Hemolytic Disease of the Fetus and Newborn

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 bilirubin 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-Lea, 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

<table>
<thead>
<tr>
<th>Reciprocal of Serum Dilution</th>
<th>Titer</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>1+</td>
<td>0</td>
</tr>
<tr>
<td>1:2</td>
<td>2+</td>
<td>0</td>
</tr>
<tr>
<td>1:4</td>
<td>3+</td>
<td>0</td>
</tr>
<tr>
<td>1:8</td>
<td>4+</td>
<td>0</td>
</tr>
<tr>
<td>1:16</td>
<td>5+</td>
<td>0</td>
</tr>
<tr>
<td>1:32</td>
<td>6+</td>
<td>0</td>
</tr>
<tr>
<td>1:64</td>
<td>7+</td>
<td>0</td>
</tr>
<tr>
<td>1:128</td>
<td>8+</td>
<td>0</td>
</tr>
<tr>
<td>1:256</td>
<td>9+</td>
<td>0</td>
</tr>
<tr>
<td>1:512</td>
<td>10+</td>
<td>0</td>
</tr>
</tbody>
</table>

#1: Strength: 3+ 3+ 3+ 2+ 2+ 2+ 1+
Score: 10 10 10 8 8 8 5

#2: Strength: 4+ 4+ 4+ 3+ 3+ 2+ 2+ 1+
Score: 12 12 12 10 10 8 8 5 3 0

#3: Strength: 1+ 1+ 1+ 1+
Score: 5 5 5 3 3 3 3 2 2 0

Amniotic Fluid Analysis

- **Amniocentesis**
  - Long needle inserted through abdominal wall and uterus
  - Fluid is aspirated from uterine cavity
  - Fluid is scanned spectrophotometrically at 350-700nm
  - Peak absorbance of bilirubin is at 450nm
  - $\Delta OD_{450}$ 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, B, and O cells
  - Perform IAT with cord serum against A, 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
  - 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