TRANSFUSION MEDICINE

NO. TM 10-4 (TM-320)

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If you wish to obtain an MOC self-assessment module (SAM) credit for this exercise, please log-in to www.ascp.org, go to My Courses, and select the appropriate course now to take your pre-test.
LEARNING OBJECTIVES

Upon completion of this exercise, the participant should be able to

- describe the pathogenic mechanism of hemolytic disease of the fetus and newborn.
- explain the signs and symptoms of hemolytic disease of the fetus and newborn.
- diagnose hemolytic disease of the fetus and newborn during pregnancy.
- recommend the optimal blood products for intrauterine transfusion to patients with hemolytic disease of the fetus and newborn.
- manage hemolytic disease of the fetus and newborn throughout the prenatal period.
HISTORY

The patient was a 37-year-old woman (gravida 2 para 1), blood type O Rh negative. Her first pregnancy had resulted in intrauterine fetal death at 37 weeks. The prenatal course of her first pregnancy included a negative antibody screen and Rhesus immune globulin (RhIG) administration at 28 weeks’ gestation, but was otherwise unremarkable. At 37 weeks’ gestation, she was found to have undetectable fetal heart tones and subsequently underwent induction of labor secondary to intrauterine fetal death. Following vaginal delivery of the stillborn fetus, her postpartum course was complicated by severe hemorrhage and disseminated intravascular coagulation, requiring transfusion of 4 units of type O Rh-negative red blood cells (RBCs). She also received a second dose of RhIG following delivery. Despite RhIG administration, her postpartum blood sample showed the presence of anti-D, anti-G, and anti-E alloantibodies. The family declined an autopsy of the fetus, and the fetal RBC phenotype was not determined.

During the patient’s second pregnancy 2 years later, baseline antibody titers for anti-D, anti-G, and anti-E alloantibodies were performed at 11 weeks’ gestation. (Results of her antibody titers throughout her second pregnancy are shown in Laboratory Results I. Antibody titers were performed in parallel with frozen samples from the prior testing date.)

At 18 weeks’ gestation, the patient presented with mild vaginal bleeding after a long walk. Ultrasound examination showed no signs of fetal distress. Laboratory tests performed at this time are shown in Laboratory Results II. However, antibody titers were not requested, and the patient was discharged. The patient failed to return to the clinic in 1 week as instructed.

At 25 weeks’ gestation, she returned to the clinic. She reported no vaginal bleeding. At this time the anti-D and anti-G titers showed a dramatic increase, and the anti-E titer showed a mild increase (Laboratory Results I).

LABORATORY RESULTS I. RBC Alloantibody Titers Up to 25 Weeks’ Gestation.

<table>
<thead>
<tr>
<th>Antibody Titers</th>
<th>11 Weeks</th>
<th>16 Weeks</th>
<th>18 Weeks</th>
<th>25 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-D</td>
<td>32</td>
<td>8</td>
<td>Not obtained</td>
<td>2048</td>
</tr>
<tr>
<td>Anti-G</td>
<td>8</td>
<td>32</td>
<td>Not obtained</td>
<td>256</td>
</tr>
<tr>
<td>Anti-E</td>
<td>4</td>
<td>16</td>
<td>Not obtained</td>
<td>32</td>
</tr>
</tbody>
</table>
**LABORATORY RESULTS II. Laboratory Data at 18 Weeks' Gestation.**

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood type</td>
<td>O negative</td>
<td>3.28-9.29 (3.28-9.29)</td>
</tr>
<tr>
<td>White blood cell count, ( \times 10^9/\mu\text{L} \times 10^9/\text{L} )</td>
<td>11.56 (11.56)</td>
<td>4.21-5.61 (4.21-5.61)</td>
</tr>
<tr>
<td>Red blood cell count, ( \times 10^9/\mu\text{L} \times 10^12/\text{L} )</td>
<td>4.04 (4.04)</td>
<td>12.3-16.3 (123-163)</td>
</tr>
<tr>
<td>Hemoglobin, g/dL, (g/L)</td>
<td>12.5 (125)</td>
<td>37.4-47.0</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>36.2</td>
<td>37.4-47.0</td>
</tr>
<tr>
<td>Platelet count, ( \times 10^5/\mu\text{L} \times 10^9/\text{L} )</td>
<td>340 (340)</td>
<td>143-398 (143-398)</td>
</tr>
<tr>
<td>Neutrophil, %</td>
<td>68.6</td>
<td>40.1-75.9</td>
</tr>
<tr>
<td>Lymphocyte, %</td>
<td>25.5</td>
<td>19.1-51.6</td>
</tr>
<tr>
<td>Monocyte, %</td>
<td>4.9</td>
<td>3.4-11.9</td>
</tr>
<tr>
<td>Eosinophil, %</td>
<td>1.0</td>
<td>0.0-6.4</td>
</tr>
<tr>
<td>Basophil, %</td>
<td>0.3</td>
<td>0.0-1.3</td>
</tr>
<tr>
<td>Wet mount: motile flagellates</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Wet mount: yeast</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Chlamydia trachomatis</em> PCR</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em> PCR</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

PCR indicates polymerase chain reaction.
PRENATAL DIAGNOSIS AND MANAGEMENT OF HEMOLYTIC DISEASE OF THE FETUS AND NEWBORN

Introduction
Hemolytic disease of the fetus and newborn (HDFN) used to be a major cause of fetal demise and death of newborns.¹ The probable first description of HDFN was in the French literature dating back to 1609 by a midwife in a set of twins.¹ One fetus was hydropic and stillborn, and the other was deeply jaundiced and died of kernicterus soon after birth.¹,² However, the clinical entity of erythroblastosis fetalis was established in 1932 by Diamond and colleagues by showing that hydrops and kernicterus were both aspects of the same condition they termed erythroblastosis fetalis.² In this condition, they described that hemolysis of fetal and neonatal RBCs resulted in extramedullary erythropoiesis, causing hepatosplenomegaly and circulation of erythroblasts.² In 1941, Levine and coworkers determined that most cases of erythroblastosis fetalis were due to isoimmunization of RhD-negative mothers by RhD-positive fetal RBCs.¹,² Currently, approximately half of Rh-positive newborns with hemolytic disease of the newborn have mild disease and do not require treatment, while approximately 20% become hydropic in utero.³,⁴ One-fourth of Rh-positive newborns with hemolytic disease of the newborn require treatment, without which they develop severe jaundice and may die of kernicterus.² If these newborns survive, they are at higher risk for deafness and mental retardation.²,⁵ The prenatal diagnosis and management of HDFN is therefore crucial in the prevention of these serious complications.

Pathogenesis
Red blood cell antigen alloimmunization can occur when foreign RBC antigens are introduced to the circulation, and anti-RBC alloantibodies are produced in the recipient.⁴ Sources of foreign RBC antigen exposure include pregnancy or blood transfusion. In the setting of blood transfusion, only ABO RhD antigens are matched between the donor and recipient.⁵ Other antigens, such as Kell, Duffy, Kidd, and Rh antigens other than the D antigen, are not routinely matched.⁵ Patients can produce alloantibodies against any foreign antigens present in transfused blood.³ In the setting of pregnancy, a fetus may possess paternally inherited blood group antigens not expressed by the mother, and exposure of fetal RBCs to the maternal circulation can happen during an episode of maternal-fetal bleeding, most commonly intrapartum and less commonly antepartum.⁴ Factors such as the volume of the fetal bleed, fetal antigen immunogenicity, and maternal immune system responsiveness influence whether alloimmunization will occur.⁶ During the course of alloimmunization, both IgG and IgM antibodies can be produced by the mother.⁶,⁷ However, only IgG antibodies cross the placenta and destroy fetal RBCs in a subsequent pregnancy, leading to HDFN.⁶ In this setting, HDFN generally does not occur during the first pregnancy because most instances of maternal-fetal bleed leading to alloimmunization occur at the time of delivery, with subsequent alloantibody formation in the postpartum period after the first pregnancy.⁶
Types of HDFN
Rh antibodies were and still are the most common cause of severe HDFN. Other alloantibodies against Kell, Duffy, Kidd, and MNSs antigens also can cause severe HDFN. The most common cause of mild HDFN is due to ABO incompatibility between the mother and fetus/newborn.

Rh HDFN
The Rh blood group system is the most immunogenic and polymorphic blood group system in humans. Antibodies to the Rh antigens (anti-D, c, C, e, E) are usually IgG, which can cross the placenta and destroy fetal RBCs. Among the Rh antibodies, anti-D and anti-c are most commonly implicated in HDFN in Western countries. This is due to the strong immunogenicity of Rh blood group antigens and the relatively higher frequency of Rh negativity in a mostly white population (approximately 15% of whites vs 8% of Hispanics, 5% of blacks, and <0.1% of Asians). The risk of RhD immunization after the delivery of the first Rh-positive child to an Rh-negative mother is 16% if the Rh-positive fetus is ABO compatible with its mother, and 2% if the fetus is ABO incompatible. ABO incompatibility confers some protection against RhD alloimmunization in this context because ABO-incompatible fetal RBCs are rapidly removed from the maternal circulation thereby reducing the likelihood of RhD antigen exposure to the maternal immune system. The routine postnatal and antenatal administration of RhIG to RhD-negative mothers has dramatically decreased the incidence of D alloimmunization in pregnancy from 14% to 0.1%. Despite the effectiveness of this prophylactic modality, RhD incompatibility and alloimmunization remains the most common cause of severe HDFN.

ABO HDFN
Maternal-fetal ABO incompatibility occurs in approximately 6.9% of all births in the United States, and is the most common cause of neonatal jaundice. Most cases result in mild hemolytic disease, with only a moderate increase in unconjugated bilirubin in the infant. However, cases of severe hemolytic disease have been reported. Because antibodies against A and B antigens are naturally occurring antibodies, ABO hemolytic disease can occur in the first pregnancy. While the majority of anti-A and/or anti-B antibodies are IgM, anti-A, B antibodies in type O blood are mostly IgG. Therefore, most cases of HDFN due to ABO incompatibility occur when the mother is type O and the infant is type A, B, or AB.

HDFN From Other Blood Groups
Warm reactive IgG antibodies against other blood group antigens such as Kell, Duffy, Kidd, and MNSs can cause HDFN. The clinical manifestations associated with HDFN due to other blood groups ranges from mild to severe.

Antibodies to the Kell blood group antigen are associated with severe HDFN with hydrops fetalis. The severity of the disease equates with the presence of the Kell antigen on both immature and mature RBCs. Subsequent antibody-antigen interaction in the fetus results in the suppression of erythropoiesis and hemolysis of mature RBCs, respectively. Although RhIG has been around for >40 years, prophylactic immune globulins to prevent alloimmunization to other blood group antigens have not been developed.
Prenatal Management of Maternal Alloimmunization

At the first prenatal visit, an ABO RhD blood group and antibody screen should be performed on the expectant mother for the presence of erythrocyte antibodies. The antibody titer should serve as a baseline and is the first step in the evaluation of an alloimmunized patient. The human antoglobulin titer (indirect Coombs) is used to determine the degree of alloimmunization.

The presence of a clinically significant maternal antibody may change maternal and fetal management during the course of the pregnancy. This management will vary depending on the likelihood for development of HDFN, which is based on presence of the antigen on fetal RBCs and the maternal immune response (monitored through periodic antibody titers). The current preferred modality for early determination of fetal blood type is by polymerase chain reaction typing of amniotic fluid. This modality is highly sensitive and specific (98.7% and 100%, respectively) but requires an invasive procedure to obtain the proper specimen and therefore is rarely performed in clinical practice. More recently, select laboratories have determined the fetal blood group genotype by molecular analysis of fetal cell-free DNA in the maternal plasma or serum in the second trimester. This test is not yet widely available.

Paternal testing should be undertaken to see if the patient’s partner is heterozygous for the offending antigen. If he is found to be negative for the antigen and there is no question of paternity, no further testing is warranted, because the fetus will not be at risk for HDFN. If paternal testing returns a positive result, zygoosity testing can be requested from the blood bank as a heterozygous state can be detected through serologic testing. If the father of the fetus is homozygous and paternity is assured, the fetus is clearly at risk for HDFN. A history of a negative offspring confirms a paternal heterozygous state. After a heterozygous paternal genotype is confirmed or if the paternal genotype is unknown, the next step in the evaluation of the sensitized patient should be to determine the RhD type of the fetus.

In the first affected pregnancy after sensitization to RhD antigen is detected, maternal titers are repeated every month until approximately 28 weeks; thereafter titers are repeated every 2 weeks. A critical titer is that titer associated with a significant risk for severe erythroblastosis fetalis and hydrops, and in most centers that level is between 1:16 and 1:32. A lower threshold (most commonly 1:8) is used for anti-Kell because Kell antibodies do not correlate with fetal status. Fetal anemia in cases of Kell sensitization is due to sensitization of antigen-positive cells with subsequent sequestration by the fetal reticuloendothelial system and erythropoietic suppression. However, for a woman with a history of a previously affected fetus or neonate, serial titer assessment is inadequate for surveillance of fetal anemia, and close fetal monitoring by Doppler ultrasound is considered the necessary standard of care.

Prenatal Diagnosis and Management of HDFN

If the maternal alloantibody titer reaches a critical titer level, or has a 4-fold increase from baseline, or the pregnant woman had a previously affected fetus, the fetus requires intensive monitoring for signs of anemia and fetal hydrops due to the high risk for HDFN.
Historically, spectral analysis of amniotic fluid was the accepted modality in monitoring fetal anemia. The $\Delta OD_{450}$ of amniotic fluid measures the level of bilirubin and thus indirectly measures the degree of fetal hemolysis. The $\Delta OD_{450}$ values are then plotted on a Queenan curve (for <27 weeks' gestational age) or a Liley curve (for 27 weeks' gestational age to term), which stratifies the degree of fetal anemia and sets indications for intruterine transfusion.

Currently, to detect fetal anemia and diagnose HDFN, a noninvasive method such as measurement of the peak systolic velocity of the fetal middle cerebral artery (MCA) by Doppler ultrasound is preferred. Increased peak velocity is an indication of increased cardiac output, a common compensatory response in a fetus with anemia due to any cause. Because the MCA velocity increases with advancing gestational age, the results are reported in multiples of median (MoMs) for the given gestational age. An MCA peak systolic velocity that is greater than 1.5 MoMs for gestational age predicts moderate to severe fetal anemia, with 100% sensitivity and a 12% false-positive rate.

As early as at 18 weeks' gestation, the MCA Doppler study can be started, but there is a higher false-positive rate after 34 to 35 weeks of gestation, and this procedure should be performed by specifically trained practitioners. However, in clinics where MCA Doppler is unavailable, spectral analysis of amniotic fluid and use of the Queenan or Liley curve is still useful.

Another method of detecting fetal anemia and diagnosing HDFN is ultrasound-directed fetal blood sampling, also known as percutaneous umbilical blood sampling (PUBS), or cordocentesis. This method allows for direct sampling to determine not only fetal hemoglobin, hematocrit, and reticulocyte count, but also blood type, direct antiglobulin test with elution test, and total bilirubin level. PUBS can be performed only after 20 weeks' gestation. If gestational age is <20 weeks or PUBS has failed because of technical difficulties, amniocentesis can be performed for measurement of the amniotic fluid bilirubin level to assess the severity of anemia and determine fetal RBC genotype. Amniocentesis can be performed as early as at 15 weeks' gestation. Both of these procedures are invasive and associated with a 1% to 2% rate of fetal loss. Therefore, fetal blood sampling is indicated only after the peak MCA Doppler exceeds 1.5 MoM or when done as part of the intratriuterine fetal transfusion (IUFT) procedure.

When the fetal hemoglobin level is $\leq 11.0$ g/dL ($\leq 110$ g/L), fetal hematocrit is $\leq 30.0\%$, or <2 SDs for gestational age, the diagnosis of HDFN is established, and an intratertinerative transfusion of RBCs is considered. IUFT can be achieved by venous transfusion via the umbilical cord vein (preferred route) or peritoneal transfusion or a combination of both. Peritoneal transfusion is associated with a higher rate of complications and is less effective.

Blood products selected for IUFT should be compatible with both the maternal and fetal blood group types as well as antigen-negative for any maternal alloantibodies. If the fetal blood type is unknown, group O Rh-negative RBCs that are antigen negative for the mother's corresponding antibodies and cross-matched compatible with maternal plasma are selected. Typically transfusion services provide RBCs that are hemoglobin-S negative to avoid potential intravascular sickling, leukocyte-reduced and/or cytomegalovirus seronegative to prevent virus transmission, RBCs <3 to 7 days old and stored in CPDA-1 to avoid high levels of potassium and to maximize RBC
survival, and irradiated to prevent transfusion-associated graft-versus-host disease.\textsuperscript{5,6} Cells are packed to a hematocrit of 75\% to 85\% to prevent volume overload in the fetus.\textsuperscript{5,6} The blood also needs to be warmed to room temperature.\textsuperscript{5} The volume of transfusion is approximately 50 mL/kg estimated body weight using ultrasonography, or volume can be estimated using formulas based on fetal hematocrit, fetal body size, and desired hematocrit increment.\textsuperscript{5,6} After a goal of a fetal hematocrit of 40\% to 50\% is achieved, subsequent transfusions are usually performed at 14-day intervals.\textsuperscript{5,6} If the goal cannot be achieved in the first attempt due to technical difficulties, a second transfusion can be done 7 to 10 days later.\textsuperscript{5,6}

If severe HDFN is detected before 20 weeks of gestation, the mother can undergo extensive plasmapheresis or intravenous immunoglobulin administration at 1 g/kg body weight weekly to limit the rise in antibody titers, prevent onset of fetal hydrops, and postpone/avoid PUBS and intrauterine transfusion.\textsuperscript{5,6} These procedures also can be used in the mother as adjunct therapy if the fetus is not responding to IUFT as anticipated.\textsuperscript{5,6}

Kell alloimmunization has a more unpredictable course, and often causes more severe fetal anemia compared with HDFN secondary to other alloantibodies.\textsuperscript{4} Doppler measurements appear to be more accurate than amniotic fluid bilirubin measurements in predicting fetal anemia in these cases.\textsuperscript{4}

**Case Summary**

Upon discovery of the dramatic rise in antibody titers, Doppler ultrasound measurement of peak blood-flow velocity of the MCA of the fetus was performed at 25 weeks' gestation, and results were consistent with severe HDFN. The fetus showed signs of hydrops fetalis. The patient underwent her first IUFT with 120 mL of Group O, D-, C-, E-antigen negative, leukocyte-reduced, irradiated, cytomegalovirus-seronegative, and <5-day-old RBCs. Type AB plasma was added to the unit to achieve the desired hematocrit of 70\%, which was specified by the obstetrician. The blood was prewarmed to room temperature and delivered in two 60-mL syringes. The fetal serologic testing of a specimen obtained by PUBS showed that the fetal RBC phenotype was group O, D- and E-antigen positive, C-antigen negative, and the fetal hemoglobin and hematocrit were 3.4 g/dL (34 g/L) and 11.0\%, respectively. The alloantibody titers throughout the pregnancy are shown in the Figure, and the anti-D

![Figure](image)

**Figure.** Red blood cell alloantibody titers up to delivery.
and anti-G titers remained high until delivery. Two more IUFTs were done at 28 and 34 weeks’ gestation.

At 36 weeks’ gestation, via planned Cesarean delivery, the patient successfully delivered a 2460-g male infant with Apgar scores of 8 and 9 at 1 and 5 minutes, respectively. The infant’s hematocrit was 46.0%, and the bilirubin was elevated at 12.2 mg/dL. The direct antiglobulin test was positive. There were no other signs of HDFN. Following phototherapy, the infant was discharged home on day 7 of life with a normal hematocrit and normal bilirubin level.

Conclusion
In a previously alloimmunized pregnant patient, even mild maternal-fetal bleeding can trigger a secondary immune response and result in severe HDFN. Close follow-up of antibody titers, monitoring of fetal status, and patient education of the importance of compliance with medical advice are crucial to early diagnosis and treatment of HDFN to prevent severe complications and fetal demise.

REFERENCES


CME DOCUMENTATION QUESTIONS

1. Which of the following antibodies if present in a pregnant woman can cross the placenta and potentially cause hemolytic disease of the fetus and newborn (HDFN)?
   A) IgA
   B) IgD
   C) IgG
   D) IgM

2. A gravida 2 para 1, RhD-negative woman presents with the following serial RhD antibody titers: 12 weeks, 1:2; 16 weeks, 1:2; 20 weeks, 1:4. What is the most appropriate next step in management?
   A) Repeat titers in 4 weeks
   B) Intraterine fetal blood transfusion
   C) Fetal middle cerebral artery Doppler ultrasound
   D) Cordocentesis to determine fetal hematocrit

3. HDFN in a blood type O, RhD-positive fetus is detected in a gravida 2 para 1, blood type O, RhD-negative mother. Blood is requested for intraterine transfusion. A type O, leukocyte-reduced, and irradiated unit is prepared and preserved in CPDA-1. The blood should also be
   A) O positive, to match the blood type of the fetus.
   B) collected within no more than 1 to 2 days.
   C) packed to a maximum hematocrit of 40% to 50%.
   D) cytomegalovirus seronegative.

4. The goal for the hematocrit for intraterine transfusion is
   A) 75% to 85%.
   B) 40% to 50%.
   C) 60% to 70%.
   D) 30% to 40%.

5. A gravida 3 para 2 woman with a history of HDFN in a previous pregnancy presents for prenatal care. Which of the following is the best choice for monitoring fetal anemia in this case?
   A) Serial maternal antibody titers every 4 weeks up to 28 weeks, then every 2 weeks until birth
   B) Fetal middle cerebral artery Doppler ultrasound starting at 18 weeks
   C) Percutaneous umbilical blood sampling every 4 weeks up to 30 weeks, then every 2 weeks until birth
   D) Amniocentesis every 4 weeks up to 24 weeks, then every week until birth

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