The ABO blood group system includes phenotypes, or subgroups, that differ in the amount of A and B antigens present on the red blood cells (RBCs). These subgroups also differ in the A, B, or H substances present in secretions (for individuals who have the secretor phenotype). B subgroups are very rare and are less frequently reported than A subgroups. Usually, B subgroups are discovered during serologic testing when there is a discrepancy between RBC and serum grouping results. Subgroups of B are usually identified by a reference laboratory using molecular and adsorption-elution methods. This report details a case of a young, healthy, pregnant woman with a B subgroup detected by a small transfusion service using adsorption-elution methods. Serology and genotyping of the ABO gene was performed at a reference laboratory where the serology was consistent with a B subgroup, but no changes were identified in ABO gene sequencing. It is important to correctly identify B subgroups in donors and recipients to help resolve ABO discrepancies and potentially prevent ABO incompatibility in blood transfusion, thus minimizing transfusion reactions. *Immunohematology* 2021;37:89–94. DOI:10.21307/immunohematology-2021-0014.

**Key Words:** blood group discrepancy, ABO blood group, B subgroup, transfusion reaction, ABO mismatch

The ABO blood group system is the most clinically significant antigen system in transfusion medicine. The ABO gene is located on chromosome 9 and consists of seven exons. Exons 6 and 7 encode for the catalytic domain of the ABO glycosyltransferases. The A blood group is formed by the addition of N-acetyl-galactosamine to the H antigen, the B blood group is formed by the addition of galactose to the H antigen, and blood group O results when no sugar is added to the H antigen.1 ABO blood groups have subgroups that are often distinguished by decreased amounts of A or B antigens on red blood cells (RBCs). A and B subgroup phenotypes can be identified based on the quantity of A or B antigens carried on RBCs and A or B substances present in secretions (for individuals who have the secretor phenotype). The most common subgroups are A1 and A2 (in Europeans, 80% of all group A and AB individuals are A1 and 20% are A2).2 Blood group A appears to have more subgroup variations than group B. In general, the serologic distinction between A1 and other subgroups of A is based on the ability of anti-A1 lectin (an extract of *Dolichos biflorus* seeds) to agglutinate A1 RBCs but not RBCs of other A subgroups.2 There are no comparable B1 and B2 subgroups, but examples of group B RBCs that react weakly or not at all with anti-B have been described.3 B subgroups are very rare and are less frequently reported.3 Usually, B subgroups are discovered during serologic testing when there is a discrepant result between RBC and serum grouping tests. Subgroups of B are difficult to classify and include B1, B2/Bweak (previously known as B2), Bweak (previously known as B1w), and B1d phenotypes, according to the International Society of Blood Transfusion (ISBT).5,6 The B subgroups can be classified by their agglutination strength with anti-B, anti-A,B, and anti-H; the presence or absence of anti-B in the serum; the presence of B substance in saliva; the results of adsorption-elution testing; and molecular studies (Table 1).2,6 An up-to-date listing of molecular variants recognized by ISBT that give rise to the observed serologic phenotypes can be found on their Web site in the table for ABO (ISBT 001) blood group alleles v1.2.4 Definitive identification of ABO subgroups is usually performed at a reference laboratory using molecular and adsorption-elution methods, which often have long turnaround times.

**Case Report**

Here we report a case in which a B subgroup was detected by an observant technologist using both automated gel and manual tube methods with conventional reagents at a busy hospital transfusion service. The facility is a 450-bed, small, community hospital and level 1 trauma center. The hospital transfuses an average of 5500–6000 total units of blood components annually. The lab received an EDTA-anticoagulated blood specimen for a type and screen order from a young healthy pregnant woman. Discrepant results (forward as group O and reverse as group B) were observed while performing routine blood ABO testing by the gel method (see Methods and Results). Repeat testing by a conventional tube method showed the same discrepancy. This 29-year-old African American woman presented to an obstetric clinic.
for routine prenatal care at 28 weeks, 4 days, gestational period for Rh(D) determination for possible Rh immune globulin (RhIG) administration. At the time of this visit, the patient had no previous history of transfusion, pregnancies, or transplantation, with current diagnosis of nonimmune, mild anemia, and a history of varicella. Her hemoglobin and hematocrit were 10.3 g/dL (normal range 11.9–16.0 g/dL) and 29.7 percent (normal range 35–47%), respectively. No blood transfusion was needed at this time. Her RBCs were typed as D–, and she received RhIG during this visit. The patient had previously tested as group O, D– with a negative antibody detection test at two other hospitals for the same pregnancy, including her most recent visit 1 month prior. About 3 months later, the patient had a spontaneous vaginal delivery with no transfusion demands. Postpartum RhIG was administered to her in a timely manner.

Methods and Results

Serologic-based ABO phenotypes were determined by agglutination and adsorption-elution testing according to standard methods and procedures described in the AABB Technical Manual. Discrepant results (forward as group O and reverse as group B) were observed while performing routine blood grouping on an automated gel-method analyzer (ORTHOVISION; Ortho Clinical Diagnostics, Raritan, NJ); antibody detection test results were negative. An attempt to resolve the discrepancy observed on the automated gel analyzer led the technologist to repeat testing using the conventional manual tube method, which showed the same discrepancy.

The manual tube method demonstrated no agglutination with the patient’s RBCs and blood grouping reagents (Ortho Clinical Diagnostics), including anti-A, anti-B, and anti-A,B (BioClone [Murine Monoclonal Blend], San Diego, CA) at immediate spin, or after 30 minutes at room temperature, but weak reactivity was noted with anti-B and anti-A,B at 4°C. The patient’s serum showed the presence of a strong anti-A, but no anti-B was detected using pooled RBCs (AFFIRMAGEN; Ortho Clinical Diagnostics) (Table 2).

Although decreased expression of A and B antigens has been observed in patients with hematologic malignancies, our patient was a healthy young woman, suggesting the possibility that the discrepant ABO typing results were due to a weak B subgroup. To explore the possibility of a B subgroup, direct antiglobulin tests (DATs) were first performed by conventional tube method with anti-IgG (Rabbit; Ortho Clinical Diagnostics) and anti-C3b/-C3d (Murine Monoclonal; BioClone, Ortho Clinical Diagnostics) reagents; all DAT results were negative.

Table 1. Characteristics of some more frequent B phenotypes

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Reagents</th>
<th>Antibodies in serum</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-A</td>
<td>Anti-B</td>
<td>Anti-A,B</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>B₂</td>
<td>0</td>
<td>++mf</td>
<td>++mf</td>
</tr>
<tr>
<td>B₃</td>
<td>0</td>
<td>wk</td>
<td>wk</td>
</tr>
<tr>
<td>B₄*</td>
<td>0</td>
<td>0/wk</td>
<td>0/wk</td>
</tr>
<tr>
<td>B₅*</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*B specificity demonstrated only by adsorption-elution methods.
0 = negative; mf = mixed field; wk = weak; pos = positive.

Table 2. Results of forward and reverse grouping and antibody detection tests

<table>
<thead>
<tr>
<th></th>
<th>RBCs with reagent</th>
<th>Serum with reagent</th>
<th>Autocontrol</th>
<th>Antibody detection test (gel method)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-A</td>
<td>Anti-B</td>
<td>Anti-A,B</td>
<td>A, RBCs</td>
</tr>
<tr>
<td>IS RT</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4+</td>
</tr>
<tr>
<td>30 minutes RT</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4+</td>
</tr>
<tr>
<td>4°C</td>
<td>0</td>
<td>W+</td>
<td>W+</td>
<td>4+</td>
</tr>
</tbody>
</table>

RBCs = red blood cells; IS = immediate spin; RT = room temperature; W+ = weak reaction.
After ensuring that the patient's RBCs were not coated with IgG and/or C3b/C3d by the DAT, adsorption was performed using washed patient RBCs and reagent anti-B (BioClone, Murine Monoclonal Blend; Ortho Clinical Diagnostics). After 1-hour incubation at 4°C, an acid elution was performed. The eluate and final wash supernatant were tested in parallel against A\textsubscript{1} and B reagent RBCs (AFFIRMAGEN), and three group O reagent antibody screening RBCs (SURGISCREEN; Ortho Clinical Diagnostics). The eluate reacted only with group B RBCs when incubated at 37°C for 15 minutes and tested using the indirect antiglobulin test (Table 3). Because the patient's saliva was not available for secretor testing, whether the patient has a B\textsubscript{weak} or B\textsubscript{si} phenotype remains unknown.

<table>
<thead>
<tr>
<th>Table 3. Adsorption-elution results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent RBCs</td>
</tr>
<tr>
<td>Eluate</td>
</tr>
<tr>
<td>Last wash</td>
</tr>
</tbody>
</table>

*Reagent antibody screening red blood cells (RBCs).

Discussion

This case showed that conventional serologic testing can be used to detect a weak B subgroup. The detection of weak ABO subgroups is very important in clinical settings, as well as in blood donor centers, because a person with weak B expression may be wrongly typed as group O when anti-B is present in the patient/donor plasma. Rare hemolytic transfusion reactions (HTRs) due to ABO incompatibility involving ABO subgroups have been reported. As Chun et al. reported, a patient with cis-A\textsubscript{1},B\textsubscript{3} interpreted as typical group A experienced adverse effects after transfusion of 4 units of group A RBCs and 4 units of group A fresh frozen plasma (FFP). Although reaction between the B antigen of the cis-AB patient and anti-B from the group A FFP is theoretically possible, the authors could not draw a definitive conclusion for the cause of hemolysis. To our knowledge, an HTR involving B subgroup has not been reported.

Theoretically, an HTR (perhaps with mild clinical effects or shortened RBC survival due to lower-level antigen expression on the patient’s RBCs) may happen in transfusions involving ABO subgroups, such as when a B subgroup recipient mistyped as group O is transfused with a group O plasma–containing blood component, or a RBC unit from a donor with weak B subgroup mislabeled as group O is transfused to a group O recipient. Chaurasia et al. emphasized that when individuals with B subgroups present as transfusion recipients, they should be transfused with group O RBC components and group B–specific plasma and platelet components. These authors report that RBCs from B subgroup donors transfused to group O recipients can result in an adverse HTR.

In the United States, as required for pre-transfusion testing by the AABB Standards for Blood Banks and Transfusion Services, the ABO group shall be determined by testing RBCs with anti-A and anti-B reagents and by testing the serum or plasma for expected antibodies with A and B reagent RBCs. If a discrepancy is detected and transfusion is necessary before resolution, only group O RBCs shall be issued.

This type of serologic discrepancy, with decreased expression of A or B antigens on RBCs, can be observed in acquired ABO subgroup phenotypes such as seen in infants, individuals with hematologic disorders, and pregnant women; however, our patient was healthy, and, although pregnant, had typed as group O in two hospitals she visited previously. These reported results indicate that anti-B was presumably detected in her plasma, although this information was not confirmed with the testing facilities. The technologist
at our transfusion service was able to “dig deep” into this case and used serologic investigation to discover a weak B subgroup. The reference laboratory’s serology tests confirmed this finding. The detection of a B subgroup is important to prevent potential ABO-mismatched transfusion reactions. Weak B subgroups are rarely detected in a hospital transfusion service setting. The ability to confirm a weak ABO subgroup phenotype generally requires molecular testing. DNA-based tests are increasingly being used at immunohematology reference laboratories to predict blood group phenotype, which helps to improve transfusion medicine practices. Reference laboratories use several methods for genotyping, including PCR followed by RFLP analysis, sequence-specific primer PCR, DNA sequencing, and array-based approaches. A notable advantage of molecular testing is its ability to identify variant alleles associated with antigens that are expressed weakly or have missing or altered epitopes, thus helping to resolve discrepant or incomplete blood group phenotyping. The disadvantages of molecular testing are mainly the longer turnaround time (days to weeks) and higher cost, compared with serologic typing.

In our patient’s case, full ABO gene sequencing did not identify any changes in the B allele that would explain the undetectable B antigen expression, raising the possibility that the patient may have a gene regulator or a promoter mutation in the non-coding regions of the B allele. Given that most weak B phenotypes are due to random missense mutations in the last ABO exon, the failure to identify a mutation makes this case somewhat unusual. Nevertheless, uncommon novel mutations are increasingly identified given the widespread availability of molecular testing, and mutations outside of the coding region are not uncommon.

Huang et al. reported the analysis of 1.4 million Chinese blood donors; 351 cases of weak ABO subgroups were identified serologically. In the 326 samples subject to DNA analysis, 44 ABO mutations were identified, of which only 10 were novel. No mutations were observed in 99 samples. A similar recent analysis of 211 ABO subgroups in Chinese blood donors detected by discrepant serologic typing revealed that 36 samples (approximately 17%) including four pregnant donors had discrepant results that were not explained by underlying disease or initial sequencing and may have an unexplained molecular basis. Mutations leading to weak subgroups may also occur in areas outside the coding region of the ABO gene. Seltzam et al. reported that weak B phenotypes can be caused by sequence variation in the CCATT-binding factor (CBF)/NF-Y region of the ABO gene, and Thuresson et al. described a hybrid between an O2 and a B allele and characterized the associated decrease in B antigen expression. Sano et al. identified a positive regulatory element in intron 1. This element was shown to enhance ABO promoter activity in an erythroid cell–specific manner, and a partial deletion in intron 1 involving the erythroid cell–specific regulatory element was associated with Bm phenotypes. The authors suggested that deletion of this enhancer-like element in intron 1 of ABO regulates transcription in the Bm allele, leading to reduction of B antigen expression on cells of erythroid lineage, but not in mucus-secreting cells. In the study of Ying et al., 75 samples of ABO variants were considered to have an unclear molecular basis, because only normal sequences were detected in their ABO coding regions. Among these samples, 32 were from individuals with infection, hematologic disorders, and other diseases. For the remaining 43 individuals with ABO subgroups, seven were associated with the erythroid cell–specific regulatory element in the first intron of the ABO gene based on the analysis of the nucleotide sequences of the partial intron 1 covering this +5.8-kb region. The other 36 samples in this group obtained from apparently healthy individuals (including four from pregnant women) might have an unclear molecular basis.

For the B subgroup patient in this case, our reference laboratory suggested that further investigation would be conducted. Most hospital transfusion service laboratories do not perform molecular-based testing. They are usually capable of serologic testing to identify A1 and A2, the most common A subgroups, but can recognize the possibility of weaker subgroups of A or B by characteristic discrepancies between RBC and plasma grouping tests. Hospital transfusion service laboratories rarely conduct a serologic investigation to detect weak B subgroup. This case, however, demonstrated that conventional serologic testing can be used to detect a weak B subgroup. The approach for detection of a weak B subgroup in the hospital laboratory setting is particularly useful when access to molecular–based reference laboratory testing is limited or if the patient needs urgent blood transfusion. The much lower cost and faster turnaround time of the conventional serology tests, in comparison to molecular-based genotyping tests, offer advantages. Serologic testing has been used elsewhere to identify and characterize ABO subgroups in a large group of blood donors and patients and B subgroups in a group of three patients and donors.

B subgroup should be suspected when serologic testing results show a discrepancy of forward as group O and reverse as group B, with a negative antibody detection test at immediate
spin. B subgroup phenotype can then be determined by using adsorption-elution testing, secretor testing for B substance, and serologic classification discussed in this report. Guidelines exist and are summarized in Table 1.12

Individuals with some B subgroups, such as B(weak) and B(i), can produce anti-B. Transfusion of group O RBCs and group B plasma—containing components to these individuals can address this type of B subgroup incompatibility due to anti-B to prevent an HTR. In our opinion, it is not clinically necessary to further differentiate between B(weak) or B(i) subgroups in these recipients. For this young healthy woman in our case with B subgroup but without identified variant alleles, further research could be conducted on the non-coding regulatory elements, such as PCR amplification and sequencing for the 5.8-kb enhancer region in the intron 1 enhancer region, and on the sequence variation in the (CBF)/NF-Y enhancer region of the ABO gene. However, it is possible that the patient’s pregnancy caused the B subgroup phenotype, with other unknown or no molecular subgroup basis. Identification of the patient’s B subgroup may help to prevent potential ABO incompatibility, if the patient needs blood transfusion. Nevertheless, further investigation to determine the molecular basis of the patient’s B subgroup phenotype at this small community hospital transfusion service is neither feasible nor necessary.

**Conclusion**

The hospital transfusion service identified, in a timely manner, a B subgroup using conventional serologic methods; these methods had the potential of preventing an ABO-mismatch transfusion reaction. This approach is also particularly useful when molecular testing by reference laboratory resources is limited or if the patient’s urgent need of blood transfusion cannot wait for the reference laboratory’s results. Nevertheless, the serologic testing approach to detect the weak B subgroup often requires a confirmation by genomic analysis because this information is crucial to donor centers and transfusion services.

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**References**


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