REVIEW

Managing a pregnancy with antibodies: a clinician's perspective

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HDN, also known as erythroblastosis fetalis, is an immune-mediated anemia, caused by maternal antibodies against a specific fetal RBC antigen. The advent of Rh immune globulin (RhIG) almost 40 years ago has decreased the incidence of D-associated HDN. Maternal isoimmunization to other RBC antigens, for which there is no prophylaxis, continues to occur. The frequency of the latter has increased compared with that of Rh disease. Historically, HDN had a high rate of perinatal mortality. Advances, including improved neonatal care, amniocentesis, fetal RBC typing, and in utero transfusion, have decreased overall fetal loss rates to 2 to 3 percent.[1] Middle cerebral artery Doppler ultrasound has reduced the need of repetitive amniocenteses for assessment of fetal anemia. Determination of free fetal DNA in the maternal circulation is a new technique that may confirm the D status of a fetus, and thus is useful in identifying the fetus, parented by a heterozygous father, who is not at risk.

Historical Review

HDN was first described in 400 BC by Hippocrates. In 1609, a French midwife reported a hydropic newborn girl who expired at birth with a twin who developed jaundice and neurologic complications and subsequently died.[2] Throughout the 19th century, the proposed cause of icterus gravis was the absence or obstruction of the bile duct. The term *erythroblastosis fetalis* connoted a hydropic stillborn by Rautmann in 1912.[3] It was not until 1931 that Buhrman and Sanford made the connection between erythroblastosis fetalis and newborn jaundice.[4] Landsteiner and Wiener described the Rh factor, and a year later Levine determined that antibodies against the D antigen were instrumental in the cause of HDN.[5,6] The subsequent 60 years advanced the care of affected pregnancies including timing of delivery, amniocentesis for fetal assessment, in utero fetal transfusion, and noninvasive modalities for determining fetal risk. Improvements made in the care for this disorder cannot be overstated. Before 1945, 50 percent of all fetuses with hemolytic disease died, accounting for 10 percent of overall perinatal mortality.[7]

RBC Alloimmunization

The antigens of the Rh system are the most common cause of immunization during pregnancy. Although more than 40 antigens belong to this system, D, C, c, E, and e are the most common. D– blood is found in about 15 percent of Caucasians and 5 percent of Africans. Other RBC antigens have been identified as the cause of HDN, including those in the Kell, Duffy, and Kidd blood group systems. HDN caused by antigens other than D is increasing in prevalence. The pathogenesis of HDN is similar, whether the disease is caused by D or another antigen, and for the purposes of this discussion D will be used.

A D– pregnant woman exposed to D antigen, usually as a result of transplacental passage, may develop alloimmunization. Events that are associated with fetal to maternal hemorrhage include delivery, abortion, molar or ectopic pregnancies, and procedures including amniocentesis, cordocentesis, chorionic villus sampling, and external cephalic version, as well as trauma and abruption. The maternal immune response is variable and is affected by the volume of fetomaternal hemorrhage, fetal zygosity for D, and ABO compatibility of mother and fetus.[8] Once a woman has developed D-specific IgG antibodies, a subsequent fetus is at risk of

hemolysis if its RBCs exhibit the offending antigen. The presence and severity of the anemia depend on both the antibody concentration and prior pregnancy history. Antibody subtype, frequency of expressed antigen, efficiency of placental immunoglobulin transport, and fetal spleen maturity all play a role in fetal RBC destruction.[9]

Fetal anemia and resultant extramedullary hematopoiesis lead to a reduction of colloid osmotic pressure and liver congestion, respectively, producing portal hypertension. The fetus may then develop abnormal fluid collections, which is termed hydrops. Other RBC antigens including C, c, E, and e can provoke similar immune responses.

Initial Workup of an Alloimmunized Pregnancy

For the purpose of consistency, Rh disease is described here. Data supporting the management of alloimmunization to antigens other than D is limited, but most authorities recommend management similar to that of D isoimmunization.[10]

Blood typing and an IAT should be performed on entry into prenatal care. The presence of a positive antibody screen suggests alloimmunization and requires characterization. Determining the antibody specificity, whether it is to D or another antigen, is necessary for two reasons: (1) some antibodies are not clinically significant and are not associated with HDN, and (2) paternal testing is available for most RBC antigens to assess for risk of transmission. For example, if the father is homozygous for D, all his offspring will be D+ and thus be at risk of HDN. Fifty-five percent of individuals are heterozygous for the antigen, which decreases the incidence of the fetus' RBCs being D to 50 percent. The difficulty with determining paternal zygosity for D is that no defined allele for the recessive condition has been identified. Therefore, serology testing for C, c, D, E, and e combined with ethnicity can provide an estimated risk of heterozygosity. Paternal testing is more helpful with other RBC antigens. In these cases, maternal sensitization may occur owing to a prior transfusion; thus the potential exists that the partner may not exhibit the corresponding RBC antigen. After phenotyping of the parental RBCs is completed, further management is dependent on the potential for the fetus' RBCs to exhibit the antigen (Fig. 1).[11]

If the father's RBCs are D+ and the couple does not have a history of an affected child, the patient may be given one of two options: (1) serial monitoring of maternal antibody titer on a biweekly or monthly basis until it reaches a critical value; or (2) offering an amniocentesis to investigate fetal D (or any other antigen) status using PCR.

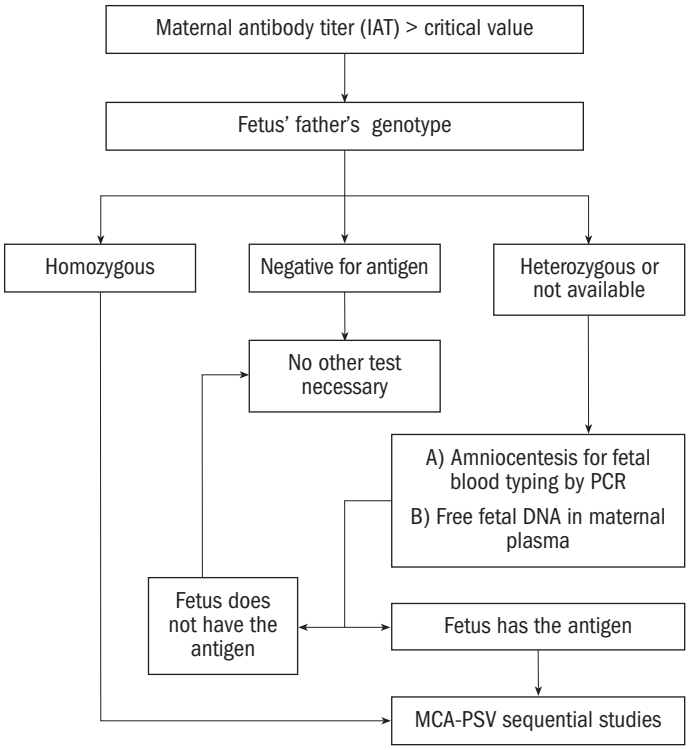

Fig. 1. Algorithm for workup in the alloimmunized pregnancy.[11]

Fetal DNA from nucleated RBCs has been isolated in maternal blood and used for fetal RhD genotyping.[12] A recent meta-analysis reported 94.8 percent accuracy of noninvasive fetal Rh determination using maternal blood. In alloimmunized pregnancies, 91.8 percent of the cases were correctly diagnosed. Free fetal DNA in maternal serum or plasma had higher diagnostic accuracy when compared with RNA or DNA extracted from maternal blood.[13] These techniques are currently not believed to be definitive. The potential error of not correctly identifying a D+ fetus in an alloimmunized mother is not completely preventable at present. Hopefully in the near future such a test will achieve 100 percent accuracy and become the preferred method for determining fetal D status and eliminating unnecessary workups for fetuses not at risk for HDN.

Estimating Risk of HDN

HDN is classified as mild, moderate, and severe.[14] The majority of cases present as mild, requiring newborn phototherapy. Only 30 percent of affected newborns have moderate and 20 percent have severe

disease. Moderate cases have anemia and may require preterm delivery and exchange transfusion. Fetuses with severe disease are hydropic. Fortunately, less than 10 percent of patients have severe disease at less than 34 week' gestational age, the time when in utero therapy would be necessary. Estimating the risk of HDN relies on prior history and maternal antibody titer. A patient without a prior history of sensitization would be expected to have a good outcome. Conversely, one with a prior affected pregnancy or certainly a prior hydropic fetus or adverse postnatal course would be at risk for an adverse outcome. Antibody titers alone are unreliable for prediction when a patient has had a prior affected fetus or infant.

Maternal Antibody Titer

Methods for determining the titer vary by institution, and the critical value for the potential of fetal anemia is usually established in each laboratory. A titer between 8 and 32 is considered critical. This applies to Rh as well as other antigens. The exception is K, in which there are affected cases when maternal titers are less than 16. In a pregnancy without a prior affected fetus, the titer is repeated every 2 to 4 weeks as long as it remains below the critical threshold. Once the value is met, serial assessments for the possibility of fetal anemia must be undertaken, by either amniocentesis or middle cerebral artery Doppler ultrasound. In cases in which there is a history of a prior severely affected fetus, serial surveillance is warranted regardless of the antibody titer.

Amniocentesis

Yellow amniotic fluid in anemic fetuses with severe hemolytic disease correlates with its high bilirubin content and is likely derived from fetal pulmonary and tracheal secretions. Liley[15] first described, in 1961, a qualitative analysis of amniotic fluid bilirubin for HDN, which is measured by spectrophotometry, absorbing light at a wavelength of 450 nm. The resulting deviation from baseline (ΔOD 450) is plotted against gestational age (Fig. 2). The graph is divided into three zones with zone I indicating a low likelihood of severe anemia, zone II intermediate, and zone III a high probability of severe fetal anemia. The prediction of fetal anemia requires repeated samplings every 1 to 3 weeks depending on the initial value. Decreasing values are reassuring, but a rising curve indicates active hemolysis. Values in upper zone II or zone III require further assessment with cordocentesis to determine fetal hemoglobin or consideration of delivery if beyond 34 weeks' gestation.

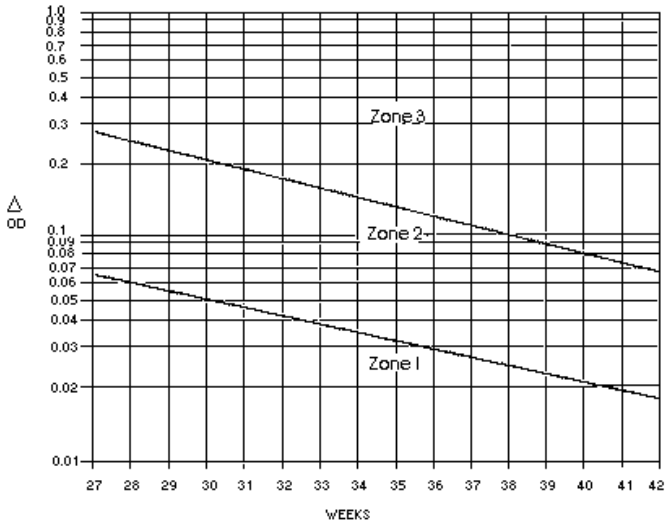

Fig. 2. Liley curve. Optical density at 450 nm is measured (ΔOD-450) and plotted against gestational age.[15]

The Liley curve was modified by Queenan using data from the early second trimester, allowing earlier diagnosis and treatment of fetal anemia in pregnancy.[16]

Amniotic fluid PCR can be used to document the fetal antigen status for D and other common antigens.[17] A number of blood banks provide this testing, usually requiring amniotic fluid or cultured amniocytes and parental blood. A fetus subsequently found lacking the gene that encodes the antigen requires no further workup or monitoring. Amniocentesis is not without risks. The common complications include infection and preterm membrane rupture. Furthermore, it may potentially cause an anamnestic maternal immune response, placing the fetus at further risk of anemia.

Ultrasound

During the past 20 years investigators have attempted to use ultrasound to indirectly screen for fetal anemia as a result of the concerns about amniocentesis complications. Parameters that can be monitored by ultrasound include umbilical vein diameter, fetal spleen or liver length, biventricular outer diameter, and placental thickness. Unfortunately, most of these poorly predict fetal anemia, and their use in current clinical practice is limited.[18] The only validated tool for fetal anemia prediction is middle cerebral artery Doppler ultrasound. Anemic fetuses have increased cardiac output, decreased blood viscosity, and thus increased flow velocity. Mari et al.[19] in 2000 showed that measurement of peak systolic velocity in the middle cerebral artery accurately predicts severe fetal anemia (Fig.3). Once the

critical antibody screening titer has been achieved, cerebral Doppler ultrasounds are performed on a weekly or biweekly basis. When peak systolic values reach 1.5 times the median of those expected for gestational age, cordocentesis is indicated for determining fetal hemoglobin level, blood type, and potential intrauterine transfusion. After 35 weeks' gestation this modality becomes less reliable, and there is an increased false-positive rate.[20] Depending on the clinical history, consideration should be given to documenting fetal lung maturity and determining the ΔOD 450 via amniocentesis at around 36 weeks.

Recently in a multicenter trial of detection of severe fetal anemia, Doppler ultrasound and amniotic fluid ΔOD 450 were compared for sensitivity and accuracy. Results revealed that Doppler was more sensitive and accurate when compared with ΔOD 450 using the Liley method and was as sensitive and accurate when compared with Queenan's method.[21] The conclusion of the trial was that Doppler can safely replace the more invasive amniocentesis for detection of fetal anemia.

Intrauterine Fetal Transfusion

Intrauterine peritoneal blood transfusion using x-ray guidance was first described by Liley in 1963.[22] Since this groundbreaking step toward intrauterine treatment of fetal anemia, many modifications of the procedure have been made with the hope of improving the safety of the procedure. The purpose of fetal transfusion is to reestablish normal fetal hematocrit with donor RBCs that lack the offending antigen(s) and suppress subsequent fetal bone marrow production. Donor blood is group O, D–. The blood is packed to a Hct of 75% to 80%, is screened for infections including CMV, and is irradiated to prevent graft-versus-host reactions. The amount of transfused blood depends on pretransfusion and desired posttransfusion hematocrits (usually 45–50%), gestational age, and hematocrit of the donor.[23]

Transcutaneous approaches can be either fetal intraperitoneal or intravascular, and both performed under ultrasound guidance. Intraperitoneal transfusion places the donor blood into the fetal peritoneal cavity and relies on the absorption of the RBCs via the subdiaphragmatic and thoracic ducts. The utility of this method is limited in fetuses with hydrops because the absorption into the lymphatics is impaired and is associated with a higher fetal death rate. Intravascular access has the advantage of direct and immediate delivery of donor RBCs into the fetal vasculature. Both the umbilical vein and the intrahepatic vein have been

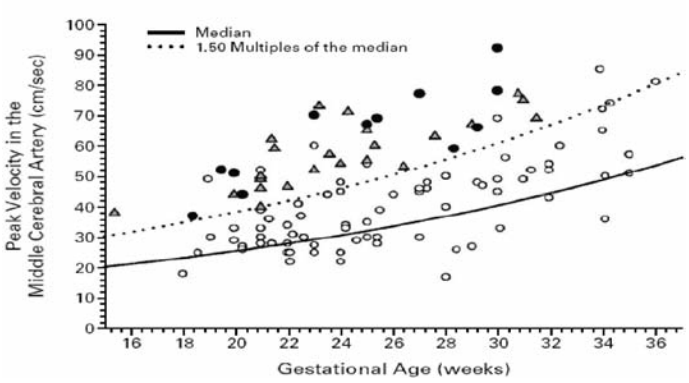

Fig. 3. Peak systolic velocity in the middle cerebral artery of pregnancies at risk for fetal anemia. Open circles indicate no fetal anemia or mild anemia. Triangles indicate moderate to severe anemia. Solid circles indicate fetuses with hydrops.[19]

used for access. Exact placement to some extent depends on fetal and placental position, ease of access to the cord root, and operator training. Free loops of cord are avoided because of the potential of tearing with fetal movement.

The timing of fetal blood sampling and potential transfusion can be challenging. The goal is to initiate the process once the fetus has moderate to severe anemia but before development of hydrops. Intravascular fetal transfusion is now considered to be a safe procedure with an overall survival rate of 89 percent and procedure-related rate of loss of 1.6 percent per procedure. Complications such as rupture of membranes, intrauterine infection, emergency cesarean section, fetal death, and neonatal death have been reported.[24]

Timing of Delivery and Intrapartum Issues

The fetal risks of HDN increase as gestation advances. Therefore an alloimmunized pregnancy is preferably delivered before the estimated due date. Actual timing of the delivery should be dictated by the estimated severity of disease, balancing the risks of ongoing hemolysis and need for in utero transfusion versus the risks of prematurity. Patients with mild or moderate disease usually are delivered at around 36 to 37 weeks, after confirmation of fetal lung maturity. Severely affected fetuses are usually delivered when the risks of transfusion outweigh the risks of long-term disability from a preterm birth, or if one can estimate the need for transfusion based on the last known fetal hematocrit after 34 weeks. If an atypical antibody is detected, the woman's blood sample should be typed and crossmatched once labor occurs. Delivery should

be preferably at an institution adept at caring for neonates with the possibility of severe anemia.

Conclusion

RhIG, Doppler ultrasonography, and intrauterine fetal transfusion have improved the care of pregnancies affected by D or isoimmunization by other antigens including K. Once thought to be a fatal disease, in utero severe fetal anemia can now be reversed with about 90 percent survival rate. The care of a mother affected by RBC alloimmunization can be challenging and time-consuming, especially in cases in which fetal RBC antigen type is unknown. The hope is that within the next decade advances in determination of free fetal DNA in the maternal blood will significantly improve care and avoid many unnecessary procedures.

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