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TITLE: Hemolytic Disease of the Fetus and Newborn

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Slide 1:

Hello, my name is <**Kerry O'Brien**>. I am the <**Medical Director of the Blood Bank at Beth Israel Deaconess Medical Center in Boston, MA. I am also an Assistant Professor in Pathology at Harvard Medical School**>. Welcome to this Pearl of Laboratory Medicine on "**Hemolytic Disease of the Fetus and Newborn**."

Slide 2:

At the end of this presentation participants should be able to: define hemolytic disease of the fetus and newborn or HDFN, discuss the pathophysiology of HDFN, be able to recognize pregnancies at risk for HDFN, and create a plan for the prenatal and postnatal management of HDFN.

Slide 3:

In HDFN, pre-existing maternal red blood cell or RBC alloantibodies target paternally inherited fetal RBC antigens. Maternal IgG antibody transports across the placenta into the fetal circulation where it binds to the corresponding RBC antigen, causing destruction by macrophages in the fetal spleen.

Slide 4:

The pathophysiology of HDFN involves several aspects. The mother must be negative for an RBC antigen. The mother must have been exposed to this foreign RBC antigen through a prior pregnancy or transfusion. She then may or may not form an IgG antibody to this foreign antigen. If she forms an antibody and then becomes pregnant with a fetus that has inherited this target antigen from its father, this fetus will be at risk for HDFN. The antibody can cross the placenta to reach the target alloantigen. Antibody coated RBCs are then destroyed by the fetal spleen.

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The fetus initially responds by increasing erythropoiesis in the bone marrow, releasing immature RBCs into circulation prematurely, a process known as erythroblastosis fetalis. As the fetal anemia worsens, erythropoiesis occurs in the fetal liver and spleen, causing organomegaly and portal hypertension. The decrease in albumin production leads to reduced plasma colloid oncotic pressure, generalized edema, ascites, and effusions known as “hydrops fetalis”. Untreated, hydrops can lead to death from high output cardiac failure as early as 18 to 20 weeks gestation.

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At the first prenatal visit, maternal ABO and Rh(D) type should be determined. An antibody screen should also be performed at this visit.

Slide 7:

Slide 7 shows an example of an abbreviated antibody screen. In this example, the patient’s serum reacts 3+ with screening cell #2. The serum does not react with screening cell #1.

Slide 8:

When the antibody screen is positive, the next step is to identify the antibody and perform a baseline titer. If the titer is not critical, repeat titers should be performed at 2 to 4 week intervals beginning at 18 to 20 weeks gestation until a critical titer is reached.

The previous titer should be stored frozen and repeated in parallel with the current specimen.

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The most common HDFN is that due to ABO. This type of HDFN is generally mild and usually involves a group O mother and a group A baby. The next most common antibodies involved in HDFN are anti-D, followed by anti-K and anti-c. It is worth noting that the incidence of anti-D HDFN has decreased dramatically since the creation of RhIG in the 1960's.

Slide 10:

Titers are reported as the integer of the greatest tube dilution with a positive agglutination reaction. Titers may vary between laboratories so they should be done where the patient is receiving care. A change in more than 1 dilution is considered significant.

Slide 11:

An example of a titration procedure is shown on slide 11. In this procedure, one starts by adding 1 mL of isotonic saline to each empty master dilution tube (tubes 2 through 2048). Next, add 1 mL of patient serum to tube #2. The contents of tube #2 are mixed and then 1 mL is taken from this tube and added to tube #4 and so on. One then transfers 2 drops of each master dilution tube into the corresponding test tubes. One drop of reagent RBC suspension is then added to each labeled dilution tube and mixed. The tubes are then incubated for 1 hour at 37 degrees Celsius and then washed 3-4 times with isotonic saline. Two drops of antihuman globulin are then added to each tube and the tubes are centrifuged. Starting with tube #2048, read and grade the agglutination towards tube #2. Add 1 drop of Coombs control cells to each negative and weak + tube and centrifuge. Reactions must be 2+ or greater for the test to be valid.

Slide 12:

For partners with a prior child with HDFN, titers are inadequate for surveillance of fetal anemia. The critical antibody titer is the level below which HDFN and hydrops fetalis are unlikely and no invasive procedures are needed. The critical titer for anti-D is 16 in the antihuman globulin phase according to the Technical Manual, 18th edition. The critical titer for all other IgG antibodies (except for anti-K) are extrapolated from the critical titer for anti-D.

Slide 13:

Anti-K is an exception. The Kell system antigens are present on early erythroid precursors. Even a low maternal anti-K titer can result in erythropoietic failure and severe fetal anemia. There is no safe titer for anti-K in pregnancy according to the American College of Obstetricians and Gynecologists Practice Bulletin Number 75.

Slide 14:

If a pregnancy is at risk for HDFN, if paternity is assured, the father of the fetus can be phenotyped for the antigen of interest. For example, in the case of maternal anti-E, the father can be phenotyped for the E-antigen and, if positive the e-antigen to determine zygosity. The father could in theory be EE, Ee or ee, putting the fetus at 100%, 50% or 0% risk of HDFN if paternity is assured. It should be noted that zygosity for the D-antigen cannot be determined by routine serologic methods. If the paternal phenotype is heterozygous or unknown, fetal genotyping can potentially be performed after obtaining fetal cells through amniocentesis or chorionic villus sampling or by using maternal serum.

Slide 15:

When a critical titer is reached, nonserologic means must be used to monitor the fetus for anemia. The most common method used for monitoring at-risk pregnancies is transcranial middle cerebral artery Doppler ultrasonography. Moderate to severe anemia can be predicted by peak systolic velocity above 1.5 times the median for gestational age. MCA Doppler has a 100% sensitivity and a 12% false positive rate.

Correct technique is critical and this test should only be performed by those with adequate training and expertise.

MCA Doppler ultrasonography has largely replaced amniocentesis with bilirubin measurement using spectral analysis at 450 nanometers. The delta OD 450 is still sometimes performed however and involves plotting the change in optical density at 450 nanometers on either a Liley graph in the late second and third trimester or the Queenan curve for earlier gestational age pregnancies (19-25 weeks). This test is not appropriate for anti-K HDFN.

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If MCA Doppler measurements are concerning for fetal anemia, cordocentesis can be performed to obtain a fetal blood sample for hematocrit testing. The goal of intrauterine transfusion is a post-procedure fetal hematocrit of 40-45%. Group O, Rh(D) negative RBCs that are negative for the antigen of interest are transfused; the RBCs must also be irradiated, CMV-safe, fresh (ideally less than 7 days old), negative for hemoglobin S and maternally crossmatch compatible. The umbilical vein is the most common site for transfusion. Intrauterine transfusion can be performed as early as 18-20 weeks gestation. There is a 1 to 2% risk of fetal mortality associated with intrauterine transfusion.

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Prior to performing an intrauterine transfusion, the volume of RBCs to be transfused must be calculated. This is done by determining the fetal and placental total blood volume by multiplying the ultrasound estimated fetal weight in grams by 0.14 mL/g. One then multiplies the total blood volume by the difference in post-transfusion and pre-transfusion hematocrit. That number is then divided by the hematocrit of the RBC unit.

An example would be for a fetus with an estimated weight of 1000g with a pretransfusion hematocrit of 15%: One first multiplies 1000 grams by 0.14 milliliters per gram. The resulting number is multiplied by the difference between 0.40 and 0.15. The result is divided by 0.85 giving an RBC volume of 41.2 milliliters to be transfused.

Slide 18:

Post-natally, close monitoring of the neonatal bilirubin is recommended. The infant may require blue-green light phototherapy. IVIG may also be needed to help control hemolysis in the infant. Double volume exchange transfusion may be needed in infants unresponsive to phototherapy and IVIG. Group O, Rh(D) negative RBCs that are negative for the target antigen that are ideally less than 7 days old are desired. The RBCs must also be CMV-safe, irradiated and hemoglobin S negative. The Group O plasma must be removed from the RBC product and replaced with AB plasma and the final product should be diluted to a hematocrit of 40-50%.

Slide 19:

In conclusion, HDFN occurs when a mother has a red blood cell antibody toward a paternally inherited fetal RBC antigen. Erythroblastosis fetalis, hydrops fetalis and death from high output cardiac failure may occur in pregnancies affected by HDFN. An antibody screen and identification performed during early pregnancy can identify at-risk patients and establish a baseline titer. Titers performed every 2 to 4 weeks starting at 18-20 weeks gestation are used for monitoring until a critical titer is attained.

Slide 20:

Transcranial MCA Doppler ultrasonography, or less commonly, amniocentesis with bilirubin measurements are useful for assessing for fetal anemia once a critical titer is reached. Intrauterine transfusions may be performed when nonserologic tests are suspicious for moderate to severe fetal anemia beginning at 18-20 weeks gestation.

Slide 21: References

Slide 22: Disclosures

Slide 23: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on **"Hemolytic Disease of the Fetus and Newborn"**