Transfusions are rarely given to obstetric patients because most are young healthy women. When transfusions are given, pretransfusion hemoglobin levels tend to be lower than in other patient groups requiring transfusion. If transfusions are necessary during gestation, potential complications for the fetus as well as the mother must be considered.

It has been estimated that 2-3% of women in the peripartum period receive allogeneic red cells. Clinical conditions that place the obstetric patient at risk include: cesarean and instrumental delivery, breech extraction, anomalies of placental implantation, oxytocin administration, polyhydramnios, previous history of postpartum hemorrhage or of bleeding disorders, as well as multiple gestations. Autologous blood collection during pregnancy remains controversial, although it may be appropriate in some of the circumstances mentioned above.

The obstetric patient, embodying the fetomaternal complex, presents special immunohematologic problems for the transfusion service. The mother may exhibit alloimmunization to antigens on fetal red cells, platelets, and/or white cells, and the fetus may be affected by maternal antibodies provoked by previous pregnancies, by previous or present transfusions, or by the ongoing pregnancy.

Hemolytic Disease of the Newborn

In hemolytic disease of the newborn (HDN), fetal red cells become coated with IgG alloantibody of maternal origin, directed against an antigen of paternal origin present on the fetal cells and absent from maternal cells. The IgG-coated cells undergo accelerated destruction, both before and after birth, but clinical severity of the disease can vary from intrauterine death to hematological abnormalities detected only if blood from an apparently healthy infant is subjected to serologic testing.
Physiologic Observations

Accelerated red cell destruction stimulates increased production of red cells, many of which enter the circulation prematurely as nucleated cells, hence the term “erythroblastosis fetalis.” Severely affected fetuses may develop generalized edema, called “hydrops fetalis,” the pathogenesis of which is not clearly defined. In HDN resulting from anti-D alloimmunization, erythropoiesis in the fetal liver may be so extensive that portal circulation is disrupted and albumin synthesis impaired, thereby adding reduced plasma colloid osmotic pressure to the hemodynamic derangements caused by anemia, cardiovascular failure, and tissue hypoxia. Without treatment, the fetus may die in utero. Intrauterine transfusion may be lifesaving in these circumstances. If live-born, the severely affected infant exhibits profound anemia and heart failure. Less severely affected infants continue to experience accelerated red cell destruction, which generates large quantities of bilirubin.

Before birth severs communication between maternal and fetal circulation, fetal bilirubin is processed by the mother’s liver. Unconjugated bilirubin is toxic to the developing central nervous system (CNS). At birth, the infant’s immature liver is incapable of conjugating the amount of bilirubin that results from destruction of antibody-coated red cells. For the live-born infant with HDN, rising levels of unconjugated bilirubin may pose a greater clinical danger than the consequences of anemia. Prematurity, acidosis, hypoxia, and hypoalbuminemia increase the liability to CNS damage. Decisions about undertaking exchange transfusion are based primarily on the bilirubin level, the rate of bilirubin accumulation and, to a lesser degree, on the severity of the anemia.

Mechanisms of Maternal Immunization

HDN is often classified into three categories, on the basis of the specificity of the causative IgG antibody. In descending order of severity these are:

1. D hemolytic disease, due to anti-D alone or, less often, in combination with anti-C or anti-E.
2. “Other” hemolytic disease, due to antibodies against other antigens in the Rh system or against antigens in other systems; anti-c and anti-K are most often implicated.
3. ABO hemolytic disease, usually due to anti-A,B in a group O woman, rarely to anti-A or anti-B.

In all but ABO hemolytic disease, maternal antibodies reflect alloimmunization by pregnancy or transfusion. Rising titers of antibody can be documented, at least in the first affected pregnancy, and the infant may be symptomatic at birth. In ABO hemolytic disease, the condition cannot be diagnosed during pregnancy and the infant is rarely symptomatic at birth.

Pregnancy as the Immunizing Stimulus

Pregnancy causes immunization when fetal red cells, possessing a paternal antigen foreign to the mother, enter the maternal circulation, an event described as fetomaternal hemorrhage (FMH). Fetomaternal hemorrhage occurs in up to 75% of pregnancies, usually during the third trimester and immediately after delivery. Delivery is the most common immunizing event but fetal red cells can also enter the mother’s circulation after amniocentesis, spontaneous or induced abortion, chorionic villus sampling, cordocentesis, or rupture of an ectopic pregnancy, as well as blunt trauma to the abdomen.

Immunogenic Specificities. The antigen that most frequently induces immu-
nization is D but, in theory, any red cell antigen present on fetal cells and absent from the mother can stimulate antibody production. One retrospective study determined that there was 0.24% prevalence of production, during pregnancy, of clinically significant antibodies other than anti-D. Because other red cell antigens are less immunogenic than D, sensitization is more likely to result from exposure to a large volume of red cells, such as during blood transfusion. Immunization to D, on the other hand, can occur with volumes of fetal blood less than 0.1 mL.

Frequency of Immunization. The probability of immunization to D correlates with the volume of D-positive red cells entering the D-negative mother’s circulation. Before the availability of immune prophylaxis against D sensitization, the incidence of anti-D formation following the first pregnancy of D-negative women who had a D-positive, ABO-compatible infant was approximately 8%. An additional 8% developed detectable anti-D during their next D-positive pregnancy, probably reflecting primary immunization during the first D-positive pregnancy and delivery, but without production of detectable levels of antibody. The small numbers of D-positive fetal red cells entering the maternal circulation during the next pregnancy constituted a secondary stimulus sufficient to elicit overt production of IgG anti-D. In susceptible women not immunized after two D-positive pregnancies, later pregnancies might sometimes be affected but with diminished frequency.

The overall incidence of D sensitization in untreated multiparous D-negative mothers of D-positive infants is about 18%. Once immunization has occurred, successive D-positive pregnancies often manifest HDN of increasing severity, although some families have a stable or diminishing pattern of clinical disease in subsequent pregnancies.

Effect of ABO Incompatibility. Rh immunization of untreated D-negative women occurs less frequently after delivery of an ABO-incompatible D-positive infant than when the fetal cells are ABO-compatible with the mother. ABO incompatibility between mother and fetus has a substantial but not absolute protective effect against maternal immunization by virtue of the increased rate of red cell destruction. With the advent of Rh immunoprophylaxis, both antepartum and postpartum, the occurrence of Rh immunization has fallen dramatically, with fewer than 1% of D-negative women exhibiting immunization.

Transfusion as the Immunizing Stimulus

It is extremely important to avoid transfusing D-positive whole blood or red cells to D-negative females of childbearing potential because anti-D stimulated by transfusion characteristically causes severe HDN in subsequent pregnancies with a D-positive fetus. Red cells present in platelet or granulocyte concentrates can constitute an immunizing stimulus; if components from D-positive donors are necessary for young D-negative female recipients, Rh immunoprophylaxis should be considered.

The risk from an allogeneic red cell transfusion of immunization to a red cell antigen other than D has been estimated to be 1-2.5% in the general hospital population. This will endanger the fetus only if the antibody is IgG and directed against an antigen also present on the fetal red cells. For a couple planning to have children, the woman should not be transfused with red cells from her sexual partner or his blood relatives. This form of directed donation increases the risk that the mother will be immunized to paternal red cell antigens or to leukocyte or platelet antigens, which could cause alloimmune cytopenias in
future children who share the same paternal antigens.

**ABO Antibodies**

The IgG antibodies that cause ABO hemolytic disease nearly always occur in the mother's circulation without a history of prior exposure to human red cells. ABO hemolytic disease can occur in any pregnancy, including the first. It is restricted almost entirely to group A or B infants born to group O mothers, apparently because the predominantly IgG anti-A,B occurs only in group O individuals.

**Prenatal Evaluation**

**Maternal History**

Invasive investigative tests, which do carry risk to the fetus, should be performed only for pregnancies where the fetus is at risk for HDN. Information about previous pregnancies or blood transfusions is essential in evaluating fetal risk. For a woman with a history of an infant with hydrops fetalis due to anti-D, there is a 90% or more chance of a subsequent fetus being similarly affected. In the first sensitized pregnancy, the risk of a hydropic fetus is 8-10%. Experience with other alloantibodies has not been as extensive as with anti-D; in one series, anti-c and anti-K were by far the most common causes of severe HDN, other than anti-D.

**Serologic Studies**

alloantibodies capable of causing HDN can be detected during pregnancy. Initial studies should be performed on all pregnant women as early in pregnancy as possible; these should include tests for ABO and D, and a screen for unexpected red cell antibodies. If the woman's cells are not agglutinated by anti-D, a test for weak D (D") should be done, and the woman should be classified as D-positive if the test for either D or weak D is positive. Very rarely, a D-positive or weak D mother produces anti-D as a result of pregnancy. If a D-negative woman has a negative initial antibody screen, the test should be repeated at 28-30 weeks' gestation before Rh immune globulin (RhIG) is given. It is seldom necessary to repeat the antibody screen on D-positive women unless there is a history of clinically significant red cell antibodies, previous blood transfusion, or trauma to the abdomen.

**Antibody Specificity.** All positive screens for red cell antibodies require identification of the antibody. The mere presence of an antibody, however, does not indicate that HDN will inevitably occur. Non-red-cell-stimulated IgM antibodies, notably anti-Le^a^ and anti-I, are relatively common during pregnancy but do not cross the placenta. IgM antibodies can be distinguished from IgG by treating the serum with 2-mercaptoethanol or dithiothreitol. (See Method 4.3.) In addition, the fetal red cells may lack the antigen corresponding to the mother's antibody; the likelihood of fetal involvement can often be predicted by typing the father's red cell antigens. The laboratory report on prenatal antibody studies should include sufficient additional information to aid the clinician in determining the clinical significance of identified antibody.

**Paternal Zygosity.** When a woman has anti-D, some obstetricians type the father to estimate the likelihood of his possessing one or two examples of the gene that determines D. A homozygote will always transmit a gene for D to the offspring, whereas in the mating of a D-negative woman and a man heterozygous for the D gene, half the offspring will be D-negative. Formulas have been devised to estimate the probability that the D-positive partner of a D-negative woman is homozygous or heterozygous.
for the D gene, based on the number of D-positive children previously born and the man’s phenotype for C, c, E, and e. (See Table 21-1.) This information may be useful in counseling a couple when the woman has anti-D.

**Typing the Fetus.** It has become possible to establish fetal D type, with the polymerase chain reaction to amplify DNA obtained from amniotic fluid and chorionic villus sampling, but this technique is still considered investigational. At many centers fetal blood is obtained by cordocentesis for Rh typing when the father is heterozygous for the D gene (RHD).

**Maternal Antibody Titer**

Antibody titrations can help in decisions about the performance and the timing of invasive investigations, especially if the antibody is anti-D. The antibody titer should be established in the first trimester to serve as a baseline, and the specimen frozen for future comparisons. Since invasive tests will not be undertaken before 16-18 weeks’ gestation, no further titration is indicated until this time. To assess the true significance of a rising antibody titer, it is important that successive titrations be performed with the same methods and with test cells of the same red cell phenotype. Testing previously frozen serum samples in parallel with a current specimen minimizes the possibility that changes in the titer result from differences in technique. Some institutions have established a critical titer for anti-D below which HDN is considered so unlikely that no further investigations will be undertaken unless and until the level is reached. Critical titers for antibodies other than anti-D have not been well defined.

**Amniotic Fluid Analysis**

A good index of intrauterine hemolysis and fetal well-being is the level of bile pigment found in amniotic fluid obtained by amniocentesis. Amniocentesis is usually performed in D-negative women who have a history of previously affected pregnancies or have an anti-D titer at or above the critical titer (usually ≥16 by the antiglobulin method).

Amniotic fluid is obtained by inserting a long needle through the mother’s abdominal wall and uterus into the uterine cavity. The aspirated fluid is scanned spectrophotometrically at wave lengths

---

**Table 21-1. Probability of Heterozygosity at D Locus in Whites and Blacks for Given Rh Phenotype and n Previous D-Positive Offspring**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>n=0</th>
<th>n=1</th>
<th>n=2</th>
<th>n=3</th>
<th>n=4</th>
<th>n=5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W/B</td>
<td>W/B</td>
<td>W/B</td>
<td>W/B</td>
<td>W/B</td>
<td>W/B</td>
</tr>
<tr>
<td>CcDe</td>
<td>0.90/0.41</td>
<td>0.82/0.26</td>
<td>0.69/0.15</td>
<td>0.53/0.08</td>
<td>0.36/0.042</td>
<td>0.22/0.021</td>
</tr>
<tr>
<td>CDe</td>
<td>0.09/0.19</td>
<td>0.047/0.10</td>
<td>0.024/0.055</td>
<td>0.012/0.028</td>
<td>0.006/0.014</td>
<td>0.003/0.007</td>
</tr>
<tr>
<td>cDEe</td>
<td>0.90/0.37</td>
<td>0.82/0.23</td>
<td>0.69/0.13</td>
<td>0.53/0.068</td>
<td>0.36/0.035</td>
<td>0.22/0.018</td>
</tr>
<tr>
<td>cDE</td>
<td>0.13/0.01</td>
<td>0.07/0.005</td>
<td>0.036/0.003</td>
<td>0.018/0.001</td>
<td>0.009&lt;0.001</td>
<td>0.005&lt;0.001</td>
</tr>
<tr>
<td>CcDEe</td>
<td>0.11/0.10</td>
<td>0.058/0.053</td>
<td>0.03/0.027</td>
<td>0.015/0.014</td>
<td>0.008/0.007</td>
<td>0.004/0.003</td>
</tr>
<tr>
<td>cDe</td>
<td>0.94/0.54</td>
<td>0.89/0.37</td>
<td>0.80/0.23</td>
<td>0.66/0.13</td>
<td>0.49/0.068</td>
<td>0.33/0.035</td>
</tr>
</tbody>
</table>

*If the father has had any previous D-negative children then the probability that he is heterozygous for the D antigen is 1.

†W=Whites, B=Blacks. (Modified with permission from Kantor.)*

---

*Copyright © 2002 by the AABB. All rights reserved.*
of 350-700 nm. Peak absorbance of bilirubin is at 450 nm. A rise in optical density from the projected baseline at 450 nm (Δ OD_{450}) is a measure of the concentration of bile pigments. This value (Δ OD_{450}) is plotted on a graph against the estimated length of gestation, because bile pigment concentration has different clinical significance at different gestational ages. Liley's system (Fig 21-1) of predicting the severity of fetal disease based on the Δ OD_{450} has been used for decades. It delineates three zones to estimate severity of disease: a high zone indicates severe disease, the low zone indicates mild or no disease, and mid-zone values require repeat determination to establish a trend. This method is applicable to pregnancies 27 weeks to term. Queenan et al have proposed a system for managing D-immunized pregnancies, based on the Δ OD_{450} from as early as 14 weeks' gestation. They identify four zones (Fig 21-2), with early invasive intervention recommended if Δ OD_{450} values fall in the top zone. With both systems, the severity of HDN is more accurately predicted with serial Δ OD_{450} measurements than with a single observation to evaluate whether readings are falling, rising, or stable. In general, the higher the pigment concentration, the more severe the intrauterine hemolysis.

Other Fetal Variables. The risk of allowing a severely affected pregnancy to continue must be weighed against the risk of premature delivery and the problems of fetal lung immaturity. Levels of surfactant, lecithin, and other pulmonary lipids inadequate to maintain stable pulmonary alveolar structures in the newborn can cause respiratory distress syndrome. Maturity of the fetal lung is most reliably estimated by determining the ratio of lecithin to sphingomyelin.
(L/S ratio). A ratio of above 2:1 indicates maturity. Respiratory distress syndrome develops in about 70% of cases in which the L/S ratio is below 1.5:1 and 40% of cases with a ratio of 1.5-2:1. If the ΔOD450 indicates severe HDN but the L/S ratio indicates dangerously immature lung development, intrauterine transfusion may be indicated. Alternative assays for assessing fetal lung maturity are available, but bilirubin interferes with the assays; therefore, they are unsuitable for use in Rh-immunized pregnancies.

**Risk of Fetomaternal Hemorrhage.** Amniocentesis may cause FMH, which can boost the titer of existing red cell alloantibody, thereby increasing the severity of HDN, or induce immunization to antigens not already implicated. When amniocentesis is performed for any reason on a D-negative woman who does not have anti-D, Rh immunoprophylaxis should be given. In most such cases, the D status of the fetus is unknown, but the likelihood is high that it is D-positive and that bleeding induced by the procedure will induce production of anti-D in a woman not previously immunized.

**Ultrasound Evaluation**

To avoid the hazards of amniocentesis, many workers have increased their use of noninvasive monitoring protocols, including ultrasound evaluation of fetal well-being. Ultrasonography allows evaluation of cardiac function and estimation of cardiac, hepatic, and splenic size, which increase with extramedullary hematopoiesis and progressive anemia. The technique can indicate the presence or absence of hydrops, but is not useful in predicting the development of hydrops. The use of ultrasound has reduced to 2% the risk of placental trauma when amniocentesis is per-
formed, by allowing delineation of the implantation site.

Percutaneous Umbilical Blood Sampling
In the early 1980’s, the use of sophisticated ultrasound equipment made it feasible to direct a needle into an umbilical blood vessel, preferably the vein at its insertion into the placenta, and obtain a fetal blood sample. In the absence of hydrops, percutaneous umbilical blood sampling (PUBS, or cordocentesis) allows direct measurement of hematologic and biochemical variables, and accurate assessment of the severity of fetal hemolytic disease. It is important to verify that the sample has truly been obtained from the fetus. This is done by evaluating hematologic and serologic findings known to differ between the mother and the fetus, such as red cell size, presence of fetal hemoglobin, and red cell phenotyping.

The fetal mortality rate has been reported to be less than 1%, but the procedure carries a high risk of FMH. Its use is recommended only for certain circumstances, such as when serial amniotic fluid determinations indicate severe HDN or when the risk of fetal disease is high but the presence of an anterior placenta makes it difficult or dangerous to perform amniocentesis.

Suppression of Maternal Alloimmunization
Several approaches to suppress maternal alloimmunization have been attempted in the last decade, of which two have limited clinical benefit in reducing maternal antibody levels: intensive plasma exchange and the administration of intravenous immunoglobulin (IVIG). Plasma exchange can reduce antibody levels by as much as 75%, but rebound usually follows. Plasma exchange has been proposed as a way to delay the need for fetal intervention when there has been hydrops fetalis before 22 weeks' gestation in a previous pregnancy and the father is known to be homozygous for the implicated gene. IVIG infusion has also decreased anti-D titers, with best results obtained when started before 28 weeks' gestation and when the fetus is not hydropic. The mechanism of IVIG effect is not clear. It may work by saturating placental Fc receptors and inhibiting the transfer of maternal antibody, by suppressing ingestion of IgG-coated red cells by the fetal reticuloendothelial system, or by introducing anti-idiotype antibodies that modify maternal antibody production. Because these therapies may have only transient benefit, if any, intrauterine transfusion is often necessary.

Intrauterine Transfusion
Intrauterine transfusion is not without risk to the fetus and should be performed only after careful clinical evaluation. Intrauterine transfusion is seldom feasible before the 20th week of gestation; once initiated, transfusions are usually administered every 2 weeks until delivery. Intrauterine transfusion can be performed by the intraperitoneal route (IPT) or the direct intravascular approach (IVT) by the umbilical vein. In many instances, IVT is the procedure of choice, but there may be problems of access that make IPT preferable; a combination may also be used.

Techniques
IPT is performed through a needle passed, with ultrasonographic monitoring, through the mother’s abdominal wall into the abdominal cavity of the fetus. Transfused red cells enter the fetal circulation by absorption from lymphatic channels that drain the peritoneal cavity. In IVT, the umbilical vein is pene-
trated under ultrasound guidance, and a blood sample taken to verify positioning in the fetal vasculature. Blood is infused directly, as either a simple transfusion or as a partial exchange transfusion, which can be particularly valuable for very severe cases of hemolytic disease of the newborn.

**Selection of Red Cells**

The red cells used should be type O, D-negative and negative for the antigen corresponding to the mother’s antibody if the specificity is not anti-D. Blood for intrauterine transfusion should be irradiated, and will often be selected or treated to reduce the risk of transmitting cytomegalovirus (CMV), especially if the mother is CMV-seronegative or her immune status is unknown. It may also be desirable to transfuse only blood that is known to lack hemoglobin S. For maximal survival of the transfused cells, blood used for intrauterine transfusion or intrauterine exchange transfusion should be as recently drawn as possible. Washed or deglycerolized preparations have been used, for their normal electrolyte levels, absence of anticoagulant or plasma, low levels of platelets and leukocytes, and low risk of CMV transmission. The hematocrit is usually 75-85%, to minimize the chance of volume overload in the fetus. Washed, irradiated maternal blood has also been used for intrauterine transfusion.

**Volume Administered**

The volume transfused may vary with the technique used as well as the fetal size and age. For IPT, a volume calculated by the formula \( V = (\text{gestation in weeks} - 20) \times 10 \text{ mL} \) appears to be well-tolerated by the fetus. The usual volume of red cells transfused by IVT is 50 mL/kg of estimated nonhydropic fetal weight, infused as 10 mL aliquots at 1- to 2-minute intervals until the desired volume is achieved. Transfusion is repeated when the concentration of circulating donor hemoglobin has fallen to a range of 9-10 g/dL.

**Postpartum Evaluation**

It is desirable to collect a sample of cord blood, preferably by syringe, from every newborn. This tube should be identified as cord blood and be labeled in the delivery suite with the mother’s name, the date, the infant’s identification, and hospital number. Samples should be stored for at least 7 days in the blood bank, where it will be available for testing if the mother is D-negative or if the newborn develops signs and symptoms that suggest HDN.

In cases of suspected HDN, samples of both cord and maternal blood should be tested, as shown in Table 21-2. When the mother is known to have antibodies capable of causing HDN, the hemoglobin or hematocrit and the bilirubin level of cord blood should also be tested. If the mother is D-negative and the infant D-positive, the mother’s blood should be tested for FMH (see later section). Tests on the mother’s blood present no special problems and can be done with routine techniques. Testing cord blood may present some special problems, which are described below.

**ABO Testing**

ABO testing on newborns relies entirely on red cell typing. ABO antibodies in
If the infant’s red cells are heavily coated with IgG antibodies, tests with anti-D may give either false-positive or false-negative results. (See Chapter 13.)

**Antiglobulin Testing**

The direct antiglobulin test (DAT) is usually strongly positive in HDN due to anti-D or antibodies in other blood groups; reactions are much weaker or even negative in HDN due to ABO antibodies. Infants who have received intratuterine transfusion may also have a weak DAT with a mixed-field pattern of agglutination. If the DAT on cord cells is positive, the antibody can be eluted from the red cells and tested for specificity. It is not necessary to make and test an eluate if the maternal serum has been shown to contain a single red cell antibody. If the mother has multiple antibodies, it is not necessary to identify the hemolyzing antibody because, if transfusion is necessary, all the clinically significant red cell antibodies in the maternal serum must be respected. (See Selection of Blood, later in this chapter.)

If the DAT is positive and the maternal serum has a negative screen for red cell antibodies, suspicion falls on ABO antibodies or on HDN due to an antibody against a low-incidence antigen not present on reagent red cells. Testing the eluate from the cord cells against A1 and B red cells aids in the diagnosis of ABO hemolytic disease. In the rare cases of ABO hemolytic disease that require transfusion, only group O red cells should be transfused.

**Pursuing ABO Antibodies.** ABO hemolytic disease may be suspected on clinical grounds even though the DAT is negative. Cord blood serum should be tested by the indirect antiglobulin technique against A1, B, and O red cells. The presence of anti-A, anti-B, or anti-A,B confirms the potential for ABO hemolytic disease. It is often possible to

---

**Table 21-2. Serologic Studies Recommended for Maternal and Cord Blood When HDN Is Suspected**

<table>
<thead>
<tr>
<th>Maternal Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO</td>
</tr>
<tr>
<td>Rh</td>
</tr>
<tr>
<td>Weak D, if apparently D-negative</td>
</tr>
<tr>
<td>Test for FMH, if mother is D-negative</td>
</tr>
<tr>
<td>and infant is D-positive</td>
</tr>
<tr>
<td>Identification of antibody, if present</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cord Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO</td>
</tr>
<tr>
<td>Rh</td>
</tr>
<tr>
<td>Weak D, if apparently D-negative</td>
</tr>
<tr>
<td>Direct antiglobulin test</td>
</tr>
<tr>
<td>Eluate from red cells, if DAT is positive</td>
</tr>
<tr>
<td>and clinical circumstances warrant</td>
</tr>
<tr>
<td>Identification of antibody in eluate</td>
</tr>
</tbody>
</table>

---

**D Testing**

Newborns who have had successful intratuterine transfusions often type at birth as D-negative or very weakly positive, because over 90% of their circulating red cells may be those of the donors. The ABO and direct antiglobulin tests may also give misleading results.
elute anti-A and/or anti-B from the infant's red cells despite a negative DAT, but this step is not necessary for the presumptive diagnosis. If transfusion is required, D-compatible group O blood should be transfused, whether or not the diagnosis has been serologically confirmed.

**Pursuing Antibodies to Low-Incidence Antigens.** If ABO hemolytic disease is ruled out, antibody against a low-incidence red cell antigen should be suspected. Testing of the eluate, with an antiglobulin technique, against the father's red cells, may provide an answer. The diagnosis can be confirmed by testing the mother's serum against the father's red cells, if they are ABO-compatible. If either or both of these tests are positive, it indicates that the father has transmitted to the child a red cell antigen to which the mother has an IgG antibody. Unless the mother has been exposed to red cells from the father or his blood relations, transfusion would be an unlikely immunizing event for a low-incidence antigen. Because there should be no difficulty in obtaining compatible blood, diagnostic studies can be performed after initial clinical concerns have been resolved.

If the DAT is positive and all attempts to characterize a coating red cell antibody are consistently negative, causes of a false-positive DAT should be considered. (See Chapter 18.)

**Exchange Transfusion**

Exchange transfusion, the indicated treatment for severe HDN, achieves several desired effects. Removal of the infant's blood reduces 1) the mass of antibody-coated red cells, the destruction of which causes bilirubin levels to rise; 2) a portion of the bilirubin that has accumulated; and 3) the number of unbound antibody molecules available to attach to newly formed antigen-positive cells. The red cells used for replacement are compatible with the antibody and provide increased oxygen-carrying capacity. Fresh Frozen Plasma, if used, restores not only albumin but also coagulation factors; albumin can be used as a replacement colloid.

**Selection of Blood**

In most cases the mother's serum is used for crossmatching and the red cells selected for transfusion are compatible with her ABO antibodies as well as the antibody(ies) responsible for the hemolytic process. Group O red cells resuspended in AB plasma are commonly used. In ABO hemolytic disease, the red cells used for exchange must be group O. If the antibody is anti-D, the red cells must be D-negative, but not every exchange transfusion requires group O, D-negative blood. If mother and infant are ABO-identical, group-specific red cells can be used. If the implicated antibody is not anti-D, D-positive red cells may be given to a D-positive infant.

Maternal serum or plasma is the specimen of choice for crossmatching in exchange transfusion. It is available in large quantity, has the red cell antibody present in high concentration, and can be accurately and completely analyzed before delivery. Maternal serum may contain antibodies directed against antigens not present on the infant's red cells, or IgM antibodies that have not crossed the placenta.

If maternal blood is not available or is unsuitable for crossmatching, the infant's serum and/or an eluate from the infant's red cells can be used for crossmatching. The eluate provides a concentrated preparation of the antibodies responsible for red cell destruction, but will not contain antibodies that may have crossed the placenta but are directed against antigens absent from the infant's red cells. The concentration of
antibody in the infant’s serum may be low, especially if most of the molecules are bound to the red cells. Use of either, or of both together, may be indicated if attempts to obtain a maternal specimen would delay therapy.

Subsequent Transfusion

Bilirubin may reaccumulate rapidly after successful exchange, partly because bilirubin in extravascular fluid will follow the concentration gradient and enter the intravascular space, and partly because the residual antibody-coated cells continue to undergo destruction. If rising bilirubin levels make a second or third exchange transfusion necessary, the same considerations of red cell selection and crossmatching apply.

Antibody Against a High-Incidence Antigen

Rarely, the mother’s antibody reacts with a high-incidence antigen and no compatible blood is available. If this problem is recognized and identified before delivery, the mother’s siblings can be evaluated for compatibility and suitability, or compatible donors can be sought through a rare donor file. If this very rare event is not recognized until after delivery, three choices are open:

1. Collect blood from the mother, if the obstetrician agrees. Remove as much plasma as possible, preferably by saline washing, and resuspend the red cells in AB plasma to the desired hematocrit. The unit must be irradiated.
2. If time permits, test the mother’s siblings or other close relatives for compatibility and suitability. Blood from family members must be irradiated.
3. Use incompatible donor blood for the exchange transfusion if the clinical situation is sufficiently urgent. The exchange will reduce the bilirubin load, the most heavily antibody-coated cells, and the number of unbound antibody molecules. Residual antibody will, however, attach to the transfused cells and one or more additional exchanges will probably be needed as bilirubin accumulates.

Rh Immune Globulin

Rh Immune Globulin (RhIG) is a concentrate of predominantly IgG anti-D derived from pools of human plasma. A full dose of anti-D (approximately 300 µg) is sufficient to counteract the immunizing effects of 15 mL of D-positive red cells; this corresponds to approximately 30 mL of fetal whole blood. RhIG is available in a reduced dose, approximately 50 µg, which is protective for up to 2.5 mL of D-positive fetal red cells. This is used primarily for first-trimester abortion or miscarriage, when the total blood volume of the fetus is less than 2.5 mL. The protective effect of RhIG on D-negative individuals exposed to D-positive cells probably results from interference with antigen recognition in the induction phase of primary immunization. Properly prepared RhIG, like other immunoglobulin preparations, has little or no risk of virus transmission.

Antepartum Administration

Widespread postpartum use of Rh immunoprophylaxis has reduced pregnancy-associated immunization to the D antigen from approximately 13% to 1-2%. This risk is further decreased to 0.1%, if RhIG is also given antepartum at 28 weeks of gestation. The American College of Obstetricians and Gynecologists (ACOG) recommends antepartum RhIG prophylaxis. When the mother receives RhIG during pregnancy, the infant may be born with a positive DAT, but there is no evidence of hemolysis. The mother’s serum will often exhibit anti-D reactivity.
There must be good communication between the patient’s physician and the staff at the institution where delivery takes place, to ensure correct interpretation of laboratory tests made at the time of delivery. The half-life of an injected dose of RhIG, in the absence of significant FMH, is approximately 21 days. Therefore, of 300 µg of anti-D given at 28 weeks, 20-30 µg could remain at the time of delivery 12 weeks later and some has remained detectable for as long as 6 months.

Antepartum RhIG is given at 28 weeks of gestation, based on the observation that, of women who develop anti-D during pregnancy, 92% do so at or after 28 weeks. Blood obtained before injection of RhIG should be tested for ABO and D, including a test for weak D; and for red cell antibodies, with identification of unexpected antibodies found. A D-negative woman who has antibodies other than anti-D remains a candidate for anti-D immunoprophylaxis.

**Postpartum Administration**

Cord blood from infants born to D-negative mothers should be tested for the D antigen. A D-negative woman with a D-positive infant should receive one full dose of RhIG within 72 hours of delivery, unless she is known to be alloimmunized to D already. The presence of residual anti-D from antepartum RhIG does not indicate ongoing protection. The need for postpartum RhIG may, if anything, be greater in such a woman than in the recently delivered mother with a negative antibody screen. There is suggestive evidence that the presence of antibody at levels around 20 µg, the level likely to persist at delivery after RhIG administration at 28 weeks, actually increases the likelihood that FMH, if it occurs, will cause immunization.

**Active vs Passive Antibody.** A few laboratory clues that may help distinguish passively administered RhIG from the anti-D of active alloimmunity is that passively acquired anti-D is entirely IgG; if a woman’s anti-D is saline-reactive or can be completely or partially inactivated by treating the serum with 2-mercaptoethanol or dithiothreitol, it has an IgM component and probably represents active immunization. Passively acquired anti-D rarely achieves an antiglobulin titer above 4, so a high-titered antibody is likely to indicate active immunization. It is desirable to obtain confirmation from the physician’s records, but RhIG should always be given when doubt cannot easily be resolved. It should also be given if there is any problem determining the Rh type.

**Postpartum Evaluation.** A sample of the mother’s blood should be drawn, preferably within 1 hour after delivery, and evaluated for FMH of a quantity greater than that for which 300 µg RhIG is immunosuppressive. If the screening test demonstrates the presence of fetal cells, the extent of PMH must be determined so that an appropriate dose of RhIG can be administered (see below). Postpartum RhIG should be given within 72 hours of delivery; if 72 hours pass without administration of RhIG, it is better to give the treatment late than not at all.

The following women are not candidates for RhIG:

1. The D-negative woman whose infant is D-negative.
2. Any D-positive woman. Very rare cases of HDN have been reported in infants whose mothers had a weak D phenotype, but routine RhIG prophylaxis is not recommended for women of the weak D phenotype.
3. A D-negative woman known to be immunized to D.

**The “Utilization Gap”**

RhIG should be given to a D-negative woman after any obstetric event that
might allow fetal cells to enter the mother's circulation: spontaneous or therapeutic abortion, ectopic pregnancy, amniocentesis, chorionic villus sampling, cordocentesis, antepartum hemorrhage, or fetal death. If pregnancy in a D-negative woman terminates before 13 weeks of gestation, a 50 µg dose of RhIG is adequate to protect against the small fetal blood volume during the first trimester. From 13 weeks until term, a full dose of RhIG should be given.

Amniocentesis

Amniocentesis can cause FMH and consequent Rh immunization. The D-negative woman who has amniocentesis at 16-18 weeks for genetic analysis should receive a full dose of RhIG at that time and a second full dose at 28 weeks of gestation, and the usual postpartum dose if the infant is D-positive. If a nonimmunized D-negative woman undergoes amniocentesis for any reason in the second or third trimester, a full dose of RhIG is indicated. If the procedure is repeated more than 21 days later, an additional full dose should be given. If amniocentesis is performed to assess fetal maturity, and if delivery is expected within 48 hours of the procedure, RhIG can be withheld until the infant is born and confirmed to be D-positive. If more than 48 hours will elapse, RhIG should be given following amniocentesis. If delivery occurs within 21 days thereafter and there is no evidence of a massive FMH, additional RhIG may not be essential, but prudent management suggests treatment at delivery.

Screening for Large-Volume FMH

Postpartum administration of RhIG may not prevent immunization if the quantity of D-positive fetal red cells entering the mother's circulation exceeds the immunosuppressive capacity of RhIG. One 300 µg dose protects against 15 mL of D-positive blood or 30 mL of fetal blood. Only 0.3% of pregnancies are estimated to sustain transplacental hemorrhage greater than 30 mL, but large FMH is an important and preventable cause of failed immunoprophylaxis. ACOG recommends postpartum testing for large FMH only for high-risk pregnancies, but Ness and colleagues showed that testing only on the ACOG criteria would miss 50% of mothers actually exposed to large-volume FMH. AABB Standards requires examination of a postpartum specimen from all D-negative women at risk of immunization, to detect the presence of FMH that requires more than one dose of RhIG.

“Microscopic Weak D.” In the past, some workers looked for D-positive red cells in the mother’s D-negative blood by examining microscopically the antiglobulin phase of the test for D (“microscopic weak D test”); mixed-field reactivity indicated substantial admixture with D-negative cells. This procedure should not be used to identify large FMH, however, because it does not adequately detect the number of cells likely to be involved. When specimens are prepared to simulate a 30-mL D-positive hemorrhage in the circulation of an average-sized D-negative woman, personnel who use this procedure often fail to demonstrate the admixture.

The Rosette Test. The rosette test effectively demonstrates small numbers of D-positive cells in a D-negative suspension. The suspension is incubated with an anti-D reagent of human origin, and antibody molecules attach to sites on D-positive cells in the suspension. Indicator D-positive cells are then added, which react with antibody molecules bound to the surface of the already-present D-positive cells and form visible agglutinates (rosettes) around them. (See Method 7.1.) This method will detect FMHs of approximately 10 mL, a sen-
sitivity that provides a desirable margin of safety for a screening test. Weak D-positive cells do not react as strongly in the rosette procedure as normal D-positive cells. If the newborn is positive for weak D, FMH can be evaluated by the Kleihauer-Betke acid-elution test, which identifies fetal hemoglobin, not a surface antigen. The rosette test gives only qualitative results; a positive result must be followed by a quantitative test such as an acid-elution procedure, enzyme-linked antiglobulin test (ELAT), or flow cytometry.

**Quantifying FMH**

Historically, quantification of FMH has been achieved by the Kleihauer-Betke acid-elution test, which relies on the differences between fetal and adult hemoglobin in resistance to acid-elution. (See Method 7.2.) Results are reported as percentage of fetal cells, but the precision and accuracy of the procedure may be poor. Because 300 µg of RhIG will protect against FMH of 30 mL of D-positive fetal blood, the number of doses of RhIG required is determined by dividing the estimated volume of fetal blood present by 30.

For example:

1. Kleihauer-Betke reported as 1.3%
2. $1.3 \times 50^* = 65$ mL of fetal blood
3. $65/30 = 2.2$ doses of RhIG required
   
   * $5000$ mL (mother’s arbitrarily assigned blood volume) $\times 1/100 = 50$

Because quantification by this procedure is inherently imprecise and because the consequences of undertreatment can be serious, it is desirable to provide a safety margin in calculating RhIG dosage. One approach is as follows:

1. When the number to the right of the decimal point is less than 5, round down and add one dose of RhIG (example: If the calculation comes to 2.2 doses, give 3 doses).
2. When the number to the right of the decimal point is 5 or greater, round up to the next number and add one dose of RhIG (example: If the calculation comes to 2.8 doses, give 4 doses).

Not more than five doses of RhIG should be injected intramuscularly at one time. For larger quantities, injections can be spaced over a 72-hour period for patient comfort; an optimal time sequence has not been established. An intravenous preparation of anti-D has been approved by the Food and Drug Administration for use in the suppression of Rh immunization.

Alternative methods used to quantify FMH include ELAT and flow cytometry; both rely on differences between maternal and fetal blood types, unlike the acid-elution procedure that is independent of blood type. Both have sensitivity at least equal to the Kleihauer-Betke technique, and provide a more objective and reproducible endpoint. Neither, however, has been readily adapted to the blood bank.

**Neonatal Immune Thrombocytopenia**

Maternal IgG antibodies to platelets can cross the placenta and cause severe antenatal thrombocytopenia. Two categories of immune thrombocytopenia are recognized, and the distinction between them is therapeutically important.

**Neonatal Alloimmune Thrombocytopenia**

The mechanism of neonatal alloimmune thrombocytopenia (NAIT) is similar to that of HDN. Fetal platelets, expressing a paternal antigen absent from the mother’s cells, may enter the mother’s circulation during gestation or delivery. If she experiences alloimmunization, the maternal IgG antibody crosses the placenta and causes neonatal thrombocytopenia.
cytopenia. The maternal platelet count remains normal. The incidence of NAIT is approximately 1 in 2000 live births.

Unlike HDN, NAIT often affects first-born children, about 60% of cases occurring in a woman’s first child. The thrombocytopenia is self-limiting, normally resolving in 2-3 weeks. NAIT varies in severity from mild thrombocytopenia with no clinical signs to overt clinical bleeding; the incidence of intracranial hemorrhage has been reported as 10-30%, with approximately half occurring in utero. Recurrence in subsequent pregnancies is frequent, with equal or increasing severity, so a woman known to be alloimmunized must receive skilled antenatal attention.

Serologic Testing

Serologic diagnosis should be sought in a woman whose infant has had NAIT. Several platelet-specific antigen systems have been associated with NAIT, with HPA-1a antigen (PlA1) accounting for approximately 80% of cases. Pregnancy, rather than transfusion, is the usual immunizing event. Approximately 2% of the population is HPA-1a negative; approximately 10% of HPA-1a-negative women with HPA-1a-positive infants become immunized. There seems to be an association between developing anti-HPA-1a and possessing the HLA phenotype DRw52a. While antibodies to HLA Class I antigens are frequently encountered in pregnancy, and platelets express Class I antigens, this is a rare cause of NAIT.

Prenatal Considerations

With a knowledge of antibody specificity and of gene frequencies, the likelihood of subsequent siblings being affected can be predicted [Table 21-3]. The recognized platelet-specific antigens occur in diallelic systems, so typing the father’s platelets indicates zygosity. If the father is heterozygous, then there is a 50% chance that subsequent offspring will have the offending antigen. In an at-risk pregnancy, the genotype of the fetus (and by inference the platelet phenotype) can be determined by DNA typing on fetal cells obtained by amniocentesis.

When the risk of NAIT is high, a fetal blood sample for platelet count determination can be obtained by cordocentesis as early as 20 weeks’ gestation. When the infant is found to be thrombocytopenic, the mother is often given infusions of IVIG in weekly doses of 1 g/kg, with or without steroids, until delivery.

Sources of Platelets. Maternal platelets are often prepared for use at delivery; as with all allogeneic components, they require testing for infectious disease markers, and high-dose IVIG may interfere with immunoassays used. It is desirable to test the mother before initiating IVIG therapy. Repeat fetal sampling is usually performed every 4-6 weeks to assess treatment. Because cordocentesis carries a risk of serious bleeding in a thrombocytopenic fetus, compatible platelets are often infused if the platelet count is low. The platelets can be collected either from the mother or from another donor whose platelets lack the corresponding antigen and whose plasma is compatible with the fetal red cells. If maternal platelets are used, the antibody-containing plasma should be removed and the platelets resuspended in compatible plasma or saline with reduced volume (see Method 9.13). All components for intrauterine transfusion must be irradiated and should have a reduced risk of CMV transmission.

Scheduling Therapy. Various strategies have been used in the management of fetal thrombocytopenia. One approach is weekly platelet transfusions, sometimes starting as early as 18-20 weeks’ gestation, and continuing until delivery. Another approach is administration
of a single transfusion just before delivery if cordocentesis reveals severe thrombocytopenia. These approaches are usually reserved for pregnancies at extreme risk for intracranial hemorrhage, because repeated cordocentesis imposes some risk. Delivery is sometimes accomplished by cesarean section to reduce the risk of intracranial hemorrhage.

Management After Delivery

Platelet counts can continue to fall after birth and should be monitored. If bleeding appears imminent, compatible platelets should be given. If compatible platelets are not available, the use of high-dose IVIG should be considered, but this treatment has induced variable responses. In patients who do respond, platelet counts usually start increasing within 24-48 hours, although longer periods of time have been seen in some patients. Because response is slow, the patient with an urgent need for transfusion and no available compatible platelets may have to receive platelets from random donors.

Thrombocytopenia Secondary to Maternal ITP

Infants born to mothers with active idiopathic (autoimmune) thrombocytopenic purpura (ITP) are often not pro-
foundly thrombocytopenic and have a smaller risk of hemorrhage than infants with NAIT. The antibody in ITP is usually IgG, which readily crosses the placenta. Some women with systemic lupus erythematosus also have circulating platelet antibodies. Occasionally, delivery of a severely thrombocytopenic infant has led to the diagnosis of previously unsuspected ITP in a moderately affected mother (postpartum platelet count 75-100,000/µL). Mild gestational thrombocytopenia can occur in which a mother with no history of autoimmune disorder has platelet counts between 100,000 and 150,000/µL. This causes little to no risk to either the mother or the fetus/neonate.

The antibody in ITP has broad reactivity against all platelets. If the infant has a high concentration of antibody, there will be uniformly short survival of platelets from random donors, from the mother, or from other family members. Responses do sometimes occur, and in the presence of hemorrhage, platelet transfusions will be used as emergency therapy. Exchange transfusion can be effective in the removal of circulating antibodies and is usually followed by platelet transfusion. Intravenous immunoglobulin therapy may also be an effective therapy for severe thrombocytopenia.

References


