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Neonatal Section

Hemolytic Disease of the Fetus and Newborn: Modern Practice and Future Investigations

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ABSTRACT

Red blood cell (RBC) sensitization occurs in some women in response to exposure to paternally derived RBC antigens during pregnancy or to nonself antigens on transfused RBCs during their lifetime. Once sensitized, future pregnancies may be at risk for hemolytic disease of the fetus and newborn. Although great strides have been made over the past few decades in terms of identifying blood group antigens and in predicting fetal anemia through the use of noninvasive monitoring, many questions remain in terms of understanding RBC alloimmunization risk factors, preventative therapies, and treatment strategies. At the present time, there is room for improvement in these areas in both developed and developing countries. Evidence-based, universal guidelines describing recommended RBC antigen matching transfusion strategies for girls or women, before pregnancy or during intrauterine transfusions, would be welcomed. A better understanding of the mechanism(s) of action of Rh immunoglobulin, first introduced more than half of a century ago and one of the most successful immunoprophylaxis therapies in existence today, would also be a large step forward. For example, answers to questions of the role(s) that fetal RBC clearance, antigen masking, antigen modulation, and immune suppression play in the effectiveness of Rh immunoglobulin may help to guide the development of novel preventative therapies during pregnancy for immunization to RhD and non-RhD antigens. Furthermore, a better understanding of the importance of anti-RhD or other alloantibody glycosylation patterns may be beneficial not only in developing such novel immunoprophylaxis therapies but also in predicting the clinical significance of existing maternal alloantibodies. One other area of need includes the development of therapies beyond intrauterine transfusions to mitigate the dangers of maternal alloantibodies to developing fetuses. We challenge physicians, scientists, and funding agencies to prioritize studies of RBC alloimmunization and hemolytic disease of the fetus and newborn and to invest in the children of our future.

Contents

Maternal alloimmunization to blood group antigens occurs through exposure to nonself antigens on red blood cells (RBCs) via transfusion or before pregnancy. RBC alloantibodies may be detrimental in transfusion

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or pregnancy settings, depending on the specificity and depending on the presence or absence of the cognate antigen(s) on transfused or fetal RBCs. In a transfusion setting, incompatible RBCs may be hemolyzed. In a pregnancy setting, fetal RBCs expressing a paternally derived antigen against which a mother is alloimmunized may be hemolyzed or may be altered such that erythropoiesis is suppressed, resulting in hemolytic disease of the fetus and newborn (HDFN).

Alloantibodies against more than 50 non-ABO blood group antigens have been implicated in HDFN, with many blood group antigens historically first identified after the birth of a hydropic infant [1]. In addition to antibodies against non-ABO blood group antigens, naturally occurring maternal isohemagglutinins, often in group O mothers, are capable of leading to anemia in fetuses expressing the A or B antigens. This anemia, although relatively common, is usually mild and rarely requires intervention. The majority of clinically significant HDFN cases are due to alloantibodies against antigens in the Rh, Kell, Duffy, Kidd, and MNS families [2–4], with 1/300-1/600 live births being affected by maternal RBC alloimmunization [5]. Of note, maternal alloantibodies against Rh antigens are significantly less likely to develop when fetal cells are ABO incompatible with maternal plasma, presumably because of the rapid clearance of fetal RBCs by maternal isohemagglutinins. Although alloantibodies to antigens in the Rh family remain a leading cause of severe HDFN worldwide, antibodies against the antigens in the Kell family are emerging to be a leading cause of HDFN in parts of the world where Rh immunoglobulin (RhIg) is widely used for immunoprophylaxis [6].

The outcomes of antigen-positive fetuses developing in utero of alloimmunized women vary depending on characteristics of the maternal antibody and the RBC antigen. Fetuses affected by maternal anti-D alloantibodies may have anemia and such severe hyperbilirubinemia that they develop kernicterus. Fetuses affected by maternal anti-Kell antibodies, however, are more likely to have anemia and reticulocytopenia but rarely have significant hyperbilirubinemia. Because many different maternal antibodies are capable of leading to hydrops fetalis and intrauterine fetal demise, the early diagnosis of at-risk pregnancies is critically important. Maternal antibody titers against antigens such as RhD are commonly reported by US transfusion medicine services. Bioassays such as chemiluminescence or monocyte monolayer assays have a higher specificity to predict fetal outcomes than maternal antibody titers [7,8], although they are not routinely used in the United States. Recent studies suggest that other antibody characteristics, including glycosylation patterns, may associate even more closely with antibody-dependent cellular cytotoxicity and fetal outcome than titer [9,10].

A better understanding of characteristics of maternal antibodies and fetal RBC antigens that result in adverse fetal outcomes would be helpful in the development of novel/targeted therapeutic interventions, as discussed in more detail below. For example, it is unclear why maternal antibodies against antigens on the KEL glycoprotein so efficiently lead to reticulocytopenia. It has been hypothesized that the expression of the Kell antigen on early fetal RBC precursors plays an important role, with direct suppression of erythropoiesis by maternal anti-Kell reproduced in cultures in vitro [11]. However, phagocytosis of RBC precursors expressing the Kell antigen has also been observed in vitro [12], making it plausible that the lack of hyperbilirubinemia observed in fetuses affected by maternal anti-Kell antibodies is due in part to immune-mediated clearance of very early RBC precursors which do not contain hemoglobin. In this review, we summarize the latest clinical approaches to HDFN and describe research that is under way as well as the topics in need of further investigation and development to continue to improve management of this disorder.

Diagnosis and Treatment

At the first prenatal visit, all pregnant women should be tested for RBC alloantibodies using the indirect antibody test. After the woman is found to have RBC antibodies, the risk of clinically significant HDFN is determined by several techniques. Using the maternal sample, serial (usually monthly) RBC antibody titration is done to assess if the fetal RBCs are acting as an immunizing stimulus. If available for testing, the paternal blood type provides inheritance information; homozygous fathers have 100% chance of passing the implicated antigen to the fetus; heterozygous fathers have a 50% chance of having an offspring with the offending blood group antigen. In the case of anti-D sensitization, serological testing cannot determine zygosity, and molecular testing for the RHD gene copy number is needed [13]. Fetal DNA can also be obtained by amniocentesis for blood group genotyping to directly determine the fetal blood type using cultured amniocytes. Because of the risk of fetal loss with the procedure, newer techniques have been developed that isolate fetal DNA from a maternal peripheral blood sample for RBC genotyping [14,15].

To determine the clinical significance of HDFN, the fetus should be monitored for well-being by ultrasonography to look for evidence of ascites (hydrops) or anemia and to monitor heart rate. Fetal anemia assessment is carried out noninvasively using ultrasonographic measurement of fetal blood flow through the large cerebral vessels, usually the middle cerebral artery. The velocity of the blood flow indicates the degree of anemia.

Although maternal alloantibody titers are used to predict the risk to the fetus, some fetuses have severe anemia despite low titers, and others have no anemia despite high titers. Pan-IgG reagents are typically used for measuring titers in the United States, but evaluating IgG subtypes may be informative for certain antibodies [16]. In one study, for example, fetal anemia correlated positively with the amount of maternal IgG1 anti-D and negatively with the amount of IgG3 anti-D bound to fetal RBCs [17]. In addition to antibody titers, some countries also use in vitro antibody-dependent cellular cytotoxicity biological assays to predict alloantibody activity [18,19].

The importance of antibody glycosylation patterns on FcγR binding avidity on clinical outcomes is increasingly being appreciated in multiple biologic systems [20]. Recent studies have described a significant association between such antibody glycosylation patterns and fetal outcomes. Maternal anti-D alloantibodies with the lowest degrees of fucosylation (as measured by mass spectroscopy), for example, have been reported to be associated with more severe fetal anemia [9]. Similarly, maternal antiplatelet glycoprotein alloantibodies with low fucosylation patterns have been shown to be more clinically significant than those with higher fucosylation patterns [21]. One potential explanation for the increase in clinical significance is high binding avidity to FcγRIIIa monocytes and FcγIIIb polymorphonuclear cells.

The treatment of a fetus affected by HDFN is focused on monitoring and support until delivery. Fetuses at gestation >16-24 weeks and found to have blood flow velocities 1.5 times the multiple of the mean are indicative of moderate to severe anemia. Periumbilical blood testing is indicated to directly sample the fetal circulation [2,22]. This is often directly followed by intrauterine RBC transfusion (IUT) if the fetus is not of acceptable gestational age for delivery. These RBC transfusions are selected as group O, negative for the offending antigen(s), leukocyte reduced, irradiated, and concentrated to ensure maximal delivery and to ensure the hematocrit of the fetus is ≥30%. Extended RBC antigen matching is done at some centers. IUT is able to preserve neurologic outcome in many children, although those with severe hydrops may develop cerebral palsy, severe developmental delay, and deafness [23]. Potential alternatives or adjunctive maternal therapies during pregnancy to decrease the severity of fetal anemia include intravenous immunoglobulin (IVIg) and plasma exchange, although the evidence to support the efficacy of these therapies is limited. Plasma exchange has been reported in women with high-titer RBC antibodies and antecedent HDFN [24]; it is given a Category II by the American Society for Apheresis, meaning that it is considered second-line therapy [25].

After delivery, infants are closely monitored for hemoglobin and bilirubin levels, as their own physiological systems must function independently from the mother. Infants affected by HDFN must be intensively observed with laboratory monitoring of hemoglobin and bilirubin to