Patient with a rare weaker subgroup-A exhibiting ABO discrepancy: a case report

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ABSTRACT

Weaker subgroups have the potential to cause problems in the immunohaematological testing in the blood banks. We report here a case of rare weaker subgroup A, detected during the usual ABO grouping. A 49-year-old lady exhibited a discrepancy in the forward and reverse groupings. In forward-grouping, there was a mixed-field reaction with anti-A and anti-A,B while there was no reaction with anti-B. The reverse grouping showed no reaction with the A1 and A2 cells, but the reaction with B-cell was 4+. Further tests were performed and revealed a weaker subgroup of A. It is crucial to properly investigate and resolve any discrepancy in ABO grouping before commencing transfusion to avoid any wrong blood transfusion. This case report may assist others in resolving the ABO blood group discrepancies.

Keywords
ABO grouping; weaker subgroup A; mixed-field reaction; ABO discrepancy

INTRODUCTION

Blood transfusion is a crucial lifesaving treatment modality used in hospitalized patients in different clinical situations1,2. ABO grouping is an essential pre-transfusion investigation performed on patients undergoing blood transfusion3. The expression or lack of ABO antigens in red blood cell (RBC) and corresponding antibodies in the plasma determines the ABO blood group. Plasma contains naturally occurring Anti-A and anti-B when the corresponding A or B antigens are lacking in the RBC membrane4,5. The ABO antigens are the carbohydrate structures present on the RBC membrane, which are also expressed on platelets, lymphocytes, most epithelial cells and endothelial cells6. The ABO gene, located in chromosome-9, codes for the glycosyltransferase enzyme that adds N-acetyl D-galactosamine (GalNAc) and/or D-galactose (Gal) sugar terminally to the H antigen to produce A and/or B antigen, respectively7. Alteration in the genes encoding the ABO blood group system can cause a diminished A or B antigen production on RBC and thus produce subgroups6,8.

In blood group-A individuals, forward grouping

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determines A-antigen, expressed on the RBC membrane and reverse grouping determines anti-B present in the plasma. The major subgroups of A are mainly as A1 and A2, which differ qualitatively and quantitatively. Among 99% of group-A individuals, 80% belong to A1(or A1B) and 20% to A2 (or A2B)4. Around 1%-8% of A2 individuals and 22%-35% of A2B individuals can produce anti-A1 antibodies, which are atypical cold IgM agglutinin, usually not clinically significant and react better at cold temperature7. A1 can be differentiated from A2 in the forward grouping based on the reaction with anti-A reagent and anti-A lectin reagent derived from Dolichos biflorus seeds. The positive reaction with anti-A and anti-A1 denotes the A1 subgroup, whereas reaction with anti-A only, not with anti-A1, denotes the A2 subgroup7. There is an abundant H antigen expressed in A2 individuals compared to the A1. This is due to the conversion of a small amount of H antigen to A2-antigen, leaving most of the H antigen detectable in the RBC. Thus, A2 red cells give a stronger reaction to anti-H4. The anti-A1 antibody produced in A2 and A2B individuals is usually a cold antibody type, but occasionally it can be reactive at 37°C and can cause a haemolytic transfusion reaction if Group-A1 red cells are transfused to them. Therefore, if subgroup-A individuals develop anti-A1, which is reactive at 37°C, the transfusion should be carried out using compatible subgroup-A or with O blood4.

Other weaker A-subgroups are less prevalent and constitute approximately 1% of those encountered in the laboratory, and these subgroups show serologically weaker reactions or no reaction with anti-A antisera compared to the reactions in the A2 individuals7,9. Less frequent weaker A-subgroups such as A3, Ax, Aend, Ay, Am, and Ael can be differentiated based on the (i) diminished A-antigen sites on RBC surfaces, (ii) varying degrees of agglutination by human anti-A and some monoclonal anti-AB, (iii) having a stronger reaction with anti-H and (iv) presence or absence of anti-A1 in plasma4,10. The serological test applied to detect weaker subgroups are forward and reverse grouping, secretor studies, adsorption-elution tests and molecular testing1. In the forward grouping, A3, Aend, Ax and Am may show weak agglutination with anti-A and/or Anti-AB, while Ay and Ael cells are not agglutinated4,7. A3 subgroups show a mixed-field appearance in the forward grouping, demonstrating agglutinates in the background of abundant free cells6. Weaker A subgroups are differentiated from the A1 group by test using anti-A lectin, which reacts only with A1 cells leaving A2 and other weaker subgroups as a non-reactive. Moreover, there may be an occasional presence of anti-A1 antibodies in the plasma of weaker subgroups9. The reverse grouping of weaker Ax subgroups shows almost always the presence of anti-A1 antibody in the plasma, whereas A3, Aend, and Ael occasionally show the presence of this antibody, while Am and Ay do not develop this antibody4,7. In India, one study among the donor population showed, the prevalence of weaker subgroup A3 and Ax was 1: 14,448 each while Aend was 1: 43,3447.

The secretor status is performed using a saliva test to detect H antigen and/or A antigen in the patient’s saliva. In subgroup A3, Am and Ay secretor individuals, A and H substances can be detected in the saliva by a saliva test4. In the adsorption-elution test, adsorption is done using polyclonal anti-A and anti-A,B derived from Group-B and Group-O individuals, respectively, followed by elution of bound antibody and subsequently testing the elute against A or B reagent red cells7,11. Serum glycosyltransferase study is a useful method to differentiate weaker subgroups where it may show the presence of A-transferase enzyme in A3, Am and Ax, but absence in Aend and Ael7,11. Molecular testing is important in confirming the ABO subgroup9.

The weaker ABO subgroups pose a challenge for immunohaematology practice in the blood bank. The weaker subgroups may have the chance to be wrongly grouped as group O due to the use of variable techniques and reagents, which can lead to a haemolytic transfusion reaction4,11. Although the subgroups are rare, they are usually discovered during resolving discrepancies between the forward and reverse group results12. Any discrepant result in ABO grouping must be identified and investigated to determine the underlying cause before the correct blood type result is released10,13. Therefore, it is very important to detect the correct blood group before transfusion to avoid any serious acute transfusion reactions. Here, we have highlighted a case report of a rare weaker Subgroup-A.

CASE REPORT

A 49-year-old lady was admitted to the Emergency Department with right intracranial bleeding. The patient was planned for transfusion due to bleeding and a low haemoglobin level. A request for group and cross-
matching was sent from the Emergency Department. The blood bank noted the ABO discrepancy in the pre-transfusion investigation (Figure-1). Her ABO grouping was performed using the tube method and gel card method. In her forward-grouping, a mixed-field reaction was observed with monoclonal anti-A, while there was no reaction with anti-B. Test with polyclonal anti-A,B derived from O donor revealed a similar mixed-field reaction. Her reverse-grouping showed a negative reaction with reagent A1 cell and A2 cell, while the reaction with reagent B-cell was 4+. Any possible technical problems were excluded. The patient has no history of a recent transfusion or transplant. The tests were repeated using the same and a new sample showing a similar reaction. Further tests of ABO grouping were performed using the cold temperature enhancement method at 4°C and prolonged incubation at room temperature and the enzyme enhancement method, but could not resolve the discrepancy. A possible weaker subgroup A was suspected in this patient. A further test was continued with anti-A1 lectin, Dolichos biflorus, which exhibited a negative reaction; while the test with anti-H lectin, Ulex europaeus, demonstrated a 3+ reaction (Figure-2). All these tests confirmed the grouping as subgroup-A, which we suspect most likely to be of subgroup A3 type, without having anti-A1 in the plasma. The summary of the serological test results is shown in table-1. The patient was transfused with group-A cross-matched compatible packed cell without any adverse reaction and was found to have achieved the targeted haemoglobin level. The family screen was planned for this patient to detect any family member with a similar blood group.

DISCUSSION

In this case report, a rare weaker Subgroup-A is presented. This patient showed a negative reaction to anti-A1 lectin, confirming the absence of an A1 antigen.

Table-1: Serological reactions demonstrated in the patient

<table>
<thead>
<tr>
<th>Forward group</th>
<th>Rh-D group</th>
<th>Reverse group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>Anti-A1 lectin</td>
<td>Anti-B</td>
</tr>
<tr>
<td>mf</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure-1: ABO and Rh D grouping by manual gel card method showed mixed-field reaction with anti-A.

Figure-2: The serological reaction of the patient with anti-A1 lectin and anti-H lectin. Patient showed a negative reaction with anti-A1 lectin (control cell: A1 and A2 cell). Anti-H lectin showed 3+ reaction (control cells: A1 and O cell).

A strong 3+ reaction with anti-H lectin detects an abundant H antigen. We suspect this case as A3 subgroup due to the strong mixed-field reaction with anti-A and anti-A,B. It is reported that A3 subgroups typically show a mixed-field appearance with anti-A and anti-A,B as small agglutinates with many free or unagglutinated cells \(^4,6\). This mixed-field pattern is demonstrated in our present case (Figure-1). Occasionally Anti-A1 may
be detected in the reverse grouping of A3 individuals. But, our patient has not developed any anti-A1. It is reported that A3 individuals possess around 35000 antigenic sites in each RBC.

The ABO discrepancy should be investigated and resolved before issuing the blood. All the test should be repeated using the same sample and a new sample. Careful history taking on the patient’s diagnosis and current medication history is important, as various malignancies and haematological disorders can alter the ABH antigens. History of a previous blood group, recent transfusion history, history of pregnancy or transplant is also very important to exclude discrepancy. Any technical problem should be excluded. For ABO discrepancy due to weak expression of antigens and antibodies, further ABO grouping tests need to be done by cold temperature enhancement method by repeating the test with prolonged incubation at low temperatures (room temperature and 4˚C). Enzyme treatment of autologous red cells and subsequent tests with monoclonal or polyclonal anti-A, anti-B, and anti-A,B can enhance antibody binding and detect weak ABO antigens and antibodies. Other methods for detecting weaker subgroups are secretor studies, adsorption-elution tests, molecular testing and serum glycosyltransferase studies. We did not perform molecular testing or serum glycosyltransferase as they are unavailable in the center. Family studies can be done to detect if other family members have this rare subgroup.

CONCLUSION
A weaker subgroup-A discovered during resolving ABO blood group discrepancy is highlighted in this case report. Identification of weak subgroups is required to ensure a safe and effective transfusion. Better techniques such as genotyping or molecular testing are better options for identifying rare blood groups. All ABO discrepancies should be resolved before issuing the blood product. A good knowledge of the ABO subgroup and an understanding of its clinical importance is crucial for blood bank personnel as well as clinicians.

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References


