

## How do I manage Rh typing in obstetric patients?

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**A** 30-year-old G2P1 female is 12 weeks pregnant and her red blood cells (RBCs) type as A+ with a negative antibody screen. The transfusion medicine service learns that the patient typed as A- during her first pregnancy and she received Rh immune globulin (RhIG). How would you proceed to clarify the patient's D type and to evaluate the need for prophylaxis to prevent hemolytic disease of the fetus and newborn (HDFN)?

For most blood group antigens, serologic testing reveals RBCs to be either positive or negative for the antigen and any discrepancy is usually related to sample or recording errors. Rh typing (D antigen), however, may be equivocal at times for additional reasons including variability in the level of detectable D antigen on some RBCs, differences in the specificity and sensitivity of reagent antibody clones, and variation in test methods. For example, some centers test patients for a "serologic weak D phenotype" by further incubating RBCs that are nonreactive with anti-D on initial testing and adding anti-human globulin (AHG).<sup>1</sup> In addition, while typically a patient who is positive for an antigen cannot make an alloantibody, some patients with altered D antigen epitopes make alloanti-D when exposed to wild-type RhD.

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**ABBREVIATIONS:** ACOG = American College of Obstetrics and Gynecology; AHG = anti-human globulin; BIDMC = Beth Israel Deaconess Medical Center; CAP = College of American Pathologists; HDFN = hemolytic disease of the fetus and newborn; OB = obstetric; RhIG = Rh immune globulin.

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The complexity of D antigen expression has led to confusion and inconsistency in transfusion medicine practice. For example, a recent College of American Pathologists (CAP) survey of more than 3100 laboratories showed there is large variation in reporting of the results when testing is performed for a serologic weak D phenotype. Approximately 47% of sites report patients with a serologic weak D phenotype as "D+," 11% report as "D-," and 30% use the term "weak D."<sup>2</sup> Clearly a lack of standardized testing and reporting leads to discrepancies when a patient is seen at different centers. While consistent practice is important for all patients, the issues are magnified in prenatal medicine with the imperative to administer RhIG when appropriate to prevent anti-D formation, as one of the primary causes of HDFN.

The complexity of Rh system typing and genetics also leads to confusion among clinicians. As one example, the American College of Obstetrics and Gynecology (ACOG) bulletin "Management of Alloimmunization during Pregnancy"<sup>3</sup> published in 2006 states "in cases of Rh-D alloimmunization in which the father is Rh positive, the probability that he is heterozygous for the D antigen can be reliably estimated by using Rh-D antisera to determine his most likely genotype." As anti-D reactivity cannot predict heterozygosity, this guidance clearly is incorrect and requires updating.

This "How do I" will discuss the current Beth Israel Deaconess Medical Center (BIDMC) approach to D typing in obstetric (OB) patients to resolve typing discrepancies, assess the need for RhIG prophylaxis, and decide on appropriate RBC products for transfusion. We perform *RHD* genotyping on women of childbearing age with D typing discrepancies or with a history of a serologic weak D phenotype. Patients are managed based on risk of alloimmunization according to their *RHD* genotype.

### SEROLOGIC WEAK D PHENOTYPE AND LIMITATIONS OF SEROLOGIC TYPING

In contrast to most antithetical antigens, which differ by one amino acid (e.g., E/e), D+ or D- reflects the presence or absence of the entire RhD protein. As such, the D antigen is composed of numerous antigenic epitopes and anti-D production in a person who lacks the RhD protein reflects a polyclonal response to multiple foreign epitopes. Immunologically, this "degree of foreignness" makes D

the most potent blood group immunogen next to A and B. The potential for ambiguity arises when an individual inherits an altered RHD gene that encodes one or several amino acid changes that weaken and/or abolish some of the D epitope(s) detected by anti-D reagents.

Current commercial anti-D reagents are monoclonal (i.e., specific for a single D epitope) or “blended” monoclonal antibodies that usually contain at most two clones. The clone(s) present in anti-D reagents from commercial sources often differ and, even if the same clone is present, the reagents may differ in formulation and potentiating agents that impact sensitivity. Blended anti-D typing reagents contain both IgM and IgG monoclonal components.<sup>1</sup> RBCs from a patient with conventional (normal) levels of D antigen will be agglutinated by the IgM antibodies in initial testing, but RBCs from some patients with depressed levels of D antigen need the addition of AHG to detect IgG-bound anti-D for visible agglutination. Alternatively, RBCs from patients with altered epitopes may only be positive with the IgG anti-D in the blended reagent and agglutinate only after addition of AHG. RBCs that are non-reactive in initial testing but agglutinate with the addition of AHG, or that react weaker than expected ( $\leq 2+$ ) on initial testing, are referred to as having a “serologic weak D phenotype” and testing RBCs using AHG is often referred to as doing a “weak D test.” However, testing with serologic reagents cannot distinguish individuals with depressed levels of D antigen (weak D) who express all major D epitopes and are not at risk for anti-D, from RBCs lacking some D epitopes (partial D) who are at risk for anti-D. As an example, current licensed D typing reagents only react with RBCs with partial DVI, the most common partial D in Caucasians, after addition of AHG (i.e., in the weak D test). Hence, RBCs of individuals who inherit RhD with amino acid changes that depress, alter, or abolish D epitopes, may or may not be classified as D+ (“Rh positive”) when tested at different facilities or in the same facility with different reagents or methods.

### RHD GENETICS

The RH genes are diverse, with well over 200 RHD gene variants reported.<sup>4</sup> RHD variant alleles are divided into four groups; “partial D alleles” encode proteins with altered or missing D epitopes putting the patient at risk for anti-D, “weak D alleles” encode proteins with changes causing reduced quantities of protein in the membrane, “DEL alleles” encode severely reduced quantities of D antigen only detected by adsorption-elution, and “inactive and deletion D alleles” that do not encode functional RhD protein.

Molecular DNA assays are capable of differentiating RHD alleles to correlate specific alleles with the risk of anti-D. Patients with RBCs expressing a serologic weak D phenotype, particularly the most common weak D Types 1, 2, and 3, are not at risk to make clinically significant

anti-D.<sup>5,6</sup> Weak D Types 1, 2, and 3 are present in the majority of people of European ancestry whose RBCs type weaker than expected with anti-D reagents,<sup>7,8</sup> and these individuals can safely receive D+ blood and are not candidates for RhIG.

In some cases the risk for anti-D is less clear. The majority of individuals with weak D type 4.0 and 4.1 do not appear to be at risk,<sup>5</sup> but some exceptions have been seen, particularly in transfused patients with sickle cell disease in which the presence of altered alleles at both RHD and RHCE loci may be a contributing factor.<sup>9</sup> The much less common weak D Types 11 and 15 have been reported to make alloanti-D,<sup>5</sup> suggesting that they have altered D epitopes. There are isolated reports of anti-D in individuals with rare weak D types, including weak D Types 47 and 21,<sup>10</sup> although to date, no anti-D made by individuals with weak D alleles have been associated with HDFN or a positive direct antiglobulin test in the neonate to the knowledge of the authors and an extensive search of the literature. More data are needed to determine the risk for production of anti-D for the equivocal or uncommon alleles.

A number of DNA-based methods are available for RHD genotyping. At present, these are research use only laboratory developed tests available at a number of reference laboratories. The turnaround time is typically 1 or 2 weeks at a cost of approximately \$200 to \$300.

### PREVALENCE OF SEROLOGIC WEAK D PHENOTYPES AND GENOTYPE CORRELATION

The prevalence of the serologic weak D phenotype is often cited as 0.3% to 1.7%<sup>11</sup> but this varies based on the ethnic diversity in the patient population being tested and method and reagents used for D typing. In Caucasians from central Europe, 0.2% to 1.0% had a serologic weak D phenotype.<sup>5,8</sup> There are no large-scale prevalence studies using current monoclonal FDA-licensed reagents in the United States, but serologic weak D phenotypes are one of the primary causes of D typing discrepancies. Wang and colleagues<sup>12</sup> typed 501 OB patients with four different commercial US reagents by automated solid phase and immediate spin tube methods. They identified 11 of 501 patients (2.2%) with discrepancies between reagents and methods. The patterns of reactivity with the reagents did not predict the partial D or weak D status determined by RHD genotyping. Even for patients with the same variant D, there can be variability when testing with a specific reagent. For example, reactivity of samples with weak D Type 4.0 with a single reagent ranged from negative to 2+. This demonstrates the limits of serology in identifying specific RHD alleles present and the risk for anti-D.

Approximately 5000 deliveries occur each year at BIDMC. We perform RHD genotyping on women of

**TABLE 1. Summary of referred samples and results of *RHD* genotyping**

<i>RHD</i>	*Weak D Type 1	*Weak D Type 2	*Weak D Type 3	*Weak D Type 4.0	New alleles D*922A, D *11bp del Exon 3	*DAR Partial	No <i>RHD</i> <i>RHCE*ceCF</i>	Total
Number OB patients	16	9	2	2	2	4	1	36
Percentage of total tested	44.5	25	5.5	5.5	5.5	11	2.8	
Risk for anti-D		NO		Majority not at risk	Unknown	YES	YES	
RhIG		Not candidate for RhIG (75%)		Candidate for RhIG	Candidate for RhIG	Candidate for RhIG	Candidate for RhIG	
RBC transfusion		D+		D-	D-	D-	D-	

childbearing age with D typing discrepancies or with a history of a serologic weak D phenotype. Our experience, from 2006 through 2013, in the OB patient population at BIDMC is summarized in Table 1. *RHD* genotyping found that 27 of 36 (75%) were weak D Type 1, 2, or 3 and not at risk for anti-D. Two were weak D Type 4.0 and two had new alleles not previously reported. In the absence of additional data we considered these patients potentially at risk and candidates for RhIG. Four had partial D (*RHD\**DAR**) and one had no *RHD* gene with this latter typing discrepancy due to a variant *RHCE* allele (*RHCE\*ceCF*) known to encode a D-like epitope reactive with some anti-D reagents. Thus, five of 36 (14%) were definitively at risk. Overall, we considered nine of 36 (25%) to be at some risk and therefore candidates for RhIG. *RHD* genotyping results were consistent in most cases with the ethnicity of the patients, in that weak D Types 1, 2, and 3 were more often found in Caucasians. Although partial D variants are more common in certain ethnic groups, with increased population admixture and migration, it would not be appropriate to diagnose the presence of weak D or partial D and risk for anti-D based on ethnicity.

**VARIATION IN TESTING AND PATIENT MANAGEMENT**

Testing for a serologic weak D phenotype is not required for transfusion recipients or OB patients,<sup>1</sup> but there is significant variation in practice. In the aforementioned CAP survey, approximately 20% of laboratories perform serologic weak D testing on all patients while 10% perform this testing only on pregnant women or women of childbearing age.<sup>2</sup> The likely perceived benefit is to avoid giving unnecessary RhIG, and if a serologic weak D phenotype is detected approximately 50% would not give RhIG to the pregnant woman with a possible D+ fetus. This approach is supported by ACOG in the “Prevention of Rh D Alloimmunization” practice guideline published in 1999, which states that patients who type as weak D “are considered D positive and should not receive anti-D immune globulin.”<sup>13</sup> In regard to transfusion practice, 64% of sur-

vey respondents would transfuse D- blood in the case of a female of childbearing age with a serologic weak D phenotype and 30% would transfuse D+ blood.<sup>2</sup>

**AN APPROACH TO PATIENT MANAGEMENT**

Management of women of childbearing age who have a serologic weak D phenotype is clearly not standardized.<sup>2</sup> The decision on the appropriate clinical approach needs to balance preventing hemolytic disease of the newborn (or costly monitoring of the pregnancy) and unnecessary RhIG administration and use of D- RBCs. Importantly, there is some risk in assuming that all OB patients with a serologic weak D phenotype do not need RhIG. Patients with RBCs positive by serologic weak D phenotype testing may have a partial D allele and be at risk for anti-D. More than 25% of sites in the CAP survey reported they had seen at least one patient in the past 12 months with a serologic weak D phenotype who made alloanti-D.<sup>2</sup> Furthermore, there have been at least seven documented cases in the literature of women with RBCs reactive in the serologic weak D test with a partial D variant who then made anti-D and had a child with HDFN.<sup>14-17</sup> In two cases, the mother was a partial DVI and the child died from hydrops fetalis. Testing women of childbearing age through the AHG phase to avoid giving unnecessary RhIG or D- RBCs puts women carrying the most common partial D variant in Caucasians, partial DVI, at risk for clinically significant anti-D.

Another approach is to identify all OB patients with a serologic weak D phenotype by testing for weak D and then reflex to *RHD* genotyping or, alternatively, simply genotype all D- OB patients. It is not clear that the additional cost of *RHD* genotyping would balance the reduced use of RhIG and D- RBCs in those women who are molecular weak D and not at risk for anti-D. Communication and appropriate follow-up would be needed. While reference laboratories provide detailed reports regarding *RHD* genotyping results and risk for anti-D, these reports are not always part of the active medical record and there can be miscommunication from provider to provider. Given

the considerable confusion in this area and current ACOG guidelines, there is the possibility that the appropriate recommendations would not be made and/or carried out.

To date, approximately 25% of women at BIDMC with D typing discrepancies involving weak D phenotypes were at risk for anti-D, and the risk of anti-D and possible HDFN in these patients drives policy. Pregnant women are not routinely typed for serologic weak D; however, if a pregnant woman presents with a typing discrepancy or has a history of typing as serologic weak D from another center, we recommend *RHD* genotyping. While awaiting genotyping results, patients are treated conservatively as D-.

Although there is no clinical data demonstrating that RhIG can prevent HDFN in women with partial D expression carrying a D+ fetus, administration of RhIG to patients with partial D antigens is generally accepted practice. Although much of the passive injected antibody would also bind the mother's RBCs, RBC tagging studies have shown that wild-type D+ cells are cleared by RhIG in individuals who type as serologic weak D.<sup>17</sup>

## THE PATIENT IN THE VIGNETTE

Our approach for the patient in the vignette is to determine whether the discrepancy is due to a molecular weak D or partial D allele by *RHD* genotyping. The result is used to guide clinical decision making in regard to administration of RhIG and transfusion of D- RBCs. If testing confirms a weak D genotype that is not associated with anti-D formation, the patient will receive D+ RBCs units and is not considered a candidate for RhIG. If testing indicates a partial D genotype, the patient is treated as D- and receives D- RBCs and is considered a candidate for RhIG (Table 1). Our approach has allowed us to determine the risk of alloimmunization for our patients with D typing discrepancies and recommend appropriate management.

## CONCLUSION

Variable or weaker-than-expected reactivity of RBCs when determining the Rh type is often indicative of the presence of an altered D antigen which may put an individual at risk for making anti-D. At BIDMC, we perform *RHD* genotyping on women of childbearing age with D typing discrepancies or a history of a serologic weak D phenotype. We do not perform routine testing for serologic weak D phenotypes on all OB patients. Our current policy is based on limited data and lack of a cost-benefit analysis and others may reasonably reach different conclusions. We acknowledge that, with our current approach, a number of women at our center with a weak D genotype are typed as D- and will receive unnecessary RhIG and D- blood even though they are not at risk for anti-D. D- RBC units are a limited resource, and although RhIG is very safe, it is

a human blood product manufactured from pooled plasma from paid donors who must be actively immunized with RBCs. Additionally, our current approach does not identify OB patients with partial D antigen expression (primarily DIIIa, DIVa) whose RBCs are strongly reactive with anti-D reagents but who would be better served as D-. With more widespread availability and decreased costs of *RHD* genotyping, it is hoped that an approach will evolve for routine *RHD* genotyping of all OB patients to more accurately determine risk of formation of anti-D. It will also be important for laboratory and hospital information systems to accommodate entry of *RHD* genotyping results and the serologic interpretation.

There is wide variation in how OB patients (and all patients) with reduced or altered D antigen expression are treated. A pregnant patient with a serologic weak D phenotype may go to one hospital and be labeled as D+ and D- at another. At one hospital, she might receive D+ blood while at another she would receive D- blood and be a candidate for RhIG. This vast difference in practice causes confusion for laboratory scientists, medical staff, physicians, and patients and suggests a need for a standardized approach. It is important that we as transfusion medicine specialists understand the limitations of serologic testing for D antigen expression and consider the use of modern molecular genetic testing.

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## CONFLICTS OF INTEREST

The authors have disclosed no conflicts of interest.

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