CASE REPORTS

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Clinically significant anti-Wr^a antibody: a report of two successfully managed patients

Klinički značajno anti-Wr^a antitelo: prikaz dva uspešno vođena pacijenta

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Abstract

Introduction. Wr^a is an antigen of the Diego blood group system. Anti-Wr^a antibody can be found in the sera of healthy individuals (naturally occurring anti-Wr^a) or can be immunostimulated after transfusion or after exposure to foreign erythrocytes during pregnancy. Commercial antibody identification panels do not routinely contain Wr^a antigen-positive erythrocytes. We present two cases of patient blood management in complex situations, such as antibody appearance to low-frequency Wr^a antigen. **Case report.** In the first presented patient, who received multiple blood transfusions, anti-Wr^a antibody was detected while solving cross-match incompatibility. In the second patient, anti-Wr^a antibody was identified during routine antierythrocyte antibody screening during pregnancy. Immunohematological testing included blood typing and antibody screening, cross-matching, antibody identification, indirect antiglobulin test (IAT), and direct antiglobulin test (DAT). Column agglutination technology with microtubes containing gel and standard tube test methodology were used in IAT and DAT. **Conclusion.** Due to the limitations of the screening test in detecting antibodies to clinically significant low-frequency blood group antigens, such as Wr^a, the recommendation when selecting erythrocytes or transfusion is to use units that are cross-match compatible by the IAT test at 37 ºC. In the case of anti-Wr^a antibody, as well as in cases of other antibodies to low-frequency antigens, immunohematology findings should be confirmed in a national or international reference laboratory that has the structure, organization, as well as technical and professional capacities to provide this service.

Key words:
antigens; diagnosis; erythrocyte; erythrocyte transfusion; pregnancy; transfusion reaction.

Apstrakt

Uvod. Wr^a je antigen Dijego sistema krvnih grupa. Anti-Wr^a antitelo se može naći u serumu zdravih osoba (prirodno anti-Wr^a) ili može biti stimulisano imunski-posredovanim mehanizmima nakon transfuzije ili nakon izlaganja stranim eritrocitima tokom trudnoće. Komercijalni paneli za identifikaciju antitela rutinski ne sadrže Wr^a antigen pozitivne eritrocite. Prikazujemo dva slučaja transfuziološkog zbrinjavanja pacijenata u kompleksnim okolnostima, kao što je pojava antitela na Wr^a antigen niske učestalosti. **Prikaz bolesnika.** Kod prvog prikazanog pacijenta, koji je više puta primio transfuziju, anti-Wr^a antitelo je otkriveno tokom rešavanja unakrsne nepodudarnosti. Kod drugog pacijenta je anti-Wr^a antitelo identifikovano tokom rutinskog skrininga antieritrocitnih antitela tokom trudnoće. Imunohematološko testiranje je uključivalo tipizaciju krvi i skrining antitela, test unakrsnog podudaranja, identifikaciju antitela, indirektni antiglobulinski test (IAT) i direktni antiglobulinski test (DAT). Tehnologija aglutinacije u karticama sa gel mikroepruvetama i standardna metodologija ispitivanja u epruveti korišćene su i u IAT-u i u DAT-u. **Zaključak.** Zbog ograničenja skrining testa u otkrivanju antitela na klinički značajne antigene krvnih grupa niske učestalosti, kao što je Wr^a, preporuka pri odabiru jedinica eritrocita za transfuziju jeste korišćenje jedinica čija je unakrsna podudarnost pokazana IAT-om na 37 ºC. U slučaju anti-Wr^a antitela, kao i u slučajevima drugih antitela na niskofrekventne antigene, imunohematološki nalaz treba da bude potvrđen u nacionalnoj ili međunarodnoj referentnoj laboratoriji koja raspolaže strukturom, organizacijom, kao i tehničkim i stručnim kapacitetima za obezbeđivanje ove usluge.

Ključne reči:
antigeni; dijagnoza; eritrociti; transfuzija eritrocita; trudnoća; transfuzija, reakcija.

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Introduction

Although often life-saving, blood transfusion carries some risk. Some red blood cell (RBC) transfusion recipients may encounter the development of alloantibodies. Alloantibodies can potentially lead to acute or delayed hemolytic transfusion reactions (HTR), cause difficulty finding compatible RBC units for future transfusion, or potentially result in hemolytic disease of the fetus and newborn (HDFN). Although finding compatible blood should not be difficult for patients with antibodies to lower-frequency antigens, these antibodies may have to be considered when multiple specificities are present or if they have the potential to cause HDFN. The specificity of antibodies, as well as how urgently blood is required, the immunological status of the patient, class and subclass of the immunoglobulin, strength and thermal amplitude of the antibody, etc., will determine whether or not antigen-negative blood is required for transfusion or whether a child runs a risk of developing HDFN.

The Wr^a blood group RBC antigen is expressed in less than 0.01% of blood donors, making it a low-prevalence antigen. The first description was made by Holman [1] in 1953. In 1995, it was assigned to the Diego system [2]. The Diego blood group system is composed of 23 antigens, including pairs of antithetical antigens: the Wright^a (Wr^a) antigen and the Wright^b (Wr^b) antigen, differing by one amino acid on the AE1 glycoprotein. Anti-Wr^a antibody is termed clinically significant because it is capable of causing acute intravascular hemolysis, resulting in HTR or HDFN [3–6]. Therefore, the detection and determination of their specificity (identification) are essential for pretransfusion testing or prenatal antibody screening. Unfortunately, current antibody screening tests cannot detect all clinically significant antibodies against low-incidence antigens, for instance, antibodies such as anti-Wr^a are likely to be missed. Anti-Wr^a antibody is often found in the sera of healthy individuals (naturally occurring anti-Wr^a) or can be immunostimulated (after transfusion of Wr^a antigen-positive donor RBC or after pregnancy-related RBC exposure). Anti-Wr^a antibody can be found in the serum of 1% of blood donors [7, 8].

Anti-Wr^a antibody in healthy donors is predominantly immunoglobulin (Ig) M. In pregnant or previously transfused patients, it can be IgG or IgM plus IgG type, with the potential to cause severe immediate or delayed HTR and severe HDFN [7, 9, 10]. Those event-causing antibodies may remain unknown because the reagent RBC panel used for antibody identification rarely expresses Wr^a antigen [11]. If the specificity of the antibody has been determined, compatible blood units can be found without difficulty because Wr^a antigen-negative RBCs are practically always available within the blood stock [12–14].

We report on the effectiveness of our clinical practice in complex situations, such as antibody appearance to low-frequency antigens. Our initial focus was based on the fact that detailed quality records of a particular case could provide adequate insight into resolving a problem and provide vital information to enhance awareness of blood safety issues. Thus, we report two cases of anti-Wr^a antibody identified for the first time in the Serbian population. The first case was detected while solving cross-match incompatibility in the repeatedly transfused patient, and the second was identified during routine RBC antibody screening during pregnancy. Both patients were from the West Bačka District of northern Serbia.

Case report

Case I

A 53-year-old female with headaches and weakness was admitted to the General Hospital Sombor, Serbia, because of the clinical manifestation of anemia: hemoglobin 79 g/L, RBC count 2.07 10^{12}/L, hematocrit 24.9% (normal references in adult non-pregnant women: hemoglobin levels 120–160 g/L, RBC count 4.2–5.4 10^{12}/L, hematocrit 36–48%). Anemia in this patient occurred as a complication of Myelodysplastic syndrome. The patient was a repeatedly transfused person who received a total of fifteen RBC units over the past four years and who had been transfused within the last 3 months.

Prior to the anemia correction of this patient, Blood Bank Sombor performed pretransfusion compatibility testing. The testing involved the patient's blood group and cross-match. The patient's blood group was determined as blood type A, RhD-positive, Rh phenotype ccDEe, Kell-negative. The complete cross-match (with a 37 °C incubation and antiglobulin phase) was performed on commercial cards with a gel matrix containing anti-IgG, anti-IgM, and anti-C3d (Cellbind Screen, Sanguin Reagens, the Netherlands), and it was positive. The results of subsequent testing were as follows: negative direct antiglobulin test (DAT), negative indirect antiglobulin test (IAT) (Screening set 1+2, Sanguin Reagens, the Netherlands), negative RBC antibody screening with enzyme-treated cells, and negative cold agglutinins blood test. There were no records of a positive cross-match in history. The sample was sent to the Blood Transfusion Institute Vojvodina (BTIV) for further testing.

BTIV performed antibody screening by the IAT method using commercial reagent RBCs (ID-DiaCell I+II, Bio-Rad, DiaMed GmbH, Switzerland) and commercial Liss-Coombs cards (DiaMed AG 1785 Cressier, Switzerland). IAT was negative as well as screening with enzyme-treated, same reagent RBCs. DAT was also negative. The antibody screening was continued using IAT, a gel technique with commercial reagent RBC panel (Column panel 16, Sanguin Reagens, the Netherlands), and an irregular anti-Wr^a antibody was identified. The strength of agglutination was 4+ (grading 1–4). The autocontrol was negative. Antisera was available for testing, so typing of the patient's RBC showed that she was Wr^a antigen-negative. We provided Wr^a antigen-negative RBC for transfusion, and cross-matches were negative. After receiving a blood transfusion, the patient did not experience hemolytic reactions. The incompatible donation from Blood Bank Sombor was not tested to confirm the presence of Wr^a antigen.

Case II

A 25-year-old female who was pregnant for the second time was admitted to the General Hospital Sombor for a routine RBC antibody screening. She was in the eighth month of pregnancy. She had never had any transfusion. However, the patient had a history of one abortion because of an unwanted pregnancy at the age of eighteen (with a different partner). It was determined that she had a blood group type A, RhD-negative, Rh phenotype ccddee. The routine antenatal antibody screening using commercial RBC reagent (Screening set 1+2, Sanguin Reagens, The Netherlands) detected that the pregnant woman had an irregular antibody. Identification of unexpected antibodies was negative. It was determined that the partner had a blood group type B, RhD positive.

Freshly collected venous samples from both the pregnant woman and the presumptive father were sent to BTIV for further testing. Antibody screening of the mother's sample using the IAT method [a gel technique with commercial reagent RBC (ID-DiaCell I+II, Bio-Rad, DiaMed GmbH, Switzerland)] was negative. No antibodies were detected by the same reagent RBC, i.e., bromelin-treated. Further antibody screening by the IAT method with the commercial reagent RBCs panel (Column panel 16, Sanguin Reagens, the Netherlands) was 2+ positive and revealed anti-Wr^a antibody. The paternal RBC was non-reactive by IAT with anti-Wr^a antibody in maternal plasma. The paternal was typed for the Wr^a antigen and found to be Wr^a antigen-negative.

The pregnancy was not complicated, and the woman delivered a full-term male newborn without jaundice. DAT for IgG antibody was negative. Antigen typing showed the newborn to be negative for Wr^a antigen.

Discussion

In the first reported case of anti-Wr^a antibody in patients, an irregular antibody was detected during anti-human globulin (AHG) cross-match. After the anti-Wr^a antibody was successfully identified, compatible blood was easily found for the patient. As the risk of HTR due to anti-Wr^a antibody was removed, the testing algorithm seemed optimal. If the reagent RBC panel had not identified the anti-Wr^a antibody, AHG cross-match negative blood would have been transfused, also without any post-transfusion issues. Contrary to the aforementioned, antibody screening as the sole part of pre-transfusion testing would be a suitable solution only if the RBC reagent contained low-frequency Wr^a antigen, on the basis of which the antibody would be detected and identified. Suppose the RBC reagent does not contain a corresponding potentially clinically significant antigen, such as the Wr^a antigen, because of its low incidence in the population. In such a case, pretransfusion testing will not reveal anti-Wr^a antibody, and the consequences are uncertain.

Many studies have focused on comparing what is more efficient during pretransfusion testing, cross-matching or antibody screening [13–15]. Unfortunately, the presence of antibodies to a low-frequency antigen can also be discovered after an incompatible transfusion, which has resulted in an HTR [16, 17].

If the AHG cross-match is part of pretransfusion testing, irregular antibodies will be detected after an unexpected positive cross-match occurs. Regrettably, the identification RBC panel without Wr^a antigen cannot reveal anti-Wr^a antibody. On the other hand, the identification RBC panel with Wr^a antigen will identify anti-Wr^a antibody and allow patients with this relatively common naturally occurring antibody to receive a blood transfusion with a low risk of receiving a non-compatible unit and HTR [14, 18].

In the second case, the irregular antibody was detected during routine prenatal screening. After identification of anti-Wr^a antibody, we were pleased to establish that the presumptive father was Wr^a antigen-negative and concluded that the child was not at risk of HDFN due to anti-Wr^a antibody. As the next purchased screening and identification panels did not have an anti-Wr^a antibody in their composition, some questions remained open, such as anti-Wr^a antibody future proving, as well as the possibility of prenatal risk assessment, prenatal monitoring, and pre- and postnatal diagnosis.

Anti-Wr^a antibody is present in pregnant women and crosses the placenta and enters the fetus, where it initiates immune destruction of fetal erythroid cells. It is restricted to IgG (mostly IgG1 and IgG3), as antibodies of other classes are not transported across the placental barrier. If the anti-Wr^a antibody is not recognized during pregnancy, the pregnancy may be at risk of HDFN, but prenatal risk assessment cannot be done, and the titer cannot be monitored. A positive DAT in a newborn should pay attention to the presence of maternal antibodies directed against antigens inherited from the father. The problem can occur in a child born with HDFN, ranging from mild to severe, whose mother has a negative antibody screening test [5, 6].

The Wr^a antigen is present in less than 1% of the population. The other study has reported a frequency as high as 1 in 1,500 in the European population and 1 in 785 in the Spanish population [3, 4]. Globally, the percentage of alloimmunized patients in the general population ranges from 1–6% after a single transfusion [3, 4, 9]. A higher percentage occurs among multitransfused patients, while the risk of alloimmunization is lower in patients receiving dialysis [19]. The frequency of Wr^a antigen and anti-Wr^a antibody in the Serbian population is unknown.

About 50% of the anti-Wr^a antibodies have the potential to be clinically significant. The use of antigen-negative RBCs ensures the safety of blood transfusion in these patients. Given the increased risk of hemolysis in patients with sickle cell disease, if the patient develops anti-Wr^a antibody, then Wr^a antigen negative units should be provided, along with a match for the full expanded phenotype.

Conclusion

One of the limitations of the screening test is the inability to detect antibodies to clinically significant low-frequency blood group antigens such as Wr^a. Therefore, when selecting red blood cells for transfusion, units that are cross-match compatible with the indirect antiglobulin test at 37 ºC are recommended. It should be pointed out that anti-

Wr^a antibody is not routinely included in antibody screening cells, but it is capable of causing severe hemolytic disease of the fetus and newborn.

In the case of anti-Wr^a antibody, as well as in cases of other antibodies to low-frequency antigens, the immunohematology findings should be confirmed in a national or international reference laboratory that has the structure, organization, technical, and professional capacities to provide this service.

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Written consent was obtained from the patients for publishing the study.

Conflict of interest

The authors declare no conflict of interest.

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