

## 38. Screening for D (Rh) Incompatibility

### RECOMMENDATION

D (formerly Rh) blood typing and antibody screening is recommended for all pregnant women at their first prenatal visit. Repeat antibody screening at 24–28 weeks' gestation is recommended for unsensitized D-negative women (see *Clinical Intervention*).

### Burden of Suffering

D incompatibility exists when a D-negative woman is pregnant with a D-positive fetus, which occurs in up to 9–10% of pregnancies, depending on race.<sup>1,2</sup> If no preventive measures are taken, 0.7–1.8% of these women will become isoimmunized antenatally, developing D antibody through exposure to fetal blood; 8–17% will become isoimmunized at delivery, 3–6% after spontaneous or elective abortion, and 2–5% after amniocentesis.<sup>1–3</sup> In subsequent D-positive pregnancies of isoimmunized women, maternal D antibody will cross the placenta into the fetal circulation and hemolyze red cells.<sup>1</sup> Without treatment, 25–30% of these offspring will have some degree of hemolytic anemia and hyperbilirubinemia, and another 20–25% will be hydropic and often will die either in utero or in the neonatal period.<sup>4</sup>

Since the introduction of routine postpartum prophylaxis in the 1960s, the crude incidence of D isoimmunization in the U.S. and Canada has fallen from 9.1–10.3 cases to 1.3 cases/1,000 total births.<sup>5–9</sup> Hemolytic disease of the fetus or newborn due to D isoimmunization (also called erythroblastosis fetalis) now accounts for only 4–5 deaths/100,000 total births,<sup>6,10</sup> although this may be an underestimate as early intrauterine deaths are not always reported.<sup>10</sup> Even before the introduction of prophylaxis, however, a decline in fetal and neonatal mortality from D hemolytic disease was occurring due to declines in both incidence and case fatality rates. It has been estimated that 30–40% of the recent decline in disease incidence is attributable to smaller family size, since the incidence of D hemolytic disease increases with increasing birth order.<sup>11</sup> Since the 1940s, the case fatality rate has fallen from about 50% to 2–6%.<sup>8,9</sup> This decline can be attributed in part to the trend toward smaller families, since the

first affected infant in a family generally has less severe disease.<sup>1,5,9</sup> The decline has also been associated with the introduction of interventions such as amniotic fluid spectrophotometry, exchange transfusion, amniocentesis, intrauterine fetal transfusion, and improved care of both the mother and the premature erythroblastotic infant.<sup>1,9</sup>

### Accuracy of Screening Tests

Hemagglutination is the established reference standard for the determination of D blood type.<sup>12</sup> The indirect antiglobulin (Coombs) test (IAGT) is the reference standard for detecting anti-D antibody in women who are sensitized to D-positive blood.<sup>12</sup> The IAGT will also detect other maternal antibodies that may cause hemolytic disease.<sup>13</sup>

### Effectiveness of Early Detection

The early detection of D-negative blood type in the pregnant woman is of substantial benefit if the patient is not yet isoimmunized and the father is not known to be D-negative. Administration of D immunoglobulin (or Rh<sub>0</sub>(D) immune globulin (human)) to an unsensitized D-negative woman after delivery of a D-positive fetus will prevent maternal isoimmunization and consequent hemolytic disease in subsequent D-positive offspring. The efficacy of D immunoglobulin prophylaxis was convincingly demonstrated in a series of controlled clinical trials in the early 1960s.<sup>14-16</sup> Despite a variety of minor flaws in study design, these trials showed that isoimmunization did not occur in any of the women who received a full dose of D immunoglobulin postpartum and who were unsensitized when it was administered. These findings led to the introduction of routine postpartum prophylaxis following licensure of D immunoglobulin in 1968. Time series studies have since shown a dramatic decline in the incidence of D isoimmunization, from 13-14% in the mid-1960s to 1-2% in the mid-1970s,<sup>7,17</sup> although as described above, at least some of this decline is probably attributable to smaller family size.

The most frequent cause of apparent failure of postpartum prophylaxis is antenatal isoimmunization, which happens in 0.7-1.8% of pregnant women at risk.<sup>1,9,18</sup> Although sample selection and other design features were not optimal, nonrandomized controlled trials have shown that the administration of D immunoglobulin at 28 weeks' gestation, when combined with postpartum administration, reduces the incidence of isoimmunization to 0.2% of women at risk.<sup>19-21</sup>

Since D isoimmunization during pregnancy is caused by transplacental hemorrhage, the risk of isoimmunization increases whenever such hemorrhage is likely to occur, including after abortion, amniocentesis, chorionic villus sampling (CVS), cordocentesis, ectopic pregnancy, fetal manipula-

tion (e.g., external version procedures) or surgery, antepartum hemorrhage, antepartum fetal death, and stillbirth.<sup>1,22-24</sup> Studies documenting the effectiveness of D immunoglobulin prophylaxis are available for only a few of these indications, however. In a nonrandomized trial of D immunoglobulin after amniocentesis, control D-negative women delivering D-positive infants were more likely to become isoimmunized than were those receiving D immunoglobulin (5.2% vs. 0%), although because of small numbers this difference was not statistically significant.<sup>25</sup> Case series describing D immunoglobulin administration after amniocentesis have demonstrated isoimmunization rates as low as 0–0.5%.<sup>26-28</sup> In a case series of D immunoglobulin after induced abortion, isoimmunization occurred in 0.4%,<sup>29</sup> compared to 2.6% among a series of patients, described by the same authors, who did not receive D immunoglobulin.<sup>30</sup> The preliminary results from a randomized controlled trial of D immunoglobulin after CVS showed that among D-negative women delivering D-positive infants, similar rates of isoimmunization were seen in both intervention (2.3%) and control (1.1%) groups; insufficient details are provided to ensure baseline comparability between the two groups, however.<sup>31</sup> D-negative women who received D immunoglobulin experienced twice as many unintended fetal losses as did controls (6.9% vs. 3.8%), but this difference was not statistically significant. Results of the completed trial confirm the preliminary findings (S. Smidt-Jensen, Rigshospitalet, Copenhagen, Denmark; personal communication, January 1995), but have not yet been published. No studies evaluating the use of D immunoglobulin after other obstetric procedures or after obstetric complications were found.

The standard postpartum dose of D immunoglobulin (300 µg) contains sufficient D antibodies to prevent sensitization to at least 15 mL of D-positive fetal red blood cells (RBCs), or approximately 30 mL of fetal blood.<sup>32</sup> A “minidose” (50 µg) prevents sensitization to 2.5 mL of D-positive fetal RBCs. For women with transplacental hemorrhages >30 mL of fetal blood, the risk of D isoimmunization developing after the full postpartum D immunoglobulin dose is 30–35%.<sup>3,33</sup> The incidence of fetal-maternal hemorrhage >30 mL is 0.1–0.7% for all D-negative pregnancies,<sup>1,33,34</sup> but it is 1.7–2.5% after complicated vaginal and cesarean deliveries,<sup>34,35</sup> and 4.5% after stillbirth.<sup>1</sup> There are several available methods for detecting excess fetomaternal hemorrhage. Acid elution (Kleihauer-Betke) is both sensitive and specific when done correctly,<sup>1,36</sup> but it is subject to substantial laboratory and technologist error.<sup>36</sup> Flow cytometry is also highly sensitive and specific, but it is technically difficult to perform.<sup>36</sup> The erythrocyte rosette test is simple to perform and highly sensitive (99–100%) for the presence of 15 mL of D-positive fetal RBCs,<sup>1,36</sup> but its specificity is low<sup>36,37</sup> so positive results must be confirmed by more specific tests such as acid elution and flow cytometry.<sup>1,36</sup>

In clinical practice, combined antenatal and postnatal prophylaxis will prevent isoimmunization in 96% of women at risk.<sup>21</sup> The remaining cases are due to failure to give D immunoglobulin when indicated, isoimmunization that occurred before the widespread availability of D immunoglobulin, administration of an insufficient dose, or treatment failure (i.e., isoimmunization occurring before 28 weeks or transplacental hemorrhage too large or too late in pregnancy to be prevented by the standard antepartum dose).<sup>3,38,39</sup> Human error causes 22–50% of these cases.<sup>6,21,39</sup> While clinicians almost always administer D immunoglobulin postpartum or after induced abortion, administration rates have been documented to be lower for other obstetric procedures and complications: 81–88% after spontaneous abortion, 36–60% after ectopic pregnancy, 31% after antepartum hemorrhage, and 14% after amniocentesis.<sup>40,41</sup>

D immunoglobulin has few adverse effects.<sup>1,42</sup> Some fetuses will become weakly direct antiglobulin-positive following antenatal administration, but resulting anemia and hyperbilirubinemia in the newborn are very rare.<sup>19</sup> All plasma for D immunoglobulin production is screened for infectious diseases as required by the Food and Drug Administration; no cases of human immunodeficiency virus (HIV) infection from D immunoglobulin have been reported.<sup>43</sup> The evidence is therefore compelling that early detection and prophylaxis of the unsensitized D-negative woman is both safe and effective in preventing isoimmunization and thus in preventing D hemolytic disease.

Early detection is also beneficial for D-negative women who are already isoimmunized and are carrying D-positive offspring, because early intervention may improve clinical outcome. Decisions to intervene depend on the validity of screening tests in predicting the degree of fetal anemia. Obstetric history, maternal antibody titers, and ultrasound are currently used to determine the need for more invasive tests during isoimmunized pregnancies, but in the absence of hydrops none of these reliably distinguishes mild from severe hemolytic disease.<sup>1,4,22,44</sup> Immunologic tests on maternal serum show promise in predicting disease severity.<sup>1,45,46</sup> In the third trimester, serial amniotic fluid spectrophotometry has been found to correctly predict disease severity (i.e., cord hemoglobin and need for neonatal therapy) in 94–99% of cases.<sup>47,48</sup> In the second trimester, however, this test has insufficient sensitivity or specificity for predicting the need for intervention.<sup>4,49,50</sup> Determination of fetal hemoglobin and D blood type by ultrasound-guided cordocentesis, which can be performed in the second trimester, quantifies the degree of anemia, can be followed by transfusion if indicated, and allows referral of those with D-negative babies to routine care.<sup>1,4</sup> Case series, however, have demonstrated complication rates of 2–7% and procedure-related fetal mortality rates of 0.5–1%.<sup>23,51,52</sup> DNA amplification in amniotic cells and chorionic villus samples appears to be

effective in determining fetal D blood type early in pregnancy, without the risk associated with invading the fetomaternal circulation.<sup>53</sup>

In the presence of severe fetal anemia, early intervention appears to offer substantial improvement in clinical outcome. Current perinatal survival after ultrasound-guided intravascular transfusion at experienced centers is 62–86% for hydropic fetuses and >90% for those without hydrops.<sup>4,54,55</sup> Once pulmonary maturity is established, the fetus can be delivered early and exchange transfusion performed with only 1% mortality risk.<sup>56</sup>

### Recommendations of Other Groups

The American College of Obstetricians and Gynecologists (ACOG)<sup>22</sup> and the U.S. Public Health Service Expert Panel on the Content of Prenatal Care<sup>57</sup> recommend D blood typing and antibody screening at the first prenatal visit and repeat D antibody screening at 24–28 weeks of pregnancy for D-negative women. Both groups recommend offering D immunoglobulin to all unsensitized D-negative women at 28 weeks of gestation, and to those at increased risk of sensitization because of delivery of a D-positive infant, antepartum hemorrhage, spontaneous or induced abortion, amniocentesis, external version procedures, or ectopic pregnancy, within 72 hours of the event.<sup>22,57</sup> ACOG also recommends D immunoglobulin administration to unsensitized D-negative women who have CVS, cordocentesis, antepartum fetal death, fetal surgery, or transfusion of D-positive blood products.<sup>22</sup> ACOG recommends measuring fetal blood cell levels in the mother when antepartum placental hemorrhage occurs.<sup>22</sup> The Canadian Task Force on the Periodic Health Examination recommends D blood typing and antibody screening at the first prenatal visit, before elective procedures such as amniocentesis and therapeutic abortion in which there is the possibility of fetal bleed, between 24 and 28 weeks if the mother is D-negative, and within 72 hours of delivery. They recommend administration of D immunoglobulin to unsensitized women at 28 weeks and postpartum, and after amniocentesis or induced abortion.<sup>58</sup>

### Discussion

Although the burden of suffering from this disease is now low, the incidence was at least 10/1,000 live births before the introduction of preventive measures in the 1960s.<sup>9</sup> There is excellent evidence for the efficacy and effectiveness of blood typing, anti-D antibody screening, and postpartum D immunoglobulin prophylaxis. Although antepartum prophylaxis offers some additional benefit, some critics argue that the total impact of antepartum prophylaxis on the incidence of D disease is relatively small, making it approximately 16 times less cost-effective than a program consisting only of postpartum treatment.<sup>2,59,60</sup> Other studies support the cost-

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50 µg) dose of D immunoglobulin if they are antibody-negative ("B" recommendation). If a D- (or D<sup>u</sup>-) positive infant is delivered, the dose should be repeated postpartum, preferably within 72 hours after delivery ("A" recommendation). Unless the father is known to be D-negative, a full dose of D immunoglobulin is recommended for all unsensitized D-negative women after elective abortion (50 µg before 13 weeks) and amniocentesis ("B" recommendation). There is currently insufficient evidence to recommend for or against the routine administration of D immunoglobulin after other obstetric procedures or complications such as chorionic villus sampling, ectopic pregnancy termination, cordocentesis, fetal surgery or manipulation (including external version), antepartum placental hemorrhage, antepartum fetal death, and stillbirth ("C" recommendation).

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(“A” recommendation). For purposes of blood typing and prophylaxis, D<sup>u</sup>- and D-negative blood types should be considered equivalent.<sup>22</sup> Unless the father is known to be D-negative, a repeat D antibody test is recommended for all unsensitized D-negative women at 24-28 weeks’ gestation, followed by the administration of a full (300

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