Detection of a weaker subgroup of A in ABO blood group system

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Abstract:
Testing to detect ABO incompatibility between a donor and potential transfusion recipient is the foundation on which all other pretransfusion testing is based. Sometimes, weak agglutination reactions may be obtained with reagent antibodies due to weak expression of A and B antigens on red blood cell (RBC) surface which may cause a discrepancy in blood group typing. Here, we report a patient showing discrepancy between RBCs (forward) and serum (reverse) typing. After doing detailed analysis, the blood type as a variant of blood Group A revealed. Subgroups of A are very rare phenotype of blood. Weaker subgroups of A blood group reported so far are mainly A<sub>3</sub>, A<sub>end</sub>, A<sub>x</sub>, A<sub>m</sub>, A<sub>y</sub>, and A<sub>el</sub>. We are reporting a case of a 35-year-old patient whose RBCs showed a discrepancy between cell and serum grouping during initial testing. Serological investigation included absorption elution tests and saliva tests after performing initial blood grouping. The serological characteristics of the patient’s red cells were similar to A<sub>3</sub> subtype. The patient was a secretor and A and H substance was present in the saliva. Serum did not show any presence of anti-A1. The weak A phenotype identified had serological characteristics similar to A<sub>3</sub>.

Keywords:
A3 subgroup, blood grouping, discrepancy, forward typing, reverse typing

Introduction

The ABO blood group continues to be the most important blood group in transfusion and transplantation due to immunogenicity.[1] It is the only blood group system in which individuals already have antibodies in their serum to antigens that are absent from their red blood cells (RBCs) without any prior exposure to RBCs through transfusion or pregnancy. Due to the presence of these antibodies, transfusion of an incompatible ABO type may result in immediate lysis of donor RBCs. This produces a very severe, if not fatal, transfusion reaction in the patient.

ABO antigens are widely expressed on human tissues and fluids, leading to their designation as histo-blood group antigens. The inheritance of ABO genes follows simple Mendelian genetics. ABO, like most other blood group systems, is codominant in expression.[2] One position, or locus, on each chromosome 9 is occupied by an A, B, or O gene.[3,4] The expression of A and B antigens on the RBCs is fully developed by 2–4 years of age and remains constant throughout life.[2] The ABO antibodies are predominantly IgM, activate complement, and react at room temperature or colder.[5] ABO antibodies produce strong direct agglutination reactions during ABO testing. The ABO system contains several weak subtypes. All are characterized by decreased A/B and a parallel increase in H-antigen expression. Weak subtypes can be associated with ABO typing discrepancies during serum grouping due to the presence of unexpected anti-A or anti-B activity. In some instances, A/B expression is so weak that red cells in forward typing can
type as Group O. Subgroups weaker than A₂ occur infrequently and are most often recognized through ABO discrepancy with unexpected reaction in the forward and reverse grouping. These subgroups of A make up 1% of those encountered in the laboratory and therefore academic interest. They are wrongly mistyped as Group O because of decreased number of A antigen sites per RBC membrane thus resulting in weak or no agglutination with human polyclonal anti-A. A₂ and other week A subgroup red cell subtypes are serologically distinguished from A₁ red cells by their lack of reactivity with the lectin Dolichos biflorus. Weak A/B subtypes have increased reactivity with Ulex europaeus, an anti-H lectin. These weak phenotypes, in majority of cases, result from expression of a variant A allele present at the ABO loci. These can be divided into two categories depending on whether the cells are agglutinated with anti-A, antiseras-Aᵢ, Aเสม, and A₂ are agglutinated, while Aเสม, Aᵢ, and A₂ cells are not. The above weaker phenotypes can be serologically differentiated from each other using the following techniques:[7]

- Forward grouping of A and H antigens with anti-A, anti-A, B, and anti-H
- Reverse grouping of ABO isoagglutinins and the presence of anti-A₁
- Testing with different batches of anti-A reagent
- Adsorption-elution experiments with polyclonal anti-A and anti-A + B from Group B and Group O individuals
- Secretor status for the presence of H and A antigen in saliva
- Molecular genotyping of ABO gene, especially exons 6 and 7 as they encode for 77% of glycosyltransferase activity.

The weak A phenotype identified in the present case had serological characteristics similar to A₁ blood type.

**Case Report**

A 35-year-old female visited obstetrics and gynecology OPD in our hospital. Blood sample was sent to the IHBT department for ABO grouping and Rh typing. Discrepancy was observed between cell and serum grouping. The patient’s red cells were nonreactive with anti-B and give mixed field pattern of agglutination with anti-A and 1+ with anti-A₁. However, it showed 2+ pattern of agglutination with anti-A, B and strong agglutination pattern with anti-H (4+). The patient’s serum showed the presence of anti-B antibody. Blood grouping pattern observed was suggestive of the presence of weaker subgroup of A, indicating Type II discrepancy.[8]

The results are summarized in Table 1.

**Blood grouping**

Blood grouping was again repeated with the same sample and technical errors were ruled out. To detect the presence of weak A antigen, heat elution was performed on the patient’s red cells. The elute showed microscopic agglutination with three different A group cells [results summarized in Table 2]. To detect the presence of soluble substances, secretor status was determined using patient’s saliva. The patient was found to be a secretor, having A and H substance detectable in the saliva [results summarized in Table 3]. These serological reactions obtained were consistent with reactivity pattern of A₁ subtype.

**Discussion**

The risk of hemolytic transfusion reaction due to transfusion of ABO-incompatible blood is 100–1000 times higher than the risk of transfusion-transmitted infections, and such a reaction may lead to serious consequences in the recipient.[9] More than 250 ABO alleles have been identified by molecular investigation.[10,11] The ABO gene, located on chromosome 9, consists of seven exons.[10] The last two exons (6 and 7) encode for the catalytic domain of the ABO glycosyltransferases.[12] Exons 6 and 7 constitute 77% of the gene, with most of the coding sequence lying in exon 7.[12,14] Amino acid substitutions, resulting primarily from deletions, mutations, or gene recombination’s within these two exons of the coding DNA of variant ABO glycosyltransferases, are responsible for the less efficient transfer of the immunodominant sugar to H substance, resulting in weak serologic reactions observed in ABO subgroups.[12] Weaker variants of A and B arise due to inheritance and expression of variant alleles at the ABO locus. Hemagglutination-based methods are used to identify these subgroups. However, due to variation in reagents and techniques used, these weaker phenotypes are often mistyped as O group. Sometimes, weaker subgroups of A may present practical problems, as if donor mistyped as Group O and transfused to a Group O recipient. This is potentially dangerous because the Group O recipient possesses anti-A, B, which agglutinates and lyses RBCs, causing rapid intravascular hemolysis. Hence, it is important to characterize these weaker

| Group | Anti-A | Anti-A₁ | Anti-B | Anti-A, B | Anti-H | Anti-D |
|-------|--------|---------|--------|-----------|--------|--------|
| A₁    | mf     | 1+      | 0      | 2+        | 4+     | 4+     |

Mf=Mixed field

**Table 1: Serological reactions observed on testing patient**

| Group | Anti-A | Anti-A₁ | Anti-B | Anti-A, B | Anti-H | Anti-D |
|-------|--------|---------|--------|-----------|--------|--------|
| A₁    | mf     | 1+      | 0      | 2+        | 4+     | 4+     |

**Table 2**

**Table 3**
subgroups as accurate determination of the ABO type would help in better management of “O” group RBCs and “AB” plasma for transfusion in discrepant cases."[13]

In this case study, the patient’s blood group was serologically identified as A\textsubscript{3} type. The red cells of A\textsubscript{3} individuals distinguish themselves by giving mixed field by anti-A sera, negative reactions with anti-B sera, but remarkably strong reactions with anti-A, B sera.

Weak subgroups of “A” are rare and require advanced techniques like “Adsorption and Elution” for their detection. Molecular testing is usually required for confirmation and exact typing of the subgroups (not available in our institute).

**Conclusion**

If any ABO discrepancy found in any donor or recipient between forward and reverse typing, that should be informed both to the laboratory staff and physicians due to the high probability of weak expression of blood group antigens. The absence of a disease process should be confirmed before subgroup investigation because ABH antigens are altered in various malignancies and other hematologic disorders. Additional special procedures such as molecular testing for mutations or serum glycosyltransferase studies for detecting the A enzyme can be performed for differentiation of weak subgroups.[6,16] Patient should be transfused with Group “O” red cell components and should receive group matched/compatible plasma and platelet components.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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**Conflicts of interest**

There are no conflicts of interest.

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