Management of Rhesus Alloimmunization in Pregnancy

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Hemolytic disease of the newborn secondary to rhesus alloimmunization was once a major contributor to perinatal morbidity and mortality. Today, rhesus immune globulin has markedly decreased the prevalence of this disease so that only one to six cases occur in every 1000 live births. The rarity of this condition warrants consideration of consultation or referral to a maternal-fetal medicine specialist. Once sensitization occurs, rhesus immune globulin is no longer effective. Evaluation for the presence of maternal anti-D antibody should be undertaken at the first prenatal visit. First-time sensitized pregnancies are followed with serial maternal titers and, when necessary, serial amniocenteses to detect fetal bilirubin by $\Delta OD_{450}$. In cases of a heterozygous paternal genotype, new deoxyribonucleic acid techniques now make it possible to diagnose the fetal blood type through amniocentesis or even from plasma/deoxyribonucleic acid analysis. When there is a history of an affected fetus or infant, maternal titers are no longer diagnostic as a screening test. Serial peak middle cerebral artery velocities using Doppler ultrasound can be used in these pregnancies to detect fetal anemia. In some situations, intrauterine transfusion is necessary through ultrasound-directed puncture of the umbilical cord with the direct intravascular injection of red cells. Perinatal survival rates of more than 90% have been reported; hydrops fetalis reduces the chance for a viable outcome by up to 25%. Immediate neonatal outcome is complicated by the need for repeated transfusions secondary to suppressed erythropoiesis. Long-term studies have revealed normal neurologic outcomes in more than 90% of cases. Future therapy will involve selective modulation of the maternal immune system making the need for intrauterine transfusions a rarity. (Obstet Gynecol 2002;100:600–11. © 2002 by The American College of Obstetricians and Gynecologists.)

With the dawn of the new millennium, medical science has made little impact on the major complications of pregnancy. The one notable exception is rhesus alloimmunization and its associated fetal/neonatal consequence—hemolytic disease of the newborn. In the early 1960s, the first successful in utero therapy was described. Later that decade, an effective method of prevention, rhesus immune globulin, was studied and introduced into clinical practice. By the end of the second millennium, new refinements in genetics and ultrasound had further advanced the care of what was once a major perinatal disease.

INCIDENCE

Although effective prophylaxis has been available since 1968, the Centers for Disease Control noted in 1991 that one in every 1000 live-born infants exhibited some effect from rhesus hemolytic disease.1 Although not considered as reliable a source of data, a 2000 review of US birth certificates indicated that 6.8 pregnancies per 1000 live births were complicated by rhesus sensitization.2
However, in the case of a large antenatal fetomaternal hemorrhage or a fetomaternal hemorrhage at delivery, maternal B lymphocyte clones that recognize the RhD antigen are established. The initial maternal immunoglobulin M anti-D production is short-lived with a rapid change to an immunoglobulin G response. Memory B lymphocytes then await a new antigenic exposure in the subsequent pregnancy. If stimulated by the RhD antigen on fetal erythrocytes, these plasma cells can then rapidly proliferate and produce immunoglobulin G antibodies and an increase in the maternal titer. Maternal immunoglobulin G crosses the placenta and destroys any RhD-positive erythrocytes, resulting in fetal anemia.

**PREVENTION OF RHESUS DISEASE**

The majority of rhesus immune globulin used in the United States is derived from human plasma that undergoes Cohn cold ethanol fractionation for purification. This process effectively inactivates the human immunodeficiency virus (HIV), hepatitis B and C viruses are more resistant to removal. Two outbreaks of hepatitis C related to the administration of rhesus immune globulin have been reported in Ireland and East Germany in the late 1970s, although none have occurred in the United States. However, screening techniques used in plasma donors today should effectively eliminate the risk from these infections. Since 1999, when nucleic acid amplification testing for HIV and hepatitis C has been added to serologic tests for these viruses, no case of post-transfusion infection with hepatitis C and only one case of transfusion-related HIV infection have been documented in over 25 million blood donations screened in the United States (personal communication, M. Brecher, 2002). Recent changes in production include the removal of the mercury-derived preservative, thimerosal, and the addition of ultrafiltration with micropore filters that remove the human parvovirus B19 particle as well as HIV and hepatitis A and C. Although once produced from the plasma of sensitized women, the decreasing prevalence of rhesus disease has necessitated the use of male donors that undergo repeated injections of RhD-positive red cells. Because rhesus immune globulin is a blood derivative, all patients should be informed of its source and give informed consent. Although the majority of rhesus immune globulin is issued from hospital blood banks, various manufacturers’ products can be purchased by private physicians for use in their offices. A recent recall by one manufacturer due to inadequate doses in the prefilled syringes warrants that careful records of lot numbers are documented in the patient’s medical chart and a general clinic logbook.

All pregnant patients should undergo an antibody screen at the first prenatal visit. Patients that are determined to be weak rhesus positive (previously D-negative) are not at risk for rhesus alloimmunization and therefore do not require rhesus immune globulin. If there is no evidence of anti-D alloimmunization in the RhD-negative woman, 300 μg of rhesus immune globulin should be administered intramuscularly at 28 weeks’ gestation. This practice has been reported to reduce the incidence of antenatal alloimmunization from 2% to 0.1%. The American Association of Blood Banks recommends that a repeat antibody screen be obtained before antenatal rhesus immune globulin, although the incidence of alloimmunization before 28 weeks is very low. The cost-effectiveness of this practice has been questioned by the ACOG. However, because antenatal sensitization occurring before 28 weeks can occasionally cause significant hemolytic disease of the newborn, it would appear prudent to repeat the maternal antibody screen in the RhD-negative patient. The maternal blood sample can be drawn at the same office visit as the rhesus immune globulin 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 because of the slow absorption from the intramuscular site. Although there are no data to provide guidance, some experts recommend that a second dose of rhesus immune globulin be given if the patient has not delivered by 40 weeks’ gestation.

Although not well studied, additional indications for the antepartum administration of rhesus immune globulin include spontaneous abortion, elective abortion, ectopic pregnancy, genetic amniocentesis, chorionic villous sampling, and fetal blood sampling. A dose of 50 μg of rhesus immune globulin is effective until 12 weeks’ gestation because of the small volume of red cells in the fetoplacental circulation. From a practical sense, most hospitals and offices do not stock this dose of rhesus immune globulin; therefore, a standard dose of 300 μg is often given. Evidence for the use of rhesus immune globulin in other scenarios that breach the fetoplacental barrier is lacking. Rhesus immune globulin should also be administered for such events as hydatidiform mole, threatened abortion, fetal death in the second or third trimester, blunt trauma to the abdomen, and external cephalic version.

Since the half-life of rhesus immune globulin is approximately 24 days, 15–20% of patients receiving it at 28 weeks will have a very low anti-D titer (usually 2 or 4) detected at the time of admission for labor at term. Three hundred micrograms of rhesus immune globulin should be administered within 72 hours of delivery if umbilical cord blood typing reveals a RhD-positive infant. This is sufficient to protect from sensitization.
caused by a fetomaternal hemorrhage of 30 mL of fetal whole blood. Approximately one in 1000 deliveries will be associated with an excessive fetomaternal hemorrhage; risk factors will only identify 50% of these. Routine screening of all women at the time of delivery for excessive fetomaternal hemorrhage should therefore be undertaken. A qualitative yet sensitive test for fetomaternal hemorrhage, the rosette test, is first performed. Results return as positive or negative. A negative result warrants administration of a standard dose of rhesus immune globulin. If the rosette is positive, a Kleihauer-Betke stain or fetal cell stain using flow cytometry is undertaken. The percentage of fetal blood cells is multiplied by a factor of 50 to estimate the volume of the fetomaternal hemorrhage. Because this calculation includes an inaccurate estimation of the maternal blood volume, the blood bank will typically indicate that additional vials of rhesus immune globulin should be administered over the calculated amount. No more than five units of rhesus immune globulin should be administered by the intramuscular route in one 24-hour period. This recommendation is based on the number and volume of injections that are required to administer this amount of rhesus immune globulin using prefilled syringes. Should a large dose of rhesus immune globulin be necessary, an alternative method would be to give the entire calculated dose intravenously using a new Food and Drug Administration-approved form of rhesus immune globulin (WinRhoSDF, Cangene Corp., Winnipeg, Manitoba, Canada). If rhesus immune globulin is 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. If delivery occurs less than 3 weeks from the administration of rhesus immune globulin used for antenatal indications such as amniocentesis for fetal lung maturity or external cephalic version, a repeat dose is unnecessary unless a large fetomaternal hemorrhage is detected at the time of delivery. Despite the widespread acceptance of similar guidelines, studies from Scotland have revealed that two-thirds of rhesus alloimmunized cases are secondary to antepartum sensitization, whereas an additional 13% are due to failure to administer rhesus immune globulin for the usual obstetric indications.7

If a patient is undergoing initial blood typing at delivery and a weak rhesus-positive result (formerly Du positive) is obtained, this may be secondary to a large fetomaternal hemorrhage causing a mixed field agglutination reaction and a false interpretation of the maternal blood type. A standard test for fetomaternal hemorrhage should be performed. If none is detected, then the weak rhesus-positive typing can be considered valid, and rhesus immune globulin is not required. The administration of rhesus immune globulin 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 rhesus immune globulin in these situations prudent. In addition, RhD sensitization would limit the availability of blood products if the patient later required a transfusion. Rhesus immune globulin is not effective once alloimmunization to the RhD antigen has occurred.

**DIAGNOSTIC APPROACH**

**Genetics**

In 1946, Fisher and Race8 proposed the concept of three genes that encode for the three major rhesus antigen groups—D, C/c, and E/e. Some 45 years later, the rhesus locus was localized to the short arm of chromosome one.9 Only two genes were identified—an RhD gene and an RhCE gene. Production of two distinct proteins from the latter gene probably occurs as a result of alternative messenger ribonucleic acid splicing. A single C to G transition in exon 5 of the RhCE gene results in formation of the ε antigen instead of the E antigen.10 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 ε antigen.11 These discoveries have resulted in major changes in the management of the RhD-sensitized pregnancy. In the case of a heterozygous father, amniocentesis can be used to detect the 50% of RhD-negative fetuses. In such situations, additional maternal or fetal testing is unwarranted as the maternal antibody will not affect the fetus. Because the RhC/c and E/e antigens are inherited in a closely linked fashion to RhD, blood banks can employ antisera to these antigens along with gene frequency tables based on ethnicity to determine the paternal zygosity at the RhD locus. In addition, mathematic modeling has been proposed to modify the incidence of heterozygosity based on the paternal history of RhD-positive offspring.12 As an example, a white partner who undergoes serologic testing with the following results—anti-D: positive, anti-C: negative, anti-c: positive, anti-E: negative, and anti-e: positive—would be considered Dce. If he had never fathered a RhD-positive child in the past, his chance of being heterozygous is 94% (Table 1). A history of previous RhD-positive offspring would decrease his chances of being heterozygous. Note that very different results for the same serologic findings cited in the above example occur if the male patient is not white. In the future, paternal zygosity testing will not use serology but instead will use quantitative polymerase chain reaction (PCR) per-
formed on genomic deoxyribonucleic acid (DNA) extracted from white blood cells.13

First described in 1993, amniocentesis is now accepted as the primary modality that is used to test the fetal blood type in cases of a heterozygous paternal genotype.14 Chorionic villus biopsy has been employed, but this should be discouraged in patients who wish to continue the pregnancy if the fetus is found to be RhD positive. Disruption of the chorionic villi during the procedure can result in fetomaternal hemorrhage and an anamnestic response in maternal titer, thereby worsening the fetal disease (Moise KJ Jr, Carpenter RJ Jr. Chorionic villus sampling for Rh typing: Clinical implications [letter]. Am J Obstet Gynecol 1993;168:1002–3). When amniocentesis is used for fetal typing, all attempts should be made to avoid transplacental passage of the needle so as to prevent this same phenomenon from occurring.

One must understand that these techniques assess the fetal genotype (DNA analysis of fetal cells in the amniotic fluid) and not the fetal phenotype (expression of the RhD antigen on the fetal red cells as determined by serology). Ultrasound-guided umbilical blood sampling can be used for serologic typing of the fetus but is associated with a four-fold or more rate of perinatal loss compared with amniocentesis. Extensive experience with the use of amniocentesis for determining the fetal blood type has revealed rare discrepancies between fetal genotype and phenotype. In the event of a PCR result that reveals an RhD-negative fetus when the fetus is RhD positive by serology, usual surveillance techniques would not be employed, and fetal loss can occur. In a review of reports of 500 amniocenteses in which four different sets of oligonucleotide primers were used, this occurred in 1.5% of cases.15 The overall sensitivity and specificity of PCR typing were 98.7% and 100%, respectively, and the positive and negative predictive values were 100% and 96.9%,15. The most likely etiology for inconsistencies is either erroneous paternity or a rearrangement of the paternal RhD gene locus. Such rearrangements have been documented in approximately 2% of individuals.16

Checking paternal blood (the source of the fetal RhD gene) with the same primers used on the amniotic fluid verifies that a gene rearrangement is not a potential source of error. For this reason, most laboratories offering fetal red cell antigen typing on amniotic fluid cells require an accompanying paternal blood sample. Hopkins17 assessed serial titers in patients with RhD alloimmunization who subsequently delivered RhD-negative offspring and noted that serial maternal titers rose by four-fold in less than 2% of cases. Therefore, if paternity is unknown or the patient’s partner is not available, a repeat maternal antibody titer should be obtained 4–6 weeks later as a confirmatory strategy. If a four-fold rise in antibody titer is noted, then an RhD-negative PCR result on amniotic fluid is suspect. Repeat amniocentesis to evaluate the ΔOD450 or fetal blood sampling to determine the fetal RhD status using serologic techniques should be considered.

Recently, an RhD pseudogene has been described in 69% of South African blacks and 21% of blacks.18 In this situation, the pregnant patient is RhD negative on serologic testing, but the entire RhD gene is present on her chromosomes. Because the fetus inherits one of its RhD genes from its mother, amniotic PCR testing would, therefore, yield a false-positive result—the fetus is RhD negative by serology but RhD positive by genotype. This could lead to unnecessary intervention with its inherent risks. For this reason, a maternal blood sample should also accompany the amniotic fluid sent for fetal RhD testing in an effort to rule out the presence of a maternal RhD pseudogene. If the maternal sample is positive for this variant, then fetal testing for the gene should also be undertaken (Figure 1).

In the future, noninvasive fetal testing for the RhD gene will be routinely available. Sorting of maternal blood for fetal cells using flow cytometry has been reported to be successful in identifying an RhD-positive fetus.19 More recently, free fetal DNA in the maternal plasma or serum has been used to detect RhD sequences.

**Table 1.** Incidence of Paternal Heterozygosity (%) Based on Serology, Ethnic Background, and Number of Previous Rhesus D-Positive Offspring

<table>
<thead>
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<th>RhD+ infants</th>
<th>White</th>
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<th>Hispanic</th>
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<td>94</td>
<td>89</td>
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RhD+ = rhesus D positive.
in the case of an RhD-positive fetus. This diagnostic test is now being employed in some centers in Europe.

**Maternal Titer**

The maternal titer is the first step in the evaluation of the RhD-sensitized patient. Old methodologies using albumin or saline should no longer be employed. The human antiglobulin titer (indirect Coombs) is used to determine the degree of alloimmunization. By convention, titer values are reported as the integer of the greatest tube dilution with a positive agglutination reaction (i.e., a titer of 16 is equivalent to a dilution of 1:16). Variation in results between laboratories is not uncommon because many commercial laboratories use enhancing media so as to not miss low titer samples. However, in the same laboratory, the titer should not vary by more than one dilution. Thus, an initial titer of 8 that returns 16 may not represent a true increase in the amount of antibody in the maternal circulation. A critical titer is defined as the titer associated with a significant risk for fetal hydrops. When this is present, further testing is required with more invasive techniques. This titer will vary with institutions based on the correlation with clinical outcome of hemolytic disease of the newborn; however, in most centers, a critical value for anti-D between 8 and 32 is used.

In Europe and the United Kingdom, the amount of circulating anti-D is compared with an international standard and reported in IU/mL. A threshold value of 15 IU/mL has been recommended for invasive testing as only mild hemolytic disease of the newborn is usually noted with anti-D levels below this level.

**Ultrasound**

Ultrasound plays a key role in the management of the alloimmunized pregnancy. It should be employed early in the pregnancy to establish the correct gestational age because this parameter becomes important in determining such normative laboratory values as amniotic fluid bilirubin levels (ΔOD450). A variety of ultrasonographic parameters have been used in an attempt to determine when fetal anemia is present. These have included placental thickness, umbilical vein diameter, hepatic length,
splenic perimeter, and polyhydramnios. Most of these have not proven reliable in clinical practice. Fetal hydrops can be detected and is usually heralded by the onset of ascites. Late findings include pleural effusions and scalp edema. Hydrops fetalis should be considered end-stage hemolytic disease because the fetal hemoglobin is often one-third of normal or less in these situations. Recently, Doppler assessment of the peak velocity in the fetal middle cerebral artery has proven accurate in the determination of fetal anemia. In one study, a value above 1.5 multiples of the median for gestational age detected all cases of anemia with a false-positive rate of only 12% (Figure 2). More recently, these investigators developed a formula for predicting the fetal hemoglobin using the middle cerebral artery peak velocity. The difference between the observed and calculated hemoglobin was lower in fetuses that exhibited moderate-to-severe anemia as compared with situations in which the fetus was mildly anemic or not anemic. This would indicate that the middle cerebral artery performs better in cases of clinically significant fetal anemia.

The fetal middle cerebral artery closest to the maternal skin should be evaluated using a minimal angle of insonation; the Doppler gate is placed over the vessel just as it bifurcates from the carotid siphon. Color Doppler aids in determining the correct location. After 35 weeks’ gestation,
there appears to be a higher false-positive rate in the detection of fetal anemia (personal communication, G. Mari, 2002). Therefore, until more data are available, it would appear prudent to use serial amniocenteses for \( \Delta OD_{450} \) assessment (see below) after this gestation. Because a learning curve is associated with performing middle cerebral artery Doppler, a center with minimal experience with this technique should initially perform these in conjunction with serial amniocenteses for \( \Delta OD_{450} \). Clearly, middle cerebral artery Doppler is proving to revolutionize the care of the RhD-sensitized pregnancy by potentially minimizing invasive diagnostic testing and may replace amniocentesis for \( \Delta OD_{450} \) in the future.

**Amniocentesis**

Since it was first introduced to clinical practice by Liley in 1961, the spectral analysis of amniotic fluid at 450 nm (\( \Delta OD_{450} \)) has been used to measure the level of bilirubin, an indirect indicator of the degree of fetal hemolysis. The original Liley curve was divided into three zones and remains useful after 27 weeks’ gestation. Extrapolated Liley curves to earlier gestational ages underestimate the level of fetal disease and should not be used. A modified curve for such gestations has been proposed by Queenan et al and involves four zones instead of three (Figure 3). If amniocentesis is used to monitor fetal disease, serial procedures are undertaken at 10-day to 2-week intervals and continued until delivery to follow trends in the \( \Delta OD_{450} \) values. As mentioned earlier, all attempts should be made to avoid transplacental passage of the needle because this can lead to fetomaternal hemorrhage and a rise in maternal antibody titer. A rising or plateauing trend of \( \Delta OD_{450} \) values that reaches the 80th percentile of zone two on the Liley curve or enters the intratuterine transfusion zone of the Queenan curve necessitates investigation by fetal blood sampling. If serial middle cerebral artery Doppler measurements are employed for fetal surveillance instead of amniocenteses, one should consider switching to amniocentesis as the primary means of assessing the fetal state after 35 weeks’ gestation because a higher false-positive rate has been reported with middle cerebral artery determinations after this gestation. After 37 weeks’ gestation, fetal lung maturity testing can be assessed as induction of labor with mature studies can be considered in lieu of subsequent amniocentesis. Fluorescence depolarization techniques (TDx-FLM, Abbott Laboratories, Abbot Park, IL) should not be employed as the value can be falsely elevated because of excess bilirubin; the lamellar body count or lecithin-sphingomyelin ratio is reliable as these assays are not affected by the excess bilirubin.

**Fetal Blood Sampling**

Ultrasound-directed fetal blood sampling (also percutaneous umbilical blood sampling, cordocentesis, and fumipuncture) allows direct access to the fetal circulation to obtain important laboratory values such as hematocrit, direct Coombs, fetal blood type, reticulocyte count, and total bilirubin. Serial fetal blood samplings have been proposed as one method of following alloimmunized pregnancies after a maternal critical titer is reached. However, because this procedure is associated with a 1–2% rate of fetal loss, it is usually reserved for patients with elevated \( \Delta OD_{450} \) values or elevated peak middle cerebral artery Doppler velocities. When used in this context, blood should be available for intravascular intrauterine transfusion if fetal anemia is detected (hematocrit less than 30% or less than two standard deviations for gestational age).

**SUMMARY OF CLINICAL MANAGEMENT**

The approach using the armamentarium of the various diagnostic tools varies based on the history of fetal or neonatal hemolytic disease (Figure 4). As a general rule, the patient’s first RhD-sensitized pregnancy involves minimal fetal/neonatal disease; subsequent gestations are associated with a worsening degree of anemia. The rarity of this condition warrants consideration of consultation or referral to a maternal-fetal specialist.

**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. Paternal blood is drawn to determine RhD status and zygosity. In cases of a heterozygous paternal phenotype, once a critical titer is reached (usually 32), an amniotic fluid sample along with maternal and paternal blood samples are sent to a DNA reference laboratory at the time of first amniocentesis to determine the fetal RhD status. If the fetus is determined to be RhD positive, serial amniocenteses for \( \Delta OD_{450} \) are initiated and repeated at 10-day to 2-week intervals. In the case of an RhD-negative paternal blood type or fetal RhD-negative genotype at amniocentesis, further maternal and fetal monitoring is unwarranted as long as paternity is assured.

If there is evidence of an RhD-positive fetus (homozygous paternal phenotype, or RhD-positive fetus by PCR testing on amniotic fluid), serial amniocenteses are continued. If a rising or plateauing \( \Delta OD_{450} \) trend into the 80th percentile of zone two of the Liley curve or a value in the intratuterine transfusion zone of Queenan curve is noted, fetal blood sampling is undertaken with blood
readied for intrauterine transfusion if the fetal hematocrit is less than 30%. If no rise in the ΔOD₄₅₀ values is detected, the last amniocentesis should be performed at 37 weeks. If the lecithin-sphingomyelin ratio or lamellar body count indicates fetal maturity, then induction at 38–39 weeks’ gestation would appear warranted in lieu of additional amniocenteses.

If serial middle cerebral artery Doppler assessments are used as the primary means of fetal surveillance, one amniocentesis should be performed in the case of a heterozygous paternal phenotype to determine the fetal RhD status. If one notes a homozygous paternal phenotype or RhD-positive fetus by PCR testing, serial middle cerebral artery testing should be continued until 35 weeks. One or more amniocentesis late in gestation for ΔOD₄₅₀ should be performed, and fetal pulmonary studies at 37 weeks’ gestation should be obtained. Induction should occur at approximately 38–39 weeks if fetal lung maturity is noted.

**Previously Affected Fetus or Infant**

If there is a history of a previous perinatal loss related to hemolytic disease of the newborn, a previous need for intrauterine transfusion, or a previous need for neonatal exchange transfusion, the patient should be referred to a perinatal 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, amniocentesis should be performed at 15 weeks’ gestation.
gestation to determine the fetal RhD status. Many centers are now using serial middle cerebral artery Doppler measurements to monitor these pregnancies at risk for fetal hemolytic disease. Testing should begin at 18 weeks' gestation and repeated every 1–2 weeks. Alternatively, serial amniocenteses for measurement of $\Delta$OD$_{450}$ can be used with the Queenan curve for reference values. If a rising $\Delta$OD$_{450}$ value into the intrauterine transfusion zone of the Queenan curve or a rising value for peak middle cerebral artery Doppler velocity greater than 1.5 multiples of the median is found, a fetal blood sampling is performed with blood readied for intrauterine transfusion if the fetal hematocrit is found to be less than 30%.

**THERAPEUTIC APPROACH**

**Intrauterine Transfusion**

Historically, the intraperitoneal transfusion remained the mainstay of fetal therapy for almost 20 years after its introduction by Liley in 1963. With the advent of real-time ultrasound for guidance, direct access to the fetal circulation by puncturing the umbilical cord at its placental insertion became commonplace. As a result, the direct intravascular transfusion has replaced the intraperitoneal transfusion in most centers. Compared with the intraperitoneal transfusion, the intravascular transfusion is clearly advantageous to the hydropic fetus where absorption of cells from the peritoneal cavity is compromised. Some centers continue to incorporate the intraperitoneal transfusion in the form of a combined procedure in conjunction with the intravascular transfusion; many European centers prefer to use the intrahepatic portion of the umbilical vein as the site of intravascular transfusion.

The source of red cells for intrauterine transfusion is typically a blood type O, RhD-negative, cytomegalovirus-negative donor. Cells are packed to a hematocrit of 75–85% to prevent volume overload. Units are irradiated to prevent graft-versus-host reaction and processed through a leukocyte-poor filter. Some centers prefer to use maternal blood as the source of red cells. Advantages include the potential to decrease the risk for sensitization to new red cell antigens associated with exposure to donor units. In addition, a fresh unit can be routinely acquired. Repeated maternal donations are possible with additional folate and iron supplementation. Such donations produce a maternal reticulocytosis that enhances the average lifespan of the donor red cells. This has the potential to decrease the total number of intravascular transfusions that are necessary. Patients must still undergo rigorous Food and Drug Administration testing for infectious markers with each donation. In addition, washing of the cells is required to remove any maternal serum containing anti-D antibody.

At the start of the intravascular transfusion procedure, an initial fetal hematocrit is determined after puncture of the umbilical cord near the placental insertion. A paralyzing agent is usually administered to cause cessation of fetal movement, although this may be omitted in cases of anterior placentaion. Our center uses vecuronium at a dose of 0.1 mg/kg of estimated fetal weight. The total amount of red cells to transfuse will depend on the initial fetal hematocrit, gestational age, and hematocrit of the donor unit. If the donor unit has a hematocrit of approximately 75%, the estimated fetal weight in grams using ultrasound can be multiplied by a factor of 0.02 to determine the volume of red cells to be transfused to achieve a hematocrit increment of 10%. A final target hematocrit of 40–50% is used; a decline of approximately 1% per day can be anticipated between transfusions. In the extremely anemic fetus, the initial hematocrit should not be increased by more than four-fold to allow the fetal cardiovascular system to compensate for the acute change in viscosity. A repeat procedure is undertaken 48 hours later to normalize the fetal hematocrit in cases of severe fetal anemia. Hydrops will usually reverse rapidly after one or two intravascular transfusions; placentomegaly is the last feature of the hydropic state to reverse. If the fetus is not severely anemic at the first intrauterine transfusion, subsequent procedures are scheduled at 14-day intervals until suppression of fetal erythropoiesis is noted on Kleihauer-Betke stains. This usually occurs by the third intrauterine transfusion. Thereafter, the interval for repeat procedures can be determined based on the decline in hematocrit for the individual fetus, usually a 3–4 week interval.

During the era of intraperitoneal transfusions, fetuses were routinely delivered at 32 weeks' gestation and often suffered complications of prematurity such as hyaline membrane disease and the need for neonatal exchange transfusions for the treatment of hyperbilirubinemia. As experience with intravascular transfusion became widespread, pregnancies were delivered at later gestational ages. Most authorities will now perform the final intrauterine transfusion at up to 35 weeks' gestation, with delivery anticipated at 37–38 weeks. Such a practice allows maturation of both the pulmonary and hepatic enzyme systems virtually eliminating the need for neonatal exchange transfusions. After a viable gestational age is attained, performing the transfusion in immediate proximity to the labor and delivery suite appears prudent so that operative delivery can be undertaken if fetal distress should occur.
Short-Term Outcome
Perinatal survival after intrauterine transfusion varies by center and the experience of the operator. Clearly, intervention before the appearance of hydrops fetalis is preferable. In one review series, overall survival was noted to be 84%. Survival of nonhydropic fetuses (92%) was markedly improved over those with hydrops (70%). Suppression of erythropoiesis is not uncommon after several intravascular transfusions. These infants are born with a virtual absence of reticulocytes with their blood volume being almost entirely comprised of donor red cells. Because exchange transfusion is rarely required, passively acquired maternal antibodies remain in the neonatal circulation for weeks. This results in a 1–3 month period in which the infant may need several top-up red cell transfusions. Weekly neonatal hematocrit and reticulocyte counts should be assessed. Threshold hematocrit values of less than 30% in the symptomatic infant or less than 20% in the asymptomatic infant have been suggested for transfusion. A neonatal trial with subcutaneous erythropoietin administered three times a week revealed a decreased need for top-up transfusions.

Long-Term Outcome
Advances in treatment techniques of the fetus with severe hemolytic disease of the newborn now allow the more moribund and severely anemic fetus to survive. Several investigations have not found hydrops fetalis to be associated with any difference in neurologic outcome compared with the nonhydropic fetus. Cerebral palsy and developmental delay is more common in fetuses with hemolytic disease of the newborn when compared with unaffected infants, although a normal outcome can be expected in more than 90% of cases. A review of the literature with unaffected infants, although a normal outcome can be expected to decline by several dilutions. However, previous experience with serial plasmaphereses alone has indicated that a rebound phenomenon occurs with antibody levels exceeding preplasmapheresis values. For this reason, the immunoglobulin G pool is replaced after the third procedure by administering a 1-g/kg loading dose of intravenous immune globulin diluted in normal saline 10% solution. The patient is premedicated with 25 mg of intravenous diphenhydramine hydrochloride. The intravenous immune globulin infusion is started at a rate of 60 mL per hour and increased by 30 mL per hour every 30 minutes to a maximum rate of 240 mL per hour. A second dose of 1 g/kg intravenous immune globulin is given the following day. The patient is then treated with a weekly dose of 1 g/kg intravenous immune globulin until 20 weeks gestation. This dosing regimen gives plasma levels of immunoglobulin G equivalent to the regimen of 0.4 g/kg daily for 5 days that has been previously used and is generally well tolerated. Typical side effects encountered during intravenous immune globulin administration include urticaria and severe headache. We have found that premedication with 1000 mg of oral acetaminophen several hours before the scheduled infusion can prevent this latter complication. In some situations, a change to a different manufacturer will produce fewer reactions in some patients. On occasion, patients will complain of a desquamation of the palmar surface of the hands; the etiology of this phenomenon is unknown. In the case of a heterozygous paternal genotype, an amniocentesis is performed at 15 weeks gestation using DNA techniques to determine the fetal red cell antigen status. Standard
noninvasive and invasive fetal testing is then used to determine the timing of the first intrauterine transfusion.

Future Treatment

Clearly, selective maternal immunomodulation will be the most promising next breakthrough for the treatment of severe hemolytic disease of the newborn. Anecdotally, cases have appeared in the literature in which an anticipated severe case of hemolytic disease of the newborn does not occur in a subsequent gestation even though the same homozygous partner is involved. Investigations using in vitro tests to simulate the fetal reticuloendothelial system have indicated a lack of phagocytosis as a result of maternal antihuman leukocyte antigen antibodies in these cases. Thus, it would appear that naturally occurring antihuman leukocyte antigen antibodies to paternal antigens that are shared with the fetus may exhibit a blocking phenomenon. An animal model for hemolytic disease of the newborn has been described in our laboratory. Purposeful immunization to paternal leukocytes has recently been demonstrated to exhibit a protective effect in the prevention of fetal anemia in the rabbit model.

SUMMARY

Great advances in the treatment of the red cell sensitization have markedly diminished the perinatal loss. Despite this, an estimated 200 fetuses die each year in the United States secondary to hemolytic disease of the newborn or the complications of treatment. Noninvasive techniques to identify the fetus at risk for hemolytic disease of the newborn are promising, and in the future, maternal immunotherapy may supplant intrauterine transfusion.

REFERENCES


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