EVERYTHING YOU NEEDED TO KNOW ABOUT HDN IN ONE CASE STUDY
ANSWERS

Questions, Pregnancies 1 & 2:

1. What immunohematologic tests are routinely performed at the first prenatal visit?
   - *ABO type; little utility, done as a routine.*
   - *Rh type; allows prediction of the need for Rh immune globulin prophylaxis.*
   - *Antibody screen; detects alloimmunization potentially causing HDN.*

2. What is RhIG? What is the goal of RhIG therapy? Why is RhIG delivered at 28 to 30 weeks gestation?
   - *Human polyclonal antibody directed against the Rh (D) factor.*
   - *Goal is to prevent alloimmunization against the antigen most often causing severe HDN.*
   - *Postnatal therapy reduces risk of alloimmunization from 16% per at-risk pregnancy to approximately 2%. Addition of an antenatal dose at 28 to 30 weeks reduces the risk to about 2 in 1000 or less. Further protection could be obtained with a second antenatal dose but is generally not considered to be cost-effective, and RhIG is in short supply.*

3. Why was RhIG not given after the second delivery? What tests are performed to determine RhIG candidacy at 28 to 30 weeks and after delivery?
   - *The infant was Rh negative so immunization by fetal-maternal hemorrhage could not occur.*
   - *At 28 to 30 weeks the ABO and Rh typing and antibody screen is repeated, but the screen need not be completed before RhIG is issued; a woman is a candidate if she is Rh negative and has not made anti-D.*
   - *After delivery in addition to a type-and-screen the neonate's blood type and a screen for FMH in excess of 30 cc is performed; a mother is a candidate if she is Rh negative, has not made anti-D, and has an Rh positive newborn. If the fetal screen is positive the bleed must be quantitated by Kleihauer-Betke test to determine the proper RhIG dose.*

4. Are there any other indications for RhIG?
   - *Any cause of FMH in an Rh negative woman including amniocentesis or CVS, ectopic pregnancy, spontaneous or induced abortion, and maternal trauma including external version (screen for excess FMH). Also can prevent immunization by transfusion.*

Questions, Pregnancy 3:

1. Why did the patient have anti-D detected at delivery? Could it have been passively administered?
   - *The differential diagnosis is between passive and active immunization. Although passively administered anti-D is frequently detected at delivery after RhIg administration, the titer is generally less than 4. This titer suggests that she is an RhIG failure.*

2. Why was postnatal RhIG given?
   - *Administration of RhIG in spite of the very probable occurrence of active maternal immunization to the D (Rh) antigen reflects uncertainty regarding the diagnosis. Passively administered RhIG cannot reverse active immunization. However, because RhIG is very safe it should be given when there is any doubt about the diagnosis.*

3. What are the known causes of failure of RhIG prophylaxis?
   - *Immunization prior to the antenatal dose*
   - *failure to provide appropriate prophylaxis at a prior pregnancy (esp. abortion or ectopic)*
   - *failure to diagnose prior pregnancy (esp. abortion).*

Questions, Pregnancy 4:

1. How are antibody titers used in monitoring alloimmunized pregnancies? Why is repeat testing of previous samples performed in parallel?
   - *Antibody titers are used only as a threshold for performing more SPECIFIC, invasive tests for fetal hemolysis, namely amniocentesis and PUBS.*
   - *Because there is significant test-to-test variability in performing blood group antibody titers, parallel testing of the previous and current maternal sample is performed to make it easier to interpret.*

2. How does amniocentesis predict the risk of significant HDN in utero? How are the results interpreted?
   - *Amniotic fluid is subjected to a measure of hemoglobin breakdown pigments (including bilirubin), the ΔOD_{450}, the magnitude of which reflects the degree of hemolysis. Unfortunately this is an indirect measure of hemolysis and there is considerable overlap between unaffected and moderately affected infants, and between moderately affected and severely affected infants.*
   - *The ΔOD_{450} is compared to norms based on gestational age. Three "zones" of values are recognized; zone I indicates lack of significant hemolysis, zone III indicates severe disease requiring immediate intervention, and zone II values are equivocal. For zone II values following trends in ΔOD may be helpful.*

3. What toxicity results from elevated bilirubin levels in the neonate? What levels of bilirubin are considered critical?
   - *Unconjugated ("indirect acting") bilirubin is lipid soluble and crosses the blood brain barrier, where it is toxic to neurons, particularly in the subcortical nuclei including the basal ganglia. The accumulation of uncomplicated bilirubin in the infant is accentuated by the immaturity of the neonatal liver and the fact that unconjugated bilirubin is not excreted in the urine..*
   - *The bilirubin levels which indicate treatment are somewhat controversial, but recommended treatment thresholds are published by the Academy of Pediatrics. A bilirubin level of approximately 20 in a newborn is considered a high risk for kernicterus and lower levels may be toxic in premature infants or those with hypoxia, acidosis, and other complications.*

4. How does ultraviolet phototherapy ameliorate bilirubin toxicity?
   - *UV light converts unconjugated bilirubin in the skin into a photoisomer that is more readily excreted by the kidneys.*

5. Why did the infant need a transfusion at age 2 months?
   - *Maternal antibody persists in the fetal circulation for a long period of time (t½ at least 21 days), and continues to destroy the neonates incompatible RBCs.*

Questions, Pregnancy 5

1. How is PUBS performed and interpreted?
   - *Percutaneous Umbilical Blood Sampling is performed under ultrasound guidance which allows visualization of the lumen of the lumen of umbilical cord vessels. A cannula is placed via through the skin into the cord vein at a point at which the vein is relatively immobilized either by its connections with the placenta or the umbilicus; a "free loop" can also sometimes be cannulated, and intra-abdominal veins of the fetus have also been used. The fetus may be paralysed so that fetal movement does not dislodge the cannula. Blood can then be withdrawn for study or transfused.*
   - *Interpretation of PUBS sampling is considerably simpler than interpretation of amniocentesis data; if the fetal hematocrit is more than 2 SD below the mean for gestational age (roughly 30) therapy (transfusion or delivery) is indicated.*

2. How is blood selected and prepared for IUT?
   - *Group O RBCs are selected since the fetus' ABO type is unknown.*
   - *RBCs must be negative for the antigen corresponding to the maternal antibody, i.e. Rh negative cells in most cases.*

- *Cells must be washed to remove donor anti-A and anti-B.*
- *Cells should be from CMV negative donors or leukocyte filtered to prevent CMV-transmission to the fetus.*
- *Cells must be irradiated to prevent transfusion-associated GVHD.*
- *Cells should be tested to ensure that they are hemoglobin S negative.*
- *Cells from "fresh" (less than 5 days from draw) to provide optimal survival.*

3. How does exchange transfusion treat HDN? How is blood selected for exchange transfusion?
   - *Exchange transfusion addresses multiple issues:*
     1) It replaces neonatal RBCs incompatible with the maternal antibody with compatible RBCs.
     2) It corrects anemia.
     3) It removes maternal antibody.
     4) It removes bilirubin.
   - *Whole blood or "reconstituted whole blood" is used for exchange transfusion, which has the following characteristics:*
     1) The RBCs must be compatible with maternal ABO antibodies as well as with the antibody causing the HDN (e.g. O mom/A baby - must use O cells).
     2) The plasma must be compatible with neonatal RBCs (e.g. O mom/A baby - must use A plasma).
     3) RBCs must be irradiated to prevent transfusion-associated GVHD.
     4) RBCs should be tested to ensure that they are hemoglobin S negative.
     5) RBCs from "fresh" (less than 5 days from draw) to provide optimal survival.
     6) RBCs may need to be "CMV-safe" if the infant is low--birthweight.

4. What are the indications for exchange transfusion?
   - *Exchange transfusion is indicated to lower bilirubin levels that are judged to be approaching the toxic threshold. However, exchange may be performed soon after delivery in infants who are severely anemic or who have high but not toxic cord blood bilirubin levels if subsequent development of toxic levels is considered inevitable, because is easier to remove RBCs that are destined to be destroyed and release bilirubin than it is to remove bilirubin once it is released.*

Questions, Pregnancy 6

1. What are the risks of PUBS and amniocentesis?
   - *Fetal trauma/precipitation of labor; The risk of fetal loss is due to amniocentesis is <1% and 1 - 5% for PUBS.*
   - *Amniocentesis and PUBS can cause oligohydramnios.*
   - *Amniocentesis and PUBS can cause FMH, increasing the rate of maternal antibody synthesis and accentuating the disease process.*

Questions, Pregnancy 7

1. Discuss the use of maternal blood for IUT (including issue of the ABO type).

   Collection of maternal blood for IUT has the advantage that her RBCs lack the antigens against which she has developed the antibodies that have crossed the placenta into the fetus. Since the fetus or newborn is not expected to have active blood group antibody production maternal RBCs which have A and B antigens the fetus lacks can be transfused. In this case the mother was group A and the fetus group O. Monitoring of the fetus for production of anti-A was performed (results not shown). When using volunteer donor blood for IUT RBCs from a CMV sero-negative donor are typically selected. If the mother is CMV sero-negative there is no issue. However, if the mother is CMV-seropositive, there might be concern that she could transmit CMV to the fetus causing an in utero infection with significant complications. leukocyte filtration can reduce this risk. More importantly, the mother's anti-CMV is expected to cross the placenta as well and has been shown to be protective against intra-uterine infection.

2. How does one determine fetal lung maturity?

   A variety of methods have been used all of which are based on measurement of lipids found in pulmonary surfactant. Classically, thin layer chromatography has been used to measure the levels of lecithin and sphingomyelin; an L/S ration of 3 suggests a moderate degree of lung maturity. Also phosphatidlyl glycerol, if present, suggests that the lungs are mature. The lamellar body number density (LBND) test can be done on standard cell counters used for complete blood counts without any special reagents. In this case an LBND of 14,000 suggests borderline lung maturity. Finally, Abbott Laboratories markets a fetal lung maturity test based on uptake of a hydrophobic dye bylipids in the amniotic fluid.

3. What toxicity of bilirubin was present in this case?

   The infant developed a central form of hearing loss, presumably due to damage of the medial geniculate body. Auditory deficits are among the earliest toxicities to develop in significant neonatal hyperbilirubinemia.