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Postpartum Rh Immunoprophylaxis

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Question 1:
What are the turnaround times for the rosette test and for the acid-elution assay that is performed in the event of a positive rosette test?

Response from Dr. Sandler:
The standard procedure for performing a rosette fetal bleed screen begins with a 1-hour wait for fetal red blood cells to mix in the maternal circulation before collecting the mother’s blood sample. The “bench time” for a rosette fetal bleed screen includes a 15-minute incubation and microscopic reading, which takes an average of 30 minutes. In 2013, one manufacturer will replace the 15-minute incubation kit with a new 5-minute incubation kit. The acid-elution assay, including 20 minutes for staining, requires approximately 60 minutes. However, “bench times” are not the same as “turnaround times.” In my experience, “turnaround times” vary widely with the efficiency of transportation from the patient’s bedside to the laboratory and competing priorities for technologists’ time. A telephone call informing the technologist that mom is waiting for the test result so she can go home with her new baby often shortens the “turnaround time” to approximate “bench time.”

Question 2:
Do you recommend that the flow cytometry test, where available, be performed instead of the acid elution test to estimate the volume of fetal—maternal hemorrhage in women with increased hemoglobin F?

Response from Dr. Sandler:
Definitely.

Question 3:
Do you recommend that a clinical history to screen for hematopoietic diseases be submitted to the laboratory along with the maternal blood sample?

Response from Dr. Sandler:
Not routinely, but if the patient has an established diagnosis of a red cell disease that might interfere with a valid test result, I recommend that it be communicated to the laboratory with the request.
Question 4:

If the mother has an increased proportion of hemoglobin F in peripheral blood, is the laboratory likely to overestimate the degree of fetal–maternal hemorrhage and to recommend a higher dose of Rh immune globulin than necessary?

Response from Dr. Sandler:

Not likely. Diseases that are associated with increased HbF in red blood cells typically cause such a large increase in the percent of F cells that the technologist is more likely to recognize that the test is invalid rather than consider the finding indicative of a large fetomaternal hemorrhage. Of course, the interpretation of an acid-elution assay is subjective and it is always possible that an individual technologist could count only the darkest-staining adult F cells as HbF-containing fetal red blood cells and arrive at an erroneous interpretation of a large fetomaternal hemorrhage.

Question 5:

Is the partial D or weak D phenotype what used to be called the RHD pseudogene?

Response from Dr. Sandler:

No. The RHD pseudogene is a technical term for one distinct RHD allele that is RhD-negative and rather frequently observed in RhD-negative African Americans. The RHD pseudogene is defined as an RHD allele that introduces a reading frameshift and premature termination of translation and a translation stop codon resulting in the complete absence of the D antigen. The RHD pseudogene has been identified to be the basis for RhD-negative phenotype in many persons of African ancestry. Historically, partial D or weak D phenotypes have been defined serologically. Weak Ds have been those antigens undetected by direct hemagglutination using anti-D typing sera without an antiglobulin (former Du) test. Greater than 80 distinct weak D types are defined molecularly. Typically, a partial D has been defined by association with formation of allo-anti-D after exposure to D antigens by pregnancy or transfusion. Most partial Ds express the D antigen in normal strength and are not weak D. As more weak Ds are genotyped, alternative definitions are emerging based on structural features, namely, the nucleotide sequence of the RHD gene. Most weak D types appear to be expressions of amino acid substitutions located in the intracellular or transmembraneous parts of the RhD protein. In contrast, partial Ds appear to have exofacial substitutions.

Question 6:

Do you have an estimate of the number of women with the partial D or weak D phenotype who would need to receive Rh immune globulin to prevent one woman becoming sensitized? How would this number needed to treat (NNT) compare with the NNT for RhD-negative women?

Response from Dr. Sandler:

No. Estimates for the prevalence of weak D phenotypes vary among geographic, ethnic, and racial populations. An estimated 0.2% to 2.0% of Caucasians may have weak D phenotypes. The percentage may be higher in persons of African ancestry. In Europeans, more than 90% of weak Ds have genotypes that are unlikely to be susceptible to alloimmunization to the D antigen. At first glance, it might appear that a genotype-based protocol for managing Rh immunoprophylaxis will not be cost effective. On the contrary, current practice results in all weak D persons being transfused with RhD-negative red blood cells and all women with a weak D phenotype receive Rh immune globulin after delivery of an RhD-positive newborn. A genotype-based protocol is likely to establish that most of these persons are not at risk of alloimmunization to the D antigen, thereby conserving inventories of RhD-negative red blood; decreasing the number of women requiring Rh immune globulin and being exposed to a blood product derived from a large pool of human blood; and reducing overall costs while increasing safety.
Question 7:

Are there any risks of the administration of Rh immune globulin to women with partial D or weak D phenotypes? You mention that the injection can cause “extravascular destruction of a few milliliters of maternal red blood cells.”

Response from Dr. Sandler:

No. Rh immune globulin is conventional gamma globulin manufactured from a large pool of plasma collected from donors with a high titer anti-D. The known risks are minimal and there is a safety record of more than four decades in the United States. Extravascular destruction of a few milliliters of maternal red blood cells occurs as an asymptomatic event. In fact, intravenous injections of significantly larger doses of Rh immune globulin have been a standard treatment for immune thrombocytopenic purpura in RhD-positive persons for many years.

Question 8:

You state that the rosette test should not be used for detection of fetal-maternal hemorrhage when the RhD type of the fetus is unknown. Do you recommend an alternative testing algorithm for fetal-maternal hemorrhage in RhD-negative women who are undergoing evaluation for trauma during pregnancy?

Response from Dr. Sandler:

Either an acid-elution or flow cytometric method using anti-HbF is the current laboratory test of choice.

Question 9:

What are the clinical benefits for “once in a lifetime” molecular testing to determine the exact RHD genotype of a woman with a partial D or weak D phenotype, compared with classifying such a woman as D-negative?

Response from Dr. Sandler:

Presently, most women with a weak D will be phenotyped by a hospital laboratory method that will categorize her as RhD-negative. If genotyped, current research suggests that she will likely be identified as having an RHD genotype that would allow her to be transfused with Rh-positive red blood cells safely and without risk of Rh alloimmunization. Given the seasonal and other serious periods of shortages of RhD-negative red blood cells, such women will not be at risk of delays being transfused while RhD-negative red blood cells are located. In case of one or more pregnancies, she would not be exposed to serial administrations of an avoidable injection of a blood product and its associated risks and costs.

Question 10:

Are there any circumstances under which an RhD-negative woman who has received recent antepartum Rh immune globulin (for example, for trauma) would not require Rh immune globulin after delivery?

Response from Dr. Sandler:

Probably not. The standard of practice for Rh immunoprophylaxis is to test for the presence of RhD-positive red blood cells in a postpartum sample of the mother’s blood—not to measure the titer of residual anti-D in the mother’s circulation. Therefore, the mother should have the standard screen for fetal red blood cells and, even if negative, she should receive the standard injection of one 300-microgram vial of Rh immune globulin.