Lab Interpretation: Beyond the Numbers

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Objectives

- Define normal ranges of hematologic values for age in pediatrics
- Explain the physiologic reasons behind variability in these ranges
- Explore case studies where this matters and why
Case #1

- Former 35 week infant is seen at 2 months of age
  - CBC shows Hgb of 9.5 gm/dl

- Is this normal or abnormal?
Fetal Erythropoiesis

Mesoblastic

- Wk 3 to 4: Progenitors of blood vessels and hemocytoblasts present in yolk sac.
- Wk 4 to 5: **First Blood Cells (Red Cells)**
  - Intravascular maturation
  - Embryonic Hb content
  - Nucleus persists with maturation
  - Increased sensitivity to EPO
  - Megaloblastic (MCV = 250fl/cell)
Fetal Erythropoiesis

Hepatic

- Week 6: Normoblastic Erythropoiesis
- Week 10: Produces > 90% of circulating red cells
- Enucleated cells
Fetal Erythropoiesis
Myeloid (Bone Marrow)

- Wk 8 to 9: Hematopoiesis
- Month 6: Principal site of production for all blood cells.
- > Birth: ↑ cell production = ↑ cell turnover
  ± ↑ volume of hematopoietic tissue
  (marrow expansion in calvaria)
Erythrocytes
Normal Values & Variants

Gestational Age

• ↑ Hb with gestational age; stabilizes at ~ 33 wks
• ↓ MCV and ↓ reticulocyte count throughout pregnancy
# Normal Values
## Term Infants

<table>
<thead>
<tr>
<th></th>
<th>Cord Blood</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>16.8</td>
<td>18.4</td>
<td>17.8</td>
<td>17.0</td>
<td>16.8</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>53</td>
<td>58</td>
<td>55</td>
<td>54</td>
<td>52</td>
</tr>
<tr>
<td>RBC count (mm$^3$ x 10$^6$)</td>
<td>5.25</td>
<td>5.8</td>
<td>5.6</td>
<td>5.2</td>
<td>5.1</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>107</td>
<td>108</td>
<td>99</td>
<td>98</td>
<td>96</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>34.0</td>
<td>35</td>
<td>33</td>
<td>32.5</td>
<td>31.5</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>31.7</td>
<td>32.5</td>
<td>33</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>3-7</td>
<td>3-7</td>
<td>1-3</td>
<td>0-1</td>
<td>0-1</td>
</tr>
<tr>
<td>Nucleated RBC/mm$^3$</td>
<td>500</td>
<td>200</td>
<td>0-5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Platelets (Th/mm$^3$)</td>
<td>290</td>
<td>192</td>
<td>213</td>
<td>248</td>
<td>252</td>
</tr>
</tbody>
</table>
Physiologic Nadir

Term infant
- 11.4  0.9 g/dL
- Usually reached by 8-12 weeks of age

Preterm infant
- Pace of hgb fall and magnitude vary directly with degree of prematurity
- In babies < 1500 g -->  8 g/dL at 4-8 weeks
Case #1

- Former 35 week infant is seen at 2 months of age
  - CBC shows Hgb of 9.2 gm/dl
- Is this normal or abnormal?
- Probably normal
Case #2

- 2.5 year-old patient undergoes hemoglobin electrophoresis
- Result returns as hemoglobin A 90%, F 10%

Is this normal or abnormal?
Oxygen-Hemoglobin Dissociation Curve
Erythropoiesis
Hemoglobin Switch

- α-chain
- γ-chain (Fetal)
- ε-chain (Embryonic)
- δ-chain (Hb-A₂)
- β-chain (Adult)

Birth
Amount of Hemoglobin F varies

- Normal adult level usually achieved by 5 years of age
  - Slowed by prematurity, trisomy 21, maternal diabetes
- HbF is unevenly distributed in normal subjects
  - F cells
- Hereditary persistence of fetal hemoglobin
  - Polymorphisms on chromosomes X and 6
Other causes of Increased HbF

- Pregnancy
  - Level increases in 2nd trimester
- Hemolytic anemias
- Acute bleeding
- Certain medications
- Other diseases
  - CML, AML M6, PNH, Diamond Blackfan anemia, Fanconi anemia
Case #2

- 2.5 year-old patient undergoes hemoglobin electrophoresis
- Result returns as hemoglobin A 90%, F 10%

- Is this normal or abnormal?
- Probably normal but important to make sure there is no clinical sign of other disease
  - Recommend full CBC
Case #3

- You receive a newborn screen in the mail from the state lab
- The results show Barts hemoglobin

- What the heck is that?
Newborn Screening for Hemoglobinopathies

1960’s Newborn screening begins in U.S.

1987 NIH recommends screening for HbSS be mandated by State Law for EVERY newborn.

2006 ACMG expert panel recommends 29 core conditions to be included in all state panels
- includes 3 hemoglobinopathies
- 2 other conditions have been added to core group since 2006

Source: Data reported from National Newborn Screening and Genetics Resource Center. Available at http://genes-r-us.uthscsa.edu.
Newborn Screening for Hemoglobinopathies

- 4 million infants are screened each year
- 12,500 diagnosed with one of the 29 core conditions
- ~2,800 with hemoglobinopathy
- Cost is about $30/infant

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Estimated no. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hearing loss</td>
<td>5,073</td>
</tr>
<tr>
<td>Primary congenital hypothyroidism</td>
<td>2,156</td>
</tr>
<tr>
<td>(excluding secondary, transient, or other)</td>
<td></td>
</tr>
<tr>
<td>Cystic fibrosis (including nonclassical)</td>
<td>1,248</td>
</tr>
<tr>
<td><strong>Hemoglobin SS (sickle cell anemia)</strong></td>
<td>1,128</td>
</tr>
<tr>
<td><strong>Hemoglobin SC (sickle C disease)</strong></td>
<td>484</td>
</tr>
<tr>
<td>Medium-chain acyl-CoA dehydrogenase deficiency</td>
<td>239</td>
</tr>
<tr>
<td>Classical galactosemia (GALT) plus variant</td>
<td>224</td>
</tr>
<tr>
<td>(excluding GALK and GALE)</td>
<td></td>
</tr>
<tr>
<td>Phenylketonuria (PKU), including clinically significant hyperphenylalaninemia variants</td>
<td>215</td>
</tr>
<tr>
<td>Congenital adrenal hyperplasia (excluding non-21-hydroxylase deficiency)</td>
<td>202</td>
</tr>
<tr>
<td><strong>Hemoglobin S/β thalassemia</strong></td>
<td>163</td>
</tr>
<tr>
<td>3-Methylcrotonyl-CoA carboxylase deficiency</td>
<td>100</td>
</tr>
<tr>
<td>Carnitine uptake defect</td>
<td>85</td>
</tr>
<tr>
<td>Very long-chain acyl-CoA dehydrogenase deficiency</td>
<td>69</td>
</tr>
<tr>
<td>Biotinidase deficiency (including partial)</td>
<td>62</td>
</tr>
<tr>
<td>Methylinomalonic acidemia (mutase deficiency)</td>
<td>50</td>
</tr>
<tr>
<td>Glutaric acidemia type I</td>
<td>38</td>
</tr>
<tr>
<td>Isovaleric acidemia</td>
<td>32</td>
</tr>
<tr>
<td>Maple syrup urine disease</td>
<td>26</td>
</tr>
<tr>
<td>Citrullinemia type I</td>
<td>24</td>
</tr>
<tr>
<td>Propionic acidemia</td>
<td>15</td>
</tr>
<tr>
<td>Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency</td>
<td>13</td>
</tr>
<tr>
<td>Methylinomalonic acidemia CblA,B</td>
<td>12</td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>11</td>
</tr>
<tr>
<td>Argininosuccinic acidemia</td>
<td>7</td>
</tr>
<tr>
<td>Beta-ketothiolase deficiency</td>
<td>7</td>
</tr>
<tr>
<td>Hydroxymethylglutaric aciduria</td>
<td>3</td>
</tr>
<tr>
<td>Multiple carboxylase deficiency</td>
<td>3</td>
</tr>
<tr>
<td>Trifunctional protein deficiency</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>11,691</td>
</tr>
</tbody>
</table>

* One of the 29 disorders listed in the screening panel (tyrosinemia type 1), and two recently approved additions (severe combined immunodeficiency and...
Newborn Screening for HbSS

- Primary aim of Newborn Screening for hemoglobinopathy is to identify HbSS individuals
- Secondary outcome is the identification of a variety of other major and minor Hb disorders:
  - $\beta$ thalassemia major
  - $\alpha$ thalassemia minor
  - heterozygotes for other variants
Hemoglobin Phenotype Nomenclature

- Hemoglobins are reported in order of quantity i.e. “FSA” = HbF > HbS > HbA

- “U” or “V” = “Unknown/Unidentified” or “Variant”. Also seen as “X”
Newborn Hemoglobinopathy Screening

Sickle Solubility Tests

- inadequately sensitive to small quantities of HbS
- cannot distinguish sickle cell disease from trait.

NEVER use as a screening tool
The Thalassemias

Beta Globin Genes

b1
b2

Hemoglobin Protein

Chromosome 11

Alpha Globin Genes

a1
a2
a3
a4

Chromosome 16
The Thalassemias

Thalassemias are named for the hemoglobin there is not enough of.

- Beta thalassemia = not enough beta chains
- Alpha thalassemia = not enough alpha chains
Hemoglobins and Their Partners
Hemoglobins and Their Partners

Alpha Hemoglobin
Hemoglobin, and Their Partners

Alpha Hemoglobin

HbF

Gamma Hemoglobin
Hemoglobins and Their Partners

Alpha Hemoglobin
Hemoglobins and Their Partners

Alpha Hemoglobin

Beta Hemoglobin

HbA
Hemoglobins and Their Partners

Alpha Hemoglobin
Hemoglobins and Their Partners

Alpha Hemoglobin

Hb A2

Delta Hemoglobin
Hemoglobins and Their Partners

Alpha Hemoglobin

Hb A2

Beta Thalassemia Trait or Disease

Delta Hemoglobin
If There’s Not Enough Alpha?

4 Gamma Chains

Barts Hemoglobin
If There’s Not Enough Alpha?

Alpha Thalassemia Trait

4 Gamma Chains

Barts Hemoglobin
If There’s Not Enough Alpha?

4 Beta Chains

Hemoglobin H
If There’s Not Enough Alpha?

4 Beta Chains

Alpha Thalassemia Major or Hemoglobin H Disease

Hemoglobin H
Newborn Hemoglobinopathy Screening

Electrophoresis
- Inexpensive, widely practiced, relatively slow.
- Low sensitivity if Hb S <10% (i.e. premies)
- Difficulty with co-migration of some Hb variants

Cellulose Acetate (Alkaline pH) can separate
Hb S, F, A and C; but not Hb A$_2$, O, E, D and G

Citrate Agar (Acidic pH) can separate those that
the alkaline gels cannot by altering the
electrophoretic properties of the proteins
Cellulose Acetate Gel

A₂  S  F  A

Normal Adult
Normal Newborn
HPFH / β₀ thalassemia
HPFH / A
β₀ thalassemia / A
Hb SF
Control
## Interpreting Screening Results

<table>
<thead>
<tr>
<th>Normal</th>
<th>Disease</th>
<th>Trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>FS</td>
<td>FAS</td>
</tr>
<tr>
<td></td>
<td>FSC</td>
<td>FAC</td>
</tr>
<tr>
<td></td>
<td>FSA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F only</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F “X”</td>
<td>FA “X”</td>
</tr>
</tbody>
</table>
Case #3

- You receive a newborn screen in the mail from the state lab
- The results show Barts hemoglobin

- What the heck is that?
- 4 gamma chains, indicating alpha thalassemia trait
Case #4

- A newborn male has some oozing from areas of venipuncture
- Platelet count 350 K
- PT 16 sec, aPTT 45 sec
- Factor VIII 75%, Factor IX 18%

- Is this normal or abnormal?
Hemostasis
Platelets

- Count, volume and survival identical to adult platelets from ≥ 18 weeks of gestation.
  - Count: 150,000 - 450,000/mm$^3$
  - MPV: 7 to 9 fL
  - Survival: 7 to 10 days
Hemostasis
Coagulation System

Coagulation Factors

- **Measurable by 10 weeks** of gestational age and ↑ thereafter
- Multiple ranges required (No true reference range for extremely premature infants)
- Do not cross the placenta
Hemostasis
Coagulation System

- Coagulation Factors
  - Vitamin K Factors (II, VII, IX and X) ↓ ↓ → ↑
  - PT
Hemostasis
Coagulation System

Coagulation Factors

- **Vitamin K Factors** (II, VII, IX and X) ↓ ↓ → ↑ PT
- **Contact Phase Factors** (XI, XII, Prekallikrein, HMWK) ↓ ↓ → ↑ APTT
Hemostasis
Coagulation System

Coagulation Factors

- **Vitamin K Factors** (II, VII, IX and X) ↓↓ → ↑ PT
- **Contact Phase Factors** (XI, XII, Prekallikrein, HMWK) ↓↓ → ↑ APTT
- **Others** (Fibrinogen, V, VIII, XIII and VWF) not decreased at birth.
## Hemostasis

### Coagulation Factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Newborns</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIc (%)</td>
<td>43.5 (27-64)</td>
<td>98.7 (70-125)</td>
</tr>
<tr>
<td>VIIc (%)</td>
<td>52.5 (28-78)</td>
<td>101.3 (68-130)</td>
</tr>
<tr>
<td>IXc (%)</td>
<td>31.8 (15-50)</td>
<td>104.8 (70-142)</td>
</tr>
<tr>
<td>Xc (%)</td>
<td>39.6 (21-65)</td>
<td>99.2 (75-125)</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>2.65 (1.68-3.6)</td>
<td>3.5 (2.5-5.1)</td>
</tr>
<tr>
<td>Vc (%)</td>
<td>89.9 (50-140)</td>
<td>99.8 (65-140)</td>
</tr>
<tr>
<td>VIIIc (%)</td>
<td>94.3 (38-150)</td>
<td>101.8 (55-170)</td>
</tr>
<tr>
<td></td>
<td>Newborns</td>
<td>Adults</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>ATIII (%)</td>
<td>59.4 (42-80)</td>
<td>99.8 (65-130)</td>
</tr>
<tr>
<td>HCII (%)</td>
<td>52.1 (19-99)</td>
<td>101.4 (70-128)</td>
</tr>
<tr>
<td>TFPI (ng/mL)</td>
<td>38.1 (22.7-55.8)</td>
<td>73.0 (50.9-90.1)</td>
</tr>
<tr>
<td>PC Ag (%)</td>
<td>32.5 (21-47)</td>
<td>100.8 (68-125)</td>
</tr>
<tr>
<td>PC Act (%)</td>
<td>28.2 (14-42)</td>
<td>98.8 (68-129)</td>
</tr>
<tr>
<td>Total PS (%)</td>
<td>38.5 (22-55)</td>
<td>99.6 (72-118)</td>
</tr>
</tbody>
</table>
## Hemostasis

### Coagulation Screening Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Newborns</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (s)</td>
<td>16.7 (12-23.5)</td>
<td>13.5 (11.4-14.0)</td>
</tr>
<tr>
<td>PT (INR)</td>
<td>1.7 (0.9-2.7)</td>
<td>1.1 (0.8-1.2)</td>
</tr>
<tr>
<td>APTTT (s)</td>
<td>44.3 (35-52)</td>
<td>33.0 (25-39)</td>
</tr>
<tr>
<td>TCT (s)</td>
<td>20.4 (15.2-25)</td>
<td>14.0 (12-16)</td>
</tr>
</tbody>
</table>
Case #4

- A newborn male has some oozing from areas of venipuncture

- Platelet count 350 K
- PT 16 sec, aPTT 45 sec
- Factor VIII 75%, Factor IX 18%

- Is this normal or abnormal?
- Normal lab values
  - Other reasons for oozing?
Case #5

- A 6 mo baby has CBC due to febrile illness
- WBC 10, normal diff
- Hgb 10.8 gm/dL
- Plt 800K

- Is this normal or abnormal?
## Reactive versus Autonomous Thrombocytosis?

<table>
<thead>
<tr>
<th>Reactive</th>
<th>Autonomous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most common (70%).</td>
<td>Less common (22%).</td>
</tr>
<tr>
<td>Cytokine driven, TPO growth factors.</td>
<td>Clonal/neoplastic, <strong>growth factor independent</strong>.</td>
</tr>
<tr>
<td>Absence of a CMPD or MDD with a medical or surgical condition likely to be associated with an increased platelet count.</td>
<td>Presence of CMPD or MDD. (Reasonable diagnosis in patient with chronic thrombocytosis, normal iron stores, and an intact spleen.)</td>
</tr>
</tbody>
</table>

- **Thrombosis 1%** & **Hemorrhage 3%**
- **Thrombosis 25%** & **Hemorrhage 25%**
Reactive Thrombocytosis

Most Common Causes:
1. Infection
2. Infection and post-op
3. Post-op
4. Malignancy
5. Postsplenectomy
6. Acute blood loss or iron deficiency
Autonomous Thrombocytosis

All rare in childhood.

Chronic myeloproliferative disorders
  Polycythemia vera
  Agnogenic myeloid metaplasia
  Chronic myelogenous leukemia
  Essential thrombocythemia

Myelodysplastic disorders - usually thrombocytopenia, but some have thrombocytosis (5q syndrome)
Extreme Thrombocytosis

- Platelets are > 1,000,000 uL.
- Reactive thrombocytosis still more common cause the autonomous thrombocytosis.
- Therefore, degree of thrombocytosis not helpful differentiating between Reactive and Autonomous Thrombocytosis.

Causes:
1. Infection
2. Postsplenectomy or hyposplenia
3. Malignancy
4. Trauma
5. Inflammation (noninfectious)
6. Blood loss
Case #5

A 6 mo baby has CBC due to febrile illness

- WBC 10, normal diff
- Hgb 10.8 gm/dL
- Plt 800K

Is this normal or abnormal?

- Normal
OUT DAMN CLOT!!

SOMETHING IS ROTTEN IN THE STATE OF COAGULATION!

WHAT CLOT FROM YONDER VESSEL BREAKS!