When should tests for unexpected antibodies be done during pregnancy?

Both the AABB's *Guidelines for Prenatal and Perinatal Immunohematology*[1] and the practice bulletin of the American College of Obstetricians and Gynecologists (ACOG) on "Management of Alloimmunization during Pregnancy"[2] advocate testing pregnant women for unexpected antibodies to red blood cell (RBC) antigens at the first-trimester visit. This testing serves to detect those alloantibodies capable of causing hemolytic disease of the fetus and newborn (HDFN). If no antibodies are detected at the initial visit, no additional testing is advocated later in pregnancy, except before administering Rh immune globulin (RhIG) prophylaxis, when there is a history of potentially significant antibodies or blood transfusions, or if blood transfusions are necessary.

However, testing for unexpected antibodies before administering RhIG prophylaxis (to prevent anti-D alloimmunization) was once mandated by wording in product circulars for RhIG distributed by Ortho Diagnostics between 1968 and 1981.[3] The statement under the subheading "Indications" read "The mother must not already be alloimmunized to the Rh_o(D) factor." This requirement was dropped once routine antenatal prophylaxis at 28 to 30 weeks' gestation was introduced,[4] first in Canada in 1977 and shortly thereafter in the United States,[5,6] because the antenatally administered RhIG anti-D can be detected at delivery, and RhIG cannot be differentiated from immune anti-D.[7,8]

Currently, no accrediting agency in the United States mandates testing before RhIG administration, although a recommendation for testing may be inferred from AABB Standard 5.20.2:[9] "Women who are pregnant or who have been pregnant recently shall be considered for Rh Immune Globulin administration when: 3) The woman is not known to be actively immunized to the D antigen."

When testing for unexpected antibodies after the woman has received RhIG earlier in pregnancy, a minipanel of three reagent D– RBC samples that between them carry all of the FDA-required antigens for antibody detection except the D antigen (i.e., r′r, r″r, and rr RBCs) can be constituted from reagent RBC panels used in antibody identification studies.[10] If used in conjunction with either a two- (R_1R_1, R_2R2) or a three- (R_1R_1, R_2R_2, rr) reagent RBC sample screening set, the passively acquired anti-D can be identified in many cases and the presence of non-D antibodies excluded.

A number of investigators have shown that testing D+ pregnant women for unexpected antibodies to RBC antigens beyond that described above is rarely clinically helpful.[11-15] However, in this issue of **TRANSFUSION**, Dajak and colleagues[16] report cases of D and non-D antibodies that were not detected at the first-trimester testing yet caused "significant" HDFN. Their data are from Croatia, where it is customary to perform additional tests for unexpected antibodies on D– pregnant women at 28 and 34 weeks' gestation, and D+ women at 34 weeks' gestation. The study involved 84,000 pregnant women encountered over a 15-year period. Unexpected antibodies to RBC antigens were seen in 1105 (1.32%). Anti-D was seen in 1.3% of D– women (n = 15,200), and the incidence of non-D antibodies was 0.2%.

Of concern were 87 pregnancies in which antibody screening tests were nonreactive at the first-trimester visit, but were reactive either during the third trimester or at delivery. Seventy-two such pregnancies involved anti-D, 14 of which were detected by screening at Week 28, 22 at Week 34, and 31 at delivery. Anti-D was seen at delivery in five cases in which no testing had been done during pregnancy.

There were 19 cases of HDFN resulting from antibodies that were not detected during the first trimester. Of these, 12 resulted in HDFN requiring exchange transfusion after delivery; the antibodies involved were D(5), c(6), and E(1). There were six cases treated with simple transfusion (three due to anti-D and one example each due to anti-c, anti-C, and anti-Ce). The authors also encountered the remarkable case of fetal death at 36 weeks' gestation due to anti-Rh17. The mother had been transfused at 21 weeks' gestation when tests for unexpected antibodies and crossmatches were nonreactive.

There were no cases in which the first-trimester testing missed non-Rh antibodies that caused intrauterine death or HDFN requiring treatment at delivery. However, there were three pregnancies with detectable antibodies at the first-trimester visit, all involving anti-K with a K+ fetus; one of these resulted in intrauterine death. No other non-Rh antibodies were implicated in HDFN.

The data of Dajak and colleagues[16] are in accord with other published reports[11-14] on the rate of alloimmunization among D+ women during pregnancy. The figures for late-onset clinically significant alloimmunization in these

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four studies were 20 in 13,143 in Adeniji and colleagues,[11] 6 in 9348 in Rothenberg and colleagues,[12] 42 in 17,568 in Heddle and colleagues, and 13 in 3012 in Andersen and colleagues.[14] The overall incidence was 0.18%, substantiating the findings of Dajak and colleagues[16] and is further supported by the report of Bowell and colleagues[15] on 70,000 pregnancies in which the incidence of late-onset alloimmunization, including anti-D, was 0.38%. Apart from the anti-Rh17 case described, none of the antibodies caused fetal demise or HDFN that required medical intervention before delivery.

It should be noted that it is not current practice in Croatia or many other countries to provide D– women with antenatal RhIG prophylaxis at 26 to 28 weeks' gestation, as is the recommended standard of care in North America. Indeed, according to the results of a 2003 international survey, antenatal RhIG prophylaxis is practiced nationally in only two other countries (United Kingdom and the Netherlands), and in Spain, Poland, and Austria it is practiced in parts of the country.[17]

Also, the authors use the term "severe HDFN" in the title and throughout the article, which they define as HDFN requiring simple or exchange transfusion or HDFN resulting in fetal or infant death. Others, especially those from countries with the resources to monitor and treat fetal hemolytic disease in utero,[18] might apply "severe" only to the latter.

Two other articles on alloimmunization are relevant to this discussion. Koelewijn and colleagues[19] studied the risk factors associated with alloimmunization during pregnancy among D+ women. They concluded that transfusion is by far the most important independent risk factor, followed by parity, major surgery, and hematologic disease. They present a flow diagram for a screening program based on preselection of women with clinical risk factors; no antibody detection tests are required in the absence of risk factors.

In a 1986 article, Howard and coworkers[7] noted that while anti-D accounted for the greatest fetal mortality and morbidity, anti-c caused the second most morbidity. They state that the production of non-D antibodies could be avoided by preselecting RBCs when transfusing premenopausal women. Koelewijn and coworkers[17] suggested matching for both K and c antigens. Blumberg[20] discussed the issue of antigen matching RBC units for patients other the chronically transfused. He was not persuaded that matching beyond ABO and D is clinically indicated for patients other than the chronically transfused and premenopausal women, but additional matching for the latter has not been widely adopted and is not mentioned in either the AABB or British Committee for Standards in Hematology guidelines. Perhaps the final word on antigen matching is yet to come from the NHLBI working group studying transfusion-induced alloimmunization.[21]

The data of Dajak and colleagues[16] clearly illustrate the detriment of not providing RhIG prophylaxis at 26 to 28 weeks' gestation. Such antenatal administration of RhIG reduced the rate of anti-D alloimmunization by pregnancy to 0.18%, down from 1.8% when given only after delivery.[4,5] Unfortunately, RhIG is not available worldwide in sufficient quantities or is deemed too costly to provide universal antenatal prophylaxis.

Should the guidelines[1] be changed with respect to the frequency and timing of tests for unexpected antibodies during pregnancy? The answer is a definite no. While there could be stronger wording for testing D– women at 26 to 28 weeks' gestation before antenatal RhIG, and for third-trimester screening of D– women who have received blood transfusions, the absence of cases in which in utero death could have been prevented by medical intervention before delivery does not support significant changes to the current guidelines.

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CONFLICT OF INTEREST

The author declares that he has no conflict of interest relevant to this manuscript.

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