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Hemolytic Disease of the Fetus and Newborn

Introduction

Hemolytic disease of the fetus and newborn (HDFN), also known as erythroblastosis fetalis, occurs when maternal IgG antibody against a foreign, paternally inherited fetal red blood cell (RBC) antigen, crosses the placenta and attaches to fetal erythrocytes. This leads to an increased rate of fetal red cell destruction, and in turn, stimulation of fetal erythrocyte production ("erythroblastosis"). The clinical manifestations of HDFN are variable, depending on the strength of the immune response and the identity of the offending RBC antigen. The severity of HDFN may range from mild subclinical hemolysis to severe anemia with hepatosplenomegaly (due to extramedullary hematopoiesis). The compensatory erythropoiesis may disrupt portal circulation and impair albumin synthesis, leading to reduction in plasma oncotic pressure, which in turn cascades to generalized edema ("hydrops fetalis"), cardiovascular failure, tissue hypoxia, and ultimately, intrauterine fetal demise. In those less severely affected in utero, accelerated red cell destruction can continue after birth, with the infant suffering the effects of hyperbilirubinemia without the benefit of maternal liver clearance. High levels of unconjugated bilirubin are toxic to the developing brain and may lead to permanent brain damage. Recent guidelines on the clinical management of hyperbilirubinemia in newborn infants born 35 weeks or greater have been published by the American Academy of Pediatrics.[2]

Types of HDFN based on maternal antibody specificity

HDFN can be divided into three categories based on the red cell antigens to which maternal immunization has occurred. The most potentially severe HDFN is caused by anti-D, where exposure to volumes of fetal blood less than 0.1 mL can immunize a D- negative (also known as Rh-negative) mother.[1] Other blood group antigens that can incite HDFN include "other" Rh antigens or antigens in other blood group systems such as anti-Jka or anti-K1. These antigens are weaker than D and usually require a larger volume exposure for sensitization. One retrospective study determined that the prevalence of clinically significant antibodies other than anti-D during pregnancy was 0.24%.[1] Finally, ABO antigens may cause HDFN when naturally occurring (i.e. no prior sensitization required for production of the antibody) anti-A,B IgG in a group O woman can enter the fetal circulation and destroy group A or B fetal red cells in any pregnancy, including the first. ABO HDFN, unlike cases of HDFN caused by antibodies directed against anti-Rh or other non-Rh antigens, cannot be diagnosed prenatally. Most infants will be asymptomatic at birth.

Maternal immunization

In general, one can predict the most severe manifestations of HDFN in those women who have been immunized with the most immunogenic red cell antigens (e.g. Rh group D antigen). Within any group of immune-naïve women exposed to the same foreign antigenic stimulus, one may expect stronger immune responses to occur in those with exposure to larger volumes of the responsible RBC antigen. Routes of maternal immunization include pregnancy and blood transfusions. During pregnancy, fetal red cells expressing a paternal antigen foreign to the mother may be introduced to the maternal circulation through a fetomaternal hemorrhage. This is a frequent occurrence in the majority of pregnancies, usually during the third trimester and during delivery. Other routes of FMH include amniocentesis, spontaneous or induced abortion, chorionic villus sampling, cordocentesis, rupture of an ectopic pregnancy, and blunt trauma to the abdomen. As for

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immunization through transfusions, even the minute amount of red blood cells present in platelet or granulocyte concentrates can incite an immune response capable of severe HDFN in future pregnancies when D-positive red cells are introduced to a D-negative woman. In these clinical situations, administration of anti-D globulin (RhoGAM®) in the appropriate dose (see below) should be strongly considered in D-negative women.The overall incidence of D sensitization in untreated D-negative mothers of D-positive infants is about 16% (1.5-2% at the time of delivery, 7% within 6 months of delivery, 7% during second affected pregnancy).[1] The rate of D immunization decreases to between 1.5% and 2% when there is coexistent ABO incompatibility between the mother and the fetus by virtue of increased RBC destruction by anti-A or anti-B.[1]

Determination of HDFN Risk

Appropriate measures during the prenatal as well as postpartum period can reduce the risk of HDFN. During the prenatal period, it is essential to gain information regarding potential immunizing events such as previous pregnancies or blood transfusions. Prenatal laboratory testing performed early in pregnancy should include determination of blood type and red cell alloantibody screen. Any positive antibody screen will be followed by determination of antibody specificity and titer, allowing for determination of HDFN risk. If the father's red cells have been typed and shown not to express the antigen, there should be no potential risk for developing HDFN. In the case of a D-negative mother with a newly identified anti-D antibody, unassociated with RhIG prophylaxis, titers can be quantitated throughout pregnancy as a measure of maternal immune response and allow for clinical decision points to be determined. A first trimester baseline titer may be obtained, with the remaining specimen frozen to be used as a comparison for future titers.

After prophylactic RhIG administration, the mother's serum will demonstrate anti-D reactivity. In the absence of significant fetomaternal hemorrhage, the half-life of an injected dose of RhIG is approximately 21 days.[1] One would expect that of a 300 mcg dose administered at 28 weeks gestation, 20-30 mcg could still remain at term 12 weeks later, and anti-D may be found in the maternal circulation for as long as six months after administration.[1] Therefore, clinical correlation with RhIG prophylaxis dosing is important for interpreting reports of anti-D reactivity.

Amniotic Fluid Analysis

In alloimmunized women or in those with an antibody titer at or above the critical level, bilirubin pigment from an amniotic fluid specimen can serve as a measure of intrauterine hemolysis. By spectrophotometric methods, the change in optical density of bilirubin pigment can be measured, and then plotted against the estimated length of gestation. Divided into three zones, and applicable from 27 weeks gestation to term, the Liley graph predicts the severity of fetal disease based on the change in optical density, with larger changes indicating severe disease and the need for clinical intervention such as intrauterine transfusion or immediate delivery.[1] Interest in this test has waned considerably over the years and it is no longer performed at Rex. Specimens are forwarded to Mayo Medical Laboratories for analysis.

Postpartum Evaluation

In cases of suspected HDFN, samples of both cord and maternal blood should be tested. If the potential for HDFN has already been established in the mother, i.e., prenatal antibody screen positive for antibody known to cause severe HDFN, the cord blood should also evaluated to determine the level of hemolysis (e.g., hemoglobin, hematocrit, bilirubin).

Newborn ABO testing

In adults when blood type is tested, two methods are used to provide a "system check" to ensure results are accurate; patient red blood cells are tested against known antibodies to determine ABO red cell antigens expressed ("forward" or "front" type), in addition to testing patient serum against known red blood cells ("reverse" or "back" type). In the usual patient, the forward and reverse types yield complementary results. For example, a patient with A red cells should react with anti-A antibody, and the serum should react with known B cells. In the newborn, cord serum is usually maternally derived, leaving only the forward typing of the newborn red cells.

Newborn D Testing

Depending on the clinical scenario, D testing may have confounding results. In those newborns with prior intrauterine transfusions, D testing will likely reflect the type of the donor, i.e., D negative. Another potential false negative D test may result from HDFN due to potent maternally derived anti-D antibodies blocking the infant's D antigen sites from binding to the test anti-D antibodies. This mechanism of false negative testing can occur in HDFN due to anti-K as well, as K antigen site density is relatively low on fetal red cells as is the amount of anti-K required to block those sites.

Newborn Antiglobulin Testing

The DAT is usually strongly positive in HDFN resulting from anti-D or antibodies to other blood groups, and much weaker or even negative in HDFN resulting from ABO antibodies. However, the strength of the DAT does not correlate with the severity of hemolysis, especially in ABO HDFN. If the DAT is positive and the maternal antibody screen is negative, ABO antibodies or HDFN caused by an antibody directed against a low-incidence antigen not present on reagent red cells may be suspected. Note that infants born to D-negative mothers who have been administered prophylactic RhIG can have positive DAT without evidence of hemolysis. ABO HDFN is restricted almost exclusively to group A and B infants of group O mothers. It may be particularly pronounced in group B African-American newborns in whom the B antigen is more developed at birth than in the general population.[3] The DAT may only be weakly positive in the newborn, because of incomplete development of A and B antigens. Preparation of eluates from DAT positive cells is no longer part of routine transfusion medicine practice in this particular clinical circumstance. Correlation with maternal ABO Rh, DAT and antibody screen is more useful. A negative newborn DAT

does not entirely exclude the possibilty of ABO HDFN, but no helpful additional information can be gained by further testing. Nonimmune causes of hyperbilirubinemia and hemolysis should still be considered in an infant with a negative DAT before concluding that it is due to ABO HDFN as other hematologic disorders might be responsible.[3]

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Quantitation of Fetomaternal Hemorrhage
(Kleihauer - Betke Discontinued)

Transplacental hemorrhage of fetal red blood cells (RBCs) into the maternal circulation has been recognized since 1954. Although small numbers of fetal RBCs frequently cross the placenta, substantial fetomaternal hemorrhage is quite uncommon, certain clinical conditions increase the risk for fetomaternal hemorrhage, such as ectopic pregnancy, genetic amniocentesis, chorion villus sampling; threatened abortion, blunt trauma to the abdomen (including motor vehicle accidents), and antepartum hemorrhage in the second or third trimester (e.g., placenta previa or abruption). Detection and quantitation of this fetomaternal hemorrhage is important in reducing the incidence of RhD alloimmunization by assuring adequate Rh Immune Globulin prophylaxis.

Fetal RBCs in maternal circulation were originally detected by the acid-elution. In this method, a blood film from the mother is fixed in alcohol and treated with an acid buffer at pH 3.3. Adult hemoglobin is soluble at this pH, but fetal hemoglobin is not. Therefore, after erythrosin staining, the fetal RBCs appear dark in a field of adult erythrocyte ghosts. This method was described by Kleihauer and Betke in 1957 - 1960, and has become known as the Kleihauer-Betke test. Similar to other antiquated laboratory tests (e.g. erythrocyte sedimentation rate), the Kleihauer-Betke (acid-elution test) is relatively crude compared to our concept of "modern" laboratory technique. One important source of error is variability in the thickness of blood smears. In one survey, the density of adult cells per mm2 in different laboratories varied from 1000 to 16,000! The accuracy of fetal cell counts in different laboratories was also assessed by distributing samples of adult blood to which known amounts of fetal red cells were added. Laboratories were asked to determine the ratio of fetal to adult red cells. When the number of fetal red cells was small (1:10,000), the difference between the estimates was ten-fold. When the fetal cells were 1:1000 or more, equivalent to 2ml or more of transplacental hemorrhage, almost all the reported values fell within 50 - 200% of the correct ones. Furthermore, the presence of an increased percentage of adult red cells containing fetal hemoglobin may cause difficulty, and hemoglobin F cells are found in one to two percent of normal adults. In about 25% of pregnant women, the level of maternal Hb F may rise above normal (0-2.0%) beginning at about eight weeks and may reach 7% with the elevation persisting until about 32 weeks. The erythrosin stained blood films from adults often contain cells staining so darkly that they are indistinguishable from cells of fetal origin. Finally, some cells display intermediate staining, creating a challenge in accurate classification. This is obviously not a very precise test by even liberal standards.

Nevertheless, laboratories have done their best to estimate the quantity of fetal RBCs. Usually, the quantity is expressed as a proportion of fetal cells to adult cells in the sample. However, to deduce the absolute quantity of fetal cells, several points need to be considered. First, fetal cells are larger so the volume will be greater than that indicated by the number present. Second, not all fetal cells stain darkly in the acid-elution method. Third, an arbitrary value for maternal red cell volume has to be assumed. A commonly used formula is to multiply the percentage of fetal cells detected by 5000 representing an arbitrarily assigned maternal blood volume. For example: If 1.3% fetal RBCs are detected, (1.3/100) x 5000 mL = 65 mL of fetal whole blood, or 32.5 mL fetal RBCs (using the standard assumption of 50% fetal hematocrit).

Due to limitations of the Kleihauer-Betke acid-elution test, alternate methods have been developed in recent years for quantitating fetal maternal hemorrhage. Quantitation of fetal hemoglobin in a maternal blood sample by flow cytometry using antibodies to fetal hemoglobin is much more accurate and precise, and avoids all the deficiencies of the acid-elution test. Rex Hospital Laboratory has now discontinued the Kleihauer-Betke test, and samples will be sent to Mayo Medical Laboratory for quantitation of fetal maternal hemorrhage by flow cytometry. The flow cytometry test will be available seven days a week. While the computer systems are being changed and health care providers become more familiar with the availability of flow cytometry for this purpose, we will accept orders for Kleihauer-Betke and automatically substitute the reference test fetomaternal bleed quantitation by flow cytometry. The fetomaternal hemorrhage quantitation will be reported the same way it has been at Rex in the past, i.e. mL of fetal red blood cells.

Determination of Rh Immune Globulin (RhIG) dosing will remain the same. To briefly review: one 300 microgram dose of Rh Immune Globulin (RhIG) protects against alloimmunization to the RhD Antigen following exposure to 15 ml of D-positive red cells (or 30 ml of fetal whole blood), and should be given within 72 hours of delivery of an RhD positive infant or potential exposure to fetal

blood antepartum. Only approximately one in 1000 deliveries will be associated with excessive fetomaternal hemorrhage (greater than 15 mL red blood cells), and risk factors will only identify 50 percent of these cases. Nevertheless, all RhD negative pregnant women should be screened for fetal cells in their circulation, and if fetal cells are found, they should be quantitated to exclude the need for an additional dose of RhIG.

The transfusion service routinely screens all D-negative postpartum women with the rosette test to detect the presence or absence of D-positive fetal cells which will form observable rosettes around indicator antibody treated Rh positive cells in the mother's sample. Once the D-positive cells are detected, they will be quantitated by flow cytometry and reported. One additional dose of RhIG is indicated for each additional 15 ml aliquot of fetal red cells. No more than five 300 microgram doses of RhIG should be injected intramuscularly in a 24 hour period. For larger quantities, injections can be spaced over a 72 hour period, or if indicated, can be given using an intravenous preparation. In these cases, no more than 600 micrograms should be given every eight hours intravenously until the total calculated dose is achieved. If anti-D immune globulin is inadvertently omitted after delivery, it should be given as soon as possible after recognition of the omission. Partial protection is afforded with administration within 13 days of the birth, and some experts recommend giving it as late as 28 days after delivery.

In summary, quantitation of fetomaternal hemorrhage will now be performed by flow cytometry at Mayo Medical Laboratory with daily testing available. Since there is a 72 hour window of time in which to administer RhIG for immune prophylaxis, quantitation of fetomaternal hemorrhage is not considered a stat test. Personal communication with area consultants indicates local tertiary care OB services find this test availability and turn around time adequate for making patient care decisions, especially since its primary usefulness is in assuring adequate Rh Immune prophylaxis. Flow cytometry will provide a significantly more accurate and precise result on which to base this decision.

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