CHAPTER 34

# Red Cell Alloimmunization

KENNETH J. MOISE JR



## KEY ABBREVIATIONS

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|---|---|
| American Association of Blood Banks | AABB |
| American College of Obstetricians and Gynecologists | ACOG |
| Cytomegalovirus | CMV |
| Circulating cell-free fetal DNA | ccffDNA |
| Deoxyribonucleic acid | DNA |
| Diphosphatidylglycerol | DPG |
| Fetal blood sampling | FBS |
| Fetomaternal hemorrhage | FMH |
| Grams per deciliter | g/dL |
| Hemolytic disease of the fetus and newborn | HDFN |
| Hemolytic disease of the newborn | HDN |
| Intraperitoneal transfusion | IPT |
| International unit(s) | IU |
| Intrauterine transfusion | IUT |
| Intravascular transfusion | IVT |
| Intravenous immune globulin | IVIG |
| Kleihauer-Betke | KB |
| Middle cerebral artery | MCA |
| Microgram | µg |
| Rhesus immune globulin | RhIG |
| Single nucleotide polymorphisms | SNPs |

## NOMENCLATURE

**Exposure to foreign red cell antigens invariably results in the production of anti–red cell antibodies in a process known as *red cell alloimmunization,* formerly termed *isoimmunization*. The expression *sensitization* can be used interchangeably with *Rhesus alloimmunization*.** The active transport of these antibodies across the placenta during pregnancy results in fetal anemia, hyperbilirubinemia, and ultimately hydrops fetalis. Before the advent of obstetric ultrasound, the perinatal effects of maternal red cell alloimmunization could be recognized only after birth in the affected neonate. Thus the neonatal consequences of maternal red cell alloimmunization came to be known as *hemolytic disease of the newborn* (HDN). Because the peripheral blood smear of these infants demonstrated a large percentage of circulating immature red cells known as *erythroblasts,* the newborn entity was also known as *erythroblastosis fetalis.* Today, ultrasound and fetal blood sampling (FBS) make the detection of the severely anemic fetus a reality. For this reason, **the term *hemolytic disease of the fetus and newborn* (HDFN) would appear more appropriate to describe this disorder.**

## HISTORIC PERSPECTIVES

The first case of HDFN was probably described by a midwife in 1609 in the French literature: a twin gestation in which the first fetus was stillborn and the second twin developed jaundice and died soon after birth.[1] In 1932, Diamond[2] proposed that the clinical entities of erythroblastosis fetalis, icterus gravis neonatorum, and hydrops fetalis represented different manifestations of the same disease. Seven years later, Levine and Stetson[3] described an antibody in a woman who gave birth to a stillborn fetus. The patient experienced a severe hemolytic transfusion reaction after later receiving her husband's blood. In 1940, Landsteiner and Weiner[4] injected red blood cells from rhesus monkeys into rabbits. The antibody isolated from these rabbits was used to test human blood samples from whites, and agglutination was noted in 85% of individuals. The following year Levine and colleagues[5] were able to demonstrate a causal relationship between Rhesus D (RhD) antibodies in RhD-negative women and HDFN in their offspring.

The advent of therapy for HDFN began in 1945 with the description by Wallerstein[6] of the technique of neonatal exchange transfusion. Later Liley[7] proposed the use of amniotic fluid bilirubin assessment as an indirect measure of the degree of fetal hemolysis. Sir William Liley's major contribution to the story of rhesus disease was the introduction of the fetal intraperitoneal transfusion (IPT).[8] He learned from a visiting fellow who had returned from Africa that the infusion of red blood cells into the peritoneal cavity of children with sickle-cell disease produced normal-appearing red blood cells on peripheral blood smear. Liley realized that he had previously inadvertently entered the peritoneal cavity of fetuses at the time of amniocentesis, based on the marked contrast in the yellow hue of the ascitic fluid as compared with amniotic fluid. He postulated that purposeful entry into the fetal peritoneal cavity could be accomplished. After three unsuccessful attempts that resulted in fetal demises, the fourth fetus was delivered at $34^{1/7}$ weeks' gestation after undergoing two successful IPTs. Early attempts at IPT used fluoroscopy for needle guidance. With the introduction of real-time ultrasound in the early 1980s, IPT became a safer procedure as fluoroscopy was abandoned. Charles Rodeck[9] is credited with the first intravascular fetal transfusion (IVT) using a fetoscope to guide the transfusion needle into a placental plate vessel. Just 1 year later, investigators in Denmark performed the first ultrasound-guided IVT using the intrahepatic portion of the umbilical vein.[10]

The 1990s saw the introduction of genetic techniques using amniocentesis to determine fetal red cell typing.[11] The turn of the century brought the noninvasive detection of fetal anemia through Doppler ultrasound of the fetal middle cerebral artery (MCA) and the use of fetal typing through cell-free DNA in maternal plasma.[12,13]

## INCIDENCE

**The advent of the routine administration of antenatal and postpartum rhesus immune globulin (RhIG) has resulted in a marked reduction in cases of red cell alloimmunization secondary to the RhD antigen.** The Centers for Disease Control and Prevention (CDC) last required the reporting of rhesus alloimmunization as a medical complication of pregnancy on U.S. birth certificates in the year 2002.[14] In that year, the most recent for which epidemiologic data are available, the incidence was reported to be 6.7 cases of rhesus alloimmunization per 1000 live births.

**Clearly, a shift to other red cell antibodies associated with HDFN has occurred as a result of the decreasing incidence of RhD alloimmunization.** In a series of over 8000 pregnant patients between 2007 and 2011, a positive screen for an antibody associated with HDFN was found in 1.2% of samples.[15] Anti-E was the most common antibody encountered; RhD antibody accounted for only 19% of the significant antibodies (Fig. 34-1).

## PATHOPHYSIOLOGY

Although the placenta was once thought to be an absolute barrier to the transfer of cells between the maternal and fetal compartments, we now appreciate that the placental interface allows for the bidirectional movement of both intact cells and free DNA. The putative "grandmother theory" of rhesus red cell alloimmunization probably occurs more commonly than first

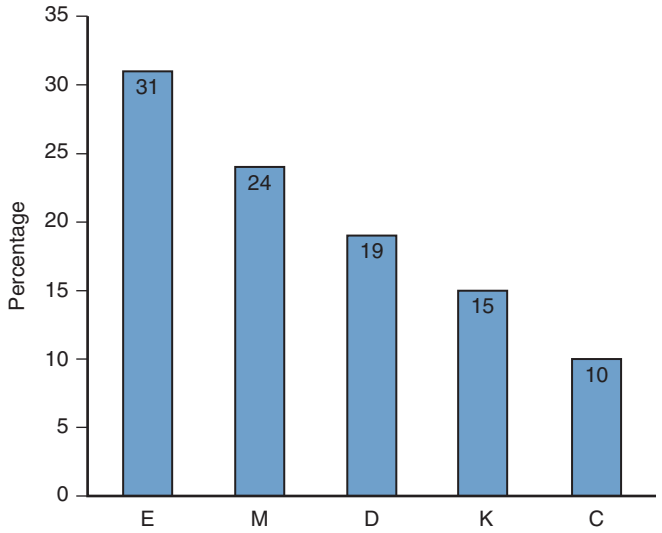


**FIG 34-1** Incidence of maternal anti–red cell antibodies associated with hemolytic disease of the fetus and newborn (HDFN) at a tertiary care institution between 2007 and 2011. E, M, D, K, and C are antibodies. (Modified from Smith HM, Shirey RS, Thoman SK, Jackson JB. Prevalence of clinically significant red blood cell alloantibodies in pregnant women at a large tertiary care facility. *Immunohematology*. 2013;29: 127-130.)

thought. In this paradigm, maternal RhD-positive red cells gain access to the circulation of the RhD-negative fetus at the time of delivery. **As many as one fourth of RhD-negative babies have been shown to be immunized in early life as a result of their delivery.**[16,17] The immune response of an Rh-negative individual to RhD-positive red cells has been characterized into one of three groups: (1) responders, (2) hyporesponders, and (3) nonresponders. About 60% to 70% of individuals are *responders* who develop an antibody to relatively small volumes of red cells; in these individuals, the probability of immunization increases with escalating volumes of cells. A small percentage of responders can be called *hyperresponders* in that they will be immunized by very small quantities of red cells. The second group of individuals (10% to 20%), *hyporesponders,* can be immunized only by exposure to very large volumes of cells. Finally, the 10% to 20% of individuals who remain appear to be *nonresponders.*

**In most cases of red cell alloimmunization, a fetomaternal hemorrhage (FMH) occurs in the antenatal period or, more commonly, at the time of delivery.** If a maternal ABO blood type incompatibility exists between the mother and her fetus, anti-A and/or anti-B antibodies lyse the fetal cells in the maternal circulation and destroy the RhD antigen.[18,19] Even if this protective effect is not present, **only 13% of deliveries of RhD-positive fetuses result in RhD alloimmunization in RhD-negative women who do not receive RhIG.** The vast majority of RhD-alloimmunized women produce an immunoglobulin G (IgG) response as their initial antibody. *Responders* may represent a group of individuals who had their initial exposure to the RhD antigen at birth because of FMH.[17] After a sensitizing event, the human antiglobulin anti-D titer can usually be detected after 5 to 16 weeks. However, approximately half of alloimmunized patients are *sensibilized.* In this scenario, an antibody screen will be negative, but memory B lymphocytes are present that can create an anti-D antibody response. When faced with the challenge of a subsequent pregnancy involving an RhD-positive fetus, the anti-D titer becomes detectable.

The anti-D immune response is the best characterized of the anti–red cell antibodies associated with HDFN. In one third of cases, only subclass IgG1 is produced; in the remainder of cases, a combination of IgG1 and IgG3 subclasses is found.[20] **Anti-D IgG is a nonagglutinating antibody that does not bind complement. This results in a lack of intravascular hemolysis; sequestration and subsequent destruction of antibody-coated red cells in the fetal liver and spleen are the mechanism of fetal anemia.** Most studies have not detected a relationship between a specific maternal human leukocyte antigen (HLA) type and susceptibility to become alloimmunized to RhD.[21] However, sensitized women with high titers of anti-D are more likely to exhibit the DQB1*0201 and DR17 alleles compared with women who have low titers.[22] Fetal sex may also play a significant role in the fetal response to maternal antibodies. **RhD-positive male fetuses are 13 times more likely than their female counterparts to become hydropic and are 3 times more likely to die of their disease.**[23]

Anemia results in several important physiologic changes in the fetus. Reticulocytosis from the bone marrow can be detected by FBS once the hemoglobin deficit exceeds 2 g/dL compared with norms for gestational age; erythroblasts are released from the fetal liver once the hemoglobin deficit reaches 7 g/dL or greater.[24] In an effort to increase oxygen delivery to peripheral tissues, fetal cardiac output increases and 2-3 diphosphatidylglycerol (DPG) levels are enhanced.[25,26] Tissue hypoxia appears as anemia progresses despite these physiologic changes. An increased umbilical artery lactate level is noted when the fetal hemoglobin falls below 8 g/dL, and increased venous lactate can be detected when the hemoglobin level falls below 4 g/dL.[27] **Hydrops fetalis, the accumulation of extracellular fluid in at least two body compartments, is a late finding in cases of fetal anemia. Its exact pathophysiology is unknown.** Enhanced hepatic erythropoietic function with subsequent depressed synthesis of serum proteins has been proposed as the explanation for the lower serum albumin levels that have been detected.[28] Colloid osmotic pressure appears decreased.[29] However, experimental animal models in which fetal plasma proteins have been replaced with saline did not produce hydrops.[30] An alternative hypothesis is that tissue hypoxia due to anemia enhances capillary permeability. In addition, iron overload due to ongoing hemolysis may contribute to free radical formation and endothelial cell dysfunction.[31] Central venous pressures do appear elevated in the hydropic fetus with HDFN. This may cause a functional blockage of the lymphatic system at the level of the thoracic duct as it empties into the left brachiocephalic vein.[29] This theory is supported by reports of poor absorption of donor red cells infused into the intraperitoneal cavity in cases of hydrops.[32]

## RHESUS ALLOIMMUNIZATION AND FETAL/NEONATAL HEMOLYTIC DISEASE OF THE NEWBORN

### Genetics

Initial concepts on the genetics of the Rh antigens proposed the presence of three distinct genes.[33] Newer DNA techniques have allowed for the localization of the Rh locus to the short arm of chromosome 1.[34] **Only two genes were identified, an *RHD* gene and an *RHCE* gene.** Each gene is 10 exons in length with 96% homology. These genes presumably represent a duplication of a common ancestral gene. Production of two distinct proteins

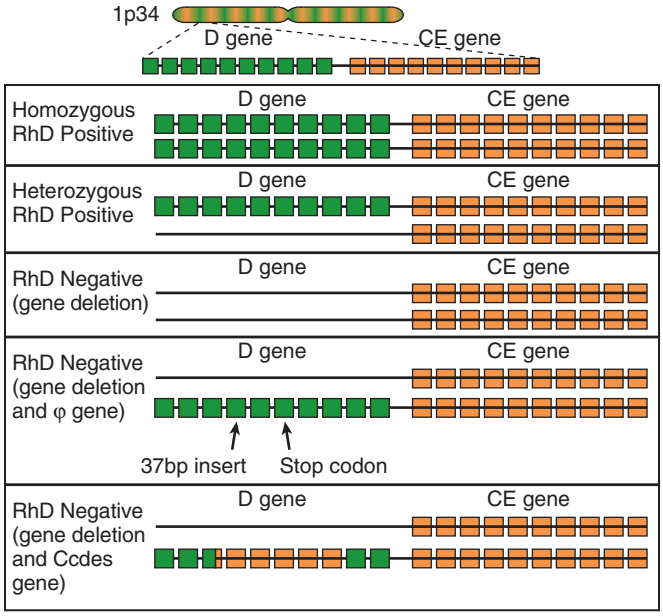


**FIG 34-2** Schematic of Rh gene locus on chromosome 1. The homozygous RhD-positive state, heterozygous RhD-positive state, RhD-negative with heterozygosity for the *RhD* pseudogene, and RhD-negative with heterozygosity for the *RHCcdes* gene are demonstrated. (Modified from Moise KJ. Hemolytic disease of the fetus and newborn. In Creasy RK, Resnik R, Iams J, eds. *Maternal-Fetal Medicine: Principles and Practice,* ed 5. Philadelphia: Elsevier; 2004.)

from the *RHCE* gene probably occurs as a result of alternative splicing of messenger RNA.[35] One nucleotide difference, cytosine to thymine, in exon 2 of the *RHCE* gene results in a single amino acid change of a serine to proline. This causes the expression of the *C* antigen as opposed to the *c* antigen.[36] A single cytosine-to-guanine change in exon 5 of the *RHCE* gene, producing a single amino acid change of a proline to alanine, results in formation of the *e* antigen instead of the *E* antigen.

The gene frequency found in different ethnic groups can be traced to the Spanish colonization in the fifteenth and sixteenth centuries. Populations native to certain land masses have a less than 1% incidence of RhD negativity—Eskimos, Native Americans, Japanese, and Chinese individuals. The Basque tribe in Spain is noted to have a 30% incidence of Rh negativity. This may well be the origin of the *RHD* gene deletion that is the most common genetic basis of the RhD-negative state in whites (Fig. 34-2). Whites of European descent exhibit a 15% incidence of RhD negativity, whereas an 8% incidence is found in blacks and Hispanics of Mexico and Central America. This latter incidence probably reflects ethnic diversity secondary to Spanish colonization of the New World.

Further study of the *RHD* gene has revealed significant heterogeneity. Several of these genetic modifications result in a lack of expression of the RhD phenotype. Although these individuals may have an aberrant RhD gene present, serologic methods do not detect the RhD antigen on the surface of the red cells. One such example is the *RHD* pseudogene, which has been found in 69% of South African blacks and 24% of American blacks (see Fig. 34-2).[37] In this situation, all 10 exons of the *RHD* gene are present. However, translation of the gene into a messenger RNA (mRNA) product does not occur owing to the presence of a stop codon in the intron between exons 3 and 4. Thus, no RhD protein is synthesized, and the patient is serologically RhD

negative. Similarly, the *RHCcdes* gene has been detected in 22% of American blacks. It appears to contain exons 1, 2, 9, and 10 as well as a portion of exon 3 of the original *RHD* gene, with other exons being duplicated from the *RHCE* gene. In the Taiwanese population of RhD-negative individuals, five different exons of the *RHD* gene were evaluated.[38] Seventeen percent of individuals had all five exons detected, and an additional 135 demonstrated the presence of at least one of the five exons tested.

## PREVENTION OF RhD HEMOLYTIC DISEASE IN THE FETUS AND NEWBORN

### History

The history of rhesus prophylaxis can be traced to three unique individuals. Vincent Freda was an obstetric resident who developed an interest in HDFN.[39] He was allowed to spend part of the fourth year of his residency at Columbia Presbyterian Medical Center in the laboratory of Alexander Weiner, one of the first investigators to identify the "Rh factor." When Freda returned to Columbia, he went on to establish a serology laboratory and later organized the Rh Antepartum Clinic in 1960. A seat on the hospital transfusion committee became vacant, and in an unprecedented move based on his interest, the chairman of obstetrics and gynecology, Howard C. Taylor, Jr., appointed Freda to this position even though he had not completed his residency. The chairman of pathology responded with the appointment of John Gorman to the committee, a resident in pathology with an interest in blood banking. It is here that these two individuals met and developed the collaboration that would one day end in the introduction of RhIG. In 1906, Theobald Smith[40] found that guinea pigs given excess passive antibody failed to become immunized to diphtheria toxin. Freda and Gorman proposed that anti-D could be used in a similar fashion to prevent alloimmunization after delivery. They enlisted the aid of William Pollack, a senior protein chemist at Ortho Diagnostics, who developed an IgG globulin fraction from high-titered donor plasma. An initial grant application to the National Institutes of Health was rejected; however, funding was secured from the New York City Health Research Council on a second attempt. This was followed by a year's negotiations with lawyers in the state capital to allow the investigators to perform their clinical trials at the Sing Sing prison in New York beginning in 1961 (John Gorman; personal communication, 2009). Nine RhD-negative male volunteers were injected monthly with RhD-positive cells for five successive months.[41] Four of the men were immunized with intramuscular RhIG 24 hours before the injection of the red cells. Four of the five controls became alloimmunized to RhD, whereas none of the treated individuals developed anti-RhD antibodies. Their second experiment involved 27 inmates at Sing Sing, 13 controls and 14 treated. Red cells were given intravenously. However, the warden of Sing Sing would not allow the investigators to return on any fixed schedule that would enable the prisoners to know the time and day of their revisit. He was concerned that this exact foreknowledge could involve the prisoners in an escape plan. The investigators gladly accepted this limitation as they reasoned that pregnant women who delivered over a weekend would probably not receive RhIG until Monday, up to 72 hours after delivery, owing to the closure of blood banks on weekends, as was commonly practiced at the time. None of the men who received RhIG were alloimmunized, whereas 8 of 13 controls developed anti-RhD antibodies. After two additional experiments at Sing Sing in this second group of individuals, Freda and Gorman[41] went on to conduct a clinical trial in postpartum women at Columbia Presbyterian Medical Center starting in March of 1964. Of the 100 patients that received RhIG, none became sensitized, as compared with a rate of 12% sensitization to RhD in the control group. In a follow-up study in these patients in their next pregnancy, none of the treated patients developed antibodies; 5 of 10 controls were alloimmunized and delivered infants affected by HDFN.

A parallel track of investigation was being undertaken by a group of British researchers in Liverpool. This group reasoned that the natural protective effect of ABO incompatibility between a mother and her fetus in preventing the formation of anti-D antibody could be used as a preventative strategy. A preparation of plasma that contained anti-D IgM was formulated and was administered intravenously to male volunteers.[42] Although initial short-term antibody studies were promising, eventually 8 of 13 treated men became immunized to RhD, compared with only 1 of 11 controls. After the publication of the initial work of Freda and colleagues[43] describing the use of a gamma globulin fraction of the plasma, the British group visited the New York investigators and obtained a sample of their gamma globulin preparation. The Liverpool group[44] began their clinical trial in postpartum women with evidence of FMH by Kleihauer-Betke (KB) stain in April 1964, and they were subsequently credited for the first publication of a successful clinical trial in women.

An observational trial in Canada was initiated and determined that the baseline rate of antenatal sensitization to RhD was 1.8%.[45] Between 1968 and 1974, a trial of antenatal prophylaxis using injections of 300 μg of RhIG at 28 and 34 weeks' gestation followed. As compared with the previous observational study, none of the women demonstrated the development of anti-D antibodies. In a subsequent investigation that involved RhIG administered only at 28 weeks' gestation, only 0.18% of women became sensitized.

In 1968, RhIG was approved by the Division of Biologics Standards of the National Institutes of Health for general clinical use in the United States as RhoGAM (Ortho-Clinical Diagnostics, Inc.). **Recommendations for use during the immediate postpartum period were set forth by the American College of Obstetricians and Gynecologists (ACOG)[46] in 1970. The Food and Drug Administration (FDA) approved the use of antenatal RhIG in 1981. Routine antenatal prophylaxis at 28 to 29 weeks' gestation was proposed by ACOG later that same year.**[47]

### Preparations

**Four polyclonal products derived from human plasma are currently available in the United States for the prevention of RhD alloimmunization.** Two of the products (RhoGAM [Kedrion Biopharma] and HyperRho S/D [Grifols USA]) can only be given intramuscularly because they are derived from human plasma through Cohn cold ethanol fractionation, a process that results in contamination with IgA and other plasma proteins. The remaining two products (WinRho-SDF, [Cangene Corporation] and Rhophlac [CSL Behring]) are prepared through sepharose column and ion-exchange chromatography, respectively. At present, all available products are subject to solvent detergent treatment to inactivate enveloped viruses; many manufacturers also use an additional micropore filtration step to further reduce the chance for viral contamination.

Additionally, thimerosal, a mercury preservative used to prevent bacterial and fungal contamination, has been removed from all RhIG products used in the United States.

The dwindling resource of plasma donors for RhIG manufacture has led to the search for a synthetic product. Several monoclonal anti-D antibodies and a synthetic polyclonal immune globulin consisting of 25 recombinant anti-D antibodies have been developed but are still being studied in human clinical trials. In the future, one of these products may replace the current polyclonal products derived from human plasma.

## Indications

**All pregnant patients should undergo determination of blood type and an antibody screen at the first prenatal visit.** In the past, all Rh negative patients underwent additional testing to see if they were *Du positive*. This terminology was later changed to classify these patients as *weak Rh positive* individuals. In one series of 500 pregnant patients, this occurred in 1% of whites, 2.6% of blacks, and 2.7% of Hispanics.[48] The recommendation in the past was that these individuals should be considered Rh positive, and RhIG was not indicated.[48] Subsequent research found that the *weak D* individuals can belong to one of two groups; some of these patients have intact D antigens that are expressed in reduced numbers on the surface of the red cells (Fig. 34-3). These individuals are *not* at risk for rhesus alloimmunization. **In others with a weak D phenotype, the individual has inherited a gene that results in a variant expression of the D antigen. In these cases, one or more of the D antigen epitopes are missing, and the patient can become alloimmunized to these missing portions of the D antigen. Severe HDFN has been reported in these cases when a maternal antibody develops to the missing epitope.[49] Although clinical trials have not been undertaken, the current recommendation is that these patients should receive RhIG.**

Confusion can arise depending on when and where the patient undergoes red cell typing. Standards from the American Association of Blood Banks (AABB) recommend that reagents that detect weak D should *not* be used for prenatal typing.[50] This guideline results in all weak D patients being called Rh negative and subsequently receiving antenatal RhIG. Newer monoclonal reagents used in an indirect antiglobulin test that can detect weak D are used at blood donor centers. These reagents are used to be sure that weak D blood is not administered to an Rh-negative recipient, resulting in a potential for alloimmunization. This will result in the same individual, now a blood donor, being called *RhD positive*—very confusing to the patient and to the clinician.

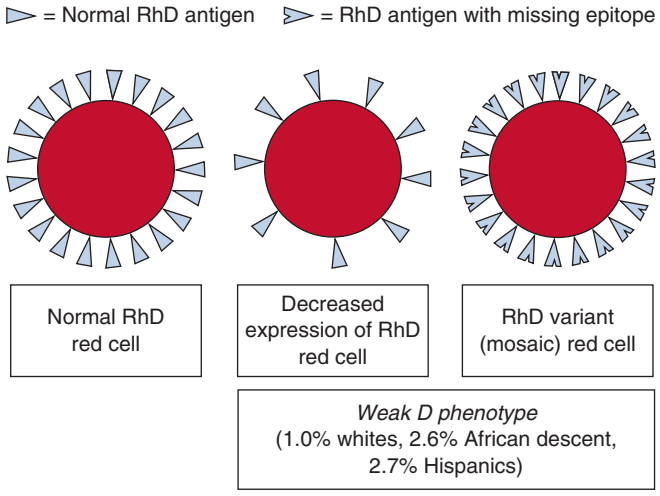


**FIG 34-3** Depiction of a normal RhD-positive red cell as well as red cells noted in individuals with weak D variants.

More recently, a work group of the AABB and the College of American Pathologists has suggested that weak D types 1, 2, and 3 can be managed as if they are RhD positive with no need for RhIG.[51] *RHD* genotyping would be required in all pregnant patients to identify this subgroup with weak D. This proposal has not yet been adopted by ACOG.

**If there is no evidence of anti-D alloimmunization in the RhD-negative woman, the patient should receive 300 μg of RhIG at 28 weeks of gestation.**[48] The 2% background incidence of RhD alloimmunization in the antenatal period can be expected to decline to 0.1%. In the United Kingdom, an antenatal protocol of administering 100 μg (500 IU) of RhIG at 28 and 34 weeks is used in primigravida women.[52] Limited resources have not allowed for extension of this protocol to all subsequent pregnancies. The issue of repeating an antibody screen at 28 weeks before the administration of RhIG is controversial. A recent study of over 2000 women found an incidence of sensitization prior to 28 weeks' gestation to occur in only 0.099% of pregnancies.[53] In addition, the authors of this study did not find the practice to be cost-effective. Although ACOG leaves the decision to repeat the antibody screen up to the obstetric provider, the AABB and the U.S. Preventative Services Task Force recommend that a repeat screen be obtained before antenatal RhIG.[50,54] If a repeat antibody screen is to be undertaken, a maternal blood sample can be drawn at the same office visit as the RhIG injection. Although the administration of the exogenous anti-D will eventually result in a weakly positive titer, this will not occur in the short interval of several hours due to the slow absorption from the intramuscular site.

A new paradigm is developing in antenatal prophylaxis. Early studies in pregnant women carrying a male fetus indicated that 3% of the circulating cell-free fetal DNA (ccffDNA) in the maternal circulation in the first trimester is fetal in origin; this increases to 6% by the third trimester.[55] The source of this DNA appears to be apoptosis of placental villi. Fetal DNA is rapidly cleared from the maternal circulation with a mean half-life of 16 minutes after cesarean delivery; after vaginal delivery, fetal free DNA is cleared by 100 hours.[56,57] The presence of fetal *RHD* DNA sequences in the maternal circulation was first reported by Lo and colleagues.[13] Clinical assays for the determination of the fetal RhD status were subsequently developed. Approximately 40% of Rh-negative pregnant women will carry an Rh-negative fetus; thus rhesus immune globulin would not be indicated in the antepartum period if this can be accurately determined.

Screening of RhD-negative pregnant patients to determine whether antepartum RhIG should be undertaken is routinely now practiced in Denmark and the Netherlands as well as in regions of Sweden, France, and England.[58] In some of these situations, ccffDNA screening was implemented as part of a new antepartum prophylaxis program because of the limited availability of RhIG. However, in the United States, plasma collected from sensitized male volunteer donors is used to manufacture RhIG. Therefore the availability of RhIG is unrestricted. Others

**TABLE 34-1** INDICATIONS FOR RHESUS IMMUNE GLOBULIN

| INDICATION | LEVEL OF EVIDENCE* |
|---|---|
| Spontaneous miscarriage | A |
| Elective abortion | A |
| Threatened miscarriage | C |
| Ectopic pregnancy | A |
| Hydatidiform mole | B |
| Genetic amniocentesis | A |
| Chorion villus biopsy | A |
| Fetal blood sampling | A |
| Placenta previa with bleeding | C |
| Suspected abruption | C |
| Intrauterine fetal demise | C |
| Blunt trauma to the abdomen | C |
| At 28 weeks' gestation unless father of fetus is RhD negative | A |
| Amniocentesis for fetal lung maturity | A |
| External cephalic version | C |
| Within 72 hours of delivery of an RhD-positive infant | A |
| After administration of RhD-positive blood component | C |

Modified from Prevention of RhD alloimmunization. American College of Obstetricians and Gynecologists Practice Bulletin 1999;4.
*A = high, B = moderate, C = low.

have argued that the potential for infection with prions and other viruses supports an ethical approach to limiting antenatal RhIG to only those patients who need it.[59] A cost-neutral strategy would appear to be the optimal approach to the implementation of ccffDNA. Several studies have determined that the break-even costs of ccffDNA testing would range from $29 to $119 for the saved doses of RhIG to offset the costs of evaluating all RhD-negative pregnant women.[60,61] In addition, even at an accuracy of 99%, as many 3000 patients in the United States would be misdiagnosed with an RhD-negative fetus, when the fetus is actually RhD positive. These cases would result in missed opportunities for the prevention of antenatal alloimmunization and an estimated 21 new cases of Rh alloimmunization annually. Continuation of the practice of obtaining cord serology at birth would allow these "misses" to be correctly diagnosed at the time of delivery when postpartum RhIG is indicated. Currently, the use of ccffDNA to guide antenatal RhIG use is not a guideline from any major U.S. organization, although this may change in the near future.

**Although not well studied, level A scientific evidence has been cited by ACOG to address additional indications for the antepartum administration of RhIG.[48] These include spontaneous miscarriage, elective abortion, ectopic pregnancy, genetic amniocentesis, chorionic villus sampling, and FBS** (Table 34-1). A dose of 50 µg of RhIG is effective until 13 weeks' gestation owing to the small volume of red cells in the fetoplacental circulation. However, most hospitals and offices do not stock this dose of RhIG because the cost is equivalent to that of the standard dose of 300 µg.

The use of RhIG in other scenarios that involve the possibility of FMH are lacking. However, most experts agree that such events as hydatidiform mole, threatened miscarriage, fetal death in the second or third trimester, blunt trauma to the abdomen, and external cephalic version warrant strong consideration for the use of RhIG.[48]

**The practice of evaluating a persistent maternal anti-D titer as an indication that additional RhIG is not required after an antenatal event is to be discouraged.** Although the precise mechanism for the protective effect of RhIG is unknown, an excess amount of exogenous antibody in relation to the volume of RhD-positive red cells in the maternal circulation is essential for effective prophylaxis. Both animal and human studies have demonstrated that a low level of RhIG can actually enhance the chance for alloimmunization.[18] In the words of Vincent Freda, "The rule of thumb should be to administer Rh immune globulin when in doubt, rather than to withhold it."

Because the half-life of RhIG is approximately 16 days, 15% to 20% of patients receiving it at 28 weeks' gestation have a very low anti-D titer (usually 2 or 4) at the time of admission for labor at term.[62] In North America, the current recommendation is to administer 300 µg of RhIG within 72 hours of delivery if umbilical cord blood typing reveals an RhD-positive infant.[50] This is sufficient for protection from sensitization due to an FMH of 30 mL of fetal whole blood. In the United Kingdom, 100 µg is given at delivery. Approximately 1 in 1000 deliveries will be associated with an excessive FMH; risk factors identify only 50% of these cases.[63] **Both ACOG and AABB now recommend routine screening of all women at the time of delivery for excessive FMH.** A qualitative yet sensitive test for FMH, the *rosette test,* is first performed. Results return as positive or negative; a negative result warrants administration of a standard 300 µg dose of RhIG. If the rosette is positive, a KB stain or fetal cell stain using flow cytometry is undertaken to quantitate the amount of the FMH. The AABB then recommends that the percentage of fetal blood cells be multiplied by a factor of 50 (to account for an estimated maternal blood volume of 5000 mL) to calculate the volume of the FMH. This volume is divided by 30 to determine the number of vials of RhIG to be administered. A decimal point is rounded up or down for values greater than 0.5 or less than 0.5, respectively. Because this calculation includes an inaccurate estimation of the maternal blood volume, one additional vial of RhIG is added to the calculation. As an example, a 3% KB stain is calculated to indicate a 150-mL FMH. Dividing this number by 30 yields five vials of RhIG with one additional vial added; therefore the blood bank would prescribe 6 vials of RhIG (a total of 1800 µg) for this patient. However, a recent survey by the American College of Pathologists of its member blood banks noted that even following these guidelines, an inadequate dose of RhIG was recommended in 9% of cases and an excessive dose was recommended in 12% of cases.[64]

No more than 5 mL RhIG should be administered by the intramuscular route in one 24-hour period. Should a large dose of RhIG be necessary, an alternative method would be to give the calculated dose using one of the intravenous (IV) preparations of RhIG now available. Doses of up to 600 µg (3000 IU) can be administered every 8 hours until the total dose has been achieved. **Should RhIG be inadvertently omitted after delivery, some protection has been proven with administration within 13 days; recommendations have been made to administer it as late as 28 days after delivery.**[63] If delivery is planned within 48 hours of amniocentesis for fetal lung maturity, RhIG can be deferred until after delivery. **If delivery occurs less than 3 weeks from the administration of RhIG used for antenatal indications such as external cephalic version, a repeat dose is unnecessary unless a large FMH is detected at the time of delivery.**[50]

Failed prophylaxis after the appropriate dose of RhIG is administered is rare. However, once postpartum administration is undertaken, the anti-D antibody screen may remain positive for up to 6 months. Anti-D that persists after this time is likely to be the result of sensitization.

Administration of RhIG after a postpartum tubal ligation is controversial. The possibility of a new partner in conjunction with the availability of in vitro fertilization would seem to make the use of RhIG in these situations prudent. In some cases, RhD-negative red cells may be in short supply if the patient presents after major trauma such as a motor vehicle accident with the need for massive transfusion. In these cases, RhD-positive blood could not be used as a life-saving alternative if the patient is alloimmunized to RhD through her previous delivery. RhIG is not effective once alloimmunization to the RhD antigen has occurred. At present, prophylactic immune globulin preparations to prevent other forms of red cell alloimmunization such as anti-K1 do not exist.

## Diagnostic Methods

### Maternal Antibody Determination

**Once a maternal antibody screen reveals the presence of an anti-D antibody, a titer is the first step in the evaluation of the RhD-sensitized patient during the first affected pregnancy.** Previous titer methodologies using albumin or saline should no longer be used because they detect varying levels of IgM antibody. The pentamer structure of this class of antibody does not allow for transplacental passage; therefore, the contribution of IgM to the titer quantitation has no clinical relevance. **The human antiglobulin titer** (indirect Coombs test) **is used to determine the degree of alloimmunization because it measures the maternal IgG response.** Most titer values in the obstetric literature are reported as dilutions (e.g., 1:32). By blood-banking convention, however, titer values should be reported as the reciprocal of the last tube dilution that demonstrates a positive agglutination reaction, that is, a final dilution of 1:16 is equivalent to a titer of 16.

Variation in results between laboratories is not uncommon because many commercial laboratories use enzymatic treatment of red cells to prevent failed detection of low titer samples. This method causes a marked elevation in titer as compared with the use of nonenzymatic treated cells. Because standard tube methodology uses red cell agglutination as the indicator reaction, subjective interpretation of end points by the laboratory technologist accounts for the variation in results. In addition, inherent subtle differences in the indicator red cell preparations may play a role because their shelf life is only 1 month, and serial titers may require the use of different reagent lots. For these reasons, serial titers should be run in tandem using stored sera from the previous draw.

In the same laboratory, the titer should not vary by more than one dilution if the two samples are run in tandem. Thus an initial titer of 8 that returns at 16 does not represent a true increase in the amount of antibody in the maternal circulation. In addition, the clinician should be aware that newer gel microcolumn assays will result in higher titers than conventional tube testing. In one study, the mean titer was 3.4-fold increased with gel technology.[65] **A *critical titer* is defined as the anti–red cell titer associated with a significant risk for hydrops fetalis.** When this is present, further fetal surveillance is warranted. This value will vary with institution and methodology; however, **in most centers, a critical titer for anti-D between 8 and 32 is usually used.**

In the United Kingdom, quantitation of anti-D is undertaken through the use of an automated technique using a device known as the *AutoAnalyzer*. Red cell samples are mixed with agents to enhance agglutination by the anti-D antibodies. Agglutinated cells are separated from nonagglutinated cells and are then lysed. The amount of released hemoglobin is then compared with an international standard; results are reported as international units per milliliter. Levels of less than 4 IU/mL are rarely associated with HDFN; a maternal anti-D level of less than 15 IU/mL has been associated with only mild fetal anemia.[66]

### Fetal Blood Typing

**Several techniques have been used to determine the fetal blood type if the patient's partner is determined to be heterozygous for the involved red cell antigen. In 50% of cases in which the fetus is found to be antigen negative, further maternal and fetal testing is unnecessary.** Historically, initial attempts at fetal testing in these cases used serology on blood obtained by ultrasound-directed cordocentesis. Unfortunately, this technique placed half of the antigen-negative fetuses at a 1% to 2% chance of procedure-related loss (see Chapter 10). Investigators went on to use chorionic villus sampling to obtain genetic material for detection of the *RHD* gene. However, the major disadvantage of this method is that disruption of the chorion villi during the procedure can result in FMH and a rise in maternal titer, thereby worsening the fetal disease.[67] Therefore this procedure should be discouraged unless the patient plans to terminate all antigen-positive fetuses detected. In 1990, amniocentesis was described as a reliable method for assessing the fetal blood type through DNA testing.[11] **This method has now been replaced in almost all countries, including the United States, by the use of fetal *RHD* determination using ccffDNA.** In addition, the test can be performed with reliable results at as early as 10 weeks' gestation.[68]

**The initial step in determining the fetal RhD type involves an assessment of paternity and paternal zygosity.** Once undertaken using serologic testing and population statistics, molecular techniques can now be used to accurately determine the paternal genotype at the *RHD* locus.[69] However, some authorities have argued that issues with paternity can be averted by omitting this step and testing every pregnancy with ccffDNA for fetal *RHD* determination.

**In a recent series of more than 1000 patients, ccffDNA testing for *RHD* was found to be accurate in 99% of cases.**[70] An *RHD* positive result on free DNA testing can be considered reliable because *RHD* positive genetic material cannot be from a maternal source. An RhD-negative result with ccffDNA is more problematic. If fetal DNA fails to amplify in a background of overwhelming maternal DNA in the plasma, an *RHD* negative result will be obtained. One internal control that can be used is the detection of the *SRY* gene found in male fetuses. The presence of this gene in free DNA indicates that fetal DNA is present, and an *RHD* negative result is reliable.[62] In the case of a female fetus, the presence of single nucleotide polymorphisms (SNPs) not found in the maternal white cells can be used as an internal control.[71] If different polymorphisms than those found in the mother are noted in the plasma sample, these are of paternal origin; thus fetal DNA is present. In this situation, the finding of an *RHD* negative fetus can be considered reliable. **In the cases with an inconclusive result, a repeat maternal**

**sample can be submitted or amniocentesis can be undertaken to determine the fetal *RHD* status.**

### Amniocentesis to Follow the Severity of Hemolytic Disease of the Fetus and Newborn

Historically, amniocentesis was routinely used in the alloimmunized pregnancy to measure the amount of bilirubin ($\Delta OD_{450}$) as an indirect indication of the degree of fetal hemolysis. Results were plotted on specialized curves first introduced by William Liley[7] and later modified by John Queenan.[72] **The advent of noninvasive testing for fetal anemia with middle cerebral artery MCA Doppler** (see below) **has now replaced serial amniocenteses for $\Delta OD_{450}$.**

### Fetal Blood Sampling

Ultrasound-directed FBS—also known as *percutaneous umbilical blood sampling, cordocentesis,* and *funipuncture*—allows direct access to the fetal circulation to obtain important laboratory values such as fetal blood type, hematocrit, direct Coombs test, reticulocyte count, and total bilirubin. Although serial FBS was once proposed as a primary method of fetal surveillance after a maternal critical titer is reached, it has been associated with a 1% to 2% rate of fetal loss and up to a 50% risk for FMH with subsequent worsening of the alloimmunization.[73] For these reasons, **FBS is reserved for patients with elevated peak systolic MCA Doppler velocities.**

### Ultrasound

**Perhaps the greatest advance in the management of the alloimmunized pregnancy has been the use of ultrasound.** Gestational age can be accurately established to evaluate fetal parameters that vary with gestational age such as the peak systolic MCA Doppler velocities. *Hydrops fetalis* is defined as the presence of extracellular fluid in at least two fetal compartments. Often, ascites is the first sign of impending hydrops, with scalp edema and pleural effusions noted with worsening anemia. **When hydrops is present, fetal hemoglobin deficits of 7 to 10 g/dL from the mean hemoglobin value for the corresponding gestational age can be expected.**[74] Unfortunately, this represents the end-stage state of fetal anemia. Survival with intrauterine transfusion (IUT) is markedly reduced in these cases. In addition, **the early second-trimester fetus can be severely anemic without signs of hydrops.**[75] Therefore many investigators have sought alternative ultrasound parameters that could predict the early onset of anemia. In one large series, fetal abdominal circumference (AC), head/abdomen circumference (HC/AC) ratio, intraperitoneal volume, intrahepatic and extrahepatic umbilical venous diameter, and placental thickness failed to accurately predict a fetal hemoglobin deficit of greater than 5 g/dL from the mean.[76] Because the fetal liver and spleen represent sites of extramedullary hematopoiesis and the destruction and sequestration of sensitized red cells in cases of severe HDFN, enlargement of these organs has been evaluated. Both splenic perimeter and hepatic length correlate with the degree of fetal anemia. However, neither has gained widespread acceptance for noninvasive fetal surveillance in red cell alloimmunization.

**The severely anemic fetus exhibits an increased cardiac output in an effort to enhance oxygen delivery to peripheral tissues.**[26] In addition, fetal anemia is associated with a lower blood viscosity that produces fewer shearing forces in blood vessels; this results in increased blood velocities. Using these principles, **Doppler ultrasound has been used to study the peak systolic velocity (PSV) in the fetal MCA to predict fetal anemia. A value of greater than 1.5 multiples of the median (MoM) for the corresponding gestational age predicts moderate to severe fetal anemia with a sensitivity of 88% and a negative predictive rate of 89%.**[12]

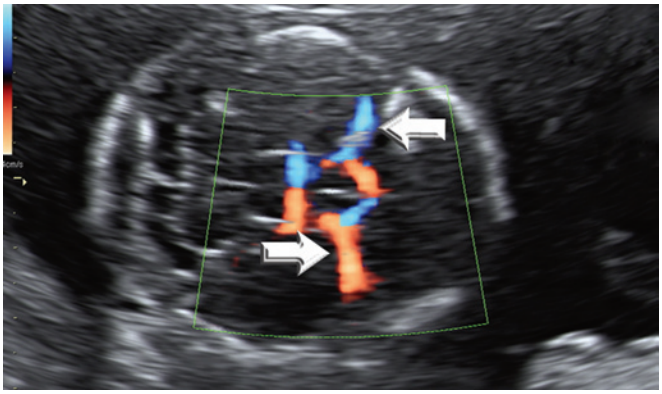

**FIG 34-4** Power Doppler image of the fetal circle of Willis. *Arrows* point to locations where the pulsed Doppler gate should be placed for obtaining the fetal peak middle cerebral artery Doppler velocity.

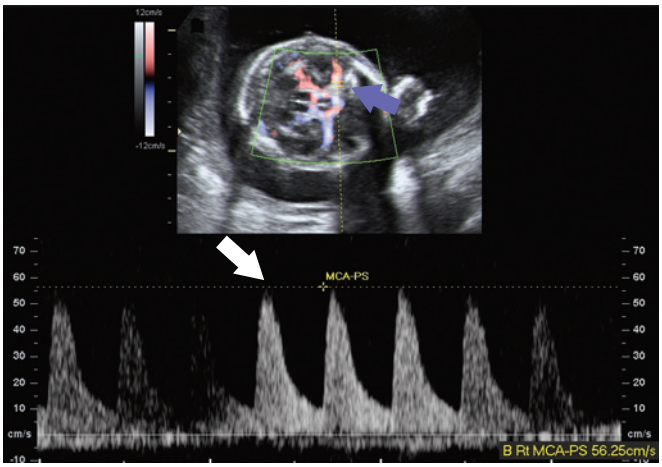


**FIG 34-5** Pulsed Doppler of the peak systolic velocity. The *blue arrow* at the top of figure indicates the location of the pulsed Doppler gate; the *white arrow* indicates the measurement using on-board software of a peak velocity of 56.25 cm/sec.

**Serial MCA Doppler studies are now the mainstay of surveillance for fetal anemia in the red cell alloimmunized pregnancy.** Careful attention to technique is paramount in using this method of surveillance. Because the anteroposterior axis of the fetal head typically lies in a transverse plane, the examiner can use either fetal MCA vessel for interrogation. First, the anterior wing of the sphenoid bone at the base of the skull is located. Color or power Doppler is then used to locate the MCA (Fig. 34-4). The angle of insonation is maintained as close to zero as possible by positioning the ultrasound transducer on the maternal abdomen (Figs. 34-5 and 34-6). The MCA vessel closer to the maternal abdominal wall is usually studied, although the posterior vessel will give equivalent results.[77] Angle-correction software is not typically used, although studies have demonstrated that its use can still result in an accurate determination of the MCA velocity.[78] The Doppler gate is then placed in the proximal MCA where the vessel arises from the carotid siphon. Measurements in the more distal aspect of the vessel will be

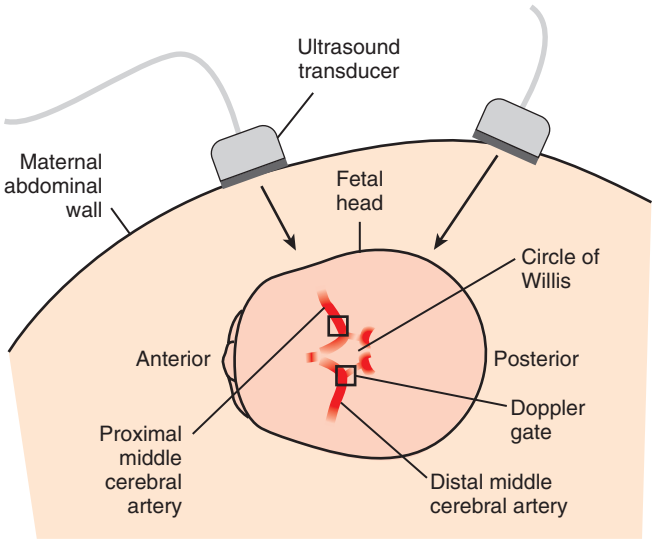


**FIG 34-6** Correct determination of the fetal peak middle cerebral artery Doppler velocity.

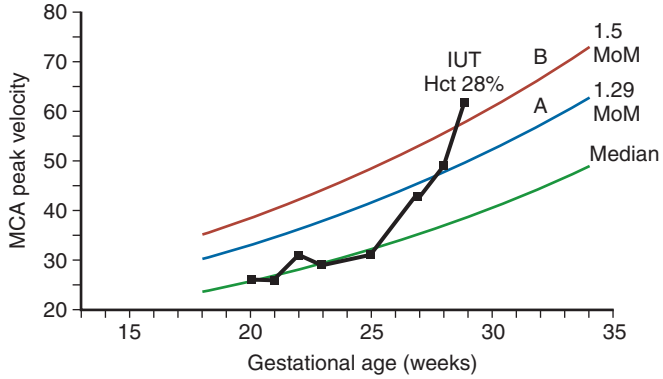


**FIG 34-7** Serial middle cerebral artery (MCA) Doppler studies in one patient who required intrauterine transfusion (IUT). Hct, hematocrit; MoM, multiples of the median.

inaccurate because reduced peak velocities will be obtained. The fetus should be in a quiescent state during the Doppler examination because accelerations of the fetal heart rate can result in a decrease in the PSV, especially late in the third trimester.[79] Several authorities have reported transient decreases in the peak MCA velocity after the administration of antenatal steroids to enhance fetal lung maturity. This effect usually lasts for 24 to 48 hours after the last dose.

MCA measurements can be obtained reliably as early as 18 weeks' gestation. Studies are repeated every 1 to 2 weeks depending on the trend (Fig. 34-7). Values should be converted to MoM using Internet-based calculators (e.g., www.perinatology.com).

## CLINICAL MANAGEMENT

The approach using the available diagnostic tools is based on the patient's history of fetal or neonatal manifestations of HDFN. **As a general rule, the patient's first RhD-sensitized pregnancy involves minimal fetal/neonatal disease; but subsequent gestations are associated with worsening degrees of anemia.**

### First Affected Pregnancy

**Once sensitization to the RhD antigen is detected, maternal titers are repeated every month until approximately 24 weeks; titers are repeated every 2 weeks thereafter** (Fig. 34-8). If paternity is assured, blood is drawn from the patient's partner to determine his *RHD* status and zygosity (DNA testing). Once a critical maternal titer is reached (usually 32), serial MCA Doppler studies are initiated at approximately 24 weeks' gestation. These are then repeated every 1 to 2 weeks depending on their trend. In cases of a heterozygous paternal phenotype or questionable paternity, ccffDNA testing should be sent to a DNA reference laboratory to determine the fetal RhD status. In the case of an RhD-negative paternal blood type or a fetal *RHD* negative genotype, further maternal and fetal monitoring is unwarranted as long as paternity is assured.

If presence of an *RHD* positive fetus is evident (homozygous paternal phenotype or *RHD* positive fetus by DNA testing), serial fetal surveillance is indicated. If an MCA Doppler returns at greater than 1.5 MoM, cordocentesis should be undertaken at an experienced referral center, with blood readied for IUT if the fetal hematocrit is less than 30%.

### Previously Affected Fetus or Infant

**If the patient has a history of a previous perinatal loss related to HDFN, a previous need for IUT, or a previous need for neonatal exchange transfusion, she should be referred to a tertiary care center with experience in the management of the severely alloimmunized pregnancy.** In these cases, maternal titers are *not* predictive of the degree of fetal anemia. In the case of a heterozygous paternal phenotype or questionable paternity, ccffDNA analysis to determine the fetal *RHD* status is indicated. **Amniocentesis can be used after 15 weeks' gestation to determine the status of the fetal red cell antigen in cases of other maternal antibodies such as anti-Kell.** Serial MCA Doppler measurements should begin at 18 weeks' gestation and should be repeated every 1 to 2 weeks.

## INTRAUTERINE TRANSFUSION

### Technique

**IUTs today are performed under continuous ultrasound guidance with direct infusions of red blood cells into the umbilical cord vessels or into the intrahepatic portion of the umbilical vein of the fetus.**[80] Some centers continue to use the intraperitoneal approach as part of a combined technique with an intravascular transfusion (IVT) in an effort to create a reservoir of red cells between procedures.[81]

Typically, a freshly donated, cytomegalovirus (CMV)-negative unit of type O, RhD-negative red blood cells is cross-matched to a maternal blood sample. Extended cross-matching to the mother can decrease the chance of new antibody formation. The unit is leukoreduced and irradiated with 25 Gy to prevent graft-versus-host reaction. It is then washed and packed to a final hematocrit of approximately 75% to 80% to prevent volume overload in the fetus.

The patient is admitted to the labor and delivery unit as an outpatient. The procedure is typically performed in the operating room, especially when a viable gestational age has been reached should an emergency delivery be necessary. The skin is prepped with hexachlorophene, and sterile drapes are applied. A long-acting local anesthetic is administered, and conscious sedation may help alleviate the patient's anxiety. A 20-gauge

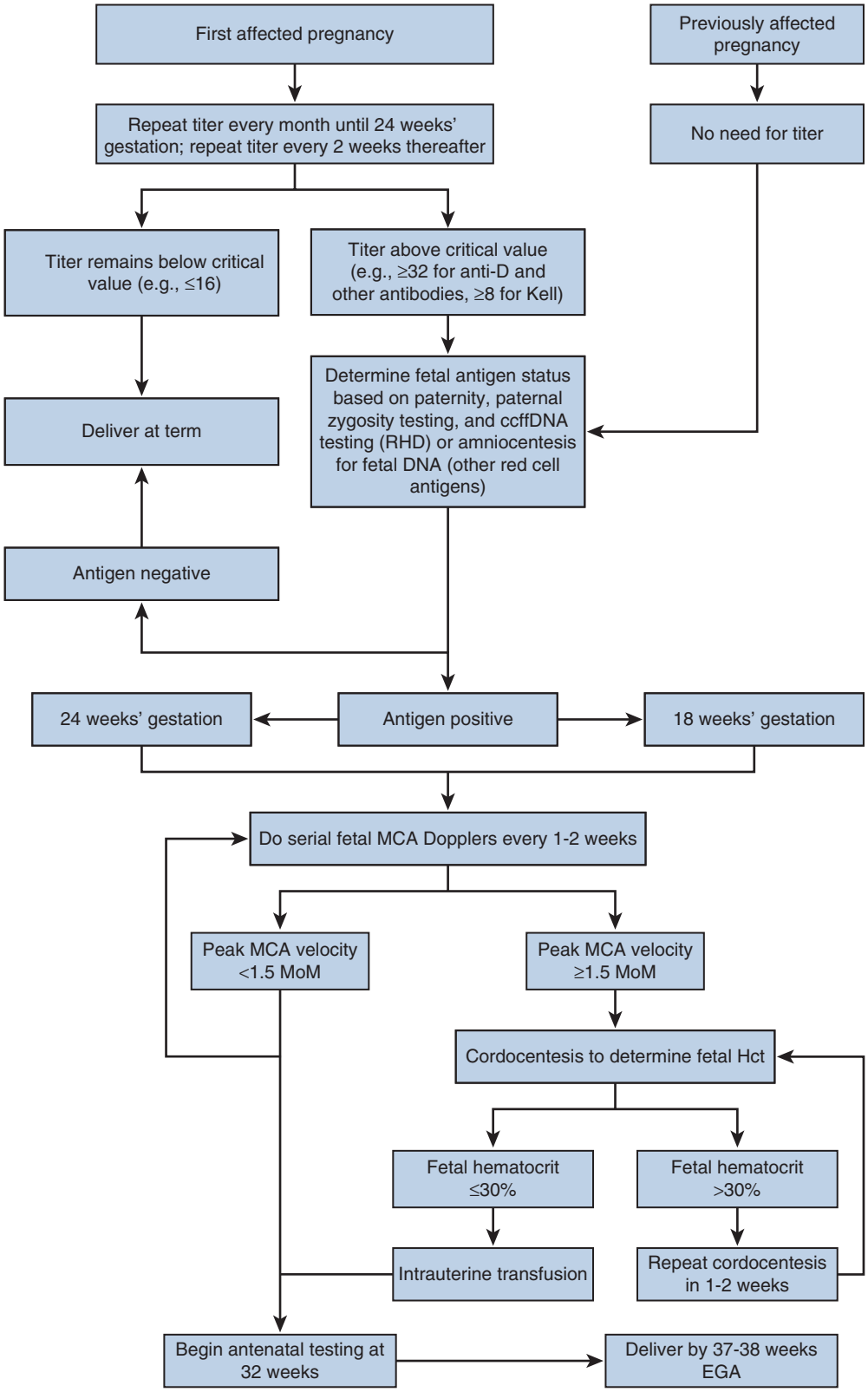


**FIG 34-8** Algorithm for clinical management of a patient with red cell alloimmunization. EGA, estimated gestational age; Hct, hematocrit; MCA, middle cerebral artery; MoM, multiples of the median.

procedure needle (a 22-gauge needle is used for gestations <22 weeks) is introduced into the amniotic cavity and then into the umbilical vein under continuous ultrasound guidance. In the case of an anterior placenta, the needle is passed through the placental mass into the cord root. With a posterior placentation, the cord insertion into the placenta is preferred because this represents a site of immobility compared with a "floating" loop of cord. A sample of fetal blood is obtained for an initial hematocrit. Optimally, the sample is processed as a spun hematocrit or through the use of an automated hemocytometer located in the operating room. A short-term paralytic agent such as vecuronium (0.01 mg/kg of estimated fetal weight [EFW]) is administered into the umbilical vein, causing cessation of fetal movement. A short-acting narcotic such as fentanyl (2 to 3 μg/kg EFW) can also be used and can be mixed with the vecuronium.[82] Paralysis is almost immediate and lasts 2 to 3 hours. The amount of packed red blood cells to be infused is based on the EFW determined by ultrasound. Using a donor unit hematocrit of 78%, a factor of 0.02 multiplied by the EFW in grams will calculate the dose of red cells to be administered to raise the fetal hematocrit by 10%.[83] Red cells are actively infused through the use of a syringe and sterile tubing connected to the donor unit. Once the predetermined volume of blood is infused, a small aliquot of blood is obtained to measure the hematocrit, as well as the percentage of fetal versus adult hemoglobin-containing red cells, through either a KB stain or flow cytometry. A final fetal hematocrit of 40% is targeted. After the first IUT, subsequent procedures can be empirically scheduled at 14-day intervals until suppression of fetal erythropoiesis is noted. This usually occurs by the third IUT. Thereafter, the interval for repeat procedures can be determined based on the decline in hematocrit for the individual fetus, usually a 3- to 4-week interval. **The PSV in the MCA has been shown to be useful in timing the second IUT.** After the second procedure, the MCA Doppler loses its validity in predicting fetal anemia, perhaps due to the changing rheology of the transfused adult red cells that make up the majority of the fetal red mass after serial IUTs.[84] The final IUT procedure is usually not performed past 35 weeks' gestation, and the patient is scheduled for delivery approximately 3 weeks later. The administration of oral phenobarbital (30 mg tid) for 10 days prior to delivery has been shown in one retrospective study to decrease the need for neonatal exchange transfusions for hyperbilirubinemia by 75%.[85]

**Severely anemic fetuses in the early second trimester do not tolerate the acute correction of their hematocrit to normal values.**[86] In these situations, the initial hematocrit should not be increased by more than fourfold at the time of the first procedure. A repeat IVT is then performed within 48 hours to correct the fetal hematocrit into the normal range.[87]

## Complications and Outcome

**Complications from IUT are uncommon.** The total procedure-related perinatal loss was 3.8% of fetuses and 1.2% of procedures in one series of over 300 procedures.[88] Survival after IUT varies with the center, its experience, and the presence of hydrops fetalis. **An overall survival rate of 91% has been reported in one series of over 1400 procedures.**[89] The presence of fetal hydrops, particularly if this does not resolve after several IUTs, has been associated with a lower rate of perinatal survival.[90] Preterm premature rupture of the membranes (PPROM) and chorioamnionitis occur rarely. Fetal bradycardia is usually transient, particularly when there is inadvertent puncture of the umbilical artery, and responds to removal of the procedure needle. Progression to fetal distress with the need for emergency delivery increases with advancing gestation and may complicate as many as 5% of procedures after 32 weeks' gestation.[91]

## Neonatal Transfusions

The practice of prolonging the gestation of the treated fetus with HDFN until near term has resulted in a virtual absence of the need for neonatal exchange transfusions. Typically, these infants are born with a virtual absence of reticulocytes with a red cell population that consists mainly of transfused red cells. The blood bank may be confused if cord blood at delivery is submitted for neonatal red cell typing—the neonate will be typed as O, RhD-negative, reflecting the antigen status of the donor blood used for the IUTs. Elevated levels of circulating maternal antibodies in the neonatal circulation in conjunction with suppression of the fetal bone marrow production of red cells often results in the need for neonatal red cell "top-up" transfusions after discharge from the nursery; this occurs in approximately 50% of infants near 1 month of age.[92] **Therefore these children should be followed weekly with hematocrits and reticulocyte counts until recovery of hematopoietic function is evident.** Typically, only one neonatal transfusion is required, although a maximum of up to three has been reported. Supplemental iron therapy in these infants is unnecessary because they have excess levels of stored iron due to previous hemolysis in utero and lysis of red cells from the IUTs. Supplemental folate therapy (0.5 mg/day) should be considered.

## Neurologic Outcome

Going forward, more data should be available to counsel the patient regarding long-term neonatal outcomes because fetuses with severe anemia and hydrops are likely to survive today secondary to the use of IVTs. **A study of almost 300 children treated with IUTs for HDFN found an overall incidence of neurodevelopmental impairment of 4.8%.**[93] Severe hydrops was associated with an elevenfold increase in neurologic problems.

Elevated levels of bilirubin have been associated with hearing loss in the neonate. Therefore, newborn screening for hearing loss would appear to be warranted in children with HDFN. Follow-up screening at 1 and 2 years of age should be considered.

# OTHER TREATMENT MODALITIES

Before the advent of the IUT, maternal plasmapheresis represented one of the few therapeutic modalities for severe HDFN. Most literature reports include single cases or relatively small case series. Despite these limitations, a review[94] of the published cases reveals a perinatal survival rate of 69%. Intravenous immune globulin (IVIG) has also been used effectively as the sole antenatal treatment for HDFN. Hydrops fetalis was less likely to occur, and the onset of anemia occurred later in pregnancies treated with IVIG. **Some experts have proposed a combined approach in patients with a previous perinatal loss in the early second trimester when technical limitations make the success of IUT unlikely.**[95] Plasmapheresis is started at 12 weeks' gestation and repeated three times in that week. The maternal titer should be expected to be reduced by 50%.

IVIG is then given to replace the globulin fraction removed by plasmapheresis in the form of a 2 g/kg loading dose after the third plasmapheresis; this is followed by 1 g/kg/week of IVIG until 20 weeks' gestation.

## FUTURE THERAPEUTIC OPTIONS

Patients with high anti–red cell titers and recurrent perinatal loss in the second trimester have few options other than artificial insemination with red cell antigen–negative donor semen, surrogate pregnancy, or preimplantation diagnosis (if the father is heterozygous). **Peptides associated with the proliferation of T-helper cells in the development of antibody to the RhD antigen and monoclonal anti-D blocking antibodies are currently being investigated to ameliorate an established anti-D response, thereby preventing severe HDFN in a subsequent pregnancy.**[96,97] Proteasome inhibitors used in in the suppression of antibodies in transplant rejection and in cases of multiple myeloma may prove useful for suppression of RhD alloimmunization prior to pregnancy.[98]

## HEMOLYTIC DISEASE OF THE FETUS AND NEWBORN DUE TO NON-RhD ANTIBODIES

**Antibodies to the red cell antigens Lewis, I, M, and P are often encountered through antibody screening during prenatal care. Because these antibodies are typically of the IgM class, they are not associated with HDFN.**[99]

However, antibodies to more than 50 other red cell antigens have been reported to be associated with HDFN (Table 34-2). **More important, only three antibodies—anti-RhD, anti-Rhc, and anti-Kell (K1)—cause significant enough fetal hemolysis that treatment with IUT is considered necessary.** In one series from a tertiary care center for IUT in the Netherlands, 85% of cases involved anti-D; 10%, anti-K1; and 3.5%, anti-c. In addition, one case each of anti-E, anti-e, and anti-Fy$^a$ was also reported.[100]

### Rhc

Anti-c antibody should be considered equivalent to anti-D regarding its potential to cause HDFN. In one report, 25% of

**TABLE 34-2** NON-RhD ANTIBODIES AND ASSOCIATED HEMOLYTIC DISEASE OF THE FETUS AND NEWBORN

| ANTIGEN SYSTEM | SPECIFIC ANTIGEN | ANTIGEN SYSTEM | SPECIFIC ANTIGEN | ANTIGEN SYSTEM | SPECIFIC ANTIGEN |
|---|---|---|---|---|---|
| **Frequently Associated With Severe Disease** | | | | | |
| Kell | -K (K1) | | | | |
| Rhesus | -c | | | | |
| **Infrequently Associated With Severe Disease** | | | | | |
| Colton | -Co$^a$ | MNS | -Mur | Scianna | -Sc2 |
| | -Co3 | | -M$^V$ | | -Rd |
| Diego | -ELO | | -s | Other Ags | -Bi |
| | -Di$^a$ | | -s$^D$ | | -Good |
| | -Di$^b$ | | -S | | - |
| Heibel | | | | | |
| | -Wr$^a$ | | -U | | -HJK |
| | -Wr$^b$ | | -Vw | | -Ht$^a$ |
| Duffy | -Fy$^a$ | Rhesus | -Be$^a$ | | -Jones |
| Kell | -Js$^b$ | | -C | | -Joslin |
| | -k (K2) | | -Ce | | -Kg |
| | -Kp$^a$ | | -C$^w$ | | -Kuhn |
| | -Kp$^b$ | | -ce | | -Li$^a$ |
| | -K11 | | -E | | -MAM |
| | -K22 | | -E$^w$ | | - |
| Niemetz | | | | | |
| | -Ku | | -Evans | | -REIT |
| | -Ul$^a$ | | -G | | -Reiter |
| Kidd | -Jk$^a$ | | -Go$^a$ | | -Rd |
| MNS | -En$^a$ | | -Hr | | -Sharp |
| | -Far | | -Hr$_o$ | | -Vel |
| | -Hil | | -JAL | | -Zd |
| | -Hut | | -Rh32 | | |
| | -M | | -Rh42 | | |
| | -Mi$^a$ | | -Rh46 | | |
| | -Mt$^a$ | | -STEM | | |
| | -MUT | | -Tar | | |
| **Associated With Mild Disease** | | | | | |
| Duffy | -Fy$^b$ | Kidd | -Jk$^b$ | Rhesus | -Riv |
| | -Fy$^3$ | | -Jk$^3$ | | -RH29 |
| Gerbich | -Ge$^2$ | MNS | -Mit | Other | -At$^a$ |
| | -Ge$^3$ | Rhesus | -C$^X$ | | -JFV |
| | -Ge$^4$ | | -D$^w$ | | -Jr$^a$ |
| | -Ls$^a$ | | -e | | -Lan |
| Kell | -Js$^a$ | | -HOFM | | |
| | | | -LOCR | | |

From Moise KJ. Hemolytic disease of the fetus and newborn. In Creasy RK, Resnik R, Iams J, eds. *Maternal-Fetal Medicine, Principles and Practice,* ed 5. Philadelphia: Elsevier; 2004.
*Ag*, antigen; *HDFN,* hemolytic disease of the fetus and newborn.

**TABLE 34-3** GENE FREQUENCIES (%) AND ZYGOSITY (%) FOR OTHER RED CELL ANTIGENS ASSOCIATED WITH HEMOLYTIC DISEASE OF THE NEONATE

| | WHITE | | BLACK | | HISPANIC | |
|---|---|---|---|---|---|---|
| | Antigen + | Heterozygous | Antigen + | Heterozygous | Antigen + | Heterozygous |
| C | 70 | 50 | 30 | 32 | 81 | 51 |
| c | 80 | 50 | 96 | 32 | 76 | 51 |
| E | 32 | 29 | 23 | 21 | 41 | 36 |
| e | 97 | 29 | 98 | 21 | 95 | 36 |
| K (K1) | 9 | 97.8 | 2 | 100 | | |
| k (K2) | 99.8 | 8.8 | 100 | 2 | | |
| M | 78 | 64 | 70 | 63 | | |
| N | 77 | 65 | 74 | 60 | | |
| S | 55 | 80 | 31 | 90 | | |
| s | 89 | 50 | 97 | 29 | | |
| U | 100 | — | 99 | — | | |
| $Fy^a$ | 66 | 26 | 10 | 90 | | |
| $Fy^b$ | 83 | 41 | 23 | 96 | | |
| $Jk^a$ | 77 | 36 | 91 | 63 | | |
| $Jk^b$ | 72 | 32 | 43 | 21 | | |

Modified from Moise KJ. Hemolytic disease of the fetus and newborn. In Creasy RK, Resnik R, Iams J, eds. *Maternal-Fetal Medicine: Principles and Practice,* ed 5. Philadelphia: Elsevier; 2004.

antigen-positive fetuses were noted to have severe HDFN, 7% were hydropic, and 17% required IUTs for therapy.[101]

## RhC, RhE, and Rhe

RhC, RhE, and Rhe antibodies are often found in low titer in the alloimmunized patient with anti-D. Their presence may be additive to the fetal hemolytic effect of anti-D.[102] When they occur alone, mild HDFN is usually the clinical course. Only a handful of case reports have indicated the need for treatment with IUT with each of these antibodies.[100,103]

## Duffy

The Duffy antigen system consists of two antigens, $Fy^a$ and $Fy^b$. Only anti-$Fy^a$ has been associated with mild HDFN.[104]

## Kidd

The Kidd antigen system consists of two antigens, $Jk^a$ and $Jk^b$. Rare cases of mild HDFN have been reported.

## Kell

The Kell antigen system includes 23 different members. Antibodies to at least nine of the Kell antigens have been associated with HDFN. The most common of these is Kell (also designated *K, K1*) and cellano (*k, K2*). Additional antibodies that have been reported to be causative for HDFN include -Penny (*$Kp^a$, K3*), -Rautenberg (*$Kp^b$, K4*), -Peltz (*Ku, K5*), -Sutter (*$Js^a$, K6*), -Matthews (*$Js^b$, K7*), -Karhula (*$Ul^a$, K10*) and -K22.[95] Unlike the case of other hemolytic antibodies, fetal anemia due to Kell (anti-K1) sensitization is thought to be secondary to not only hemolysis but also to suppression of fetal erythropoiesis.[105]

**The majority of cases of K1 sensitization are secondary to previous maternal blood transfusion, usually as a result of postpartum hemorrhage in a previous pregnancy. Because 92% of individuals are Kell negative, the initial management of the K1-sensitized pregnancy should entail paternal red cell typing and genotype testing.** If the paternal typing returns K1-negative (kk) and paternity is assured, no further maternal testing is undertaken. The majority of Kell-positive individuals will be heterozygous (Table 34-3). Amniocentesis can be used to determine the fetal genotype in these cases because ccffDNA for fetal Kell typing is currently only available in Europe. **A lower maternal critical antibody value of 8 has been proposed to begin fetal surveillance.[106] Serial MCA Doppler studies have proven effective in detecting fetal anemia.[107]**

### KEY POINTS

- Alloimmunization to the RhD, Kell (K1), and Rhc red cell antigens is the main cause for severe HDFN.
- Despite the widespread use of RhIG, approximately six cases of RhD alloimmunization occur annually per 1000 live births in the United States.
- *Hydrops fetalis* is defined as extracellular fluid in two fetal compartments; it represents the end-stage of fetal anemia in HDFN.
- The rhesus D, C, c, E, and e antigens are coded by two genes located on the short arm of chromosome 1.
- The rule of thumb should be to administer RhIG when in doubt, rather than to withhold it.
- A critical maternal antibody titer can be used in the first affected pregnancy to decide when to begin further fetal testing.
- The fetal peak systolic MCA Doppler velocity can be used to determine the onset of fetal anemia.
- In the case of a heterozygous paternal phenotype for a particular red cell antigen, fetal typing can be undertaken through ccffDNA in maternal plasma for the *RHD* gene; fetal DNA typing for other red cell antigens can be obtained through amniocentesis.
- Intravascular fetal intrauterine transfusions are the mainstay of fetal therapy with an overall perinatal survival of greater than 90%.
- Except in cases of alloimmunization to Kell antigens, irregular red cell antibodies in pregnancy should be managed in a similar fashion to RhD.

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