thorough analysis of the patient's clinical history, previous blood group or transplant or transfusions history is extremely important.

Keywords: Blood grouping, ABO discrepancies, Anti-M

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Introduction

ABO blood group is the most clinically significant blood group in transfusion services in view of its ability to cause severe transfusion reaction due to incompatible ABO blood group transfusion[1]. This is the most important primary investigation to be done in the blood transfusion service as a pre-transfusion investigation. Therefore, any ABO discrepancies must be resolved before proceeding towards the next step[2]. In ABO grouping, forward grouping or red cell antigen grouping and reverse grouping or serum isoagglutinin grouping are required to match with each other as they serve as a check on the other[2]. Any mismatch between the forward (cell) grouping and reverse (serum) grouping is recognized as the ABO discrepancies. The main reasons for ABO discrepancies are clerical errors, problems related to red cells and plasma or procedure-related problems[3]. In resolving the ABO discrepancy, it is important to confirm the patient's details, patient's medical history,

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history of medication, as well as any sensitizing event such as previous transfusion, transplant or history of pregnancy[3]. It has been mentioned that 4.3%[3] to 10%[4] of ABO discrepancies are due to the presence of cold reacting alloantibodies in the patient. Alloantibodies are most often developed as a result of exposure to non-ABO red blood cell (RBC) antigens following transfusion[5], pregnancy or transplantation[6]. There are naturally occurring alloantibody such as anti-M which are usually not reactive at 37°C, and are considered to be clinically insignificant. However, rarely, these antibodies are reactive at 37°C or at the antiglobulin phase of testing and can lead to hemolytic transfusion reactions and hemolytic disease of the newborn[7]. In this present case report, we are highlighting a rare case of naturally occurring clinically significant anti-M in the patient's plasma causing ABO discrepancy and the serological tests that were carried out to determine its presence.

Case Report

A 2-year-old girl was admitted with the diagnosis of right canine space cellulitis, and planned for incision and drainage (I&D) and extraction of teeth under general anaesthesia. She had underlying iron deficiency anaemia and upper respiratory tract infection. Her haemoglobin was 10.4 g/dl. Group screen hold (GSH) was requested as a pre-operative procedure. Patient had no previous history of blood transfusion or transplantation.

In the laboratory testing, ABO blood grouping done by conventional test tube technique showed discrepancy in forward and reverse grouping. Forward grouping showed A blood group while reverse grouping revealed as O with 4+ reaction with both A cell and B cell. (Table 1). Repeat testing with new sample also showed similar results by tube method and gel-card method as well as with pre-warm technique at strict 37°C. We continued with the antibody screening as a pre-transfusion investigation. Antibody screening with commercially available three cell panel (Bio-Rad ID-DiaCell I-II-III Asia, gel card technique) was found positive and subsequent testing of antibody identification (11 cells panel, Bio-Rad ID DiaPanel, ID DiaPanel-P, gel card technique) showed presence of anti-M which was reactive at 37°C and at anti-human globulin (AHG) phase. Patient's Rh phenotype was identified as CDe/CDe (R1R1) and MN red cell phenotyped as NN. Direct Coomb's test (DCT) was negative with polyspecific anti human globulin (Bio-Rad ID-Card "LISS/Coombs") by gel card technique.

In view of the ABO discrepancy, further testing was performed. Any possibility of technical error was excluded. We excluded the subgroup of A by testing the patient's red cells with anti-A1 lectin which showed strong (4+) reaction. Presence of any autoantibody was ruled out as DCT and autocontrol were negative. In view of presence of anti-M antibody in the patient's plasma, we suspected that the reverse grouping reagent A cell having M antigen cross reacted with the patient's anti-M antibody. Subsequently, by doing the phenotype of the reagent A cell it turned out to be positive for M antigen. This confirmed that the positive reaction on the A cell in reverse grouping was due to anti-M rather than anti-A. Repeat reverse grouping using M-antigen negative A-cell showed no reaction and matched the forward and reverse grouping.

The anti-M detected in the patient's plasma was found to have a wide thermal range, be reactive at room temperature, 37°C and AHG phase. We confirmed the reactivity at 37°C by using strict pre-warm condition for 37°C followed by antiglobulin (AHG) phase. As this anti-M was reactive at 37°C and AHG phase, it was considered as clinically significant antibody. Crossmatch with M-antigen negative packed cell was compatible. The operation of the patient went out without any complication or excess bleeding and the patient did not require any packed cell transfusion during the procedure.

Table 1: Results of ABO blood grouping

	Anti-A	Anti-B	Anti D	A cell	B cell	O cell
M-antigen positive reagent 'A cell'	4	0	4	4	4	0
M-antigen negative 'A cell'	4	0	4	0	4	0

Discussion

In this case report, we have described a case of a 2-year-old girl who showed ABO discrepancy where reverse ABO grouping showed an unexpected reaction due to anti-M present in her plasma. It is reported that the common causes of ABO discrepancies in the reverse grouping are the presence of cold autoantibodies (50.7%), weak or missing antibody (25·4%), cold-reacting alloantibody (4·3%), warm autoantibody (2·2%), anti-A1 antibody (2·2%), Bombay phenotype

(1·5%), transplantation (0·7%) and rouleaux formation (0·7%)[3]. Here, in this case, we have excluded any sensitizing events such as transfusion or transplantation, any technical error, subgroup or presence of any autoantibody and other relevant causes. All these exclusions are very important to resolve the ABO discrepancy. The anti-M antibody identified in this case is a naturally occurring but clinically significant antibody as it was reactive at 37°C and at AHG phase.

The MNS blood group system was discovered in 1927 following the discovery of the ABO system. The Anti-M antibody is a naturally occurring saline agglutinin, mostly IgM type, usually reacts below 37°C and is considered clinically insignificant. But they may be of IgG type or an IgG component can be present in 50-80% of cases[8]. However, anti-M antibody reactive at 37°C or at AHG phase, is considered to be clinically significant[9]. Rarely anti-M antibody is reactive at 37°C or at the AHG phase of testing and can lead to hemolytic transfusion reactions and hemolytic disease of the newborn (HDN)[7]. In this present case, anti-M was reactive at 37°C and at AHG phase and considered clinically significant.

To determine the thermal range, it is necessary to carry out the test at strict 37°C temperature conditions to rule out false positive reactions. Test results negative at 37°C should be evaluated before cooling to prevent false positive results. A negative result may turn positive if the test is permitted to cool prior to evaluation and be misinterpreted as positive due to the binding of IgM at the room temperature[3]. Cold alloantibodies causing ABO discrepancies are usually resolved after doing the test at strict pre-warm temperature[7]. In this present case report, the test carried out by strict pre-warm condition for 37°C and AHG phase showed positive results indicating the anti-M antibody is reacting at 37°C and is clinically significant.

Anti-M does not agglutinate with enzyme-treated red cells and shows a dosage effect where anti-M gives a stronger reaction with homozygous M-antigen than heterozygous. Due to the dosage effects, heterozygous units may be compatible on crossmatch. Therefore, during crossmatch, care should be taken in case of clinically significant anti–M and M-antigen negative blood must be transfused instead of heterozygous compatible units in order to avoid the delayed hemolytic transfusion reaction[7]. The frequency of anti-M in routine blood donors is 1 in 2500 to 5000 as detected with saline-suspended cells at room temperature by microplate technique, and testing with homozygous M+N- cells and heterozygous M+N+ cells respectively[10,11]. There are only a few case reports describing Anti-M that cause blood group discrepancy and crossmatch incompatibility[7,11,12].

Conclusion

This case report highlights a rare example of naturally occurring clinically significant anti-M reactive at 37°C and AHG phase causing discrepancy in ABO grouping. Any ABO discrepancies need to be resolved before transfusion. Therefore, careful interpretation of the results is extremely important. To resolve the discrepancy, it is important to investigate the patient's clinical history, history of previous blood group record, history of any sensitization events and any technical error and should follow proper serological test.

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