# Hemolytic Disease of the Fetus and Newborn

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## Abstract

Hemolytic disease of the fetus and newborn (HDFN) is the result of immune-mediated destruction of fetal or newborn red blood cells when such cells contain antigens that are not present in the maternal blood. HDFN is now the preferred term that replaces the historic term erythroblastosis fetalis. Sensitization of the mother to fetal-newborn red blood cells requires fetomaternal hemorrhage in most cases except in ABO incompatibility where naturally occurring antibodies against A and B antigens are present in mothers with O blood type. The most common antigen involved in HDFN is Rhesus D. Kell 1 HDFN is rare but commonly associated with severe anemia and lower titers of anti-Kell antibodies in maternal serum in severely affected infants. Prevention of Rhesus D HDFN with anti-D immunoglobulin during pregnancy, delivery, and fetal-maternal events that predispose to fetomaternal hemorrhage, have markedly decreased the incidence of the disorder but may not be available in low-income countries. An algorithm is available to manage affected pregnancies by using antibody titers, fetal middle cerebral artery velocities, intrauterine transfusions, and timed delivery. Infants who have mild to moderate anemia may tolerate normal labor, but severely affected infants may require transfusion or exchange transfusions at birth, and the delivery team needs to be prepared. Delayed anemia in the transfused infants is still a concern, and the infants need to be closely followed after delivery. Phototherapy has largely replaced exchange transfusion in the management of hyperbilirubinemia. With appropriate early detection and multidisciplinary planning, infants who have HDFN can be delivered in a timely manner with appropriate planning for postnatal resuscitation and postnatal therapy resulting in good neonatal outcomes.

## Objectives

After completing this article, readers should be able to:

1. Recognize the symptoms and signs associated with hemolytic disease of the fetus and newborn (HDFN).
2. Understand the pathophysiology of HDFN.
3. List the red blood cell antigens most commonly associated with HDFN.
4. Identify the fetus at risk for HDFN.
5. Discuss the clinical management of fetus/newborn affected by HDFN.

## Abbreviations

| | |
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| FMH: | fetomaternal hemorrhage |
| HDFN: | hemolytic disease of the fetus and newborn |
| IAT: | indirect antiglobulin testing |
| MCA: | middle cerebral artery |
| pRBC: | packed red blood cell |
| RBC: | red blood cell |
| RhD: | Rhesus D |

## Introduction

Advances in prevention and detection have markedly decreased the incidence of hemolytic disease of the fetus and newborn (HDFN). The disorder, caused by red blood cell (RBC) incompatibility between infant and mother, and its multiple clinical manifestations were first brought together in 1932 in the landmark article by Dr Louis K. Diamond, where he coined the term erythroblastosis fetalis, based on the morphology of the peripheral blood smear seen in infants who have severe diseases. HDFN is now the preferred term encompassing all infants who have alloimmune

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hemolysis, whether or not erythroblasts are present. The work of Landsteiner and other investigators defined RBC antigens and their role in transfusion reaction by 1940, and Levine et al in 1941 gathered enough cases to document that HDFN was indeed caused by blood group incompatibility between a mother and her infant. HDFN is the result of immune destruction of fetal or newborn RBCs. Maternal antibodies develop when fetal RBCs express cell surface antigens that are not present on the maternal RBCs. For this process to occur, fetal RBCs must enter the maternal circulation secondary to fetomaternal hemorrhage (FMH). The exception to this rule is ABO incompatibility because type O mothers have naturally occurring anti-A and anti-B antibodies. HDFN should be considered in the differential diagnosis of postnatal early, severe, or prolonged jaundice. It also should be considered in the differential diagnosis of neonatal anemia, in particular, if it is severe or associated with hydrops fetalis. The presence of maternal RBC antibodies and/or a positive direct antibody testing in the infant are diagnostic for HDFN.

A comprehensive maternal history is essential for the proper diagnosis of HDFN. Particular emphasis should be placed on uncovering maternal events that predispose to FMH. A maternal history of a previous pregnancy, particularly a complicated pregnancy, history of hydrops fetalis, a miscarriage, early termination of pregnancy, blood transfusion, or clinical documentation of FMH, should alert the clinician to the possibility of HDFN in an infant with anemia and/or jaundice.

The greatest advances in this disorder have been the introduction of very effective prevention strategies, the aggressive use of phototherapy for jaundice, and the introduction of noninvasive techniques to monitor the affected fetus.

## Differential Diagnosis

The two main signs of HDFN are anemia and hyperbilirubinemia. The anemia is hemolytic and the bone marrow is reactive with reticulocytosis and often presents immature RBCs, erythroblasts, in the peripheral blood; hence, the original term erythroblastosis fetalis was given to the disorder. Other types of non–immune-mediated hemolysis can result in anemia and hyperbilirubinemia. These include RBC membrane defects such as hereditary spherocytosis and hereditary elliptocytosis. Although these disorders are hereditary, their phenotype is variable, and the RBC defects may go undiagnosed well into adulthood. Clues that can be suggestive of undiagnosed RBC membrane defects include family members who are intermittently jaundiced, intermittently have scleral icterus, or intermittently have dark urine. These episodes generally occur in association with acute illness. Family members who have a history of splenectomy unrelated to trauma or early gallbladder disease may suggest an undiagnosed inherited RBC membrane defect.

Hemoglobinopathies also may contribute to neonatal hemolysis. Hemoglobinopathy screening in the United States has been available in most states for over a generation, and today all 50 states screen for these disorders and they are diagnosed at birth. Nonetheless, recent immigrants to the United States may have previously undiagnosed hemoglobinopathies. Review of the blood smear, newborn screening, confirmatory hemoglobin electrophoresis, and potentially maternal and paternal hemoglobin electrophoresis should clarify this diagnosis.

RBC enzyme defects such as glucose-6-phophate dehydrogenase can lead to hemolysis and anemia. Significant and prolonged hyperbilirubinemia and even kernicterus have been described in infants who have glucose-6-phophate dehydrogenase deficiencies, particularly in the Philippines, Africa, and Greece. Hemolysis can occur from disorders of glycolysis such as pyruvate kinase deficiency. Here too, family history and ethnic background can be key factors in identifying the risk for RBC enzyme defects.

## Mechanisms of Maternal Exposure

All individuals with blood type O naturally express antibodies to A and B RBC surface antigens. No previous exposure to blood group antigens is necessary for antibody development. These antibodies are immunoglobulin G type and cross the placenta. Individuals who have blood type A have antibodies against type B and vice versa. However, these antibodies are predominantly immunoglobulin M class and do not cross the placental barrier.

For all other blood group antigens, maternal exposure to blood group antigens is necessary for the development of antibodies. The most frequent cause of maternal sensitization is FMH. FMH can occur in the first trimester, but is most common in the third trimester. Numerous fetal events or procedures may be associated with previously underappreciated risk for FMH. See the Table for a listing of events that may be associated with FMH.

Women who have a known history of transfusion represent only a small proportion of all pregnant women. However, women who have a previous transfusion history represent half of the pregnancies affected by non-Rhesus D (RhD) HDFN. Routine blood typing and cross-match screen for ABO and RhD blood types, but none of the other blood types involve HDFN. As more children survive previously serious childhood illnesses such as cancer and congenital heart disease, we are likely to see an increase

**Table. Mechanisms of Maternal Exposure to Red Blood Cell Antigens**

1. ABO group
   Innate antibody production, no previous exposure needed
2. Known fetal-maternal hemorrhage
   a. Placental abruption
   b. Other placental bleeding or injury
   c. Fetal surgery
   d. In utero transfusion
   e. Delivery of previous infant
   f. Delivery of previous infant affected by HDFN
3. Risk for unappreciated fetal-maternal hemorrhage
   a. Ectopic pregnancy
   b. Abnormal placental insertion
   c. Spontaneous abortion
   d. Induced abortion
   e. Fetal demise
   f. Amniocentesis
   g. Cordocentesis
   h. Chorionic villus sampling
   i. Maternal abdominal trauma
   j. Fetal version maneuvers
   k. Delivery of previous infant requiring exchange transfusion or phototherapy
4. Known maternal transfusion
5. Maternal history with potential for unappreciated maternal transfusion
   a. Prolonged hospital stay as an infant
   b. Survivor of childhood cancer
   c. Repair of craniosynostosis in childhood
   d. Correction of congenital heart defect
   e. Major surgical procedure in childhood or adulthood
   f. Abdominal surgery in childhood or adulthood
   g. Splenectomy for unclear indication (may suggest maternal red cell defect)
   h. Return to operating room within 7 days of delivery of an infant

in HDFN from maternal transfusion. In fact, the mother may be unaware of her own blood product exposure, because treatment may have occurred during her early childhood. See the Table for a listing of maternal history elements that raise concern for unappreciated exposure to RBC antigens.

The ever expanding utilization of reproductive technologies leads to unexpected risks for HDFN. The battle with infertility is highly personal in nature. That, combined with our fragmented health-care system and highly mobile society, leads to significant opportunity for utilization of egg donor, sperm donor, or embryo adoption to go undisclosed to the health-care team at the time of delivery. Even when a family is willing to disclose use of egg donor, sperm donor, or embryo adoption, ABO blood type of the donors may or may not be known to the pregnant woman. This circumstance can set up otherwise uninheritable combinations of RBC antigens. For example, an O− (negative) mother with an O− (negative) husband could deliver an AB+(positive) infant who is at risk for HDFN from ABO and Rh mismatch.

## Red Blood Cell Antigens Most Frequently Involved in Hemolytic Disease of the Fetus and Newborn

Rhesus (RhD) incompatibility is the best described cause of HDFN. Despite international efforts aimed at prevention of HDFN in infants of Rh-negative mothers, Rh remains the most commonly identified RBC antigen causing HDFN.

RhD-negative denotes the lack of D antigen on the RBC surface. Eleven percent to 35% of white populations are RhD-negative because of gene deletion. In contrast, most East Asian and African populations that lack RBC surface expression of RhD have a grossly intact gene. In Africans, a 37-bp insertion results in the insertion of a stop codon leading to a prematurely shortened protein product.

A special circumstance exists with an RhD variant called weak D. There is both an altered protein sequence and decreased cell surface protein expression. Serologic testing will identify this person as RhD-negative. Although more sensitive testing can demonstrate the presence of the protein on the cell surface, importantly, these apparently RhD-negative mothers will not form anti-D antibodies even if they are transfused with RhD-positive blood.

The second most common RBC antigen associated with HDFN is the ABO blood group. HDFN due to an ABO blood group mismatch occurs almost exclusively in infants with mothers of blood type O. Hemolysis is more common with anti-A than with anti-B. The clinical presentation for HDFN due to ABO blood group is predominantly hyperbilirubinemia without severe anemia. Phototherapy is generally sufficient for most of these infants.

Another major cause of HDFN is Kel1 antigen of the Kell antigen system. Kell is a glycoprotein containing 15 antigens and their antithetical variants. It is the first erythroid-specific antigen known to be expressed during erythroid development. Clinically, anti-Kel1 HDFN is manifest by more severe anemia and reticulocytopenia. Hyperbilirubinemia is less severe than with other RBC antigens. Anti-Kel1 antibody is rare and is only found in 0.1% of pregnant women. However, most of these women have developed antibody because of previous transfusion exposure. Fortunately, only 9% of people of European

descent and 2% of people of African descent express Kel1. Because of the early erythroid lineage expression of Kell when Kel1 is present, anti-Kel1 is associated with a lower critical antibody titer (1:8) than anti-RhD or antibodies to other RBC antigens.

A variety of other RBC antigens have been described as causing HDFN. For a detailed listing of RBC antigens, the severity of HDFN associated with each antigen, and reference citation, see Table 6.1 in de Alarcon, Werner, and Christensen's *Neonatal Hematology*.

## Clinical Management: Prevention of Hemolytic Disease of the Fetus and Newborn Due to Rhesus D

The first approach to prevention of HDFN due to an RhD-positive fetus born to an RhD-negative mother is administration of one postnatal dose of anti-RhD. Between 1968 and 1983, both maternal immunization and perinatal infant deaths were reduced by 90%. Subsequently, clinical trials were performed demonstrating that the administration anti-RhD at 28 weeks' gestation in addition to the postnatal dose could further decrease the frequency of immunization from the remaining 2% down to 0.2%. Similarly, an appreciation rose for other events that led to FMH effectively immunizing the mother further against RhD. We now know that fetal and pregnancy events such as ectopic pregnancy, spontaneous or induced abortion, fetal demise, and maternal abdominal trauma can result in maternal immunization from FMH. Invasive medical procedures such as amniocentesis, cordocentesis, and chorionic villus sampling are associated with an increased risk of immunization to RhD. Recommendations are now in place for immune prophylaxis in the event of planned or unscheduled events that may increase FMH and therefore increase maternal exposure to RhD. For a listing of such events, see the Table.

Anti-RhD prophylaxis policies vary slightly between developed countries (such as the United States, England, Mexico, Canada, and Australia). In third world countries, access to anti-RhD may still be extremely limited both owing to medical infrastructure reasons as well as socioeconomic reasons such as minimal prenatal care and delivery at home.

In the United States, the Preventative Services Task Force current consensus recommendations are available at http://www.uspreventiveservicestaskforce.org (then search the site for "Rh incompatibility"). Current US recommendations include the proposal that all pregnant women should have antibody screening and RBC antigen typing for ABO and RhD at the first prenatal visit. RhD-negative mothers with no antibody present at the first screen should have repeat screening at 24 to 28 weeks. If they remain negative, they should receive 1,500 IUs (= 300 $\mu$g) anti-RhD immunoglobulin at 28 weeks' gestation unless the father of the infant is also known be RhD-negative. Second, women at high risk for FMH, such as previous transfusion, obstetrical complications in which anti-RhD was not administered, or the woman had sustained injuries, should continue to be screened for antibody development into the second and third trimesters. An additional dose of anti-RhD is recommended for women experiencing obstetrical complications with a risk for FMH or undergoing diagnostic testing that can increase FMH. This anti-RhD dose is trimester-dependent. For events in the first trimester, the recommended dose is 250 IUs (= 50 $\mu$g). For events in the second or third trimester, the recommended dose is 1,500 IUs (= 300 $\mu$g). Last, the postnatal dose of 1,500 IUs is administered to all RhD-negative mothers. This dose should prevent immunization when as much as 15 mL of newborn blood enters the maternal circulation. Women experiencing high-risk problems or maneuvers, such as abruption, manual removal of the placenta, or multiple infant gestations may be at risk for higher volume FMH. An effort should be made to quantitate the FMH and administer additional anti-RhD, if necessary (when estimated to be >15 mL).

## Clinical Management: Identifying Pregnancies at Risk for Hemolytic Disease of the Fetus and Newborn

Monitoring is dependent upon previous pregnancy history, whether the infant from a previous pregnancy was clinically affected, which RBC antigen is involved, what is the father's genotype and phenotype, how high is maternal antibody, and whether there is clinical evidence of fetal anemia. The first level of monitoring is phlebotomy for serial determination of anti–RBC antigen antibodies or indirect antiglobulin testing (IAT).

An algorithm has been developed by Moise for monitoring of pregnancy in an RhD-negative woman with an RhD-positive fetus or RhD-positive father. This algorithm can also be applied to assessing risk for fetal anemia due to other RBC antigens.

A first pregnancy of an RhD-negative mother is monitored by repeated anti–RBC antibody titers. If the titer remains low, serial monitoring continues and the infant is delivered at term. If the titer crosses above a critical threshold, then infants are monitored by serial fetal middle cerebral artery (MCA) velocities. An increased MCA velocity correlates well with fetal anemia.

For pregnancies with previously affected infants, the current fetus is monitored with serial MCA velocities

by using Doppler ultrasound to monitor for development of fetal anemia.

## Clinical Management: Intrauterine

Once a pregnancy is identified as being at risk because of positive or rising IAT, serial Doppler ultrasound MCA velocities are utilized to monitor for fetal anemia. If mild anemia is detected, serial monitoring by ultrasound continues until there is adequate lung maturity or term delivery.

Where severe anemia is suspected, cordocentesis may be utilized to confirm severe anemia (hematocrit <30% or hemoglobin <10 g/dL). In the event of severe anemia, an intrauterine transfusion may prevent progression to a severely ill, hydropic infant. Packed red blood cells (pRBCs) that are negative for the RBC antigen involved in the HDFN are used for transfusion. Additional pRBC specifications include leukodepletion, cytomegalovirus-negative donor, and irradiated products to prevent transfusion-associated graft versus host disease.

## Clinical Management: Postnatal

Delivery, either preterm or term, should be scheduled in a perinatal level 3 center with interdisciplinary obstetric, maternal-fetal, and pediatric services available. Assessment of fetal lung maturity and risk for subsequent respiratory distress must be taken into account in preparation for delivery. This includes consideration of glucocorticoids to accelerate lung maturity, when appropriate, and planning for personnel and equipment for resuscitation of the infant experiencing respiratory distress.

A mildly or moderately anemic fetus will often tolerate labor adequately. A severely anemic fetus may not. ABO matched (if ABO typing has been done with previous cordocentesis) or O-type blood that is also negative for the antigen responsible for HDFN should be available to the resuscitating team in the delivery room. This pRBC product should be leukodepleted, cytomegalovirus-negative, and irradiated.

The most severely ill infants may require an exchange transfusion, which involves replacing the native infant RBCs with appropriately antigen-negative RBCs to prevent further hemolysis. This is accomplished by replacing a total of 25 to 50 mL/kg pRBCs. Once vascular access has been established, 5 mL/kg aliquots can be removed and replaced over several minutes. This cycle is repeated until the targeted volume is replaced.

Many anemic but more stable infants may be transfused with ABO-matched pRBCs that are negative for the antigen contributing to the HDFN at 10 mL/kg over a 2- to 3-hour period; 3 mL/kg pRBCs are needed to increase the hemoglobin 1 gm/dL (hematocrit 3%).

Exchange transfusion was the mainstay of therapy early in management of RhD HDFN to minimize kernicterus from hyperbilirubinemic infants. Now, many hyperbilirubinemic infants who have HDFN will respond adequately to early phototherapy with blue light "bilirubin lights," often in combination with "bilirubin blankets." These infants may only require early phototherapy in combination with transfusion to address mild to moderate anemia.

HDFN from some RBC antigens may result in delayed or prolonged postnatal anemia. Therefore a pediatric hematologist should be consulted to help monitor for the need for delayed or repeated pRBC transfusion.

## Conclusions

RhD remains the most significant antigen contributing to HDFN. However, prenatal anti-D prophylaxis has significantly decreased the incidence of severe HDFN. At this time, antibody prophylaxis is only available for HDFN due to RhD. Appropriate prenatal screening with IAT and Doppler ultrasound has greatly improved infant outcomes by allowing early identification of pregnancies at risk for HDFN. With appropriate early detection and multidisciplinary planning, these infants can be delivered in a timely manner with appropriate planning for postnatal resuscitation and postnatal therapy resulting in good neonatal outcomes.

**American Board of Pediatrics Neonatal-Perinatal Content Specifications**

- Know the diagnostic evaluation and perinatal management of fetal-maternal blood group incompatibility.
- Know the etiology and pathophysiology of hemolytic anemias in the neonate.
- Know the clinical and laboratory features of hemolytic anemia in the neonate.
- Know the management of hemolytic anemia in the neonate.

## Suggested Reading

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