4. Hemolytic disease of the newborn

Hemolytic disease of the newborn (HDN) used to be a major cause of fetal loss and death among newborn babies. The first description of HDN is thought to be in 1609 by a French midwife who delivered twins—one baby was swollen and died soon after birth, the other baby developed jaundice and died several days later. For the next 300 years, many similar cases were described in which newborns failed to survive.

It was not until the 1950s that the underlying cause of HDN was clarified; namely, the newborn's red blood cells (RBCs) are being attacked by antibodies from the mother. The attack begins while the baby is still in the womb and is caused by an incompatibility between the mother's and baby's blood.

By the 1960s, trials in the United States and the United Kingdom tested the use of therapeutic antibodies that could remove the antibodies that cause HDN from the mother's circulation. The trials showed that giving therapeutic antibodies to women during their pregnancy largely prevented HDN from developing (1). By the 1970s, routine antenatal care included screening of all expectant mothers to find those whose pregnancy may be at risk of HDN, and giving preventative treatment accordingly. This has led to a dramatic decrease in the incidence of HDN, particularly severe cases that were responsible for stillbirth and neonatal death.

This chapter will discuss the causes of HDN and how the disease can be treated or minimized, if not prevented entirely.

**Maternal antibodies cross the placenta and attack fetal red blood cells**

During pregnancy, some of the mother's antibodies are transported across the placenta and enter the fetal circulation. This is necessary because by the time of birth, newborns have only a primitive immune system, and the continuing presence of maternal antibodies helps ensure that they survive while their immune system matures. A downside to this protection is that by targeting fetal RBCs, maternal antibodies can also cause HDN.

A major cause of HDN is an incompatibility of the Rh blood group between the mother and fetus. Most commonly, hemolytic disease is triggered by the D antigen, although other Rh antigens, such as c, C, E, and e, can also cause problems.

Pregnancies at risk of HND are those in which an Rh D-negative mother becomes pregnant with an RhD-positive child (the child having inherited the D antigen from the father). The mother's immune response to the fetal D antigen is to form antibodies against it (anti-D). These antibodies are usually of the IgG type, the type that is transported across the placenta and hence delivered to the fetal circulation.

HDN can also be caused by an incompatibility of the ABO blood group. It arises when a mother with blood type O becomes pregnant with a fetus with a different blood type (type A, B, or AB). The mother's serum contains naturally occurring anti-A and anti-B, which tend to be of the IgG class and can therefore cross the placenta and hemolyse fetal RBCs.

HDN due to ABO incompatibility is usually less severe than Rh incompatibility. One reason is that fetal
RBCs express less of the ABO blood group antigens compared with adult levels. In addition, in contrast to the Rh antigens, the ABO blood group antigens are expressed by a variety of fetal (and adult) tissues, reducing the chances of anti-A and anti-B binding their target antigens on the fetal RBCs.

Less common causes of HDN include antibodies directed against antigens of the Kell blood group (e.g., anti-K and anti-k), Kidd blood group (e.g., anti-Jka and anti-Jkb), Duffy blood group (e.g., anti-Fya), and MNS and s blood group antibodies. To date, antibodies directed against the P and Lewis blood groups have not been associated with HDN.

**Sensitization occurs during the first pregnancy**

Sensitization to an antigen occurs when the immune system encounters an antigen for the first time and mounts an immune response. In the case of HDN caused by Rh incompatibility, an Rh D-negative mother may first encounter the D antigen while being pregnant with an Rh D-positive child, or by receiving a blood transfusion of Rh D-positive blood. Once a mother has been sensitized to the D antigen, her serum will contain anti-D. The direct Coombs test (see below) confirms the presence of anti-D and hence that the mother has been sensitized.

Only a small amount of fetal blood need enter the mother's circulation for sensitization to occur. Typically, this occurs during the delivery of the first-born Rh D-positive child. Fetal-maternal hemorrhage is common during labor and is increased during a prolonged or complicated labor, which in turn increases the risk of sensitization. Sensitization can also occur earlier in the pregnancy, for example during a prenatal bleed or a miscarriage. It may also occur during medical procedures, such as a termination of pregnancy or chorionic villus sampling.

The risk of sensitization to the Rh D antigen is decreased if the fetus is ABO incompatible. This is because any fetal cells that leak into the maternal circulation are rapidly destroyed by potent maternal anti-A and/or anti-B, reducing the likelihood of maternal exposure to the D antigen.

**HDN occurs in subsequent pregnancies**

Initially, the maternal anti-D that is formed at the time of sensitization is of the IgM type, which can not cross the placenta. In subsequent pregnancies, a repeat encounter with the Rh D antigen stimulates the rapid production of type IgG anti-D, which can be transported across the placenta and enter the fetal circulation. Once in the fetal circulation, anti-D attaches to the Rh D antigens found on the fetal RBCs, marking them to be destroyed.

The rate of hemolysis determines whether the nature of HDN is mild, moderate, or severe. In mild cases, the small increase in the rate of hemolysis is tolerated by the fetus. At birth and during the newborn period, symptoms include a mild anemia and jaundice, both of which may resolve without treatment.

In cases where there is a greater increase in the rate of hemolysis, the level of bilirubin may still remain low during the pregnancy because of the ability of the placenta to remove bilirubin from the fetal circulation. However, after birth the neonate's immature liver is unable to metabolize the increased amount of bilirubin that instead accumulates in his or her blood. Within 24 hours of birth, the level of bilirubin may rise dramatically. If levels continue to rise, bilirubin may enter the brain to cause kernicterus, a potentially fatal condition that leaves permanent neurological damage in the babies that survive.

An even greater rapid and prolonged destruction of RBCs leads to severe anemia in the fetus. The liver, spleen, and other organs increase their production of RBCs to compensate for their loss. The drive to produce RBCs causes the liver and spleen to increase in size (hepatosplenomegaly), and liver dysfunction can occur. Immature RBCs (erythroblasts) spill into the circulation, giving rise to the alternative name of
this disease, erythroblastosis fetalis. A complication of severe HDN is hydrops fetalis, in which the fetal tissues become swollen (edematous). This condition is usually fatal, either in utero or soon after birth.

The Coombs test detects Rh incompatibility between mother and fetus

To detect HDN, the presence of maternal anti-Rh IgG must be identified. In vivo, these antibodies destroy Rh D-positive fetal RBCs, but in vitro, they do not lyse cells or even cause agglutination, making them difficult to identify. Therefore, the Coombs test is used. This test uses antibodies that bind to anti-D antibodies. The test is named for Robin Coombs, who first developed the technique of using antibodies that are targeted against other antibodies.

Direct Coombs test: diagnoses HDN

The direct Coombs test detects maternal anti-D antibodies that have already bound to fetal RBCs. First, a sample of fetal RBCs is washed to remove any unbound antibody (Ig). When the test antibodies (anti-Ig) are added, they agglutinate any fetal RBCs to which maternal antibodies are already bound. This is called the direct Coombs test because the anti-Ig binds "directly" to the maternal anti-D Ig that coats fetal RBCs in HDN.

Indirect Coombs test: used in the prevention of HDN

The indirect Coombs test finds anti-D antibodies in the mother's serum. If these were to come into contact with fetal RBCs they would hemolyse them and hence cause HDN. By finding maternal anti-D before fetal RBCs have been attacked, treatment can be given to prevent or limit the severity of HDN.

For this test, the mother's serum is incubated with Rh D-positive RBCs. If any anti-D is present in the mother's serum, they will bind to the cells. The cells are then washed to remove all free antibodies. When anti-Ig antibodies are added, they will agglutinate any RBCs to which maternal antibodies are bound. This is called the indirect Coombs test because the anti-Ig finds "indirect" evidence of harmful maternal antibodies, requiring the addition of fetal RBCs to show the capacity of maternal anti-D to bind to fetal RBCs.

Preventing HDN

Determine Rh status of the mother

As part of routine prenatal or antenatal care, the blood type of the mother (ABO and Rh) is determined by a blood test. A test for the presence of atypical antibodies in the mother's serum is also performed. At present, Rh D incompatibility is the only cause of HDN for which screening is routine.

In the United States, the frequency of Rh D-negative status varies from about 17% in Caucasians to about 7% in Hispanics and Blacks. The frequency is much lower in people of Asian descent (including people from China, India, and Japan), averaging about 2% (2).

If the mother is not sensitized, reduce the risk of future sensitization

To find out whether a pregnant Rh D-negative mother has been sensitized to the Rh D antigen, an indirect
Coombs test is done (see above). If anti-D is not found in the mother's serum, it is likely that she has not been sensitized to the Rh D antigen.

The risk of future sensitization can be greatly reduced by giving all unsensitized mothers anti-D Ig, which "mops up" any fetal RBCs that may have leaked into the maternal circulation, reducing the risk of first-time exposure to the D antigen.

Usually, Rh D-negative mothers receive on injection of anti-D Ig at about 28 weeks gestation, which is about the time when fetal RBCs start to express the D antigen, and mothers receive another dose at about 34 weeks, a few weeks before labor begins during which the risk of fetomaternal hemorrhage is high. A final dose of anti-D Ig is given after the baby has been delivered. In addition, anti-D Ig is given to cover other events during the pregnancy that may lead to sensitization, e.g., antepartum bleeds and pre-eclampsia.

This prophylaxis regime against Rh D sensitization is effective. However, currently, there is no routine prophylaxis for HDN caused by incompatibility of other blood group antigens.

If the mother is sensitized, determine whether the fetus is at risk and monitor accordingly

Once the presence of maternal anti-D has been confirmed, the next step is to determine whether the fetal RBCs are a target, i.e., confirm the Rh status of the fetus. If the father is homozygous for the D allele (D/D), the fetus will be D positive. If however the father is heterozygous (D/d), there is a 50:50 chance that the fetus is D positive, and the only way to know the blood type for sure is to test a sample of fetal cells taken from the amniotic fluid or umbilical cord.

If the fetus is Rh D-positive, the pregnancy is carefully monitored for signs of HDN. Monitoring includes regular ultrasound scans of the fetus and monitoring of the amount of anti-D in the mother's serum. Active hemolysis is indicated by a rise in anti-D. If a fetal blood test confirms fetal anemia, depending upon its severity, a blood transfusion can be done in utero to replace the lysed fetal RBCs.

Blood transfusions may also be needed to correct anemia in the newborn period. During this period there may also be a sharp rise in the level of bilirubin in the neonate, which can be lowered by phototherapy and exchange transfusions.

References