

Case Report**A Rare B-Subgroup Causing ABO Discrepancy in Blood Grouping: A Case Report**

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Abstract

This is a 24-year-old gentleman with underlying status epilepticus, admitted for septic shock secondary to meningoencephalitis. On pretransfusion investigation, his blood grouping showed ABO discrepancy. His forward grouping showed negative reaction with anti-A, and anti-B while his reverse grouping showed strong 3+ reaction with A1 cell and no reaction with B cell. Repeated tests with new samples also showed similar results. Subsequently, test was repeated at room temperature and 4°C with prolonged incubation and with enzyme treated cells. Forward grouping showed the same result while the reverse grouping showed enhancement with A1 cell (4+). We suspected B subgroup and proceeded with anti-H lectin test which showed 4+ reaction. Adsorption and elution study confirmed the presence of antigen B on patient's RBCs. Therefore, it is necessary to resolve all discrepancies to ensure safe transfusion practice.

Keywords: B subgroup, ABO discrepancy, Adsorption and elution, anti-H lectin

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Introduction

The ABO blood group system, discovered by Karl Landsteiner in 1901 defined the distinctiveness of an individual by the presence of red blood cell (RBC) antigens on the RBC membrane. A naturally occurring antibody is present in the plasma when the corresponding antigen is absent in the RBC membrane¹. An inverse reciprocal relationship exists between the presence of antigen on RBC and the presence of antibody in sera². ABO grouping is performed as an important pretransfusion test in blood bank that consists of forward and reverse grouping which must tally with each other. Forward grouping is performed by using known antisera anti-A, and anti-B to identify the RBC antigens present on the membrane. While the reverse grouping detects the ABO antibodies present in the patient's plasma by using known reagent RBCs, namely A and

B cells¹. ABO discrepancies occur when there is mismatch between forward and reverse grouping or mismatch occurs between previous and present results. Discrepancies can occur as a result of clerical errors or technical problems or intrinsic causes associated with the RBC or plasma. Any discrepancy must be resolved before transfusion to ensure safe transfusion practice³.

B subgroup is a rare occurrence, discovered during resolving ABO blood group discrepancy⁴. They are found less frequently than A subgroup². The inheritance of B subgroups is considered to be due to mutation in the alleles at the B locus, resulting in the changes in the transferase enzyme activity that add B-sugar to the precursor H antigen^{1,5}. The B subgroup is distinguished by decreased quantity of B antigens on RBC and by the presence of B substances in secretions of the secretor individuals⁴. The different phenotypes in B subgroups include

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B₃, Bm, Bx and Bel. Detection of B subgroup is usually performed by the reaction strength of ABO grouping with monoclonal anti-B and anti-AB and anti-H, the presence or absence of ABO isoagglutinin anti-B in the serum, detection of B substance in saliva, the adsorption-elution studies with anti-B antisera, presence of B transferase in serum and molecular technique¹. The B₃ and Bx phenotype shows a mixed field reaction and weak reaction respectively with anti-B and anti-AB antisera while Bm and Bel are not agglutinated. Normal amount of B substance is present in the saliva of B₃ and Bm secretors, while in the Bx secretor some amount of B substance is present. However, Bel secretor phenotype does not contain any B substance in saliva. Bel phenotype must be determined by the adsorption and elution test. A weak anti-B may be present in Bx^{1,6}. Identification of ABO subgroups is important to resolve discrepancy and to prevent severe transfusion reactions. In this case report we highlight a rare case of B subgroup detected during resolving ABO discrepancy.

Case report

This is a 24-year-old gentleman, with underlying epilepsy, admitted for septic shock secondary to meningoencephalitis. His haemoglobin on admission was 14.4g/dl and a group screen and hold (GSH) request was sent to the blood bank. On testing, using automated analyzer IH 500, his blood grouping showed ABO discrepancy between forward and reverse grouping. His forward grouping showed a negative reaction with anti-A and anti-B while his reverse grouping showed strong 3+ reaction with A1 cell and no

reaction with B cell. The antibody screening and direct antiglobulin test was negative. Taking into account the ABO discrepancy, a new sample was requested. Test was repeated with previous sample and new sample which showed similar discrepant results. All clerical errors and technical errors were excluded. Further investigations were proceeded to resolve the discrepancy. Forward and reverse grouping was repeated using column agglutination technology (CAT) at 22°C room temperature (RT) and at 4°C with prolonged incubation of 30 minutes as well as tested with enzyme treated cells. Forward grouping showed the same result while the reverse grouping showed enhancement of reaction with A1 cell (4+). Reactions with O cell was negative. Therefore, B subgroup was suspected. The test with anti-H lectin, *Ulex europaeus*, showed 4+ reaction, which indicate no transformation of H antigen to B antigen. Further, adsorption and elution study was done. Adsorption of patient's washed RBC with reagent antisera anti-B was done at 4°C for 1 hour. Subsequently, the eluate was prepared by Lui Freeze thaw elution and tested with B and O reagent cells that showed weak positive reaction with B cell and negative reaction with O cell. The anti-B detected in the eluate confirms presence of a weak B antigen on tested RBCs. Non-reactivity with O cell signifies the validity of the test. With all the supplementary tests done, patient's blood group was concluded as B subgroup. We could not proceed with secretor study and genotyping as they are not available at our center. However, we suggested to the patient to do these tests at referral center together with the family study to detect any family member having a subgroup.

Table-1: List of tests performed on patient to resolve the ABO discrepancy.

ABO grouping	Forward group		Reverse group			Lectin	Adsorption-elution	
	Anti-A	Anti-B	A cell	B cell	O cell	Anti-H	Eluate tested with	
							B cell	O cell
IH -500	0	0	3+	0	-	Positive (4+)	1+	0
Prolonged at RT	0	0	3+	0	0			
Prolonged at 4°C	0	0	4+	0	0			
Enzyme treated cell	0	0	4+	0	0			

Discussion

In this case report, a rare subgroup of B is highlighted which showed discrepant results in the forward and reverse ABO grouping. The forward

group looks like group O while the reverse group showed group B. Test with anti-H lectin revealed an increased amount of H antigen as there is little conversion of H antigen to B antigen. By using

the conventional serological testing of adsorption-elution, presence of weak B antigen in the patient's RBC was detected and subgroup B was confirmed. Earlier, few cases of B subgroup were reported³⁻¹⁰. Although the secretor study and more advanced molecular study are available as a better option for the detection of ABO subgroup, the suitability of the adsorption-elution test is demonstrated here which can be used by blood bank laboratories without the confirmatory tests⁴.

In this present case, we have excluded other causes of discrepant results such as haematological diseases, extreme age or any transfusion/transplant event. Medication and pregnancy history are also important to rule out causes of discrepancy^{1,4}. Frequency of B subgroup is around 2.3% to 4%.^{3,9} and is reported to be more common among Indian population⁵. It is better to inform the subgroup individuals about their blood group status and if possible, a special blood group card to be issued explaining their respective donor and recipient status^{5,7}.

Identification of ABO subgroups is important for both patient and donor. Although there is no reported haemolytic transfusion reaction (HTR) involving B subgroups, it is possible to develop HTR if the discrepancy is not resolve. Weak

subgroup B donors may be mistyped as group O and subsequently if transfused to group O patient containing anti-A,B, or recipient mistyped as O and transfused with O plasma, haemolytic transfusion reaction can ensue⁴. Therefore, any discrepant result must be resolved to provide safe transfusion practice¹¹. Patients with B-subgroup should be transfused with group O RBC and group B matched/compatible plasma and platelet components^{4,7}. In our patient, packed cell was not transfused, however, fresh frozen plasma and cryoprecipitate were transfused due to coagulopathy and compatible AB plasma was given.

Conclusion:

In this case report, we highlighted the rare B subgroup detected during resolving the ABO discrepancy. The conventional adsorption-elution method is an effective way to detect the subgroup and any discrepant results in ABO grouping should be resolved to ensure safety of transfusion.

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