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Scientific Comment

Impact on patient of the detection of weakly expressed RhD antigens in blood donors☆

Helio Moraes-Souza [a,b,*], *Vitor Mendonça Alves* [a]

[a] *Universidade Federal do Triângulo Mineiro (UFTM), Uberaba, MG, Brazil*
[b] *Hemocentro Regional de Uberaba/Fundação Hemominas, Uberaba, MG, Brazil*

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Due to blood transfusions, pregnancy, and organ/tissue transplants or grafts,[1] red blood cell alloimmunization may lead to serious complications, such as hemolytic transfusion reactions,[2–4] which have been regarded as one of the most frequent causes of transfusion-related deaths in recent years according to reports from the Food and Drug Administration (FDA).[5]

Rh, the most complex blood group system, has the highest clinical importance after the ABO group.[6,7] The D antigen, found in Rh-positive individuals, is the most important in the system as it is the most immunogenic.[7,8] Therefore, given the risk of alloimmunization, RhD-positive red blood cells must not be transfused to RhD-negative patients except in emergencies involving massive hemorrhage when RhD-negative units are not available.[7]

The RhD protein has 417 amino acids divided into seven intracellular segments, twelve transmembrane segments and six extracellular segments.[9] Amino acid substitution in the intracellular or transmembrane segment of the RhD protein leads to weakening of the D antigen expression in the membrane of red blood cells resulting in a weak D phenotype.[6–13] Hence, weak D testing must always be performed in blood donors with absent reactivity in the screening test, and if the results are weakly positive, the donors must be classified as RhD-positive in order to prevent anti-D alloimmunization in RhD-negative patients who receive transfusions of packed red blood cells.[6–8,10]

From the 1980s, the introduction of monoclonal anti-D reagents in the United States have helped to increase weak D antigen detection by using serological methods. In routine laboratory investigations, blood samples are found to have this phenotype when the reactivity of red blood cells with anti-D serum is absent or weakly positive (≤2+) in the screening test, yet with moderate or strong agglutination when antiglobulin serum is added,[10] or when RhD genotyping is demonstrated by molecular methods.

Nonetheless, in some cases, standard serological methods may not detect red blood cells with the weakly expressed D antigen and thus, RhD-positive donors are erroneously typed as RhD negative,[11,13] despite the improvement in the sensitivity of detection that has been observed using different monoclonal antibody reagents.

A study by Schmidt et al.[14] showed positive results for the RhD antigen in 5.9% of 152 blood donors – who had been

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* *Corresponding author at*: Universidade Federal do Triângulo Mineiro (UFTM), Av. Getúlio Guaritá, 250, Abadia, 38025-440 Uberaba, MG, Brazil.
E-mail address: helio.moraes@dcm.uftm.edu.br (H. Moraes-Souza).
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previously identified as RhD negative in routine examinations – when using anti-D IgG monoclonal antibodies (clones ESD1, MS-26 and TH-28) in gel cards. In the same study, the authors evaluated another population of 4897 donors from different regions of Minas Gerais state, Brazil, who had all been previously phenotyped as D negative in the indirect antiglobulin test (IAT), with clones MS-26 and TH-28, and 70 (1.43%) individuals showed weak reactivity with ESD1 anti-D antibodies. Molecular analysis were performed for 39 of these 70 individuals with RHD positivity confirmed by a multiplex RHD genotyping assay, whereas other molecular techniques such as polymerase chain reaction-sequence-specific primers (PCR-SSP) and RHD BeadChip were effective in specifically identifying different RHD variants in 48.71% of these 39 RHD positive samples. However, it would be important that the negative samples of ESD1 anti-D clone were also subjected to molecular analysis so as to establish the level of sensitivity of this antiserum.

Costa et al.[15] found, by molecular methods, that 18 (3.4%) of 520 donors previously classified as RhD negative in São Paulo, Brazil, carried different RHD variants. Moreover, the authors observed that four (22.2%) of these 18 individuals had a negative IAT, even though 11 different monoclonal anti-D antibodies (including clone ESD1) were used, whereas the other individuals showed a weak or very poor reaction to five of these antibodies, as well as negative reactions to six of these antisera.

It is consensus that molecular techniques allowed the start of a new stage of blood donor typing, including several strategies to identify RhD variants that are potentially immunogenic in apparently RhD-negative donors, as shown in several studies.[11–17] Furthermore, in some of these studies, these variants were also found to be associated with the C and E antigens of the Rh system,[11,12,14,15] although some RhD subtypes were found in ccee individuals.[13] Techniques have now identified more than 200 variants of the D antigen, including weak D, D partial and DEL types.[16] Of this total, over 80 belong to the weak D phenotype (11), with subtypes 1, 2 and 3 being the most prevalent.[10] It is known that 0.2–1% of European and American Caucasian individuals are carriers of this phenotype.[10]

Advances in serological and molecular techniques have made the detection of the different variants of RhD increasingly more accurate in recent years. Donors who were once typed as RhD negative have been reclassified as RhD positive, hence increasing transfusion safety for RhD-negative patients. Therefore, the adoption of improved serological methods and in particular molecular techniques is key to the routine of immunohematology laboratories to standardize tests to identify weakly expressed D antigens and other RhD variants. This procedure allows the correct classification of the RhD status of blood donors, thus minimizing the risk of anti-D alloimmunization and hemolytic reactions, as well as increasing transfusion safety of RhD-negative patients, including women of childbearing age, receiving transfusions of packed red blood cells. Therefore, hemolytic disease of the fetus and newborn by antibodies directed against D antigen is prevented.

Conflicts of interest

The authors declare no conflicts of interest.

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