Overview of Hemolytic Disease of Newborn
What is HDN?

• Destruction of the RBCs of the fetus and newborn by antibodies produced by the mother
• Only IgG antibodies are involved because it can cross the placenta (not IgA or IgM)
Pathophysiology
Pathogenesis

* Mother sensitized to paternal RBC antigen by FMH→ maternal alloantibody crosses placenta → IgG binds to fetal rbc→ rbc destroyed by splenic macrophages→ fetal marrow erythropoiesis (erythroblastosis fetalis)→ extramedullary hematopoiesis in liver→ Decr. albumin→ high output failure→ edema (hydrops fetalis)

*extensive hemolysis→hyperbilirubinemia (kernicterus)

*Severe disease can occur between 18-20 weeks’ gestation

Rh antigens does not fix complement

Hydrops Fetalis

- Multiple causes linked by cardiac failure
- Decreased circulation oncotic pressure, increased venous capillary pressure cause generalized edema, ascites
- Increased interstitial fluid, effusions

100% mortality!
Immune Hydrops

- NRBC in fetal placental vessels
- EMH with HSM, polyhydramnios, effusion, ascites
- Jaundice, unconjugated hyperbilirubinemia
Hydrops due to Anemia
Results in high output fetal heart failure

- Thalassemia (world’s commonest cause of hydrops)
- Twin-twin transfusion, chronic fetomaternal hemorrhage
Hydrops due to Anemia
Results in high output fetal heart failure

- Infection
  - Syphilis, CMV
  - Parvovirus B19 infects erythroid precursors; inhibits maturation, resultant anemia. Nucleated RBC with inclusions in placenta, marrow.
Hydrops - Other Causes

- Hypoproteinemia: Congenital nephrotic syndrome
- Intrauterine heart failure: arrhythmia
- Structural abnormalities that interfere with fetal circulation
- Chromosomal abnormalities: X0, T21, 18, 13
- Storage disease: MPS I and VII, Niemann-Pick, I-cell Disease, Gaucher, Sialidosis
Kernicterus
Bilirubin Encephalopathy

- Yellow discolored nuclei (hippocampus, basal ganglia, thalamus, CN nuclei, dentate), neuronal necrosis.
- Lipophilic Br crosses blood brain barrier, injures brain cells, possibly by interfering with mitochondrial function.
- Unconjugated Br 12mg/dl can cause kernicterus in premie, <20mg/dl OK at term.
Bilirubin

• Hemoglobin is metabolized to bilirubin
  – Before birth, “indirect” bilirubin is transported across placenta and conjugated in maternal liver (“direct”) where it is excreted
  – After birth, the newborn liver is unable to conjugate the bilirubin
    • Unconjugated (“indirect”) bilirubin can reach toxic levels (18-20 mg/dL)
    • This is called **kernicterus** and can lead to permanent brain damage
Sensitization occurs as a result of seepage of fetal cells into maternal circulation as a result of a fetomaternal hemorrhage:

- Takes 0.1 – 1 mL of D+ rbcs
- Placental membrane rupture (7%)
- Trauma to abdomen
- Delivery (>50%)
- Amniocentesis
- Abortion
• Without prophylaxis D alloimmunization is 16% of D neg. mothers
  – 2% sensitized in 1\textsuperscript{st} pregnancy
  – 7% w/n 6 months
  – 7% during 2\textsuperscript{nd} pregnancy
  – Reduced to 1.5% if ABO incompatible

• History - presence of anti-D
  – + for hydrops - 90\% chance hydrops in subsequent fetus
  – 10\% [for mom’s w/ anti-D] chance hydrops
Etiology
• ABO - most common (ABO>D>K), usu. mild
  – typically group O mothers - often have IgG anti-A, anti-B or anti-A,B
  – Can occur during 1st pregnancy
  – 65% of HDN

• Rh - most severe (D > c > E)

• Others - K, Fy, Jk (usu. mild)
  – anti-Kel1 associated with suppression of fetal erythropoiesis, low bilirubin and poor correlation with anti-Kel1 titres
  – alloimmunization rate - 0.08 - 0.4%

Prenatal Evaluation
Serologic testing on mother

- **ABO and Rh testing**
  - Test for D antigen (test for weak D if initially negative)

- **Antibody Screen**
  - To test detect for IgG alloantibodies that react at 37°C

- **Antibody ID**
Prenatal Evaluation

• Alloantibody screen – 1st trimester (again at 28 wks for RhD(-) mother)
  – alloantibody identification
  – alloantibody titre (if known to cause HDN) starting 16-18 wks

• Serial alloantibody titres - q 4 - 24 w then q 2 w***
  – “critical titre” - usu. 16 or 32 at AHG for anti-D; 8 for anti-Kel1

*** varies from place to place

Geaghan SM. Diagnostic laboratory technologies for the fetus and neonate with isoimmunization. Semin Perinatol 2011; 35:148.
0.5 mL plasma

0.1 mL 2% rbc

1:1 → 0.1 mL → 1:2 → 1:4 → 1:2048

37°C → wash → add anti-IgG → look for agg.

RBC

- May choose to use strongest expressed antigen rbcs
  - R2R2
- May choose rbc that are similar to what you would find in fetal circulation
  - R1r

AABB Tech Manu. 17th ed.
The agglutination reactions for each dilution are given a corresponding score; scores are added:

- 4+  12
- 3+  10
- 2+  8
- 1+  5
- w+  3

Example:

\[
\begin{align*}
&3+ &+3 &+3 &+2 &+2 &+2 &1+ \\
&10 &10 &10 &8 &8 &8 &5 \\
&\text{Total} &59
\end{align*}
\]
Testing of father

- serologic Rh(D) type – can’t determine zygosity
  - C/c E/e typing can indicate probable genotype
  - D homozygosity – 40% W and B; 87% Asian

- zygosity - DNA amplification technique
  - Europeans – low false neg rate
  - Africans/Asians – depending on specific test, may miss D pseudogenes (67% Africans), hybrid D/CE genes, etc.

Fetal typing

- amniocentesis – false neg rate 1 – 1.5%
- free fetal DNA – maternal serum/plasma

Geaghan SM. Diagnostic laboratory technologies for the fetus and neonate with isoimmunization. Semin Perinatol 2011; 35:148.
Response to Rising/Critical Titers
High output decreases velocity… Good correlation w/ velocity and fetal hematocrit and aminocentesis
• Fetal ultrasound - for evidence of anemia

  – early signs - ↑ placental thickness, ↑ umbilical v. diameter, hepatosplenomegaly, polyhydramnios

  – *hydrops* – ascites, skin edema, pleural effusion occurs when HgB < 7 g/dL

Amniocentesis & Cordocentesis

- About 18-20 weeks’ gestation
- Cordocentesis takes a sample of umbilical vessel to obtain blood sample
- Amniocentesis assesses the status of the fetus using amniotic fluid
  - Fluid is read on a spectrophotometer (350-700 nm)
  - Change in optical density ($\Delta$OD) above the baseline of 450 nm is the bilirubin measurement
Analysis of amniotic fluid (example)
• The ΔOD is plotted on the Liley graph according to gestational age
• Three zones estimate the severity of HDN
  – Lower: mildly or unaffected fetus (Zone 1)
  – Midzone: moderate HDN, repeat testing (Zone 2)
  – Upper: severe HDN and fetal death (Zone 3)
Liley graph

A ΔOD of .206 nm at 35 weeks correlates with severe HDN.

Cordocentesis or Percutaneous umbilical blood sampling - PUBS

- Needle in umbilical vein
- Direct assessment of anemia, hemolysis (DAT, Hb, retics, bili)
- Risks - FMH, fetal demise
  - (1.2%)
Prenatal Management
HDN: Management

- mother
  - IVIg - best begun < 28 weeks and before **hydrops**
- fetus
  - intrauterine transfusion – Hct < 30%
  - intraperitoneal or -vascular
  - group O RBC, target antigen negative, crossmatch compatible (maternal serum)
  - HbS neg, CMV reduced risk, irradiated, leukoreduced, frozen or < 7 d
  - delivery
What to do before birth?

- Intrauterine transfusion is done if:
  - Amniotic fluid ΔOD is in high zone II or zone III
  - Cordocentesis has hgb <100 g/L
  - Hydrops is noticed on ultrasound
- Removes bilirubin
- Removes sensitized RBCs
- Removes antibody
• **Intrauterine transfusion:** Volume to transfused can be calculated:

\[
\text{US-estimated fetal weight} \times (0.14 \text{ mL/g}) \times (40\% - 15\%) \times \frac{85\%}{\text{Hematocrit of RBC unit}} = 41.2 \text{ mL}
\]

It would take 42 mL of blood to increase the hct 25%
Neonatal Jaundice: Prediction of Severity

- Neonates placed into risk categories on basis of:
  - Total Serum Bilirubin (TSB) levels
  - Risk factors:
    • HDN
    • G-6-PDH deficiency
    • asphyxia
    • lethargy
  • sepsis
  • acidosis
  • albumin < 3 g/dL
  • temperature instability

- Stepwise interventions
  - Phototherapy
  - IVIg
  - Exchange Transfusion - removes Rh(D) + RBCs, anti-D and bilirubin
### Indications for Phototherapy: AAP*

<table>
<thead>
<tr>
<th>T Bili (mg/dL)</th>
<th>12h</th>
<th>24h</th>
<th>48h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Risk</td>
<td>12</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>≥ 38w no risk factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium Risk</td>
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<td>13</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>35-37w no risk factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Risk</td>
<td>8</td>
<td>11</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>35-37w + risk factors</td>
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</tbody>
</table>

Indications for IVIg

- Indication
  - T Bili rising despite phototherapy
  - T Bili within 2-3 mg/dL of threshold for exchange transfusion

- Dose
  - 0.5 – 1.0 g/kg over min. 2 h
  - May repeat x 1 at 12 h

### Indications for Exchange Transfusion: AAP*

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<th>T Bili (mg/dL)</th>
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<th>24h</th>
<th>48h</th>
</tr>
</thead>
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<tr>
<td>Low Risk</td>
<td>19</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>Medium Risk</td>
<td>16.5</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>High Risk</td>
<td>15</td>
<td>17</td>
<td>18.5</td>
</tr>
</tbody>
</table>

- ≥ 38w no risk factors
- ≥ 38w + risk factors
- 35-37w no risk factors
- 35-37w + risk factors

Postpartum testing
• ABO – forward only

• Rh grouping

• DAT- might be negative if hemolysis
  – +/- Elution
Prevention
RhIg (RhoGAM®-IM or WinRho- IV or IM) is given to the mother to prevent immunization to the D antigen

- “Fools” mom into thinking she has the antibody

- RhIg (1 dose) is given at 28 weeks’ gestation

- RhIg attaches to fetal RBCs in maternal circulation and are removed in maternal spleen; this prevents alloimmunization by mother

- May cause a positive DAT in newborn (check history)

DO NOT GIVE IF THE MOTHER ALREADY HAS ANTI-D
RhIg is administered:

- at 28 weeks gestational age (dose 300 µg),
- at delivery, if the neonate is D-positive, weak-D positive, or D untested (minimum dose of 300 µg, further dosing determine by fetomaternal hemorrhage [FMH] testing), and
- following perinatal events associated with FMH, such as abortion, ectopic pregnancy, amniocentesis, chorionic villus sampling, external cephalic version, abdominal trauma, and antepartum hemorrhage (minimum dose of 300 µg, further dosing determine by FMH testing if >20 weeks gestational age).
Whole-blood derived platelets = 0.5 ml of RBCs $\rightarrow$ 1 dose will cover 30 whole blood plt units.

Apheresis platelet product contains less than 2 ml of RBCs $\rightarrow$ 1 dose will cover 7 apheresis platelet products.

<table>
<thead>
<tr>
<th>Vial size</th>
<th>IU</th>
<th>Whole blood (ml)</th>
<th>RBCs (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 µg</td>
<td>250</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>120 µg</td>
<td>600</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>300 µg</td>
<td>1500</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>1000 µg</td>
<td>5000</td>
<td>100</td>
<td>50</td>
</tr>
</tbody>
</table>
To determine how much to give… 1\textsuperscript{st} determine if there is FMH
If you know the fetal bleed volume (mL), then just take that volume divide by 30.
Rosette Test

- A qualitative measure of fetomaternal hemorrhage - detects fetal RBC in mother’s blood

Fetomaternal Hemorrhage:
- <1 rosette per 3 lpf = 1 dose of RhIg
- >1 rosette per 3 lpf = Quantitate bleed
Reagent anti-D binds to baby’s RhD pos RBC
Indicator RBC form a rosette around the baby’s RHD pos RBC coated with anti-D
Positive indicates greater than 10 mL RBC present
Kleihauer-Betke acid elution

- Quantitates the number of fetal cells in circulation
  - Fetal hemoglobin is resistant to acid and retain their hemoglobin (appear bright pink)
  - Adult hemoglobin is susceptible to acid and leaches hemoglobin into buffer ("ghost" cells)
\[
\frac{\text{# fetal cells}}{\text{total cells (2000)}} \times 100 = \% \text{ of fetal cells}
\]
Flow cytometry

- Used in some centers rather than KB
- Samples many more cells and is considered more accurate.
<table>
<thead>
<tr>
<th>TABLE 35.2 Calculation of RhIg Dosing</th>
</tr>
</thead>
</table>

% Fetal RBCs in maternal circulation is determined by Kleihauer-Betke test or flow cytometry.

Maternal blood volume (ml) = $70 \text{ ml/kg} \times \text{ maternal weight (kg)}$, or 5000 ml if maternal weight unknown

Fetal bleed (ml) = % fetal RBCs $\times$ maternal blood volume

Dose of RhIg = fetal bleed (ml)/30 ml per dose (300-µg vial)

If the number to the right of the decimal point is $<5$, round down and add one dose of RhIg (e.g. $2.3 \rightarrow 2 + 1 = 3$ vials).

If the number to the right of the decimal point is $\geq 5$, round up and add one dose of RhIg (e.g. $2.6 \rightarrow 3 + 1 = 4$ vials)
• An easier and equally accurate way!
  – KB%[DO NOT CHANGE TO DECIMAL] x 5/3 = number of vials.
  – If KB is 2%, then [2x5]/3 =3.33
  – Note: “5” is mom’s BV in L; tweak if necessary…
Considerations

- **RhIg** is of no benefit once a person has formed anti-D.
- It is VERY important to distinguish the presence of anti-D as:
  - Residual RhIg from a previous dose OR
  - True immunization from exposure to D+ RBCs
- RhIg is not given to the mother if the infant is D-negative (and not given to the infant)
End