



437 Team: Obstetrics and Gynecology

Rhesus Isoimmunization

Objectives:

- Describe the pathophysiology and diagnosis of alloimmunization
- Describe the use of immunoglobulin prophylaxis during pregnancy for the prevention of alloimmunization
- Discuss the management of a patient with Rh-D sensitization in pregnancy

References:

- Kaplan USMLE step 2 CK - Obstetrics and Gynecology
- Online Meded videos
- Team 435

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Alloimmunization

Definition: When a pregnant woman develops **antibodies to foreign red blood cells (RBCs)**, most commonly against those of her **current or previous fetus(es)**. It is rarely caused by transfusion of mismatched blood.

Pathophysiology: The initial response to exposure to Rh antigen is the **production of immunoglobulin M (IgM)** antibodies for a short period of time, followed by the **production of IgG antibodies** that are capable of crossing the placenta. If the fetus has the Rh antigen, these antibodies will coat the fetal red blood cells and cause hemolysis.

The most common RBC antigens are of the Rh system (C, c, D, E, e) (**most common is big D**).

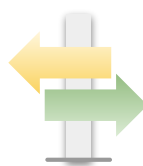
- Antibodies to RBC antigens are detected by **indirect Coombs test** (atypical antibody test [AAT]). The concentration of antibodies is reported in dilutional titers with the lowest level being 1:1, and titers increasing by doubling (e.g., 1:1, 1:2, 1:4, 1:8, 1:16, 1:32...1:1,024, etc.).
- **Hemolytic disease of the newborn (HDN)** is a continuum ranging from hyperbilirubinemia to erythroblastosis fetalis. HDN is caused by maternal antibodies crossing into the fetal circulation and targeting antigen-positive fetal RBCs, resulting in hemolysis. When severe, this can result in anemia, fetal hydrops, and even death.

Risk Factors:

Alloimmunization most commonly occurs when **fetal RBCs enter** the mother's circulation transplacentally at delivery. It can also occur if a woman is transfused with mismatched RBCs.

Other pregnancy-related risk factors are:

1. amniocentesis,
2. ectopic pregnancy,
3. D&C,
4. abruptio placentae,
5. and placenta previa.



Protective Factors:

ABO incompatibility decreases the risk of maternal alloimmunization from foreign RBCs. Naturally occurring anti-A and anti-B antibodies rapidly lyse foreign RBCs before maternal lymphocytes are stimulated to produce active antibodies

Requirements (all must be present).

- **Mother** must be **antigen-negative**.
- **Fetus** must be **antigen-positive**, which means the **father** must also be **antigen +ve**.
- Adequate fetal RBCs must cross over into the maternal circulation to stimulate her lymphocytes to produce antibodies to the fetal RBC antigens.
- Antibodies must be associated with Hemolytic disease of the newborn (**Erythroblastosis fetalis**).
- Significant titer of maternal antibodies must be present to cross over into the fetal circulation and lead to fetal RBC hemolysis (**>1:16**)



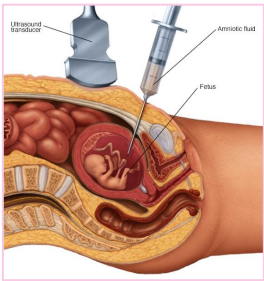
NOTE

Fetal blood type may be determined by amniocentesis or percutaneous umbilical blood sampling (PUBS). If the fetus is RBC antigen-negative, there is no fetal risk.

Detecting Fetomaternal/Transplacental Hemorrhage

- **Kleihauer-Betke test** quantitates the volume of fetal RBCs in the maternal circulation by differential staining of fetal and maternal RBCs on a peripheral smear. This can assess whether more than one vial of RhoGAM needs to be given when large volumes of fetal-maternal bleed may occur (e.g., abruptio placentae).
- It is dependent on the fact that adult hemoglobin is more readily eluted through the cell membrane in the presence of acid than is fetal hemoglobin (**HbF**).
- # of fetal cells counted/ # of maternal cells counted = Estimated fetal blood volume (mL)/ Estimated maternal blood volume (mL)
- **Rosette test** is a qualitative screening test for detecting significant fetomaternal hemorrhage (≥ 10 mL).

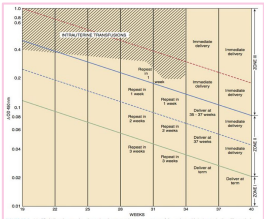
Techniques to Evaluate Fetal Rh Status



→ Amniocentesis

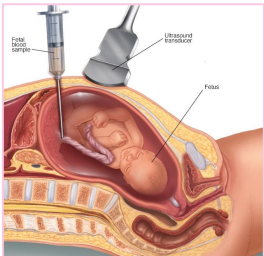
→ Free fetal DNA in maternal serum

→ U/S (we may see hydrops*)



→ MCA doppler (**most valuable to detect fetal anemia**)

→ Amniotic fluid spectrophotometry (**best to estimate fetal bilirubin concentration**)**



→ Liley chart or modified Liley chart (**Queenan chart**)

→ Percutaneous umbilical blood sampling (PUBS) → we can measure fetal Hb, Hct, blood gases, pH, and bilirubin levels.

*Both the placenta and the fetal liver are enlarged with hydrops. Fetal hydrops is easily diagnosed by the characteristic appearance of one or more of the following: ascites, pleural effusion, pericardial effusion, or skin edema.

**There is an excellent correlation between the amount of biliary pigment in the amniotic fluid and the fetal hematocrit, beginning at 27 weeks' gestation.

- The incidence of fetomaternal hemorrhage with amniocentesis → **8.4-11%** per procedure.
- The incidence of fetomaternal hemorrhage with PUBS → as high as **40%**.

Management

1 Determine if the fetus is at risk for anemia

- **Fetal risk is present only if**
 - (a) atypical antibodies are detected in the mother's circulation,
 - (b) antibodies are associated with HDN,
 - (c) antibodies are present at a significant titer ($>1:8$), and
 - (d) the father of the baby (FOB) is RBC antigen-positive.
- **No fetal risk is present if**
 - (a) the AAT is negative,
 - (b) antibodies are present but are NOT associated with HDN,
 - (c) antibody titer is $\leq 1:8$, or
 - (d) the FOB is RBC antigen-negative.
- **If the atypical antibody titer is $\leq 1:8$, management is conservative. Repeat the titer monthly as long as it remains $\leq 1:8$.**

2 Assess if the fetus is anemic using Doppler ultrasound

Doppler ultrasound measures peak flow velocity of blood through the fetal middle cerebral artery (MCA). As fetal anemia worsens, the peak systolic velocity rises. Doppler MCA ultrasound is the **procedure of choice** since it is **non-invasive** and has a **high correlation with fetal anemia**.

3 Intervene if the anemia is severe

This is diagnosed when amniotic fluid bilirubin is in Liley zone III or PUBS shows fetal hematocrit to be $\leq 25\%$ or MCA flow is elevated.

- If gestational age < 34 weeks, perform intrauterine intravascular transfusion.
- If gestational age ≥ 34 weeks, perform delivery.

How to Manage the Baby?

- Intrauterine transfusion (fresh **O Rh-** blood and packed RBCs, repeat transfusions are scheduled at 1 to 3 week intervals, last transfusion should be between 32 and 34 weeks).
- Intraperitoneal transfusion* (RBCs are absorbed via the diaphragmatic lymphatics, non-hydrotic fetuses absorption should occur in **7 to 9 days**, in hydroptic ones it's variable)
- Maternal Plasmapheresis
- Phenobarbital (Has been shown to **induce fetal liver enzyme** activity and maturation, this is used 2-3 weeks before delivery)

*Formula for intraperitoneal transfusion:
Volume = [GA (wks) - 20] x 10

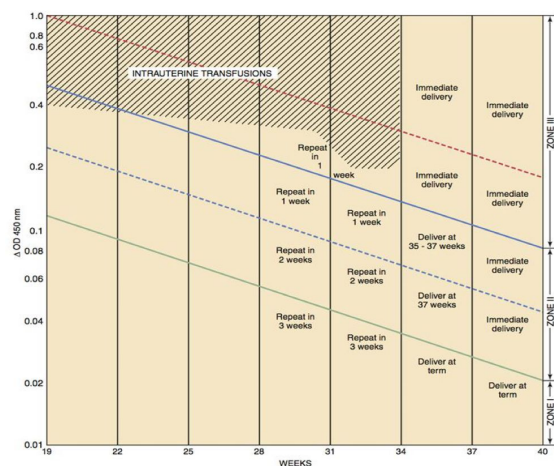


FIGURE 15-2 Modified Liley chart used to determine the appropriate management of the patient with isoimmunization. The optical density at a wavelength of 490-nm (ΔOD 490-nm) level in the amniotic fluid at a given weeks' gestation determines whether fetal transfusion or delivery is advisable.



Timing of delivery

- Fetuses are evaluated at least twice weekly from **24 to 28 weeks** for fetal well-being (NST, modified biophysical profile) and fetal growth.
- Delivery is performed if gestational age is **>34 week**.
- If delivery is expected to occur before 34 weeks' gestation (or if amniocentesis suggests an immature lung profile), **betamethasone** should be given at least 48 hours before delivery to enhance fetal pulmonary maturation.

Prevention



RhoGAM is pooled anti-D IgG passive antibodies that are given IM to a pregnant woman when there is significant risk of fetal RBCs passing into her circulation. The passive IgG antibodies attach to the foreign RBC antigens, causing lysis to occur before the maternal lymphocytes become stimulated.

- **RhoGAM** is routinely given to Rh(D)-negative mothers at **28 weeks**, and **within 72 h** of chorionic villus sampling (CVS), amniocentesis, or D&C. It is also given **within 72 h** of delivery of an Rh(D)-positive infant. About **300 mcg** of RhoGAM will neutralize 15 ml of fetal RBCs or 30 mL of fetal whole blood.
- All pregnant women who are RhD -ve and Anti D -ve and experience → (spontaneous or induced abortion, ectopic pregnancy, significant vaginal bleeding, abdominal trauma, or external cephalic version) should receive **50 to 100 µg before 12 week of gestation and 300 µg after 12 week**.
- RhO-GAM is probably not necessary for “complete” molar pregnancy, but necessary for “partial” molar pregnancy.



Irregular Antibodies:

- Kell Antibodies can elicit a strong **IgG** reaction similar to Rh isoimmunization.
- In Kell isoimmunization, the anemia is due to more of **suppression of hematopoiesis** rather than hemolysis.
- The predictor of anemia in this case is still the **MCA PSV**. (Like in Rh)

Important notes all over the lecture

- ★ Fetus can compensate for mild anemia that is caused by hemolysis. So we only intervene if severe.
- ★ If a woman has a previous pregnancy with fetal hydrops, there is a 90% chance of it occurring again in the next pregnancy (at the same time or earlier in the pregnancy).
- ★ Only direct measure of fetal anemia is PUBS = Cordocentesis.
- ★ Increasing levels of bilirubin will lead to fetal kernicterus which leads to cerebral palsy.
- ★ In Intrauterine transfusions, the goal is to transfuse fresh group O, Rh- negative packed red blood cells/ Overall survival rate following intrauterine transfusion is about 85%.



Physiology & Immunology

- Alloimmunization can only happen in antigen negative mom.
- If the mom has antigen negative and the dad has antigen positive, the baby will have antigen positive.
- In the **first pregnancy**, the **baby is not at risk** because the mom's immune system has never recognized the positive antigen. At the end of pregnancy, there will be maternal-fetal blood mixing, the primary response of the mom's body build IgM which can't cross the placenta and affect the baby.
- In the **subsequent pregnancy**, the **baby is at risk** because the mom's immune system has matured its response and convert IgM to IgG which can easily cross the placenta and attack baby's blood leading to fetal anemia.
- Antibodies in right type and sufficient quantity are necessary to cause harm for the baby; there are 3 antibodies:
 - 1- **Lewis > Lives** (no fetal anemia).
 - 2- **Duffy > Dies** (causes fetal anemia), treated by Rhogam D which is Rh D immunoglobulin.
 - 3- **Kal > Kills**
- There must be a sufficient titers > **1:8** (this is not a ratio, the bigger the number after the colon, the more antibodies).

Diagnostic Workup

- **FIRST:** Determine mom Rh status; if positive STOP. If negative check the dad Rh status; if negative STOP. If positive or unknown, the baby at risk.
- **SECOND:** Determine mom Rh-antibodies status; if Rh-Ab is negative (such as in 1st pregnancy) give prophylaxis. if less than 1:8, they can't cause harm. If more than 1:8, do a Transcranial Doppler (TCD) study.
- **THIRD:** If the TCD study doesn't show increased blood flow, then even though the mom immune response is targeting the baby, it is not bad enough to consider. If the blood flow increased, there is fetal anemia and you have to make your decision based on the gestational age (GA). We assume that the delivery of oxygen is based on the amount of Hb and the cardiac output, if Hb falls in baby, the cardiac output has to increase to compensate. So, increased velocity and flow is an indication of fetal anemia.
- **FOURTH:** if baby > 32 weeks, delivery. If baby < 32 weeks, do Percutaneous Umbilical Blood Sampling (PUBS) test to draw the Hb (low Hb is confirmatory of fetal anemia). Also, it allows you to transfuse.

Prophylaxis

- Prophylaxis is given for a mom who is Ag (-) and Ab (-) exposed to a baby which is Ag (+) at time of maternal- fetal mixing (delivery, postpartum hemorrhage, C section or any obstetric procedure).
- **Rh (D) Ig will be given at 28 weeks (third trimester) and within 72 hours of maternal-fetal mixing.** The immunoglobulin will bind to baby's antigen. So, mom never sees the antigen and never develops the antibodies.
- After giving the prophylaxis once, no need to give it again in future pregnancies.

Teaching case (video case)

A 32 year-old P1101 woman and her new husband present for prenatal care at 20 weeks gestation. Her past obstetric history is significant for a first child delivered at term following an abruption. Her second child died of complications of prematurity following in utero transfusions for Rh alloimmunization. Her initial prenatal labs this pregnancy indicate her blood type as A negative and an antibody screen positive for anti-D with a titer of 1:256. You discuss any additional evaluation needed, her risks in this pregnancy, and the plan of management with her and her husband.



1. What is Rh alloimmunization and what are the red cell antigens involved?

- Occurs when any fetal blood group factor (in this case the Rh antigens) inherited from the father is not possessed by the mother. Antepartum or intrapartum fetal-maternal bleeding may stimulate an immune reaction in the mother.
- Most cases of Rh alloimmunization causing significant hemolytic disease in the fetus or newborn are the result of **D antigen** incompatibility

2. What are the risk factors for Rh alloimmunization?

- Any clinical situation that could lead to **fetal-maternal hemorrhage**. (mixing)
- **Obstetric procedure:** pregnancy termination, chorionic villus sampling, amniocentesis, external cephalic version.
- Threatened abortion, ectopic pregnancy, abortion.
- Delivery of an Rh+ neonate to an Rh- mother (cesarean or vaginal delivery)—**most common cause of alloimmunization**.
- Multifetal gestation/ Abdominal trauma.
- Bleeding placenta previa or abruption / Manual removal of placenta.
- Spontaneous fetal-maternal hemorrhage has been detected to 10% of cases of alloimmunization.
- **Partial molar pregnancy bc there are fetal parts**

3. What is the mechanism for RhoGAM prophylaxis against Rh disease? What is the dose of RhoGAM? What is the recommended schedule for RhoGAM administration?

- **Exogenous IgG** (Rho(D) immune globulin) suppresses the maternal immune response through **central inhibition**. The Rh D IgG coated fetal RBCs are sequestered in the maternal spleen and these antigen antibody complexes inhibit the primary immune response (B cell transformation to plasma cells) and antigen specific B cell proliferation.
- **300 micrograms** of anti-D immune globulin can prevent Rh D alloimmunization after an exposure to up to 30 mL of Rh D-positive blood or 14 mL of fetal cells.
- In the U.S. for Rh-mothers, the recommended immunoprophylaxis regimen using anti-D immunoglobulin is:
 - **300 mcg dose at 28 week EGA.**
 - **Second 300 mcg dose should be given if delivery has not occurred within 12 weeks of the initial dose.**
 - **Within 72 hours after delivery of an Rh+ neonate.**
 - **After first trimester pregnancy loss, threatened abortion, or elective termination.**
 - **After invasive antepartum procedures or external cephalic version or trauma.**
 - **After second or third trimester bleeding.**

4. Could this patient's Rh alloimmunization have been prevented?
What are the ways in which alloimmunization might be diagnosed?
Is there any further blood work that should be obtained before you counsel this patient on her risks in this pregnancy?
What are some ultrasound findings that may suggest Rh disease?

- Administration of an adequate dose of RhoGAM within approximately 72 hours prevents an active maternal antibody response to the fetal antigens. The extent of fetal to maternal hemorrhage can be estimated using the **Kleihauer-Betke test**.
 - Maternal antibody screen is recommended at the first prenatal visit, at 28 weeks gestation, at the time of any event in pregnancy associated with possible fetal-maternal hemorrhage, and postpartum. Positive antibody screens should be evaluated for strength of antibody response (titer) and type of antibody. A **critical titer** that may be associated with fetal hemolytic disease is most often **between 1:16 and 1:32**.
 - The paternal antigen status for the specific maternal antibody should be assessed to determine if the fetus is at risk. This assessment is accomplished by performing direct genotype testing of the father. If paternal testing is not possible, fetal antigen assessment can be accomplished through genetic analysis of fetal cells obtained through amniocentesis.
 - Ultrasound findings consistent with severe fetal anemia include elevated peak velocity of the middle cerebral artery and evidence of hydrops fetalis (fetal subcutaneous edema, pleural and/or pericardial effusions, and ascites).
- Negative first pregnancy > nothing happens then alloimmunization occurs
 - first visit do blood grouping +Ab titer (1:16 or more is significant titer), look at paternal antigen.
 - MCA at 16 weeks then every 2 weeks to delivery if titer is significant
 - titer every 2 weeks if titer is not significant
 - if severe titer and anemia (hemolysis) > intrauterine transfusion
 - hydrops fetalis: swelling in 2 cavities (plural, pericardium, ascites , subcutaneous)
 - Any minor trauma, bleeding or any pregnancy events >give RhoGAM