Update on HDFN: new information on long-standing controversies

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Hemolytic disease of the fetus and newborn (HDFN) results from maternal IgG antibodies that cross the placenta to the fetal circulation during gestation and cause RBC destruction and complications before birth (HDF), or anemia and hyperbilirubinemia after birth (HDN), or both. In its most severe form, HDF produces hydrops fetalis, which is characterized by total body edema, hepatosplenomegaly, and heart failure and can lead to intrauterine death. Before discovery of Rh immunoglobulin (RhIG), HDFN from anti-D was a significant cause of perinatal mortality or long-term disability. Routine administration of RhIG to D– women during pregnancy and shortly after the birth of D+ infants effectively reduced the incidence of HDFN caused by anti-D. Maternal alloimmunization to other RBC antigens in the Rh, Kell, and other blood group systems can not be routinely prevented and these antibodies can also cause HDFN. Advances in prenatal care, noninvasive monitoring, and intrauterine transfusion have improved the outlook for affected pregnancies to the extent that even hydrops fetalis can be reversed and effectively treated in many cases. This review will provide an update on the current issues in prevention and treatment of HDFN, emphasizing recent insights into long-standing controversies regarding maternal weak D phenotypes and D alloimmunization, noninvasive fetal diagnosis and monitoring of affected pregnancies, and recent treatment guidelines. ***Immunohematology* 2006;22:188–195.**

The incidence of D alloimmunization in pregnancy decreased from 14 percent to between 1 and 2 percent following the introduction of postnatal prophylaxis with Rh immunoglobulin (RhIG) in the late 1960s and, after 1979, was further reduced to 0.1 percent with the addition of routine antenatal RhIG prophylaxis.[1–3] Smaller family size in recent decades has also contributed to the decline in the number of cases, but hemolytic disease of the fetus and newborn (HDFN) caused by anti-D continues to occur in about 6.7 of 1000 live births in the United States, which likely reflects inadvertent failure to administer RhIG prophylaxis, inadequate prenatal care, or antenatal sensitization prior to RhIG administration at 28 weeks' gestation.[4,5]

More than 50 different specificities of RBC antibodies have been implicated in HDFN, but most cases of severe fetal anemia that require treatment in utero are caused by anti-D or anti-c (Rh system), or anti-K (Kell system) (Table 1).[1,2,6] The epidemiology of HDFN in different ethnic and racial populations directly reflects the frequency of blood group alleles in the population and the likelihood of incompatibility and consequent maternal alloimmunization.[6,7] Incompatibility with respect to the D antigen occurs in about 10 percent of all pregnancies among Caucasians and African Americans; in contrast, the D– phenotype is extremely rare among Asian women and HDFN caused by anti-D is seldom encountered in these populations.

In a study of 17,568 screened pregnancies, the prevalence of new antibody production was 0.24 percent (95% CI, 0.17–0.32).[8] Anti-D is still one of the most common antibodies found in pregnant women, but other antibodies have surpassed anti-D in some studies. In one large series, anti-K was detected at a rate of 3.2 per 1000 maternal samples compared with anti-D at 2.6 per 1000.[9] In another recent study of 1133 Dutch women with positive antibody screens, anti-E was the most common antibody detected (23%) followed by anti-K (18.8%), anti-D (18.7%), and anti-c (10.4%).[10]

Severe hemolytic disease requiring intrauterine transfusion was caused by anti-D, -K, or -c, in 85 percent, 10 percent, and 3.5 percent of HDF cases, respectively.[11] Rh and Kell antibodies are more likely associated with severe hemolysis than are other antibodies, but HDFN associated with these and other blood group antibodies can demonstrate a broad spectrum of symptoms, ranging from mild anemia and hyperbilirubinemia in an infant to life-threatening complications before birth. About one-half of D+ infants with detectable maternal anti-D in their serum are unaffected or only mildly affected and require no treatment; whereas 20 percent are severely affected in utero.[1,2] About one-half of these severely affected

Table 1. Probability of causing severe HDFN associated with RBC antibodies[2,36] *

Blood group	Highest likelihood of severe HDFN	Rare cases of severe HDFN	Usually associated with mild disease	Not a cause of HDFN
MNS		M, S, s, U, Mi^a, Vw, Mur, Mt^a, Hut, Hil, M^v, Far, s^D, En^a, MUT	M, S, s, U, Mt^a, Mit	N
Rh	D, c	C, E, f, Ce, C^w, C^x, E^w, G, Hr$_0$, Hr, Rh29, Go^a, Rh32, Be^a, Evans, Tar, Rh42, Sec, JAL, STEM	E, e, f, C^x, D^w, Rh29, Riv, LOCR	
Lutheran			Lu^a (rare), Lu^b	
Kell	K	k, Kp^a, Kp^b, Ku, Js^a, Js^b, Ul^a, K11, K22	Ku, Js^a, K11	K23, K24
Lewis				Le^a, Le^b
Duffy		Fy^a	Fy^b (rare), Fy3 (rare)	
Kidd		Jk^a	Jk^b (rare), Jk3	
Other		Di^a, Wr^a, Rd, Co^a, Co3, PP1P^k Vel, MAM Bi, Kg, JONES, HJK, REIT	Di^b, Sc3, Co^b, Ge2 (rare), Ge3, Ls^a Lan, At^a, Jr^a JFV, HOFM	P1, Wr^b, Yt^a, Yt^b, Sc1, Sc2, CH/RG, CROM, KN, JMH, I Jr^a HLA: Bg^a, Bg^b, Bg^c

* For some of the antibodies listed, the information is based on a very small number of examples, sometimes only one, resulting in overlap between categories.

fetuses have significant hemolysis before 34 weeks' gestation and require intrauterine transfusion. A similar spectrum of disease severity is observed with anti-c, anti-K, and anti-Fy^a, with severe disease affecting as many as 7 percent, 38 percent, and 16 percent of susceptible fetuses, respectively.[1,2] Subsequent pregnancies are more likely to be severely affected than are first pregnancies because of the anamnestic immune response.

Weak D and RhIG Prophylaxis

To prevent maternal alloimmunization, RhIG should be given to all D– women who do not have detectable anti-D at 28 weeks' gestation and within 72 hours of delivery or other potentially sensitizing event.[12] Testing for ABO, D, and unexpected antibodies should be performed on samples from all pregnant women in the first trimester, but the need to test D– pregnant women for weak expression of D has been controversial.[13,14] Considerable variability in practice exists within the United States even though such testing is not required by AABB or the American College of Obstetricians and Gynecologists (ACOG).[12,13]

The majority (90%) of individuals with a weak D phenotype are weak D type 1, 2, or 3 and express normal, but reduced, quantities of D antigens on the RBC surface; these individuals cannot be immunized to make anti-D. The remaining 10 percent of individuals with a weakened expression of D express aberrant D proteins and may not be recognized as having a partial D phenotype until they develop anti-D. Most Caucasian D+ individuals with anti-D belong to partial D category DVI which occurs in 0.02 percent to 0.05 percent of Caucasians.[14] Alloimmunization to the D antigen and HDFN caused by anti-D can occur in women with partial D antigen expression.[15,16] Denomme et al.[15] evaluated partial D, weak D types, and novel *RHD* alleles among 33,864 screened multiethnic patients and found 54 typing discrepancies which represented mutated *RHD* alleles. Among these cases, 10 of 25 (35%) obstetric patients were assigned D+ status, but expressed a D variant known to permit anti-D alloimmunization (e.g., DAR, DFR, and DAU, and categories DIIIa, DVa, and DVI).

The frequency with which partial D individuals develop anti-D in the general population is not known, but it may be higher than previously recognized, as demonstrated in a recent CAP survey that found that one-third of transfusion services reported at least one patient whose RBCs were of a weak D phenotype with anti-D formation in a 12-month period.[17] Although severe HDF in women whose RBCs are of a weak D phenotype is rare, antibodies have been more frequently reported in women with a partial DVI phenotype, and fatal HDF has occurred in pregnancies of such alloimmunized women.[16]

AABB does not require testing for weak D in pregnancy, and, if an IAT is not performed, most women with partial D will be classified as D– and will be candidates for RhIG. In the CAP survey, however, 58.2 percent of transfusion services routinely performed testing for weak D in patients whose RBCs were negative in direct testing with anti-D reagents.[17] AABB Standards require designating the RBCs of individuals with positive tests for weak D as D+, and the ACOG advises against administering RhIG to women with

RBCs of known weak D phenotypes.[12,18] However, the CAP survey found that only 50 percent of transfusion services reported weak D as D positive, and 71.1 percent of transfusion services that routinely test for weak D give RhIG to these pregnant women.[17]

On the basis of the demonstrated risk of D alloimmunization in women with a partial D phenotype, some experts have cautioned that if pregnant women are tested for weak D, an appropriate anti-D reagent or technique should be used that will not detect DVI so that these women are assigned D– status for transfusion and Rh prophylaxis.[14–16] The alternative is to not test pregnant women (or transfusion recipients) for weak D, which would result in most of these individuals being classified as D– and candidates for RhIG prophylaxis. This presents a challenge to countries that do not have a sufficient supply of RhIG and need to more accurately distinguish those individuals with D variants who are not at risk of anti-D alloimmunization from those who are at risk and should receive RhIG prophylaxis. Not surprisingly, a recent survey showed that international practices on testing for weak D and RhIG administration diverge from practice in the United States, and 8 of 10 countries perform further testing for weak D or D variants if the woman's RBCs type as D– or if the typing results are anomalous, to limit administration of RhIG to women with partial D at risk for developing anti-D.[19] The relative effectiveness of RhIG in preventing the sensitization of partial D women compared with D– women is not yet known.

Infants born to D– women should have their blood type determined using a reagent and a method that detects DVI and weak D, and RhIG should be administered to the mother if the infant's RBCs type as D+ (including weak D) within 72 hours of the delivery.[12,13] In addition, routine screening for fetomaternal hemorrhage (FMH) should be performed after a D– woman delivers a D+ infant to identify bleeding in excess of 15 mL of RBCs (30 mL whole blood), an event that would require administration of additional postpartum RhIG.[12] If the woman's RBCs are of a weak D phenotype, the rosette test may yield a diffusely positive result, and Kleihauer-Betke or other method may be needed to determine the extent of FMH.[12] Significant (> 30 mL whole blood) FMH complicates less than 1 percent of deliveries and is more likely following Cesarean sections or operative vaginal deliveries, but can occur following uncomplicated vaginal deliveries as well.[20,21]

Noninvasive Fetal Rh Genotyping

The initial type and antibody screen that is performed for all pregnant women not only identifies D– women who are candidates for RhIG, but also identifies alloimmunized women that require further monitoring for HDF during the pregnancy. If a clinically significant maternal antibody is present, the blood type of the biologic father should be determined to assess whether the antigen could be present on the RBCs of the fetus. Paternity must be certain, however, to draw meaningful conclusions. Serologic studies of RBC antigen expression may be informative. If the biologic father's RBCs lack the antigen, the infant is not at risk for HDF during pregnancy and no further fetal testing is necessary. If the father's RBCs have double-dose expression of the implicated antigen, the trait will be inherited and the fetus should be monitored for HDF; if they have single-dose expression, his offspring have a 50 percent chance of inheriting the blood group antigen allele. Because no antithetic allele for D exists, paternal zygosity cannot be definitively predicted by serologic means. The most probable combination of haplotypes can be predicted, because *RHD* inheritance is closely linked to *RHCE,* and the probability that the father is heterozygous for the *D* allele can be estimated from a model that takes into account the serologic results as well as the ethnic background and the number of previous D+ children.[7] Tables of gene frequencies in Caucasians, African Americans, and Mexican Americans have been published to estimate the likelihood of paternal heterozygosity for the *D* allele.[7,22]

If paternal testing indicates that the father may carry an antigen to which a clinically significant antibody could be made, fetal testing should be performed to determine if the allele is present. Most RBC polymorphisms can now be tested for by molecular analysis at the DNA level, including the complex *RHD* and *RHCE* loci (for D, C, c, E, and e antigens), as well as the Kell (for K and k antigens), Duffy (for Fy^a and Fy^b antigens), and Kidd (for Jk^a and Jk^b antigens) loci.[2] The Rh system is by far the most complex of the human blood group systems, and considerable genetic diversity underlies the aberrant *RH* alleles associated with the D– phenotype.[10,23,24] The most prevalent D– genotype in Caucasian populations is the complete deletion of *RHD;* in African blacks and African Americans, other variant *RHD* genes are more likely to be found.[2] The *RHD* pseudogene, which contains a 37-bp insertion in exon 4 and results in no

detectable transcription of the gene, is found in 66 percent of D– black Africans and 24 percent of D– African Americans.[23]

Similarly, a variant *RHD-CE-D* gene encodes the r's (dCce^s) haplotype (phenotype) that underlies serologic D negativity in 22 percent of D– African Americans. These variant *RHD* alleles have important implications for prenatal diagnosis of fetal blood type in different populations because the presence of one of these genes in the fetus can lead to a false positive result (i.e., the fetus is predicted to be D+ by molecular methods but is found to be D– by serology after birth) and unnecessary prenatal intervention. A maternal blood sample should be analyzed in parallel with the fetal sample. False negative results (i.e., the fetus is predicted to be D– but is found to be D+ by serology at birth) have been attributed to erroneous paternity or rearrangement at the paternal *RHD* gene locus. If no paternal sample is available, a predicted D– fetal blood type determined by molecular methods should be treated with caution, and the pregnancy should be monitored to ensure that the titer of maternal anti-D does not increase.[2]

The most common method to obtain fetal DNA for molecular testing is still amniocentesis in the United States because noninvasive alternatives are not yet available. Amniocentesis is performed at 24 weeks' gestation and is relatively safe but is associated with a pregnancy loss rate of about 0.3 percent.[25] This risk is avoided with noninvasive methods to obtain fetal cells or fetal cell-free DNA from the maternal circulation and maternal blood has been used routinely for determination of fetal *RHD* status in Europe.[10,26] Fetal DNA can be isolated from maternal plasma as early as 32 days' gestation and constitutes 3 to 6 percent of the plasma DNA pool in the second and third trimesters.[10] Cell-free fetal DNA is rapidly cleared and does not persist into subsequent pregnancies in contrast to the potential for long-term persistence of fetal leukocytes that can complicate analysis of cellular fetal DNA.[27] Several groups have demonstrated 96 to 100 percent accuracy in predicting the RhD phenotype with over 200 pregnancies tested.[10,19,26] False positives were the result of the presence of a pseudogene or D variant; false negatives resulted from the failure to isolate sufficient fetal DNA. When negative results are obtained (e.g., no RhD-specific signal is detected), the presence of fetal DNA in the plasma should be confirmed by another fetus-specific DNA sequence from a highly polymorphic paternal antigen or from the Y chromosome for male fetuses (e.g., *SRY*). There are no reported strategies for Kell testing or Rh testing other than for *RHD* using a sample of maternal blood, which likely reflects the difficulty of developing a sensitive assay for subtle allelic differences that will still be specific in the presence of an excess of maternal DNA.[10,26]

Monitoring Affected Pregnancies

When a clinically significant antibody is detected in a woman's first pregnancy, maternal antibody titers are typically determined each month until approximately 24 weeks' gestation. Although the concept of a critical titer has been challenged, most laboratories consider the titer of anti-D that is associated with a significant risk of severe HDF to be 32. Maternal antibody titers are only useful in a first pregnancy, not in assessing subsequent antigen-positive pregnancies. The utility of antibody titers in monitoring Kell-sensitized pregnancies is limited because the severity of intrauterine disease may not correlate with maternal antibody titers, and severe HDF has occurred with low anti-K titers.[2] Conversely, most other RBC antibodies are less likely to cause severe disease than anti-D and anti-K, and higher thresholds for antibody titers during pregnancy have been used.[7] Because the antibody titer will depend on laboratory technique, there can be considerable variability in titers when the same sample is analyzed by different institutions; however, serial assessment of titer by an individual institution should reliably reflect trends when careful attention is given to consistent and appropriate laboratory methods. For all RBC antibodies implicated in HDF, a fourfold increase in antibody titer is considered a significant change that warrants further diagnostic investigation.

The most significant advance in monitoring alloimmunized pregnancies has been the recent demonstration that the severity of fetal anemia can be predicted by a noninvasive method, Doppler ultrasound, which obviates the need for serial amniocentesis to measure bilirubin concentration (ΔOD_{450}) in most cases.[28] In the presence of significant fetal anemia, the velocity of blood flow through the middle cerebral artery (MCA) increases and the change can be detected by Doppler ultrasound. In a prospective study of 165 fetuses with maternal RBC alloimmunization (anti-D, -c, -E, or -Fy^a), an increase in peak velocity in the MCA expressed as more than 1.5 multiples of the median (MoM) had a sensitivity of 88 percent and a specificity of 82 percent for severe fetal

anemia.[28] The performance of Doppler ultrasound was better than that of amniocentesis using the Liley curve, but similar to that of amniocentesis with Queenan's method. Doppler ultrasound has also been used to manage Kell-sensitized pregnancies and is preferred to amniocentesis which is often unreliable when HDF is caused by anti-K because these antibodies not only cause hemolysis but also suppress erythropoiesis as reflected by falsely reassuring ΔOD_{450} values in the setting of profound fetal anemia.[29] Fetal blood sampling to directly measure fetal hematologic parameters is generally undertaken after Doppler MCA ultrasound suggests the presence of severe or worsening fetal anemia.

Intrauterine Transfusion for HDF

Prenatal monitoring of maternal antibody titers and fetal MCA velocity with Doppler ultrasound may indicate the need for fetal blood sampling and intrauterine transfusion. If a woman with a rare null phenotype has corresponding RBC antibodies, the identification of antigen-negative blood for transfusion is a major challenge facing the transfusion service. Washed maternal RBCs are a potential source of antigen-negative RBCs that can be collected and used for intrauterine transfusion to the fetus. Blood donation during pregnancy may risk premature labor and fetal intrauterine growth restriction but is usually well tolerated and must be weighed against the need for antigen-negative blood for the fetus. Recently, ABO-incompatible maternal blood was successfully used for intrauterine transfusion when ABO-compatible RBCs which lacked the implicated antigen were not available.[30] A pregnant woman with the rare homozygous type D– (RH:-17), characterized by the complete absence of C, c, E, and e antigens and the elevated expression of D on the surface of RBCs, demonstrated antibodies against the high-incidence antigen Rh17. Her second infant was severely anemic at birth, and a hydropic fetus was identified early in the course of her third pregnancy. ABO-compatible, Rh17– RBCs were not available; consequently, washed maternal RBCs (group B) were used for seven intrauterine transfusions to the group O fetus prior to 36 weeks' gestation. At birth, the infant's RBCs typed group B, D+ and had a negative DAT with no evidence of mixed field agglutination. A Kleihauer-Betke test on cord blood indicated the presence of adult Hb and no detectable fetal Hb. Phototherapy was initiated at birth but was discontinued after the first day because the indirect bilirubin was 55 μmol/L at birth and remained stable, while the initial Hb (143 g/L) improved by day 3 (209 g/L). Exchange transfusion was not required; the infant was reported to be healthy at 2 weeks of age with no evidence of neurologic impairment. The case demonstrates that ABO incompatibility is not a deterrent to intrauterine transfusion of maternal blood because anti-A and anti-B are not present during gestation and are usually absent or only weakly detectable at birth. ABO-mismatched transfusion is an option for rare cases when antigen compatible or group O RBCs cannot be obtained for intrauterine transfusion. Maternal blood should be washed to remove antibody, leukoreduced to lower the risk of CMV transmission, and irradiated to prevent GVHD.

Evaluation of Infants at Risk for HDN

Immunohematologic testing of infants born to women with potentially significant RBC antibodies should include ABO and D typing as well as a DAT.[13] If HDN is suspected on clinical grounds, but the DAT and maternal antibody screen are negative, the possibility of incompatibility should be investigated by testing the mother's serum or an eluate prepared from the infant's RBCs against the biologic father's RBCs. A negative DAT does not rule out the possibility of immune-mediated hemolytic anemia and may reflect a low-antigen density on fetal RBCs or low avidity of the offending antibody under the reaction conditions.[1] However, when the infant's DAT is negative, neonatal hyperbilirubinemia should not be attributed to ABO incompatibility; other possible causes of hemolysis should be evaluated.[31]

Routine immunohematologic testing of infants born to women with negative antibody screens is not necessary, except to determine the need for RhIG for D– women.[13] Regardless, many institutions continue to perform ABO and D typing and a DAT on all newborns; others selectively test infants born to group O, D+ mothers. The latter strategy, which is intended to identify infants at risk of developing ABO HDN, has an extremely poor predictive value and is not recommended because it will miss cases of hemolysis resulting from nonimmune causes.[13,32] In one study, the positive predictive value of a routine cord DAT was only 23 percent and the sensitivity only 86 percent.[32] Functional assays that evaluate antibody-mediated RBC destruction, such as the erythrophagocytosis assay, may be useful adjuncts to predict the severity of HDN but are technically demanding and suffer from

limitations similar to those of the DAT.[33] Instead of reliance on laboratory screening for HDN or tests to predict its course, all newborns should be followed for jaundice in the first week of life with directed use of serum bilirubin and other laboratory testing, as appropriate.

Treatment of HDN

Hyperbilirubinemia does not occur before birth because the bilirubin that results from the immune-mediated destruction of fetal RBCs is transported across the placenta and eliminated by the maternal liver. After birth, however, serum bilirubin can accumulate to dangerous levels with ongoing hemolysis that poses a direct threat of brain damage because the infant's liver function is not fully developed. Hyperbilirubinemia also occurs in otherwise healthy infants, resulting in neonatal jaundice which is the most common indication for treatment in newborn infants.[34] The degree and duration of hyperbilirubinemia that place an infant at risk for bilirubin encephalopathy and kernicterus have been debated for years. Recently, a prospective, blinded study compared 140 term and near-term infants with total serum bilirubin levels of 25 mg/dL (428 μmol/L) or more to 419 control subjects and found no significant differences between the two groups in neurodevelopmental outcomes.[35] However, the subgroup of infants with hyperbilirubinemia resulting from immune-mediated hemolytic disease had lower IQ scores than the control group. This observation corroborates previous studies that suggested that hemolysis enhances the risk of bilirubin-induced central nervous system injury, although it is not clear why. Factors potentiating bilirubin toxicity in the setting of hemolytic disease may include acid-base disturbances, asphyxia, free heme groups, and other byproducts of hemolysis or drugs that displace bilirubin from albumin and other plasma-binding protein. The recent clinical practice guidelines from the American Academy of Pediatrics treat infants with jaundice caused by immune-mediated hemolysis more aggressively than infants with physiologic jaundice, for every given serum unconjugated bilirubin concentration.[34] Phototherapy is the mainstay of treatment for neonatal jaundice, but exchange transfusion will be necessary when phototherapy fails to adequately decrease bilirubin concentration or when the initial serum bilirubin concentration places the infant at high risk for kernicterus. Treatment of jaundiced preterm infants and jaundice on the first day of life in any infant requires individualized treatment decisions. Treatment decisions after the first 24 hours are guided by gestational age, bilirubin concentration, and the rate of its increase (> 0.5 mg/dL/hr), as well as the presence of comorbid factors such as hemolysis, asphyxia, significant lethargy, temperature instability, sepsis, or acidosis.[34] Immediate exchange transfusion is recommended if an infant shows signs of acute bilirubin encephalopathy (e.g., hypertonia, arching, fever, high pitched cry) or if total serum bilirubin is 25 mg/dL (428 μmol/L) or more.[34] Severe reactions related to the procedure have been reported in about 5 percent of infants and include citrate-related arrhythmias, bleeding caused by dilutional coagulopathy or thrombocytopenia, catheter-related infection, and bacterial sepsis.[34] The mortality rate among term infants within six hours of exchange transfusion was estimated as 3 to 4 per 1000.[34]

Infants who respond to phototherapy alone or those who receive intrauterine transfusion may not require exchange transfusion. However, these infants may need straight RBC transfusions (also referred to by some clinicians as booster or top-off transfusions) during the first 1 to 3 months of life for late-onset anemia resulting from ongoing low-grade hemolysis or erythropoietic suppression. In general, the transfusion decision should be guided not only by the Hb concentration but also by the reticulocyte count and, most importantly, by the infant's condition, particularly when the infant is lethargic, feeding poorly, or not thriving.

Conclusion

The advent of RhIG prophylaxis to prevent D alloimmunization in pregnancy represents one of the most significant medical advances in modern times, reducing the risk of D alloimmunization from 14 percent in the late 1960s to 0.1 percent with the routine use of antenatal and postpartum RhIG administration.[1-3] Further improvements in prenatal care, noninvasive monitoring, and intrauterine transfusion have provided effective treatment to even the most severely affected infants with hydrops fetalis caused by anti-D or other clinically significant, maternal RBC antibodies. Women with RBCs of known partial D phenotypes may be at significant risk of alloimmunization and may benefit from RhIG administration. Noninvasive fetal diagnosis using maternal DNA is possible for predicting the presence of *RHD,* although

not yet routine in the United States. Doppler ultrasound, now in widespread use, allows for monitoring of affected pregnancies without the potential complications associated with amniocentesis or fetal blood sampling. Infants with hyperbilirubinemia associated with HDN are at greater risk of neurologic complications than infants without hemolysis, and the current practice guidelines from the American Academy of Pediatrics identify HDN as a significant risk factor that requires more aggressive treatment at any given level of hyperbilirubinemia than in the absence of hemolysis.

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