Diagnosing Hemolytic Disease of the Newborn

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A Rh negative, pregnant female presented to a major medical center for possible Rh alloimmunization. This female had nine previous pregnancies, including three spontaneous abortions, four live births, and two fetal demises. Because of poor prenatal care, the immunization Rh immune globulin was administered to only the first two pregnancies. After much laboratory testing and treatment, this tenth pregnancy also ended in fetal demise.

ABBREVIATIONS: HDN = hemolytic disease of the newborn; IDAT = indirect anti-globulin test; IUFT = intrauterine fetal transfusion; PUBS = percutaneous umbilical blood sample; RBC = red blood cells.

INDEX TERMS: HDN; pregnancy.

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ETIOLOGY OF HDN

Erythroblastosis fetalis, more commonly known as hemolytic disease of the newborn (HDN), is an immune response disorder. It is caused mostly in pregnancies with blood incompatibilities, such as ABO or Rh, with Rh being the most common. It occurs in approximately 10% of pregnancies.[1] The fetal red blood cells (RBCs), contain an antigen such as A, B, or D that the mother's red blood cells do not have. For example, an Rh– woman who has no D antigen present on her RBCs is pregnant with an Rh+ fetus that does have the D antigen present on its RBCs. The four most common antibodies are anti-D, anti-c, anti-E, and anti-Kell, which is the highest significance clinically.[2] In this case study, the antibody of concern is anti-D, which is associated with Rh incompatibility. This is determined by the mother's rising IDAT testing of anti-D antibodies.

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According to Dr Lopez-Plaza, only 17% of Rh– mothers will become immunized if exposed to fetal Rh+ cells.[3] This often happens when the placenta breaks from the mother's uterus during delivery because fetal RBCs can enter maternal circulation. When the fetal RBCs get into maternal circulation there is a reaction because the mother's immune system does not recognize the fetal antigens. She will start to build up antibodies against the fetal cells, a process called alloimmunization.

The severity of alloimmunization can be affected by the subclass of antibodies produced.[2] IgG antibodies are more severe than IgM antibodies. Antibodies made by the mother are IgM at the first exposure; however, IgG are produced after eight to ten weeks of secondary exposure, such as a second pregnancy. Unlike the IgM antibodies, the IgG antibodies can pass through the placenta and return to the fetus causing an antibody-antigen reaction with the fetal blood. This leads to the shortened life of the fetal RBCs. This does not usually occur in the first pregnancy because the fetal RBCs are not usually introduced into maternal circulation until birth. The maternal immune system builds up IgM antibodies, which remember the fetal antigens. If the woman becomes pregnant again with a fetus that has the same antigens, the IgM antibodies react and cause the production of IgG antibodies. These IgG antibodies will attack that fetus's RBCs. It is this second fetus that can suffer from the disorder of HDN. The antibody titer usually increases with each pregnancy; therefore, the higher the number of pregnancies, the increased risk and severity of HDN.

A CASE STUDY OF HDN

A 31-year-old white, Rh–, pregnant female presented at a major medical center for possible Rh alloimmunization. This was the patient's tenth pregnancy. Her pregnancy history is shown in Table 1.

It was presumed that the pregnancy in 1982 was the first Rh sensitization caused by the breech pregnancy of 1978. In 1982, the patient did not receive prenatal care. The baby born from that pregnancy was immediately sent to a children's hospital for transfusions and treatment of a collapsed lung. The baby born in 1988 weighed only one pound and four ounces. Like many of his siblings, he died of intrauterine fetal demise secondary to fetal edema. Hematopoiesis was found in his liver and spleen. The current pregnancy was considered high-risk because of the patient's previous history.

The patient's doctor followed standard precautions for women at risk for HDN caused by Rh alloimmunization. Major tests done were weekly ultrasounds, non-stress tests, and amniocentesis starting at 20 weeks. The patient's indirect anti-globulin test (IDAT),

better known as the antibody screening test, was determined to be 1:512 for the anti-D antibody. During the ultrasound, the fetus showed fetal ascites, which are protein and electrolyte fluid collections under the skin, and also increased fluid accumulation in the fetal abdomen. There were many non-stress tests done at the hospital also, and all of them showed the baby to be non-reactive. The amniocentesis at 20 weeks resulted in a multiple of the median, or MoM, value of 1.1 showing that the baby was not under great risk for genetic anomalies. Another IDAT and a delta-OD were performed on this amniotic fluid. The IDAT was 1:4096 for anti-D and 1:1 for anti-C. This confirmed that the antibody was anti-D and the degree of hemolysis was worsening. The patient's Delta-OD, which measures the bilirubin from broken down RBCs in the amniotic fluid, was 0.055 mg/dL. This was not very helpful in determining the severity because the fetus was less than 28 weeks old, and the bilirubin had not started decreasing from normal high levels. A percutaneous umbilical blood sample (PUBS) was collected next. Upon receipt of the results, the doctor performed an intrauterine transfusion of packed Rh (-) RBCs. The PUBS results are indicated in Table 2.

Because of the benefits of this transfusion, two more were performed at a later date; however, later ultrasounds depicted worsening fetal swelling.

Despite all efforts, the fetus was stillborn and therapeutically aborted at 24 weeks gestation. An autopsy was performed to confirm the primary diagnosis. The primary cause of death was recorded to be severe hydrops fetalis, also known as massive edema. This is usually associated with severe HDN. Other symptoms that corroborate these findings are: autolysis in the spleen, decreased hematopoiesis with no white pulp in the spleen, marked autolysis in the liver with decreased liver function activity, bilateral renal hemorrhage, large pale placenta, placental areas of necrotic villi, and increased fibrin. Because of the fetal ascites, the abdomen was extended and there was slight scalp edema. The autopsy report stated that the mother's Kleihauer-Betke test, a test for fetal hemoglobin, was 0.37% providing evidence that there was a recent fetal-maternal hemorrhage.

Table 1. Pregnancy history

1976	Spontaneous abortion at 12 weeks	Rh immune globulin given
1978	Spontaneous abortion at 5 months	Rh immune globulin given
1978	Breech ~ Pre-eclampsia at 7 months	No Rh immune globulin given
1979	Vaginal delivery at 8 months (Rh–)	No Rh immune globulin given
1981	Vaginal delivery at 9 months (Rh–)	No Rh immune globulin given
1982	Vaginal delivery at 9 months (Rh+)	No Rh immune globulin given
1983	Intrauterine fetal demise with Rh alloimmunization	
1985	Spontaneous abortion at 13 weeks	No Rh immune globulin given
1988	Vaginal delivery at 20 weeks ~ presumed Rh alloimmunization	

Table 2. Percutaneous umbilical blood sample (PUBS) results

PUBS results:	Pre-Transfusion	Post-Transfusion
Hemoglobin	3.1 g/dL	11.9 g/dL
Hematocrit	9.0%	32.6%
MCV	159.9 fL	92.2 fL
RBC morphology:	Slight anisocytosis and poikilocytosis; few macrocytes and spherocytes.	

CLINICAL SYMPTOMS OF HDN

Other than the obvious RBC lysing, HDN causes a variety of problems in the affected fetus. Those symptoms include skin edema, subcutaneous edema, fetal ascites, jaundice, enlarged umbilical vein, enlarged placenta, and enlarged spleen and liver.[4] The most dangerous of these symptoms is hydrops fetalis, in which the fetus accumulates fluid.[5]

All these symptoms are in some way related to the hemolysis of the fetal RBCs. Because the fetus is trying to rapidly compensate for and remove the hemolyzed cells, the liver and spleen become enlarged, thus causing swelling and accumulation of fluid in the fetal trunk.[5] The fetus' reticuloendothelial system, which contains the macrophages in the spleen, tries to remove the RBCs with attached antibodies.[2] This contributes to the enlarged spleen.

All of the RBC lysing leads to a buildup of bilirubin. Under normal circumstances, the fetal bilirubin would be transported across the placenta to be excreted by the maternal liver; however, the effects of the disease on the placenta hinder this. These symptoms can range from mild cases to severe ones, which could lead to death.

TESTS TO DIAGNOSE HDN

There are many tests that are done to diagnose HDN; some are standard, while others are still under investigation. The first test that should be done is a blood typing of the pregnant patient to check for Rh factors. If the patient were Rh+, there would be no risk of anti-D alloimmunization. However, if the mother were Rh–, the next step would be to perform an IDAT. This test is done on the patient's serum to detect anti-D antibodies by agglutination. A patient without these antibodies would have a normal, non-agglutinating result, while the patient with these antibodies would have aggluti-

nation of the RBCs. Antibodies are then identified and titered. Titers are done to look for the highest dilution that causes a reaction. These titers must be performed exactly the same during each test. Any positive above 1:8 is indicative of HDN risk, and also that the antibodies are a result of a previous pregnancy or transfusion.[6] If the titer is less than or equal to 1:16, the titer should be repeated monthly.[2]

Doctors would then perform periodic ultrasounds to detect fluid accumulation, which are caused by severe anemia.[5] Periodic non-stress tests would also be performed. These tests assess the viability of the fetus and the functional ability of the placenta.[7] This is a non-invasive test that monitors fetal movements by monitoring the changes in the fetal heart rate. These tests determine if the fetus is 'reactive' or 'non-reactive'. A fetus is considered reactive if there are at least two measurements of increased heart rate in a 10-minute period.[7] If there are less than two changes of heart rate in the 10-minute period, it is considered 'non-reactive'. A non-reactive fetus would support the laboratory's evidence of HDN. A reactive fetus is a good indicator of a healthy baby.

Next, the doctor would check for a fetal-maternal hemorrhage. This can be detected by one of three procedures. The procedure most commonly performed is the Kleihauer-Betke test, which is an acid elution of maternal blood. Acid elution starts with a smear of whole, fresh maternal blood on a glass slide. It then goes through a series of steps including fixative, buffer, acid, and stain. This test detects fetal hemoglobin, pink stained cells, among the normal maternal hemoglobin A, clear cells. Anything above 0% pink cells is considered a positive test.[1] It is specific and sensitive, but subject to laboratory error. Flow cytometry is another test that is both sensitive and specific for fetal cells; however, this test is difficult to perform. The third method is the erythrocyte rosette test, which is simple and sensitive, but demonstrates low specificity, so it requires a confirmation method.[1]
The amniocentesis that is performed at 20 weeks is used to detect two things: bilirubin concentration and a multiple of the median screen (MoM). This MoM screen is used to predict the risks of genetic defects, such as Down's syndrome, trisomy 18, and neural tube defects. It is difficult to assign a 'positive' or a 'negative' value to this test because of the amount of overlap in the graphs. Although this test aids in the prediction of other disorders, it does not have any effect on the tests performed in HDN.

The more important test of amniotic fluid is for the presence of bilirubin. This test is called the Delta-OD 450 and results will vary based on gestational age. The amniotic fluid is filtered in the laboratory, and the absorbance is measured and compared to the absorbance of a water blank. Absorption is read between 365 and 700 nm. Bilirubin is absorbed at 450 nm. To determine the concentration of bilirubin, the doctor must compare the delta-OD number with the Liley Graph for Zone location. Zone definitions are explained in Table 3.

As noted in Table 3, zone 1 is the least severe and zone 3 the most critical. The amniocentesis can be performed every two to three weeks. The delta-OD is usually tested multiple times during pregnancy to compare results. A decreasing trend is favorable. Using these multiple results, the doctor can determine the status of the fetus and possible precautions for the baby's delivery.

Another test to assess fetal condition is a percutaneous umbilical blood sample, or PUBS. This blood sample is taken directly from the umbilical vein to monitor the fetal antibody levels, blood type, blood counts, indices, bilirubin levels, and also the compensatory response mechanism of the fetus.[5] This is a much more invasive procedure; however, much information is learned from this fetal blood sample. The low hematocrit and RBC count indicates the degree of hemolysis and helps evaluate fetal anemia, which is confirmed by the low fetal hemoglobin. Mean corpuscular volume, better known as the MCV, is also monitored by the PUBS. MCV measures the size of the RBC. A high MCV is indicative of HDN. The RBC morphology and high MCV are from the extramedulary hematopoiesis. Extramedulary hematopoiesis is the production of RBCs in non-traditional places, such as the spleen and liver, to compensate for the rapid RBC destruction.

In addition to the tests shown in Table 4, there are other, non-conventional ways to detect fetal blood type as well. One test suggested is the direct DNA testing via the amniotic fluid.[9] By using this method, one can not only determine the presence of the D antigen, but also the status of the c and E antigens as well. Some doctors prefer this procedure to the PUBS testing which is more risky to the infant, because it is 99% accurate.[9] This testing can either be based on amino acid sequencing or by exon sequences.[9,10] In the exon testing, maternal blood is also required. In the future, diagnostic testing may further reduce the risks to the fetus; however, currently, the risks must be weighed against survival for the baby.

TREATMENT OF HDN

There are not many options for the treatment of HDN. In very mild cases, the treatment of choice is to place the baby under UV lights as soon as it is delivered. For more severe cases, however, the classic treatment of HDN is an intrauterine fetal transfusion,

Table 3. Liley graph

Zone 1	Mild or unaffected. Pregnancy may proceed to term.
Zone 2	Moderate. Repeat delta-OD every 1 to 2 weeks to monitor. Pregnancy may proceed to 37 weeks.
Zone 3	Severe. Requires treatment. Induce labor if lungs are mature.[5,8]

or IUFT.[5] The IUFT is the introduction of fresh RBCs into the fetal blood via the umbilical vein. All this takes place while the fetus is still in the uterus. The mother is in an operating room in case the need for an emergency cesarean arises.[5] The purpose of this procedure is to infuse enough RBCs to raise the fetal RBC count and hemoglobin enough for the baby to stay healthy and remain in the mother's uterus until mature enough to live on its own. Some of the risks of IUFT are as follows: reduced heart rate, bleeding from the umbilical vein, blood clot, infection, rupturing of the amniotic sac, and introduction of more fetal RBCs into maternal circulation.[5] The optimal time for the IUFT is 32 to 34 weeks gestation.[5] In the case study, the IUFTs were performed between 20 and 24 weeks gestation because of the severity of the disorder. This could be a reason for the lack of success of the procedure in the case study. The fetus was not developed enough and had symptoms too severe to take on the infused red blood cells as its own.

PREVENTING HDN

Although there are limited treatment opportunities, there is a very reliable prevention for Rh alloimmunization. First, mothers should have good pre-natal care throughout the pregnancy, including prediction of HDN risks, which could aid in the prevention. These risks are determined by maternal blood type, family history, and IDAT results.

The prevention of HDN is relatively simple. It is the administration of Rh immune globulin. Rh immune globulin is usually given intramuscularly as an injection of anti-D IgG. This has an immunosuppressive effect on the maternal immune system because of interference with D antigen identification.[3] This type of immunization has certain limitations: antigen frequency, specificity for the D antigen only, capacity of the mother to be immunized, ability to reach the baby, the amount of antigen received by the mother, and the route administered.

Table 4. The laboratory's role in diagnosing HDN is summarized below in order of chronology

1. Blood typing of mother's blood sample
2. IDAT and titer of mother's blood sample
3. Periodic ultrasounds
4. Periodic non-stress tests
5. Fetal-maternal hemorrhage testing
6. Amniocentesis
7. Percutaneous umbilical blood sample

Rh immune globulin immunization can be given intramuscularly or intravenously. Rh immune globulin was introduced in the 1960s, lowering the frequency of anti-D alloimmunizations from nine to ten cases per 1000 total births to 1.3 cases per 1000 total births.[1] Now, due to both decreased family size and Rh immune globulin, this disorder only causes an approximate four to six deaths per 100,000 total births.[1] After years of clinical studies, Rh immune globulin became a routine injection for Rh–/IDAT– women in 1968.[1]

Rh immune globulin should be given both at 28 weeks and within 72 hours post delivery or abortion. It was also reported "the administration of D immunoglobulin at 28 weeks gestation, when combined with postpartum administration, reduces the incidence of alloimmunization to 0.2% of women at risk".[1] This is amazing success for the prevention of such a difficult disorder to treat. The standard dose of D immunoglobulin is 300 mg. This is enough to immunize against 30 mL of fetal blood being introduced into maternal circulation.[1] There are very few side effects to this immunization. This is an excellent argument for all women to seek prenatal care for each pregnancy.

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