EDUCATIONAL COMMENTARY – RhD AND RHIG IN OBSTETRIC PATIENTS

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Learning Objectives

On completion of this exercise, the participant should be able to

- describe the variable forms of the D antigen;
- explain the variations in testing methods for identifying the D antigen;
- discuss the significance of D variants in obstetric patients; and
- describe the role of RHIG.

Note: Rh terminology distinctions:

1. Antigens are designated as: D, C, E, c, e.
2. Rh genes are indicated by italics and capital letters: RHD and RHCE.
3. Alleles of the RHCE genes are designated according to the antigens they encode – RHCE*ce, RHCE*Ce, RHCE*cE and RHCE*CE.

RhD positive or RhD negative? The question resounds at many testing facilities. Serologic discrepancy in RhD typing, a result of the intrinsic properties of the protein, can produce inconclusive serologic results and lead to disagreement among testing personnel. Disparity in testing methods and reagents undermines standardization and leads to the potential for alloimmunization in RhD-negative patients. When the results of the initial RhD testing are unexpected or are less than expected, an altered form of D, a variant, should be suspected. Disconcerting in any patient testing, a variant is of vital concern for obstetric patients, in whom it may lead to hemolytic disease of the fetus and newborn (HDFN). Results of RhD testing help determine the need to administer anti-D immune globulin RhIG.

The Rh group is extremely immunogenic, being highly hydrophobic and intertwined 12 times in the cell membrane. The antigens of the Rh group are composed of D, C, E, c, and e alleles. These are encoded by two genes: RHD, which gives rise to the D antigen, and RHCE, which gives rise to Cc and Ee antigens. RHD is the more immunogenic. It possesses many polymorphisms and protein variants, which determine its complexities and its clinical significance. As little as 0.03 mL of transfused D-positive red blood cells may elicit an immune response in a D-negative recipient.1 The RhD antigen became most noted in its discovery as the cause of fetal death due to severe jaundice and is the most serious complication in pregnancy.
The variable phenotypic expressions of the RhD antigen can result from single-nucleotide polymorphism, to major gene arrangements, to the relative positions of the Rh alleles on the chromosome. For example, the expression of D is decreased when Ce is positioned in trans to RhD, as in the D haplotype Dce/Ce. In contrast, higher levels of D are seen in persons with the DcE/DcE genotype. Ethnicity can be predictive of Rh phenotype and the variable expressions of the D antigen.

D-negative phenotypes in most persons of European descent, for example, have a deletion of the entire RHD gene. D-negative phenotypes in 66% of individuals of African descent are primarily caused by inactivating mutations in RHD. An RHD mutation that causes extremely low levels of RhD occurs in 10-30% of individuals of Asian ethnicity. In comparison, D-positive phenotype individuals have the conventional and predictable unaltered RhD protein.

The D antigen expresses many epitopes. These were initially discovered in D-positive individuals who formed anti-D. Numerous D epitopes are highly conformational transmembrane linear amino acid sequences. Any alteration in the protein sequencing or missing epitope portion results in variable expression of the D antigen. Some of these D variant forms are the culpable ones we encounter in routine serological testing and are responsible for causing the formation of anti-D. The 3 main variant forms being considered herein are weak D, partial D, and DEL phenotypes.

Weak D, also known as Dw, is defined as a quantitative D variant resulting from a reduced expression of the antigen. It appears to be caused by a defect in the molecular structure of the gene as a result of an altered intracellular amino acid and/or by a positional gene interaction of RHD and the Ce alleles. Serologic testing to expose weak D requires the appropriate typing reagent. Weak D is most notably not agglutinated by IgM and must be tested through the antihuman globulin (AHG) phase with immunoglobulin G (IgG). This requires a monoclonal blended reagent that will detect all IgM and IgG expressions of RHD. There are 53 types of weak D expressions; types 1, 2, and 3 are the most common, accounting for 90% of weak D in individuals of European ethnicity. Most D-positive individuals tested will produce strong (3+ - 4+) reactions. Individuals with a weak reaction (<2+) at initial testing are considered a serologic weak D phenotype and should undergo further thorough evaluation. Most important are obstetric patients who have a weak reaction at initial testing with these types of reagents. At this phase it is difficult to ascertain the Rh status of the patient and its clinical significance without further molecular studies. These patients may possess a partial D, a weak D, or both, and potentially be alloimmunized to an Rh positive neonate. It is vital that conservative actions are employed by recommending the administration of RhIG.

The second category of D variants is the partial D, also known as D mosaic. This is a qualitative defect resulting from altered or missing protein portions of the D epitopes. These are due to hybrid genes in which portions of the RHD gene are replaced by other alleles in the Rh group such as CE. This
substitution generates new antigens, potentially placing the individual at risk for being alloimmunized. In contrast to weak D, the changes or mutations occur on the exterior surface of the \textit{RHD} membrane. A classic example of a partial D phenotype is observed in individuals who are test as Rh positive but who have also formed anti-D. These individuals possess the \textit{RHD} gene with changes in the protein sequencing that lead to altered, depressed, or abolished D epitopes. They may be classified as D-negative or D-positive when tested at different facilities depending on the reagents used. Individuals with partial D may also exhibit weak D, placing them in a category of partial weak D variant.

The third category of D variants is the DEL allele, (also known as D\textsubscript{el}), D-elution. Found mainly in individuals of Asian ethnicity, they are the product of several \textit{RHD} mutations that acutely reduce the expression of the D antigen. These alleles encode extremely low levels of the D antigen and are not detectable by routine serological testing. The antigen can be adsorbed out and small amounts may be detected after elution. Individuals with DEL appear RhD-negative but may be stimulated to form an anti-D. The DEL can only be discovered with RHD phenotyping and scrupulous adsorption-elution studies.

Why do we care whether a person is partial D or weak D? It matters most notably in prenatal testing. Individuals with partial D can make anti-D when exposed to Rh-positive red blood cell transfusions, or in the case of an obstetric patient with an RhD-positive fetus. Mothers with partial D who have not yet formed antibodies to anti-D need RhIG prophylaxis to prevent HDFN. RhIG is an immune globulin used in the prevention of Rh immunization. RhIG, introduced in 1968 under the brand name RhoGAM (Ortho Clinical Diagnostics), has been used safely by millions of women worldwide. RhIG is manufactured from human plasma containing anti-D. A single dose of RhoGAM containing 300\textmu g (1500 IU) is a sufficient amount of anti-D to suppress the immune response for up to 15 mL of Rh-positive red blood cells (approximately 30 mL of whole blood).

Weak D moms may or may not be alloimmunized depending on the type of weak D. Obstetric patients who are serologic weak D may also have partial D. Distinguishing between the two variants is difficult using current typing reagents and requires further evaluation through the use of molecular methods. Molecular assays are capable of differentiating \textit{RHD} alleles and identifying the need to administer RhIG and/or the transfusion of Rh negative blood. If Rh typing is inconclusive or doubtful, administering RhIG should be considered. In addition, molecular testing only needs to be done once to obtain the permanent Rh status. Based on current studies a patient with weak D type 1, 2, or 3 can safely receive a transfusion with Rh positive red blood cells and an obstetric patient would not be a candidate for RhIG. This represents a cost savings for patients and testing facilities.

In contrast, testing of neonates who are RhD-negative on initial testing must be extended through the AHG phase to detect weak D, thereby determining the mother’s status for RhIG. For blood donors, the
blood centers would extend testing of individuals who are RhD-negative and label them as RhD-positive when their weak D testing results are positive. For most patients we would not carry the testing through to this phase, and would classify them as RhD-negative as potential recipients a red blood cell transfusion. It is not assumed that weak D phenotypes may or may not be alloimmunized. To be certain further molecular studies such as RhD phenotyping are needed. Some facilities may choose to report these patients as D-negative and they would receive RhD-negative blood if indicated. For obstetric patients with an RhD-positive neonate, administration of RhIG is encouraged. These patients have approximately a 16% rate of being alloimmunized if not treated with RhIG.

To summarize, we cannot distinguish between weak D and partial D based on current testing reagents and methods. This is the number one cause of D typing discrepancies with the potential of missing the partial weak D mother who would then be at risk of forming anti-D. Historically, the obstetric patient with an RhD typing weaker than expected has not been addressed. Managing these patients can be done using the algorithm in the Figure on the next page.
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Figure. Markov-based decision tree model depicting “The financial implications of RHD genotyping of pregnant women with serologic weak D phenotype” presented at the 2014 AABB annual meeting in Philadelphia, PA. This can assist providers in their decision whether to administer RhIG prophylaxis. Reprinted with permission from Aaron Tobian, MD, PhD, Johns Hopkins Medical Institutions.

References
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