Neonatal Hematopoiesis and RBC Disorders

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Pediatric Hematology
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Objectives

• Review normal erythropoiesis in the fetus and neonate and regulation of fetal hemoglobin

• Outline the differential diagnosis of neonatal RBC disorders with a focus on the clinical and laboratory findings

• Discuss common presentations of intrinsic red cell disorders in neonates
Which of the following infants is most likely to be diagnosed with a primary hematologic disorder (i.e. need ongoing follow-up in my office)?

A. A full-term male with a Hb of 7.5 gm/dL at birth (MCV 108)
B. A one week old with a newborn screen that shows Hb FAS
C. A full-term Caucasian male with a peak bilirubin of 21 mg/dL whose mom is AB+
D. A 26 week AA female whose father has a history of G6PD deficiency
RBC Disorders in the NICU

• Anemia is a common finding in the NICU
• Differential is broad
• Hospitalized preterm infants receive more PRBC transfusions than any other patient group
• >80% of ELBW infants receive at least one PRBC transfusion
How RBC Disorders Present?

• Anemia on a CBC
  – May be an expected or incidental finding
• Abnormal RBC indices
• Abnormal newborn screens
• Hyperbilirubinemia
• Screening because of family history
What is Normal?

Christensen et al, Semin Perinatol 2009
What is Normal?

Christensen et al, Semin Perinatol 2009
Hemoglobin Switching

<table>
<thead>
<tr>
<th>Types of cells</th>
<th>Megaloblast</th>
<th>Macrocye</th>
<th>Normocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organs</td>
<td>Yolk sac</td>
<td>Liver</td>
<td>Spleen</td>
</tr>
</tbody>
</table>

Part in the total synthesis of globin, %

- α-similar globin chains
- β-similar globin chains

Prenatal age (weeks): 6, 12, 18, 24, 30, 36
Postnatal age (weeks): 6, 12, 18, 24, 30, 36, 42

Birth
How to Approach Anemia

• Are other cell lines involved?

• What is the MCV?

• What is the reticulocyte count?

• What does the peripheral blood smear show?
Microcytic Anemia

• Iron Deficiency
  – Iron supplementation for preterm infants
• Thalassemia
  – Beta-thalassemia less likely in the neonatal period
• Chronic Inflammation
  – Disorders of iron transport (e.g. TMPRSS6)
• Rare disorders
  – Unstable hemoglobins, sideroblastic anemia, copper deficiency
Hemoglobin Genes

Beta Globin Gene Cluster
Chromosome 11

- epsilon
- gamma
- delta
- beta

5' -> Hb F -> 3'

Alpha Globin Gene Cluster
Chromosome 16

- Zeta 2
- Zeta 1
- Alpha 2
- Alpha 1

5' -> 3'
# Alpha Thalassemia

## Table. Alpha-thalassemia syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Number of alpha-globin genes affected</th>
<th>Clinical features</th>
<th>Hemoglobin pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silent carrier ($\alpha^+$)</td>
<td>1</td>
<td>No or minimal anemia</td>
<td>1%–2% Hb Barts ($\gamma_4$)</td>
</tr>
<tr>
<td>Thalassemia trait ($\alpha^+$)</td>
<td>2</td>
<td>Mild anemia</td>
<td>5%–10% Hb Barts ($\gamma_4$)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hypochromic microcytic</td>
<td></td>
</tr>
<tr>
<td>HbH disease ($\alpha^0 + \alpha^+$)</td>
<td>3</td>
<td>Moderate anemia</td>
<td>10%–30% HbH ($\beta_4$)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hypochromic microcytic</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>RBC inclusion bodies</td>
<td></td>
</tr>
<tr>
<td>Hydrops fetalis ($\alpha^0$)</td>
<td>4</td>
<td>Death in utero or at birth</td>
<td>97% Hb Barts ($\gamma_4$)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Severe anemia</td>
<td>3% HbH ($\beta_4$)</td>
</tr>
</tbody>
</table>

RBC indicates red blood cells.
# Beta Thalassemia

## Classification of β Thalassemia (Genetic)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Genotype</th>
<th>Clinical Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>β thal minor/trait</td>
<td>β/β+, β/β0</td>
<td>Silent</td>
</tr>
<tr>
<td>β thal intermedia</td>
<td>β+/β+, β+/β0</td>
<td>Moderate</td>
</tr>
<tr>
<td>β thal major</td>
<td>β0/ β0</td>
<td>Severe</td>
</tr>
</tbody>
</table>
Macrocytic Anemia

- Drug-induced, esp. AEDs
- Down’s syndrome
- Liver disease
- Bone marrow failure syndromes
- Hypothyroidism (in adults)
- B12 or folic acid deficiency (rare)
- Dyserythropoietic anemia (very rare)
Diamond-Blackfan Anemia

- Clinical features: macrocytic anemia, reticulocytopenia, normal bone marrow cellularity with a paucity of erythroid precursors
- About 50% of patients have congenital abnormalities
- Genetics: AD (sporadic or inherited) mutations in ribosomal proteins
- 9 genes described, many patients have unknown mutations
- Testing: red cell ADA (elevated in 80% of patients)
- Treatment: steroids, chronic transfusions, HSCT
- Some increased risk of malignancies – AML, MDS, colon, osteosarcoma, female genital cancers
Fanconi Anemia

- Clinical features: marrow failure, congenital anomalies (75%), and family history suggestive of cancer predisposition
- Pathogenesis: defective DNA repair leading to chromosomal fragility
- Genetics: AR (99%; 12 genes) and X-linked (1%; FANCB)
- Testing: Chromosomal breakage studies
  - Increased chromosomal breakage following exposure of patient’s lymphocytes to mitomycin C or diepoxybutane (DEB)
  - If blood is negative, can test fibroblasts if you have a high clinical suspicion
Normocytic Anemia, elevated retic

• Blood loss vs. hemolysis
• Blood loss is more common in the NICU
  – Obstetrical causes (e.g. placental abruption)
  – Feto-maternal hemorrhage (Kleihauer-Betke test on mom)
  – Twin-twin transfusion
  – Internal hemorrhage
  – Iatrogenic blood loss
    • Probably the most common cause of anemia (and transfusion) in preterm infants
Normocytic Anemia, elevated retic

• Hemolysis
  – Intrinsic
    • Membrane, Hb, and enzyme defects
  – Extrinsic
    • Immune-mediated, esp. alloimmune processes
    • Microangiopathic causes (DIC, congenital TTP)
    • Congenital heart disease
    • Kasabach-Merritt syndrome
    • Drug or infection-induced
    • Vitamin E deficiency (of historical interest mostly)
Hereditary Spherocytosis

- Most common cause of non-immune hemolytic anemia in the Caucasian population
- Can be due to AD, AR, or sporadic mutations
- Varying degrees of anemia
- Consider an evaluation if a patient has jaundice in the first 24 hours of life or early gallstones
- Clue: elevated MCHC (>36%) on a CBC
- Increased osmotic fragility
# HS – Clinical Variability

**Table 1**

Classification of hereditary spherocytosis

<table>
<thead>
<tr>
<th></th>
<th>Carrier</th>
<th>Mild Spherocytosis</th>
<th>Moderate Spherocytosis</th>
<th>Severe Spherocytosis&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>Normal</td>
<td>11–15</td>
<td>8–12</td>
<td>6–8</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>≤3</td>
<td>3–6</td>
<td>≥6</td>
<td>≥10</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0–1</td>
<td>1–2</td>
<td>≥2</td>
<td>≥2</td>
</tr>
<tr>
<td>Spectrin content (% of normal)</td>
<td>100</td>
<td>80–100</td>
<td>50–80</td>
<td>40–60</td>
</tr>
<tr>
<td>Peripheral smear</td>
<td>Normal</td>
<td>Mild spherocytosis</td>
<td>Spherocytosis</td>
<td>Spherocytosis</td>
</tr>
<tr>
<td>Osmotic fragility fresh blood</td>
<td>Normal</td>
<td>Normal or slightly increased</td>
<td>Distinctly increased</td>
<td>Distinctly increased</td>
</tr>
<tr>
<td>Incubated blood</td>
<td>Slightly increased</td>
<td>Distinctly increased</td>
<td>Distinctly increased</td>
<td>Distinctly increased</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values in untransfused patients.


HS – morphologic variability

Fig. 2. Peripheral blood smears in hereditary spherocytosis. (A) Typical hereditary spherocytosis. Characteristic spherocytes lacking central pallor are seen. (B) Severe, recessively inherited spherocytosis. Numerous small, dense spherocytes and bizarre erythrocyte morphology with anisocytosis and poikilocytosis associated with severe hemolysis are seen.

# HS – genetic variability

## Table 1: Erythrocyte Membrane Proteins Involved in HS

<table>
<thead>
<tr>
<th>Protein</th>
<th>Gene</th>
<th>Chromosomal Location</th>
<th>Percentage of HS Cases</th>
<th>Typical Severity</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankyrin-1</td>
<td>ANK1</td>
<td>8p11.2</td>
<td>40–50</td>
<td>Mild to moderate</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>Band 3</td>
<td>SLC4A1</td>
<td>17q21</td>
<td>20–35</td>
<td>Mild to moderate</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>β-spectrin</td>
<td>SPTB</td>
<td>14q23-24.1</td>
<td>15–30</td>
<td>Mild to moderate</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>α-spectrin</td>
<td>SPTA1</td>
<td>1q22-23</td>
<td>&lt;5</td>
<td>Severe</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Protein 4.2</td>
<td>EPB42</td>
<td>15q15-21</td>
<td>&lt;5</td>
<td>Mild to moderate</td>
<td>Autosomal recessive</td>
</tr>
</tbody>
</table>
HS – Diagnosis

Obtain CBC & Blood Smear

High MCHC/MCV ratio (>0.36)  
- Likely to have autosomal dominant HS

Intermediate (35–36) or normal (<35) MCHC/MCV ratio
- Spherocytes  
  - Might have autosomal dominant HS
  - EMA flow
  - Incubated osmotic fragility
  - Hematology consultation
- No spherocytes  
  - Less likely to have autosomal dominant HS (but sometimes spherocytes are not prominent on blood films of neonates who have HS)

Christensen et al, Pediatrics 2015
**Evaluation of HS***

*Family studies can also be very helpful*

**Christensen et al, Pediatrics 2015**

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**TABLE 2 Laboratory Evaluation for HS in a Jaundiced Neonate**

<table>
<thead>
<tr>
<th>HS Ratio (MCHC/MCV)</th>
<th>EMA Flow(^a)</th>
<th>Incubated Osmotic Fragility</th>
<th>DNA Sequencing(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonates with HS will generally have a high MCHC and a low MCV, producing an elevated ratio (&gt;0.35)</td>
<td>EMA dye binds stoichiometrically to band 3 and Rh-related membrane proteins. Decreased fluorescence intensity of EMA-tagged erythrocytes due to loss of membrane proteins is seen in HS. Decreased EMA binding is also seen in other disorders such as hereditary pyropoikilocytosis and congenital dyserythropoietic anemia II</td>
<td>HS erythrocytes are more susceptible to osmotic lysis than normal erythrocytes due to decreased membrane surface area. Incubation overnight stresses the already fragile HS erythrocyte, accentuating the defect. Spherocytes from any cause, including ABO incompatibility, will yield a positive result on osmotic fragility testing</td>
<td>Not needed to diagnosis most cases of HS. However, it can establish the diagnosis in difficult cases. Consider sequencing of relevant genes when family history is negative and severe DAT-negative hemolysis is idiopathic</td>
</tr>
</tbody>
</table>

\(^a\) Available in many reference laboratories.  
G6PD

- X-linked inheritance
- Most common in African Americans and those of Mediterranean decent
- Hemolysis occurs in response to oxidative stress
  - Avoid fava beans, naphthalene, sulfa drugs
  - [http://g6pddeficiency.org](http://g6pddeficiency.org)
- G6PD assay may not be reliable in the setting of an acute hemolytic event
## Other RBC Enzyme Defects

Table 1. Hemolytic anemia: associated symptoms and benefit of splenectomy in enzyme-linked hemolytic anemias

<table>
<thead>
<tr>
<th>Involved enzyme</th>
<th>Apparent degree of residual activity, %</th>
<th>Severity of hemolysis</th>
<th>Associated symptoms</th>
<th>Splenectomy</th>
<th>Blood picture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hxokinase</td>
<td>17-180</td>
<td>Mild to severe</td>
<td>Transient bone marrow aplasia</td>
<td>Inconsistent; recommended in severe cases</td>
<td>Macrocystosis</td>
</tr>
<tr>
<td>Glucose phospho isomerase</td>
<td>4-60</td>
<td>Mild to severe</td>
<td>Some cases with neuromuscular impairment</td>
<td>Recommended; variable response</td>
<td>Macrocystosis</td>
</tr>
<tr>
<td>Phosphofructokinase M</td>
<td>8-62</td>
<td>Mild to moderate</td>
<td>± Myopathy, hyperuricemia, arthritis</td>
<td>Not recommended</td>
<td>Basophilic stippling</td>
</tr>
<tr>
<td>Aldolase</td>
<td>5-16</td>
<td>Mild to severe</td>
<td>Myopathy; ± mental retardation</td>
<td>Recommended in a severe case</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>Triosephosphate isomerase</td>
<td>5-20</td>
<td>Moderate</td>
<td>Always neuromuscular impairment; frequent infections; near death before 5 yr of age</td>
<td>Not recommended⁵</td>
<td>Some target cells and small contracted cells</td>
</tr>
<tr>
<td>Phosphoglycerate kinase</td>
<td>0.7-15†</td>
<td>Moderate</td>
<td>± Convulsions, mental retardation, rhabdomyolysis</td>
<td>Inconsistent; mostly beneficial</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>50-100</td>
<td>Mild to severe</td>
<td>Not described</td>
<td>Recommended; variable response</td>
<td>Macrocystosis; shrunken, spiculated cells⁶</td>
</tr>
<tr>
<td>Pyrimidine 5'-nucleotidase</td>
<td>5-15</td>
<td>Moderate; usually no transfusions</td>
<td>None</td>
<td>Partially beneficial</td>
<td>Basophilic stippling</td>
</tr>
</tbody>
</table>
Figure 1  Maternal alloimmunization during pregnancy. Abnormal exposure of the maternal immune system to antigens present on fetal blood cells can result in antibody formation. Subsequent transmission of these maternal antibodies into the fetus can cause destruction of the fetal blood cells carrying the cognate antigen.
Maternal Alloimmunization*

*Minor Blood Group Antigens: “Kell Kills, Duffy Dies, but Lewis Lives”

<table>
<thead>
<tr>
<th>Table 2 Hemolytic Disease of the Newborn</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood groups</strong></td>
</tr>
<tr>
<td>Mother</td>
</tr>
<tr>
<td>Infant</td>
</tr>
<tr>
<td><strong>Clinical features of hemolytic</strong></td>
</tr>
<tr>
<td>disease in the newborn</td>
</tr>
<tr>
<td>Occurrence in first-born</td>
</tr>
<tr>
<td>Severity in subsequent pregnancies</td>
</tr>
<tr>
<td>Stillbirth/hydrops</td>
</tr>
<tr>
<td>Severe anemia</td>
</tr>
<tr>
<td>Jaundice</td>
</tr>
<tr>
<td><strong>Laboratory tests</strong></td>
</tr>
<tr>
<td>Direct Coombs’ test</td>
</tr>
<tr>
<td>(infant)</td>
</tr>
<tr>
<td>Reticulocyte count</td>
</tr>
<tr>
<td>Maternal antibodies</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

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*Maternal Alloimmunization*

*Minor Blood Group Antigens: “Kell Kills, Duffy Dies, but Lewis Lives”*
**Blood Typing**

<table>
<thead>
<tr>
<th>Blood type</th>
<th>Donor incidence</th>
<th>Abs present</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>44%</td>
<td>Anti-B</td>
</tr>
<tr>
<td>B</td>
<td>8%</td>
<td>Anti-A</td>
</tr>
<tr>
<td>AB</td>
<td>4%</td>
<td>Neither Anti-A nor B</td>
</tr>
<tr>
<td>O</td>
<td>44%</td>
<td>Both Anti-A and B</td>
</tr>
</tbody>
</table>
Normocytic Anemia, decreased retic

- “Normal (?)” finding in preterm infants
- Primary marrow disorder
- Acute blood loss or hemolysis
- Acute infections or inflammation
  - Includes congenital infections (e.g. parvovirus B19)
- TORCH infections (e.g. Rubella)
- Anemia or prematurity
Anemia of Prematurity

• RBC production naturally decreases after birth as a result of increased tissue oxygenation due to the onset of breathing and closure of the ductus arteriosus

• Reduced production of erythropoietin

• Confounded in premature infants by blood loss from phlebotomy, reduced RBC lifespan, iron depletion, and increased oxidative stress

• Leads to exaggerated physiologic nadirs
Evaluation of Hyperbilirubinemia

- Fractionate the bilirubin and check a CBC to narrow the differential
- Indirect Hyperbilirubinemia
- Direct Hyperbilirubinemia
- Consider additional hematologic evaluation for indirect hyperbilirubinemia requiring phototherapy >2 days, peak bilirubin levels >20 mg/dL, or hyperbilirubinemia in the first 24 hours of life
Neonatal Hyperbilirubinemia

Problematic Neonatal Jaundice?
Is phototherapy needed for >2 days? To evaluate potential underlying causes, obtain blood type on mother and infant baby, DAT, CBC with peripheral blood smear, and reticulocyte count.

DAT positive
- In ABO hemolytic disease, if the jaundice is severe or atypical, consider the possibility of a coexisting condition; consider additional diagnostic testing and sequencing

DAT negative
- RBC enzymology or other intrinsic defect
  - G6PD
  - Pyruvate kinase other

Suspicious for HS? (MCHC/MCV >0.36)
- EMA flow incubated osmotic fragility

Pathogenesis still unclear?
- Additional diagnostic testing
- Next-generation DNA sequencing
- Hematology consultation

Christensen et al, Pediatrics 2015
Newborn Screen Interpretation

• Performed in all 50 states to identify SCD
• Considerable variation in methodology and implementation
• Identification of Hb variants
  – Is the patient symptomatic?
  – Is Hb A present
• Common patterns (order matters)
  – SCD (FS, FSA, FSC), SCT (FAS), beta-thal major (fetal only), alpha-thal (Barts)
Treatment of RBC Disorders

• PRBC transfusions
  – Goal is to maintain adequate oxygen carrying capacity
  – Remember to irradiate blood products in cases of suspected T-cell dysfunction/deficiency or BW<1500 gms

• EPO
  – Will not provide an immediate increase in oxygen delivery
  – Best used in combination with iron (and other substrates as needed)
Early EPO Administration???

- Meta-analysis of early (started before 8 days of age) EPO administration in preterm (<37 weeks) and/or LBW (<2500g) neonates
- 23 studies enrolling 2074 infants from 18 countries
- RR for “use of one or more RBC transfusions” 0.80 (95% CI 0.75-0.86)
- RR of >stage 3 ROP 1.71 (95% CI 1.15-2.54)
- Early EPO not recommended

Ohlsson and Aher, Cochrane Database 2006, updated 2012
Conclusions

• Anemia is a common clinical finding in the NICU with a broad differential diagnosis
• Most cases are acquired, but primary hematologic disorders are likely underdiagnosed
• A systematic approach to the evaluation and treatment of RBC disorders seems desirable
• Improved treatments for neonatal RBC disorders are required