Open Access Case Report

Weaker B Blood Group Demonstrating Discrepancy Between Forward and Reverse Grouping

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Abstract

This study focused on issues occurring in the case of blood group typing and more particularly in cases of discrepancies in grouping forward and reverse. Particular emphasis is put on B blood group phenotype, the weaker one, which can cause differences in ABO blood typing. Such discrepancies are frequently seen in weak A and B blood group phenotypes in which forward and reverse grouping do not agree. Both complete forward and reverse blood grouping should be performed to determine the ABO blood type of an individual and avoid the risk of hemolytic transfusion reactions, including delayed hemolytic reactions. The use of prolonged incubation at different temperatures, as well as adsorption and elution techniques to confirm the presence of specific antigens on the red cell membrane will resolve these discrepancies. This study points out the problems associated with the weaker B phenotypes and the necessity of proper blood typing procedures for safe and accurate transfusions.

Keywords: Weaker B phenotype, blood typing, elution, adsorption, forward and reverse grouping, ABO discrepancies, transfusion safety.

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Introduction

The knowledge to accurately determine an individual's ABO blood type is of great importance in transfusion medicine, as its correct determination will affect blood transfusion and organ transplant safety and efficacy. There are two important techniques for blood typing using forward grouping and reverse grouping. In the forward grouping, the red blood cells are tested for the presence of specific antigens whereas, in the reverse grouping, it screens for the complementing antibodies in the plasma.[1] It turns out that these two methods correlate generally and give you a good blood type. Although, differences might occur between the forward and reverse groups when weak or variant antigens are present, so that it becomes difficult to conclude on the right blood group.[2]

Weak B phenotypes serve as one of the known examples. These are most often due to genetic polymorphisms in the ABO gene that cause a reduction or deformed expression of B antigen on red blood cells. Such genetic variations lead to a lower number of B antigen copies on the cell surface which cannot be detected in forward grouping tests. A patient with weak B antigen might be misclassified as blood group O, although he/she has the B antigen in his/her red blood cell. The misclassification can cause life critical mistakes in clinical applications such as incorrect transfusions or incompatible organ transplants.[3, 4]

In cases where there is blood group discrepancy, the issue is usually resolved by advanced serology techniques like elution and adsorption. An antibody interfering adsorption is used to remove any interfering antibodies from plasma so that the blood type is not misclassified because of interference from antibodies.[5] The use of elution, a technique of removing antibodies bound to red blood cells to confirm the presence or absence of specific antigens, e.g. B antigens for weak B phenotypes, is discussed.[5,6] Accurate identification of these methods is very important in order to correctly identify blood types minimizing misdiagnosis and consequently patient safety during transfusion and transplantation. These serological techniques combined with comprehensive reverse grouping provide a reliable method of authenticating true ABO blood type of a patient also in the presence of weak or rare phenotypes.

Methodology

Patient is a female aged 25 years who was admitted for a routine preoperative assessment of the procedure she was scheduled to go through. Blood typing was done in accordance to the preoperative protocol to assure compatibility for any possible transfusions. Forward grouping done with standard anti A, B and anti D reagent

resulted in the patient being assigned as O type. If the patient's red blood cells are agglutinated by the anti-A reagent but no agglutination occurs in the anti-B reagent well, the patient is known to have B antigens and this result is concluded to mean that the patient has B antigen without A antigen.

The discrepancy that was observed concerns cells of A1 and B reagent were reverse grouped. In the test of antibodies were found in the plasma, which indicated that the blood type was of group B. The contradictory results from the O (forward) and the B (reverse) groups brought both doubt and the utilization of further testing methods to address the discrepancy and to conclusively figure out the patient's actual ABO blood group.

Laboratory Investigation

Further testing was conducted to resolve the discrepancy in blood group in order to address the discrepancy in forward and reverse grouping results. A new supply of anti-B reagent was obtained and the patient's red blood cells retested using this, so that the reagents used in the retest were both fresh and not expired nor defective. To improve accuracy to the test, the authors used enhanced techniques such as longer incubation periods, both 4°C and 37°C. These incubation temperatures were selected to optimize the reaction conditions for antigen- antibody reactions and make it more likely that any weak reactions that had not been detected in the previous testing will be seen.

This second round of testing yielded mild and sporadic results of agglutination with the anti-B reagent, hinting that the patient may possess a very weak expression of the B antigen on her red blood cells. But the agglutination was not enough for the definite classification and the very slight reactivity suggested that the patient may have a weak B phenotype. To prove this and resolve the blood group discrepancy, more advanced serological techniques like elution and adsorption were used. These methods were specially designed for removal of interfering antibodies and detection of the B antigen in a more refined manner and thus clarifying patient true ABO blood group.

Adsorption Technique: By adsorbing the patient's washed red blood cells with known commercially available monoclonal Antisera B, incubated at 37°C for one hour. A careful adsorption of this process was performed so that all free B antibodies adsorbed to the patient's red blood. adsorption, the sample were washed at least for 6 times and last wash was checked in the reverse grouping method to confirm all available B antibodies are attached to red cells and all other free antibodies which are not associated with red cells had been removed.

Strong ionicity of this outcome strongly suggested that this initial weak reactivity observed in the forward grouping test resulted because the patient possessed a red blood cell which expressed a weaker B antigen. The adsorption procedure also helped to confirm that the discrepancy in blood typing was not the result of the antibody interference but the consequence of decreased expression of the B antigen. Consistent with this finding was the diagnosis of weak B phenotype, which can lead to subtle agglutinations that would be misinterpreted as blood type if fully investigated by using any advanced technique.

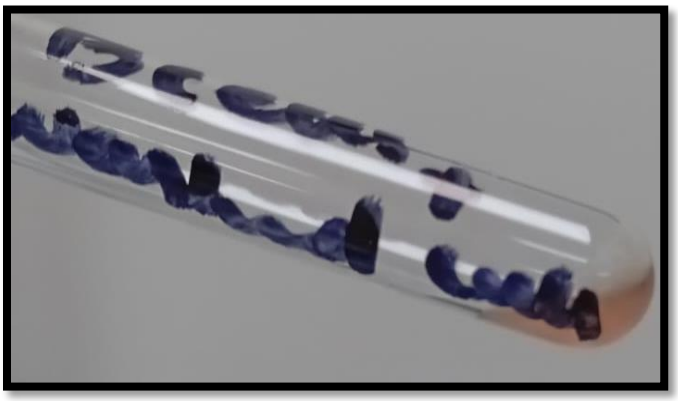

Figure 1: No Reactivity with B cells after adsorption.

Elution Technique: An elution was performed in order to verify B antigens on the patient's red blood cells. This technique consists of treating the patient's red blood cells so carefully that antigen cannot be detected because the antigen itself is being tested and not the antibodies on the cell surface. The eluate, which contained the antibodies removed from the cells after technique of heat elution at 56°C for 10 minutes, was then checked in reverse grouping.

The eluate was showing agglutination , indicating Anti B antibody in eluate and B antigen on surface of adsorbed red cells of patient. Though not a strong reaction as would be seen in typical B group blood, the agglutination was still sufficient to confirm that the patient in fact made the B antigen, just not in as strong of a form. This

diagnosis was reinforced by this finding and, in addition, the initial blood group discrepancy was attributed to reduced expression of the B antigen rather than a misidentification or error in testing.

Figure 2: B antigen reactivity seen on elute.

The combination of these results confirmed that the patient had a weak B blood group, in spite of the initial forward grouping suggesting group O.

Discussion

It is demonstrated that both forward and reverse grouping are vital to determine the ABO blood type of a patient. The need for accurate blood typing is indispensable; it is a standard clinical practice in case of transfusion medicine and transplantation of organs, as it leads to safe practices and helps prevent severe clinical complications. That is, in this case the patient would have been mistakenly labeled as blood group O if only the forward grouping is performed. Given this misclassification, there would have been serious clinical consequences and include transfusion reactions or organ transplant rejections due to similar blood type being mistakenly transfused to a recipient giving rise to life threatening hemolytic reactions, delayed hemolytic transfusion reactions.[1,2] For this reason, both forward and reversing blood grouping is really necessary to get an accurate and complete blood type diagnosis.

Weak phenotypes such as the weak B blood group are caused by the frequent occurrences of genetic differences, e.g. mutations in the ABO gene. The presence of these genetic mutations can result in lower expression of the B antigen on red blood cells in turn resulting in a discrepancy between forward and reverse grouping results. The causes of reduced expression of B antigen are usually allelic variations leading to weak expression of antigen, which are often encountered in rare ABO subgroups such as weak B and A3.[3] The diagnosis of the weak B phenotype is difficult as the decreased antigen density on red blood cells may not be detected using standard forward grouping techniques. As a result, the individual is misclassified as blood group O. Such misidentifications reinforce the need to employ both forward and reverse blood grouping techniques to identify discrepancies in an early and correct manner.[4,5]

Resolving discrepancies in forward grouping results will require adopting Reverse grouping algorithm. This time, the negative result by forward grouping was corrected by the reverse grouping test, which detected anti B antibodies in patient's plasma and determined the true blood type to be B. Reverse grouping is most beneficial when the antigen being tested for is expressed at low levels, since it makes it possible to identify antibodies that are not detectable via the forward test. Confirmation of blood type using this method is an important tool for identifying blood type in cases of weak or partial antigen.[6]

Advanced serological techniques such as adsorption and elution are necessary when discrepancies arise in order to determine the precise blood type. Free anti-B antibodies are removed from plasma by adsorption whereby they are permitted to bind to red blood cells that are known to have B antigens. It removes any interfering antibodies that may be present in the plasma so that when the plasma is retested, blood type will be more precisely defined.[7] Elution removes antibodies that were bound to the red blood cells, so as to confirm the presence of the weak B antigen. Forward grouping results undetectable for patient blood, which was confirmed by this combination of adsorption and elution, of the weak expression of B antigens on the patient red blood cells.[8]

With the use of these advanced methods, along with reverse grouping, blood typing is accurate, even when the patient has a weak or partial ABO blood group. If these techniques would not have been used, then there could have been adverse clinical outcomes such as hemolytic transfusion reaction or organ rejection in the course of transplantation. Increased awareness of weak and partial blood group phenotypes among healthcare professionals is required in this case. Knowing that weak or variant phenotypes that are misclassified as one of the common blood types is possible can prevent errors and risks.[9,10]

Conclusion

This case highlights the importance of such blood typing, particularly in situations in which weak ABO phenotypes are involved. To accurately determine blood types, both forward and reverse grouping is undertaken as well as sophisticated serological procedures, including adsorption and elution. This approach prevents misidentified transfused blood type, it is particularly important for transfusion medicine and organ transplantation because mismatched transfused blood types often cause severe complications.

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