Hemolytic Disease of the Fetus and Newborn

Reviewed: 6/24/15

HDFN

- Fetal RBCs are coated with maternal alloantibody
  - Directed against ag inherited from the father that is absent from the mother
- IgG-Coated RBCs are destroyed
  - Before and after birth
  - Severity ranges from intrauterine death to asymptomatic (serologic detection only)

Three prerequisites for HDFN

- Mom lacks antigen (exposed through pregnancy or transfusion)
- Fetus possesses antigen; inherited from father
- Mom has built an IgG antibody
  - Sensitization depends on:
    - Recognition of foreign antigen
    - Responder
    - Ag is immunologic
    - Amt. of bleed
    - ABO compatibility

Physiologic Observations

- Extensive erythropoiesis in fetal liver can produce multiple problems
  - Disruption of portal circulation
  - Impaired albumin synthesis
    - Both can reduce plasma colloid osmotic pressure
- Leads to severe anemia which causes:
  - Cardiovascular failure
  - Tissue hypoxia
  - Death in utero

Definitions

- Erythroblastosis fetalis
  - Accelerated RBC destruction stimulates increased production of RBCs
  - Enter circulation as nucleated cells
- Hydrops fetalis
  - High-output cardiac failure
  - Generalized edema
  - Effusions - The seeping of serous, purulent, or bloody fluid into a body cavity or tissue.
  - Ascites - An abnormal accumulation of serous fluid in the abdominal cavity.

Bilirubin

- Fetal bilirubin is processed by maternal liver before birth
- Infant liver is immature at birth
  - Cannot conjugate amount of bilirubin that results from destruction of ab-coated RBCs
- Unconjugated bilirubin is toxic to CNS
  - Kernicterus

Complications of HDFN

- Rising levels of unconjugated bilirubin biggest risk
  - Decision to perform exchange transfusion driven by bili levels
- CNS damage caused by:
  - Prematurity
  - Acidosis
  - Hypoxia
  - Hypoalbuminemia

HDFN Categories

- Rh HDFN
  - Anti-D alone, or in combination with
  - Anti-C or anti-E
- "Other" HDFN
  - Other antigens in Rh system
    - Anti-c
  - Antigens in other systems
    - Anti-K1
- ABO HDFN
  - Anti-A,B in group O woman

ABO HDFN

- Can occur in any pregnancy
- Group A or B infants born to group O mothers
  - O persons can make IgG anti-A,B
  - ABO IgG abs occur without history of prior exposure
- Group A or B mothers
  - Produce IgM antibody in response to an incompatible fetus
  - Very small amounts of IgG antibody produced

ABO vs. Rh HDFN

ABO-HDFN

- Most common
- Can't be diagnosed
- Can affect 1st baby
- Weak-neg. DAT
- Occurs in "O" moms
- Slight rise in bilirubin (treat w/phototherapy)

Rh-HDFN

- Not due to RhIg
- Followed w/titers
- Immune exposure (2nd child)
- Very strong DAT
- Can affect any Rh=
- Need to exchange (but not always)

Immunization

- Fetomaternal hemorrhage (FMH)
  - Can occur in third trimester
  - Delivery – **most common**
  - Amniocentesis
  - Spontaneous or induced abortion
  - Chorionic villus sampling
  - Cordocentesis
  - Rupture of ectopic pregnancy
  - Blunt trauma to abdomen

Avoiding HDFN

- D antigen is very immunogenic
  - Exposure to less than 0.1 mL of blood can cause sensitization
- Anti-D causes most severe HDFN
- How can Rh HDFN be avoided?
  - Use of Rh immune globulin – RhIG
  - Will be discussed in later lecture

Effect of ABO Incompatibility

- Rh immunization of untreated D-neg mothers occurs less frequently after delivery of an ABO-incompatible D-pos infant
  - Protective effect of naturally occurring abs
  - Fetal RBCs are destroyed by anti-A or anti-B

Transfusion Stimulus

- Avoid giving D-pos RBCs to D-neg females of child-bearing age
- Platelets and granulocytes
  - RhIG should be considered if D-pos components must be used
- Avoid directed donations from sexual partner or his blood relatives
  - Increases immunization risk by exposure to paternal RBC ags, leukocytes and plts

Prenatal Evaluation

- Maternal History
  - Previous pregnancies
    - History of hydrops fetalis due to anti-D
      - 90% or greater chance that subsequent fetus will be similarly affected
    - History of ABO HDFN
      - Cannot discern risk to subsequent infants
      - Why?

Serologic Studies

- Early pregnancy
  - ABO/D, weak D if D neg
  - Antibody screen
- 28 weeks
  - D neg women with initial neg absc:
    - Repeat absc
    - Administer RhIG

Positive Antibody Screen

- Identify antibody
  - Presence of ab does not mean HDFN will occur
    - Not all antibodies are risk to fetus
      - Anti-Le^a, anti-I
    - Baby may lack ag
      - Fetal involvement may be predicted by typing father's RBC antigens
      - Fetal type
        - Sample from amniotic fluid, chorionic villus sampling
        - PCR testing

Maternal Antibody Titer

- Titration studies can aid in treatment decisions
  - Establish baseline in first trimester
  - Repeat at intervals determined by clinician
    - Usually not repeated until 16-18 wks
- Use is controversial
  - No established critical titers for abs other than anti-D
  - Represents a non-invasive means to monitor pregnancy

Titrations

- Successive titrations
  - Performed with same method
  - Use test cells of same phenotype
  - Test previously frozen sample in parallel
- Critical Titers - examples
  - Anti-D – 16 to 32
  - Anti-K1 – 8
  - Must be established by each facility

Method

- Ab is determined by testing serial twofold dilutions of the serum against selected RBCs
  - Some select a homozygous cell because optimal reactivity will be seen
  - Some use a heterozygous cell because this more accurately reflects phenotype of fetus
- Variations in technique are unavoidable
  - Testing samples in parallel helps explain any noted differences in results

Interpreting Results

- Titer is reported as reciprocal of dilution level
  - Significance depends on institutional critical titers
- Significant difference in titer is three or more dilutions
- Scoring system is used by some to represent the strength of agglutination
  - Difference of 10 or more between different samples is considered significant

Examples of Antibody Titers, Endpoints, and Scores

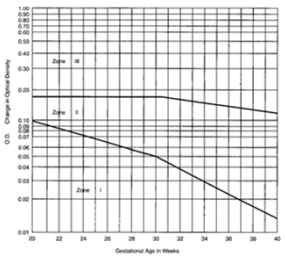
		Reciprocal of Serum Dilution											
		1	2	4	8	16	32	64	128	256	512	Titer	Score
#1	Strength	3+	3+	3+	2+	2+	2+	1+	±	±	0	64 (256)	
	Score	10	10	10	8	8	8	5	3	2	0		64
#2	Strength	4+	4+	4+	3+	3+	2+	2+	1+	±	0	128 (256)	
	Score	12	12	12	10	10	8	8	5	3	0		80
#3	Strength	1+	1+	1+	1+	±	±	±	±	±	0	8 (256)	
	Score	5	5	5	5	3	3	3	2	2	0		33

Amniotic Fluid Analysis

- Amniocentesis
  - Long needle inserted through abdominal wall and uterus
  - Fluid is aspirated from uterine cavity
  - Fluid is scanned spectrophotmetrically at 350-700nm
    - Peak absorbance of bilirubin is at 450nm
  - ΔOD_{450} value is plotted on Liley graph against estimated gestation length

Liley Graph

- Zone 1 – Mild or no disease
- Zone 2 – Repeat determination need to establish trend
- Zone 3 – Severe disease



Fetal Lung Maturity Test

- Also called lecithin sphingomyelin test:
  - Done on amniotic fluid to determine LUNG MATURITY
  - Measures the ability of the alveolar spaces to inflate and allow oxygen to be transported in to the blood
  - Used to determine if baby can be delivered

PUBS

- Percutaneous Umbilical Cord Sampling
  - Needle is inserted into umbilical blood vessel to obtain fetal blood sample
    - Hemo and biochemical tests can be done on sample
  - Risk of fetal mortality – 1.2%
  - High risk of FMH

Intrauterine Transfusion

- Begun in 20th week of gestation for severely affected infants
  - Intraperitoneal – IPT
  - Intravascular – IVT – through umbilical vein
- Interval depends on
  - Presence or absence of hydrops
  - Gestational age
  - Amount of blood infused

Selection of RBCs for IUT

- Group O, D-neg or neg for ag corresponding to mother's ab
- Irradiated
- CMV neg
- Lack Hgb S
- Fresh as possible

Postpartum Evaluation

- Cord blood studies
  - Performed on infants with risk of HDFN
    - Rh-pos baby born to Rh-neg mother
    - A or B infant born to O mother
  - Sample
    - Labeled as cord blood with mother's name, date, infant's id, and hospital number
  - Testing
    - ABO/D
    - DAT

ABO/D Testing

- Forward typing only
  - ABO antibodies in cord serum are of maternal origin
    - If ABO HDFN suspected, test cord serum for AHG-reactive ABO abs
- D Typing
  - False negative reactions can be seen
    - Infant RBCs heavily coated with anti-D

DAT

- Rh or other HDFN
  - Strongly positive
- ABO HDFN
  - Strength of DAT does not correlate with severity of hemolysis
- IUT
  - Weakly pos
  - Mixed field reaction

Testing

- Positive DAT
  - Perform elution
  - Test for specificity
  - Not necessary if maternal serum contains a single RBC ab
- Positive DAT – mother's absc neg
  - Consider ABO HDFN
  - Antibody to low-incidence antigen

Notes on Cord Blood Testing

- Wharton's Jelly
  - Presence can cause false pos reaction – much like rouleaux
  - Weak, sticky, nebulous reactions are noted
  - Recommended to wash all cell suspension made from cord blood sample 4x's
  - If washing does not remove reactivity, request a heelstick recollection

Evaluation of ABO HDFN

- Group O mom – infant A or B
- DAT may be negative
  - Can confirm by testing the cord eluate against A_1, B, and O cells
  - Perform IAT with cord serum against A_1, B, and O cells
- If transfusion necessary:
  - Group O, D-compatible RBCs

Antibody to Low-Incidence Antigen

- DAT pos, mother's absc neg
  - R/O ABO HDFN
  - Test eluate or maternal serum against father's RBCs
    - Maternal serum must be ABO compatible
    - If pos, indicates that infant has ag that mother lacks, causing her to make IgG ab
- Should be no difficulty in obtaining blood, if needed

Antibody to High Incidence Antigen

- Can be difficult to find blood
  - Mother
    - Can freeze RBCs
  - Mother's siblings
    - Irradiate any components from blood relatives
  - Rare donor file
- If compatible blood cannot be found, may have to use least incompatible in urgent situation

Exchange Transfusion

- Removal of ab-coated fetal RBCs
- Removal of maternal ab
- Removal of bilirubin
- Replacement of RBCs – treats fetal anemia

Specimen

- Mother's serum is specimen of choice
  - Available in large quantities
  - Decreases volume of blood taken from infant
  - RBC ab is present in high concentrations
  - Can be analyzed prior to delivery
  - Can also cause problems
    - Presence of other abs
    - Presence of IgM abs
- If maternal serum unavailable, use:
  - Infant's serum
  - Eluate from infant's red cells
    - Use of eluate is preferable
    - Concentration of ab can be low in serum

Exchange Components

- RBCs IRRADIATED!!
  - Crossmatched with mother's serum
  - ABO compatible
  - Compatible with any other additional abs
    - Group O red cells resuspended in AB plasma commonly used
- FFP
  - For replacement of coag factors
  - PF24 can be used – contains ↓ V and VIII
- Platelets
  - Should be monitored and tx'ed as necessary

Subsequent Transfusion

- Bilirubin can accumulate rapidly after exchange, despite phototherapy
  - Bilirubin in extravascular fluid reequilibrates by entering intravascular space
  - Ab coated cells continue to hemolyze
- If additional txns necessary, same considerations for RBC selection and xmatching apply