

XI. Neonatal and Obstetrical Transfusion Practice

A. Introduction

1. *The neonatal period is generally considered to extend from birth to 4 months of age.*
2. Indications for transfusion of infants differs with weight, gestational age, circumstances of delivery and with subsequent maturation.
3. Ill newborn infants are more likely to receive transfusions than hospitalized patients of any other age.
 - a. The amount of blood removed for laboratory samples to monitor their progress may be quite substantial.
 - b. Each time blood is drawn the nurse notates it on the chart, once the amount becomes significant the baby must be transfused.
4. Supplying blood banks should be capable of providing components tailored to satisfy the specific requirements of these tiny recipients whose small blood volumes and impaired organ functions provide little margin of safety.
 - a. Must minimize the number of donors the baby is exposed to.
 - b. The blood center supplies a unit of blood with satellite bags attached.
 - c. When an order is received for transfusion the technician squeezes over the necessary amount from the primary bag into the satellite bag (usually 15 - 30 cc), seals the tubing and removes the satellite bag.
 - d. The primary bag retains its original expiration date. The satellite bag will have a 24 hour expiration date once it has been entered.
 - e. Infants can receive transfusions from the same donor unit for 28 days or until all of the blood has been used up.

B. Fetal and Neonatal Erythropoiesis

1. Appropriate transfusion practice requires knowledge of neonatal physiology and careful clinical observation.
2. As the embryo develops, the predominant sites of hematopoiesis change; at about the 9th week of gestation, hematopoiesis shifts from the wall of the yolk sac to the liver, and at about the 24th week from the liver to the bone marrow.
3. Hematopoiesis is regulated by gradually increasing erythropoietin levels stimulated by low oxygen tensions during intrauterine life.
4. At 40 weeks (full term), normal infants have a cord blood hemoglobin of 19 +/- 2.2 g/dL. Neonates of lower birth weight have lower normal hemoglobin levels.

5. *Fetal red cells present at birth have a **life span of 45-70 days** and contain **53-95% hemoglobin F** (fetal hemoglobin).*
 - a. Red cells rich in *hemoglobin F* are physiologically well adapted to low intrauterine oxygen tensions; their *high oxygen affinity* allow fetal red cells to acquire oxygen from maternal RBC throughout pregnancy and release it to their tissues.
 - b. However, the *high oxygen affinity results in poor tissue oxygenation after birth*.
 - c. As hemoglobin A (adult hemoglobin) replaces hemoglobin F after birth, oxygen delivery to the tissues remains satisfactory despite a physiologic fall in hemoglobin concentration.
 - d. The oxygen dissociation curve (ability of hemoglobin to release oxygen to the tissues) shifts to the right, reflecting improving oxygen delivery to the tissues.
 - e. *Premature infants have lower hematocrits and a greater percentage of hemoglobin F in their RBC than term newborns.*
6. *As tissue oxygenation in the newborn improves, levels of erythropoietin decline and erythropoiesis diminishes.*
 - a. This decline in RBC produces a "*physiologic anemia of infancy*" and is a normal, expected process.
 - b. Despite hemoglobin levels that would indicate anemia in older children and adults, the normally developing infant usually maintains adequate tissue oxygenation.

C. Unique Aspects of Neonatal Physiology

1. Introduction

- a. Differences between newborns and adults may dictate differences in transfusion practice.
- b. Newborns are small and physiologically immature.
- c. Those requiring transfusion are often **premature, sick and unable to tolerate minimal stresses**.

2. Infant size

- a. Full-term newborns have a blood volume of approximately 85 ml/kg (a 6.6 lb baby has a total blood volume of approximately 225 mLs); premature infants have an average blood volume of 100 ml/kg.
- b. As survival rates continue to improve for infants weighing 1000 g (2.2 lbs) or less at birth, blood banks are increasingly asked to provide blood components for patients whose total blood volume is less than 100 mLs.
- c. The small blood volumes of neonates and the need for frequent laboratory test makes replacement of **iatrogenic blood loss** the most common indication for transfusion of these patients.

3. Infants do not compensate for **hypovolemia** as well as adults. This will cause diminished cardiac output, resulting in poor tissue perfusion, low tissue oxygenation and metabolic acidosis.

4. The infant's **bone marrow** responds more slowly than adult marrow to anemia. If hemolysis is occurring due to maternal antibody there may be no increased erythropoiesis for 2-3 weeks in the newborn.

5. Cold stress (**hypothermia**) in the newborn causes exaggerated effects, including increased metabolic rate, hypoglycemia, metabolic acidosis and a tendency to apneic episodes that may lead to hypoxia, hypotension and cardiac arrest. Blood for transfusion should be warmed if given in large amounts, small amounts reach RT in about 20 minutes and does not need to be warmed.
6. Infants are **immunologically immature**, and antibodies present in their plasma originate almost entirely from the maternal circulation.
 - a. **IgG is the only immunoglobulin class that crosses the placenta.**
 - b. Passively acquired antibody is conserved during the neonatal period due to slow catabolism by the fetus.
 - c. Infants exposed to an infectious process in utero or shortly after birth may produce small amounts of IgM detectable by sensitive techniques, but they rarely form RBC antibodies of either class during the neonatal period.
7. *Graft versus host disease (GVHD)*
 - a. GVHD has been reported in newborns who received intrauterine transfusion followed by postnatal exchange transfusion.
 - b. *The lymphocytes given during intrauterine transfusion may have induced **host tolerance**, so that the lymphocytes given in subsequent exchange transfusion were not rejected in the normal way.*
 - c. GVHD is not felt to be a significant clinical problem for **immunologically normal newborns** who receive multiple exchange transfusions.
 - d. *Irradiation of blood kills immunologically competent lymphocytes* and some neonatal nursery units provide irradiated blood for low birth weight, low gestational age or septic premature neonates based on the belief, not yet universally accepted, that such infants are immunologically more vulnerable to GVHD.
 - e. *Blood for intrauterine transfusion should be irradiated.*
 - f. *Any directed donor blood from a relative should be irradiated.*
8. Metabolic problems
 - a. Because immature kidneys have reduced glomerular filtration rate and concentrating ability, the newborn may have difficulty excreting potassium, acid and/or calcium loads.
 - b. Acidosis or hypocalcemia may also occur postransfusion because the immature liver metabolizes citrate in the banked blood inefficiently.
 - c. Studies have shown older units do not affect the infant for routine transfusion purposes.
9. *2,3-Diphosphoglycerate (2,3-DPG)*
 - a. Tissue oxygenation is poor in sick newborns due to the high percentage of hemoglobin F. *Hemoglobin F does not release oxygen to the tissues like adult hemoglobin.*

- b. *Infants with respiratory distress syndrome (RDS) or septic shock have decreased levels of 2,3-DPG, and alkalosis and hypothermia can further increase the oxygen affinity of hemoglobin, shifting the dissociation curve to the left making oxygen even less available to the tissues.*
- c. *Since 2,3-DPG levels decrease in stored blood, newborns should be given the freshest blood available, less than 5 days old if possible, to ensure that the transfused RBC have adequate 2,3-DPG levels.*
- d. *There is controversy in the field about the practice of using fresh blood. It is currently felt by some in the blood bank field that it is of greater importance to decrease the number of donor exposures rather than give fresh blood. These institutions allow CPD donor units to be put on hold for the infant for 21 days.*

10. Cytomegalovirus (CMV) Infection

- a. *Infection by CMV may occur in the perinatal period, either in-utero or during birth, by breast feeding or by close contact with mothers or nursery personnel.*
- b. *CMV may also be transmitted by transfusion. The virus seems to be associated with leukocytes in blood and components.*
- c. *Infection in newborns is extremely variable in its manifestations, ranging from asymptomatic seroconversion to death.*
- d. *Symptomatic infection may produce pulmonary, hepatic, renal, hematologic and/or neurologic dysfunction.*
- e. *Epidemiology and prevention of post transfusion CMV in neonatal patients have been under intense investigation. The following observations have been noted:*
 - 1) *Where CMV rate is high, symptomatic infection is low.*
 - 2) *Babies of seropositive moms are unlikely to develop CMV.*
 - 3) *Premature infants who are born of seronegative mothers, weigh less than 1200 grams and who require multiple transfusions are at risk for symptomatic postransfusion infections.*
 - 4) *The risk of CMV increases with number of donor exposures.*
 - 5) *Risk of transmission decreases by using seronegative donors or components depleted of leukocytes.*
- f. *Standards states that, in geographic areas where postransfusion CMV transmission is a problem, blood with minimal risk of transmitting CMV be used for newborns weighing less than 1200 g, born to mothers who lack CMV antibodies or whose antibody status is unknown. (NOTE: Most blood banks make it standard practice to transfuse all infants with CMV negative blood).*

D. Hemolytic Disease of the Newborn (HDN)

1. Introduction

- a. **In HDN, red cells of the fetus become coated with IgG alloantibody of maternal origin, directed against an antigen of paternal origin present on the fetal cells.**
- b. *The IgG coated cells undergo accelerated destruction, both before and after birth.*
- c. *Clinical severity of the disease is extremely variable, ranging from intrauterine death to a condition that can be detected only by serologic tests on blood from an apparently healthy baby.*

2. Pathophysiology
 - a. Shortened RBC survival causes fetal hematopoietic tissue to increase production of new RBCs, many of which enter the circulation prematurely as nucleated red cells (NRBCs).
 - b. Organs containing hematopoietic tissue increase in size, **particularly the liver and spleen** (*hepatosplenomegaly*).
 - c. If increased hematopoiesis cannot compensate for the immune destruction, *anemia becomes progressively more severe*.
 - d. The severely affected fetus may develop high output cardiac failure with generalized edema, a condition called ***hydrops fetalis***, and death may occur in utero.
 - e. If live-born, the severely affected infants exhibits heart failure and profound anemia.
 - f. Less severely affected infants continue to experience accelerated red cell destruction, which generates large quantities of bilirubin.
 - g. Before birth severs the communication between maternal and fetal circulation, fetal bilirubin is processed by the mother's liver, but after birth the neonatal liver must conjugate and excrete large quantities of unconjugated indirect bilirubin, a substance toxic to the developing central nervous system.
 - h. The immature liver is deficient in **uridine diphosphoglucoronyl transferase** (the enzyme necessary to conjugate bilirubin for excretion) and accumulation of ***unconjugated bilirubin constitutes a severe threat to the infant***.
 - 1) *Total plasma bilirubin levels approaching 20 mg/dL can cause mental retardation or death.*
 - 2) Bilirubin, once its concentration is excessive can accumulate in the lipid-rich tissue of the central nervous system, this is clinically known as ***kernicterus***.
 - I. The threat is increased by prematurity, acidosis, hypoxia and hypoalbuminemia (albumin is necessary, along with the enzyme, for conjugation of bilirubin).
 - j. For the live-born infant with HDN, rising bilirubin levels may be a greater clinical problem than the loss of oxygen carrying capacity resulting from continuing hemolysis.
 - k. *The decision of when or whether to undertake exchange transfusion is based primarily on **bilirubin accumulation**, and on anemia and severity of hemolysis only to a lesser degree.*
3. *HDN is classified into **three** categories based on serologic specificity of the causative IgG antibody. In descending order of severity these are:*
 - a. **Rh (D) hemolytic disease, due to anti-D alone or more rarely in combination with anti-C or anti-E. THIS IS THE MOST SEVERE TYPE and may result in fetal death.** With anti-D, the second infant will be severely affected, and future pregnancies will usually result in stillbirth due to the increasing pathogenicity of the antibody.
 - b. **"Other HDN" due to antibodies against other antigens in the Rh system**, such as anti-c, anti-E, or anti-e, **or the antigens in other blood groups systems**, such as anti-K, anti-Fy^a and many others. Baby is usually mildly affected but severe HDN has also been documented.

- c. **ABO HDN, due usually to anti-A,B in a group O woman, but rarely to anti-A or anti-B. THIS IS THE MOST COMMON FORM, BUT ALSO THE MILDEST.**
 - d. *In HDN other than ABO, maternal antibodies result from immunization by previous pregnancy or transfusion. Rising titers of antibody can be documented during the pregnancy by performing titration studies, and the baby may be symptomatic at birth.*
 - e. *In ABO HDN the immunizing stimulus is seldom known, the condition cannot be diagnosed during pregnancy and is rarely symptomatic at birth.*
4. Maternal immunization due to pregnancy.
- a. When immunization results from pregnancy, the antigenic stimulus is entry into the mother's circulation of fetal red cells possessing **paternal antigen** which the mother does not possess which she recognizes as foreign.
 - b. The antigen that most frequently induces immunization is D, but any red cell antigen present on fetal cells and absent from the mother can, in theory, stimulate antibody production.
 - c. Small numbers of fetal cells enter the mother's circulation during the last half of pregnancy, but they are rarely sufficient to induce immunization.
 - d. Most immunizations result from the fetomaternal hemorrhage that occurs during placental separation at delivery.
 - e. In approximately half of all recently delivered women, small quantities of fetal cells are detectable in a post-delivery blood specimen.
 - f. **Immunization to D can occur with volumes of fetal blood less than 0.1 ml.** The incidence of immunization to D correlates with the volume of D-pos RBCs entering the D-neg mother's circulation.
 - g. Although the usual immunizing event is delivery, primary immunization does occasionally occur **during pregnancy or after amniocentesis, miscarriage, abortion, chorionic villus sampling, cordocentesis, blunt trauma to the abdomen or rupture of an ectopic pregnancy.**
5. Maternal Immunization due to Transfusion.
- a. In *D-neg* women who receive transfusions of *D-pos* RBCs, subsequent pregnancies with a D-pos fetus are likely to be complicated by severe HDN.
 - b. *It is **extremely important** to avoid transfusing D-pos blood to D-neg women who might subsequently become pregnant.*
 - c. The RBCs in platelet or granulocyte concentrates can constitute an immunizing stimulus, and D-neg women who receive platelet therapy should be considered candidates for Rh prophylaxis if the donors are D-pos.
 - d. *Directed donor transfusion from husband to wife should be avoided* if the couple plan to have children, as this form of directed donation will likely increase the risk of alloimmunization of the mother.

6. ABO Antibodies

- a. The IgG antibodies that cause ABO HDN nearly always occur in the mother's circulation without a history of prior immunization by foreign transfused RBCs.
- b. ABO HDN can develop in any pregnancy including the first, but it is restricted almost entirely to group A or B babies born to group O mothers.
- c. *This is apparently attributable to the pathogenic antibody, anti-A,B being present only in group O individuals.*

7. HDN other than ABO

- a. The *first baby is not affected* or is mildly affected if this pregnancy is the immunizing event.
 - 1) The antibodies produced in a primary response are IgM and do not cross the placenta.
 - 2) A sufficient titer of IgG may not develop during the pregnancy, resulting in an asymptomatic or mildly affected infant.
- b. In "other" HDN, the first baby will not be affected if this pregnancy is the immunizing event.
 - 1) The severity of future babies being affected is extremely variable.
 - 2) The pathogenicity of the antibody will vary from individual to individual, and so will the HDN which it will cause.
 - 3) *Most "other" HDN cause only mildly symptomatic HDN.*

E. Prenatal Tests

1. Serologic Studies

- a. Alloimmunization that could result in HDN can be diagnosed during pregnancy with suitable serologic tests.
- b. Initial studies on **all** pregnant women should include tests for **ABO and D (including weak D), and a screen for unexpected antibodies.**
- c. *All positive antibody screens require identification of the antibody and evaluation of the clinical significance of the antibody identified.*
- d. The mere presence of an antibody does not indicate that HDN will inevitably occur, clinical significance based on history of the antibody as it relates to HTR and HDN.
- e. IgM antibodies existing without known red cell stimulation, notably anti-Le^a and anti-I, are relatively common during pregnancy but do not cross the placenta.
- f. Treating the serum with **2ME or DTT** will aid in distinguishing IgM antibodies from IgG.
- g. The fetus may be antigen negative for the mother's antibody.
- h. The results of prenatal antibody studies should be reported with sufficient additional information to indicate when a reported antibody may or may not be clinically significant.

- i. When the woman has anti-D, some obstetricians test the father to estimate the likelihood of his being homozygous or heterozygous for alleles determining the presence of the D antigen. A homozygote will always transmit a gene for D to the offspring, whereas in a heterozygous father, half of the children will be D-neg.

F. Amniotic Fluid Analysis

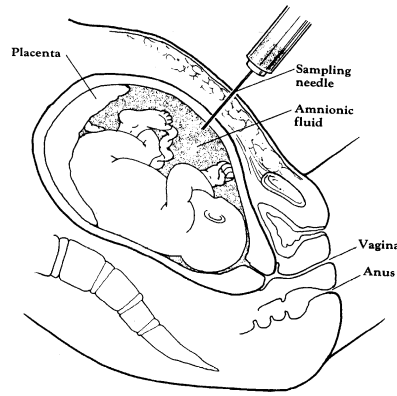


Illustration of amniocentesis.

1. During gestation, the *clinical history and amniotic fluid analysis can be used to assess the probable severity of HDN.*
2. Anti-D HDN rarely occurs in the first pregnancy, but once a woman has *anti-D*, *HDN tends to become more severe with each succeeding D-pos pregnancy.*
3. Information about the severity of the disease in previous infants is somewhat helpful in predicting severity in subsequent infants.
4. In a women with anti-D, the severity of fetal HDN correlates modestly with the maternal antibody titer.
5. **A better index of intrauterine hemolysis and fetal well being is the level of bile pigment in the amniotic fluid**, obtained by amniocentesis, or doing an H&H on the infant's blood obtained by direct fetal blood sampling via the umbilical vein.
6. Amniocentesis is usually performed in D-neg women with a history of previously affected pregnancies **or** with anti-D titers above 16.
7. Amniotic fluid is obtained by inserting a long needle through the mother's abdominal wall and uterus into the uterine cavity. *The aspirated fluid is scanned spectrophotometrically for a change in optical density at 450 nm, to measure the concentration of bile pigments.*
 - a. This value is plotted on a semilogarithmic graph against the estimated age of gestation, because bile pigment concentration has clinical significance at different gestational ages.
 - b. In general, *the higher the pigment concentration, the more severe the intrauterine hemolysis.*

8. *The risk of allowing a severely affected pregnancy to continue must be weighed against the risk of premature delivery and the problems of fetal lung immaturity.*
 - a. Respiratory Distress Syndrome (RDS) may result when there is *inadequate surfactant lecithin* and other pulmonary lipids to maintain stable pulmonary alveolar structures in the newborn.
 - b. *Maturity of the fetal lung is assessed by determining the ratio of lecithin to sphingomyelin (L/S ratio) and levels of phosphatidylglycerol (PG) in the amniotic fluid.*
 - c. If the change in optical density at 450 nm indicates severe HDN but the L/S ratio indicates the lungs are not sufficiently mature to prevent RDS, intrauterine transfusion may be indicated.
9. Amniocentesis and direct fetal blood sampling may be complicated by fetomaternal hemorrhage (FMH), which can cause immunization of susceptible mothers.
 - a. If amniocentesis has been performed on an D-neg woman who is **not** already sensitized to the D antigen, *Rh immunophylaxis should be given.*
 - b. In most cases the D status of the fetus will be unknown, but the likelihood is high that it is D-pos and the bleeding induced by amniocentesis could induce anti-D in a non immunized women.
 - c. ***Cordocentesis*** is the process where by a needle is inserted into the baby's umbilical cord allowing the drawing of fetal blood for laboratory analysis (previously called percutaneous umbilical blood sampling - PUBS)
10. Due to the hazards of amniocentesis and cordocentesis, the noninvasive monitoring protocols, including greater use of ultrasound evaluations of fetal well-being, are being recommended.

G. Intrauterine Transfusion

1. Intrauterine transfusion **carries a high risk to the fetus** and should be performed only after **careful** clinical evaluation.
2. Intrauterine transfusion is *rarely feasible before the 20th week of gestation.*
3. Once initiated, the series is usually *administered every two weeks* until delivery.
 - a. It is performed through a needle passed with radiographic monitoring, through the mother's abdominal and uterine wall into the fetal abdominal cavity.
 - b. The transfused red cells enter the fetal circulation by absorption from lymphatic channels draining the peritoneal cavity.
 - c. Blood can also be infused directly into the umbilical vein by a procedure called *percutaneous umbilical blood transfusion* (PUBT) or by cordocentesis.
4. For maximum survival, *transfused cells less than 5 days old should be used.*
5. Washed or deglycerolized frozen red cells have normal electrolyte levels, contain no anticoagulant or plasma, have very low levels of platelets and leukocytes, have a low risk of CMV and is the component of choice.

6. A hematocrit of 80% or greater is desirable to minimize the chance of volume overload in the fetus.
7. The red cells **must be group O, D-neg and must be compatible with the mother's serum, lacking any antigens to which she may have antibodies.**
8. The volume transfused ranges from 75-175 mL depending on the fetal size and age.
9. Blood for intrauterine transfusions **should be irradiated because of the potential risk of GVHD.**
10. Newborns who have had successful intrauterine transfusions *often type at birth as D-neg* or very weakly positive, because at birth over 90% of their circulating RBCs may be those of the donors. Similarly, the ABO grouping and DAT may give misleading results as well.

H. Intrauterine Exchange Transfusion

1. New medical techniques have been developed for performing intrauterine exchange transfusion.
2. Under ultrasound guidance, the *umbilical vein is cannulated* and a blood sample taken for immediate H&H measurement and verification of catheter location in the fetal circulation.
3. The as yet unborn baby can thus undergo and *exchange transfusion*.

I. Laboratory Investigation During the Neonatal Period

1. Introduction

- a. A sample of cord blood, preferably collected by syringe, to *avoid contamination by Wharton's jelly* and maternal blood and debris, should be obtained on every newborn.
- b. This tube should be identified as cord blood and be labeled in the delivery suite with the mother's name, hospital number and date.
- c. Samples should be stored in the blood bank for at least 7 days. The cord sample is then available for testing if the newborn develops signs and symptoms suggestive of HDN.

2. Postpartum testing

- a. *ABO, D (including test for weak D) and antibody screen on the mother.*
- b. *Identification and evaluation of alloantibodies detected in maternal sample.*
- c. ABO testing on the cordblood or babies blood relies entirely on cell grouping because antibodies in the babies serum or plasma are of maternal origin. **Reverse grouping is not done.**
- d. Determination of the baby's D type (including Du) must be done but may be difficult.
 - 1) **False negative due to "blocked D"**, all D antigen sites are coated with maternal anti-D, no sites left for attachment of reagent anti-D.
 - 2) **False positive** due to contamination with **Wharton's jelly**.

- e. A DAT *should be performed* on the cord cells.
 - 1) If the DAT is positive, the antibody can be eluted from the cells and tested for specificity.
 - 2) The DAT usually gives strongly positive results in HDN due to anti-D or antibodies in other blood groups; reactions are much weaker or negative in HDN due to ABO.
 - f. *If the DAT is positive and the maternal serum has a negative antibody screening test, suspicion falls on ABO HDN or HDN due to antibody against a low-incidence antigen.*
 - g. *ABO hemolytic disease may be suspected on clinical grounds even though the DAT is negative.*
 - 1) In many cases it is possible to elute anti-A or anti-B from the infant's cells despite a negative DAT.
 - 2) If transfusion is required, Group O, D compatible blood should be transfused
3. Laboratory Diagnosis of Severity of **ABO HDN**
- a. Hemolytic disease caused by ABO incompatibility is **clinically mild, with jaundice most frequently developing within 24 hours of birth**. The laboratory profile of this form of HDN consists of (MEMORIZE) the following:
 - 1) Type and screen on mother
 - 2) Direct antiglobulin test on infant
 - 3) ABO/D type of infant
 - 4) Antibody elution testing from cord red blood cells
 - 5) Bilirubin assay on infant
 - 6) CBC and Reticulocyte count on infant
 - b. *ABO grouping most commonly reveals the mother to be group O and the baby to be group A or B.*
 - c. The DAT may be either *negative or weakly positive*. The lack of consistency in this test may be due to:
 - 1) A weak antigen-antibody reaction, which causes antibody to be removed during the washing phase of the DAT,
 - 2) an antibody titer that may be too low to be detectable,
 - 3) variation in fetal ABO antigen development.
 - d. **Antibody elution of cord blood may reveal the presence of immune anti-A or anti-B**. An eluate is often more useful than the DAT in assessing HDN caused by ABO incompatibility.
 - e. The bilirubin assay may exceed the normal values.
 - 1) Hyperbilirubinemia may also be seen in conditions such as premature birth or maternal diabetes.
 - 2) *HDN is judged to be clinically significant (phototherapy treatment) if the peak bilirubin level reaches 12 mg/dL or more.*

- f. Hemoglobin may be slightly lower than in ABO compatible infants.
 - 1) Normal hemoglobin concentrations range from 15 to 20 g/dL.
 - 2) *The peripheral smear may reveal RBCs abnormalities such as hypochromia, microspherocytosis, and reticulocytosis. Immature nucleated RBCs may be increased.*
4. Treatment of ABO HDN
- a. *Except in the extremely rare cases of severe HDN produced by ABO incompatibility, phototherapy is the usual treatment.*
 - b. Phototherapy uses ultraviolet light that reacts with bilirubin near the surface of the skin.
 - 1) This process slowly decomposes/converts bilirubin into a nontoxic isomer, **photobilirubin**, which is transported in the plasma to the liver.
 - 2) There the molecules are rapidly excreted in the form of bile without being conjugated.
 - c. *The need for exchange transfusion is rare in cases of ABO incompatibility because of the generally mild nature of this type of HDN.*
 - 1) One of the risks of exchange transfusion in these cases is the triggering of yet more hemolysis due to a technician transfusing a baby with adult A or B cells that will interact with circulating maternal IgG anti-A,B in the baby's circulation.
 - 2) ***The blood for exchange must be compatible with baby AND mother. If mother is Group O than blood for transfusion must be Group O and D compatible with the infant.***
5. Laboratory Diagnosis of HDN due to "Other" Blood Group Antibodies.
- a. When performing a type and screen on the mother **the antibody screen is positive.**
 - b. A panel is performed, on the mother's serum and **the antibody is identified**, and it is determined that it is a *clinically significant antibody (IgG)*. More than 40 antigens have been identified as causing HDN.
 - c. The DAT performed on the infant's cord blood is positive. The strength of reaction may vary from weakly to strongly positive.
 - d. **An eluate is performed on the cord cells**, the eluate is tested against a panel of RBCs (including A and B cells if mother is O and baby is A or B). **The specificity of the antibody is determined.**
 - e. Bilirubin levels are determined and evaluated.
 - f. CBC and reticulocyte count are determined and evaluated.
6. Treatment
- a. If the antibody is detected prenatally, antibody titers and amniocenteses may be indicated.
 - b. Depending on the antigen strength on the baby's cells and other variables, **the severity of the disorder can range from mild to severe.**

- c. **Most cases of HDN due to "other" blood group antibodies simply require phototherapy.**
- d. **In some rare instances exchange transfusion is necessary. The blood for exchange must be compatible with baby and mother, and lack the antigen(s) to which the mother has produced antibody(ies).**

J. HDN Caused by D Incompatibility

1. History

- a. In the early 1930's Diamond, Blackfan and Baty made the classic observations that *kernicterus*, *hydrops fetalis and anemia* in the newborn represented different grades of clinical severity of the same unknown process. Unfortunately, they did not suspect that this syndrome was a manifestation of hemolytic anemia affecting the fetus. This process was originally referred to as *erythroblastosis fetalis*.
- b. In 1938 Darrow suggested that this process was a hemolytic anemia caused by the transfer of immune bodies from the mother to the fetus. Because she had no data to support her hypothesis, she incorrectly selected fetal hemoglobin as the causative agent.
- c. The developmental work on the Rh factor (D antigen) conducted by the major investigators, Landsteiner and Wiener, led Levine to establish that the D antigen was the immunizing agent in the blood of the fetus.
 - 1) He speculated that the D antigen was not present in the mother but inherited by the fetus from the father.
 - 2) In 1941, Levine concluded, based on experimental and case study data, that the majority of cases of HDN in the fetus/newborn resulted from immunization of an D negative mothers by the D positive erythrocytes of the fetus.

2. Types of Responders to Immunization to the D Antigen

- a. The immunization of D negative mothers depends on both the dosage of D positive RBCs and the mother's ability to respond to these foreign D antigens.
- b. About one third of all D negative persons are classified as nonresponders. Nonresponders fail to form anti-D despite repeated injections of D-pos RBCs.
- c. In addition to the nonresponder status, two other categories exist: responders and hyperresponders.
 - 1) *Responder and hyperresponders* differ in terms of the type and quantity of anti-D antibody that they produce.
 - 2) *Hyper-responders* produce extremely high titers of *both IgM and IgG* types of anti-D.
- d. The normal pattern of immunization in a D-neg mother involves primary immunization due to previously D-pos pregnancy or blood transfusion which stimulates the production of low titered anti-D.
 - 1) Subsequent antigenic stimulation such as fetal-maternal hemorrhage in a woman pregnant with a D-pos fetus elicits a secondary response.
 - 2) Characterized by the predominance of increasing titers of anti-D of the IgG class.

- e. Levine observed as early as 1943 that a **condition of HDN due to ABO incompatibility between the mother and fetus reduced the chance of maternal immunization the D antigen.**
 - 1) D-neg women with group O blood and ABO incompatible, D-pos babies were more strongly protected due to the ABO antibodies (especially anti-A,B) in the maternal circulation.
 - 2) This antibody causes destruction of the baby cells before they can be recognized as foreign by the mother.
3. Signs, Symptoms and Physiology of HDN Caused by anti-D
- a. Except in rare instances, *the firstborn D-pos infant of a D-neg mother seldom shows clinical signs of HDN* and may simply have a positive DAT.
 - b. If severe HDN is observed in a firstborn D-pos infant, it must always be suspected that the mother was previously immunized before the pregnancy by means of a previous blood transfusion, abortion, etc.
 - c. When the mother has been previously immunized and subsequently produces anti-D of the IgG type in response to a D-pos fetus, the following activities occur:
 - 1) The passage of IgG anti-D into the fetal circulation induces RBCs hemolysis and anemia of varying degrees of severity.
 - 2) *This anemia may produce heart failure and hypoxia in the unborn child, causing stillbirth or rapid accumulation of bilirubin in the newborn.*
 - d. Once an alloantibody such as anti-D has been identified in maternal serum, a rising antibody titer is evidence of a presently active immune response.
 - 1) The severity of HDN, however, does not correlate well with maternal anti-D titer.
 - 2) The discrepancy observed between the severity of HDN and the antibody titer may be due to differences in the nature of IgG antibodies.
 - 3) Fetal hemolysis is essentially due to antibodies of the IgG1 and IgG3 subclasses.
4. Laboratory Diagnosis of HDN Due to Anti-D
- a. *A detectable antibody titer is rarely observed before 28 weeks gestation in the first immunizing pregnancy.*
 - 1) When anti-D develops during the first pregnancy, it is most commonly detected at about the 35th week of gestation or later.
 - 2) The titer is usually low.
 - b. In subsequent pregnancies amniocentesis may be performed in cases where the serum titer of anti-D is 16 or higher in a woman with a history of a previous child who needed an exchange transfusion, or a progressive rise in anti-D to 64 without a history of an affected fetus or child.
 - c. In such cases, an initial amniocentesis would be done at 24 to 28 weeks of gestation or 6 to 8 weeks before the gestational age of previous fetal loss due to anti-D. Two sequential amniotic specimens are required to assess change in the status of the fetus.

- d. ***The identification of HDN postpartum due to anti-D is characterized by the following test results:***
- 1) D blood typing (baby is D or weak D positive)
 - 2) Cordblood DAT is positive and the mother demonstrates a positive IAT with anti-D identified as the antibody.
 - 3) Eluate performed on cord cells reveals the presence of anti-D.
 - 4) Hemoglobin levels of baby may be moderately to severely decreased.
 - 5) Infant bilirubin levels of 20 mg/dL or more at birth are present in severely affected full term infants, and lower levels in premature infants are present in severely affected infants.
 - 6) CBC on infant indicative of severe anemia.

5. Treatment

- a. In the severely affected infant ***exchange transfusion*** is needed to save the infant.
- b. Removing the babies plasma **reduces the load of accumulated bilirubin and the number of unbound maternal antibody molecules.**
- c. Replacement with donor plasma restores albumin and any needed coagulation factors.
- d. **Antibody coated cells, whose destruction would further raise the bilirubin load, are removed and replaced with RBCs compatible with the maternal antibody, which will have a normal survival rate.**
- e. The immediate effectiveness of a two-volume exchange transfusion is 45-50%. *The baby's plasma bilirubin tends to **rise or rebound** after an exchange transfusion because:*
 - 1) of the entry of bilirubin from the extravascular spaces and tissues and,
 - 2) partly because of continued production of bilirubin from residual maternal antibody coating newly released RBCs.
- f. Additional exchanges are necessary if the bilirubin level threatens to exceed 20 mg/dL in a full term infant.
- g. Phototherapy is used as an adjunct in this situation.

K. Exchange Transfusion

1. Exchange transfusion, originally used almost exclusively as treatment for HDN, has recently been advocated as adjunctive therapy for a variety of life-threatening diseases affecting newborns including RDS, DIC and sepsis.
2. The procedure carries a *mortality rate of approximately 1%* and there may be substantial morbidity.
3. Exchange transfusion is often quantified (eg, two-volume, single-volume) to reflect the effect on the infant's total blood volume.
4. ***Blood selected for exchange transfusion should be as fresh as possible, preferably less than 7 days old, negative for hemoglobin S and CMV negative.***
5. ***The blood must lack all antigens to which the mother has antibodies.***

6. **To crossmatch blood for exchange transfusion, the serum or plasma of either the mother or infant may be used.**
 - a. *The mother's serum has the advantage of being available in large quantity, having the antibody present in high concentration, and is capable of being accurately and completely analyzed before birth.*
 - b. If the infant is to be transferred to another facility, a specimen of maternal blood should accompany the child.

7. **If the maternal blood is not available, or if it is unsuitable for immediate use in crossmatching, either the infant's serum or, if necessary, an eluate from the infant's cells, or both can be used for crossmatching.**
 - a. The eluate provides a concentrated preparation of the antibodies responsible for RBCs destruction, but will not contain antibodies against antigens absent from the infant's cells.
 - b. The serum may not contain a high concentration of the antibody if most of the molecules are bound to the RBCs.
 - c. Neither the infant's serum nor eluate alone is ideal for crossmatching. Use of either or both may, however, be preferable to delaying transfusion while a maternal specimen is obtained or while the antibodies in the mother's serum are separated and identified.

8. *Selection of blood for HDN due to anti-D*
 - a. ABO HDN use group O, D specific
 - b. For anti-D use D negative
 - c. Not every exchange requires O negative. If mom and baby are of the same ABO group, group specific RBCs can be used, if the antibody is not anti-D, D specific may be used.

9. *When in doubt, a useful consideration to keep in mind to prevent further damage to the infant is that the blood selected should be **negative for antigens which the mother has antibodies to and be crossmatch compatible with baby and mother.***

L. Obstetrical Considerations

1. *Rh Immune Globulin (RhIg) is a concentrated solution of IgG anti-D derived from human plasma.*
 - a. A 1-ml full dose vial, containing **300 ug of anti-D**, is sufficient to **counteract the immunizing effects of 15 ml of D-pos RBCs; this corresponds to 30 ml of fetal whole blood.**
 - b. RhIG, like other immunoglobulin preparations, does not transmit hepatitis or HIV infection.
 - c. The protective effect of RhIG on D-neg individuals exposed to D-pos RBCs probably results from interference with antigen recognition in the induction phase of primary immunization.
 - d. RhIG was initially utilized experimentally. RBCs were coated with anti-D in-vitro then injected into D-neg individuals. After repeated injections of the D-pos coated cells, D-neg individuals failed to produce anti-D.
 - e. This was a major breakthrough in the prevention of hemolytic disease due to anti-D.

- f. The frequency of HDN caused by anti-D has declined dramatically in the U.S. since the introduction of widespread postpartum administration of RhIG. In 1968, RhIG began to become available to postpartum D-neg women who had delivered an D-pos infant.
 - g. In a relatively short time, the incidence of immunization with the *demonstration of anti-D dropped dramatically dropped from 8% after the first pregnancy, with an additional 8% developing anti-D during their second pregnancy, to 1 to 2%.*
2. Antepartum administration
 - a. It was discovered that even though an D-neg woman was identified and given RhIG after giving birth to an D-pos child, there were still **RhIG "failures"**, the woman came in with a second pregnancy and testing revealed that she had become sensitized to the D antigen.
 - b. *The risk of 1% immunization is decreased further to 0.1% if RhIG is given antepartum at 28 weeks gestation, **in addition to the postpartum dose.***
 - c. The American College of Obstetricians and Gynecologists has recommended RhIG antepartum prophylaxis.
 - d. When antepartum RhIG is given, good communication must exist between the patient's physician and the blood bank staff of the hospital where the patient will be delivered, so that laboratory tests made at the time of delivery will be correctly interpreted.
 3. Testing at Delivery.
 - a. Standards does not require that blood from D-neg women be tested at the time of delivery for the presence of unexpected antibodies, but it is standard practice in the field to perform a Type and Screen on all women admitted for delivery, abortion or any invasive obstetric procedure.
 - b. The D test on the mother should include D^u if there is not immediate agglutination with anti-D.
 - c. *Cord blood from infants born to D-neg mothers should have an ABO and D type performed.*
 - d. *The mother's antibody status and the infant's clinical condition should dictate whether or not a DAT should be done.* In some hospitals it is routine to perform ABO, D and DAT testing on all D-neg and Group O mothers.
 4. Women who **are not candidates for RhIG** include the following (**MEMORIZE**):
 - a. *D-neg women who have D-neg infants.*
 - b. *D-pos women.* Although one case of HDN has been reported involving an infant whose mother was of the D^u phenotype, this is extremely rare and does not justify routine RhIG prophylaxis in women of the D^u phenotype.
 - c. *D-neg women known to be immunized to D.* Since the presence of anti-D in the postpartum specimen may actually be due to prenatal passive immunization, postpartum RhIG may be indicated. **An accurate history is essential in such cases.**

5. Evaluation of anti-D in the Postpartum Specimen

- a. The woman who has received antepartum RhIG often has anti-D present in an antibody screening test done at delivery. **Such a woman still should receive postpartum RhIG.**
- b. Only if the anti-D present at delivery **is known** to be the result of active immunization can administration of RhIG be omitted.
- c. *There are some laboratory clues that may help distinguish the origin of the antibody.*
 - 1) Passively administered RhIG is IgG. If a woman's anti-D is saline-reactive or can be inactivated by treating the serum with 2-ME or DTT, it probably represents active immunization.
 - 2) Passively acquired anti-D is usually weakly reactive, **rarely achieving a titer above 4**. High-titered anti-D is likely to be of maternal origin.
 - 3) **However, confirmation should always be sought from the physician's records.**
 - 4) **RhIG should be given whenever there is doubt that cannot be resolved.**

6. Administration of RhIG.

- a. The antepartum dose of RhIG is given between *28 and 30 weeks of gestation*, a recommendation based on the fact that, of women who develop anti-D during pregnancy, 92% do so at 28 weeks or later.
- b. A sample of blood for laboratory testing should be obtained **before** injection of RhIG.
- c. *Tests on the specimen drawn at 28-30 weeks should include:*
 - 1) ABO, D including D^u.
 - 2) Antibody screen.
 - 3) Identification of antibody, if present. The presence, in an D-neg woman, of antibodies other than anti-D does not preclude giving RhIG.
- d. The anti-D from an injected dose of RhIG may remain detectible *for as long as 6 months*.
- e. The half-life of an injected dose is approximately 23 days; of the 300 ug of antibody given at 28 weeks, 20-30 ug should remain at the time of delivery 12 weeks later.
- f. Postpartum RhIG should be injected **within 72 hours** of delivery, whether or not RhIG has been given during the pregnancy.
- g. It is **extremely important** that RhIG administration not be omitted or delayed because of uncertainty in interpreting an antibody screening test.
- h. If for some reason RhIG was not given within 72 hours, **later administration should not be withheld.**

7. The "Utilization Gap"

- a. *Administration of RhIG is indicated, but sometimes inadvertently omitted, after several common events(MEMORIZE):*
- 1) abortion/miscarriage
 - 2) ectopic pregnancy
 - 3) antepartum hemorrhage
 - 4) fetal death
 - 5) amniocentesis
 - 6) chorionic villus sampling
 - 7) cordocentesis
 - 8) blunt trauma to the abdomen
- b. During amniocentesis fetomaternal hemorrhage may occur causing D immunization of the mother.
- c. *The D-neg woman who has amniocentesis at 16-18 weeks for genetic analysis should receive a 300 ug dose of RhIG.*
- 1) A second antenatal dose should be given 12 weeks later (28 weeks) as usual.
 - 2) A third dose is given after delivery if the infant is D-pos.

8. Detection and Quantitation of Fetomaternal Hemorrhage (FMH)

- a. Postpartum D immunization can occur despite RhIG administration if the quantity of D-pos fetal RBCs entering the mother's circulation exceeds the 30 ml of whole blood covered by a single 300 ug dose of RhIG.
- b. The incidence of transplacental hemorrhage greater than 30 ml has been estimated at only about 0.3%, but large fetomaternal hemorrhage is an important and preventable cause of failure of immunoprophylaxis.
- c. Standards requires that a postpartum specimen be examined from all D-neg women at risk of immunization to detect the presence of fetomaternal hemorrhage larger than that for which one dose of RhIG provides protection.
- d. The micro Du used to be the only screening procedure available. After Coomb's the mother's Du was examined microscopically.
- 1) *Small, tight, clumps of cells (mixed field)* were the only indication that a FMH had occurred.
 - 2) This test was very insensitive and required skill to detect bleeds around 30 mLs.
 - 3) *In a CAP survey 12% of labs failed to detect D-pos cells in a specimen simulating a hemorrhage of approximately 30 ml.*
- e. The rosette test is the most practical method to use as a screening test for the detection of a large FMH.
- 1) *The rosette test utilizes D-pos indicator cells to form easily identified rosettes around individual D-pos fetal cells that may be present in the maternal circulation.*
 - 2) This method will detect FMHs of approximately 10 ml. Such sensitivity provides a margin of safety that is desirable for a screening test.

- 3) The rosette test gives only *qualitative results*, and positive results must be followed by the quantitative Kleihauer-Betke (KB) acid elution test or enzyme linked antiglobulin test (ELAT) to quantitate the hemorrhage.
 - f. Weak D positive (D^u) cells from the infant do not react as strongly in the rosette procedure as normal D-pos cells. **If the newborn types as weak D positive, then a KB acid elution test should be done routinely.**
9. Kleihauer-Betke Acid Elution
- a. *This technique utilizes the fact that fetal hemoglobin is resistant to acid elution, whereas adult hemoglobin is not.*
 - 1) When a thin blood smear is exposed to an acid buffer, the *adult RBCs lose their hemoglobin* into the buffer so that only the stroma remains.
 - 2) *Fetal RBCs are unaffected* (bright, pink and refractile) and retain their hemoglobin.
 - 3) ***The number of fetal cells counted in 2000 adult cells is calculated out into a percentage.***
 - b. The percentage of fetal cells in the maternal blood film is used to calculate the approximate volume of FMH.
 - c. The precision of the procedure is poor, even in highly experience hands, and safety factors are added to the procedure to compensate for the lack of precision.
 - d. *To calculate the volume of fetomaternal hemorrhage, this percentage of fetal cells is multiplied by 50 (5000 ml represents the mother's arbitrarily assigned blood volume). Since one 300 ug dose of RhIG will protect against a transplacental hemorrhage of 30 ml of D-pos fetal blood, the volume of fetal blood should be divided by 30 to determine the number of vials required.*
 - e. **Example:**
 - 1) K-B reported as 1.3%
 - 2) $1.3 \times 50 = 65$ ml of fetal blood
 - 3) $65/30 = 2.2$ doses of RhIG required.
 - 4) When the number to the right of the decimal point is less than 5, round down and add one dose of RhIG: 2.2 doses - give 3 doses.
 - 5) When the number to the right of the decimal point is 5 or greater, round up to the next number and add one does of RhIG: 2.8 doses - give 4 doses.
 - f. Not more than five doses of RhIG should be injected at one time into each buttock. If more than five doses are required, the injections may be spaced over a 72-hour period. An optimum time sequence for these injections has not been established.
 - g. A recent paper addresses the issue by comparing the KB technique with flow cytometry. The authors found the KB test to be accurate and precise with hemorrhages of 25 ml or greater. However, flow cytometric determinations appear to be more accurate.

***Exam 5 Online
Lecture Topic XI
Labs 8 and 9***