Disclosures

• I have no actual or potential conflict of interest in relation to this program
Objectives

• Explain what all those numbers on a CBC mean
• Discuss common causes of anemia in childhood
• Develop a rational diagnostic strategy in the approach to anemia
• Keep you awake since this is the last talk
The CBC

• LOTS of values on a CBC and all give great amount of information

• Able to help form a differential diagnosis as to the cause of the anemia
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>12.43</td>
</tr>
<tr>
<td>Hgb</td>
<td>10.1</td>
</tr>
<tr>
<td>Hct</td>
<td>28.9 L</td>
</tr>
<tr>
<td>Platelet</td>
<td>TNP *</td>
</tr>
<tr>
<td>Abs Imm Gran</td>
<td>0.29 H</td>
</tr>
<tr>
<td>Abs Neut</td>
<td>3.77</td>
</tr>
<tr>
<td>Abs Lymph</td>
<td>6.15</td>
</tr>
<tr>
<td>Abs Mono</td>
<td>1.76</td>
</tr>
<tr>
<td>Abs Eos</td>
<td>0.39</td>
</tr>
<tr>
<td>Abs Baso</td>
<td>0.07</td>
</tr>
<tr>
<td>% Imm Gran</td>
<td>2.3 *</td>
</tr>
<tr>
<td>% Neutro</td>
<td>30.3</td>
</tr>
<tr>
<td>% Lymph</td>
<td>49.5</td>
</tr>
<tr>
<td>% Mono</td>
<td>14.2</td>
</tr>
<tr>
<td>% Eos</td>
<td>3.1</td>
</tr>
<tr>
<td>% Baso</td>
<td>0.6</td>
</tr>
<tr>
<td>Differential Method</td>
<td>Auto Diff</td>
</tr>
<tr>
<td>RBC</td>
<td>3.11</td>
</tr>
<tr>
<td>MCV</td>
<td>92.9</td>
</tr>
<tr>
<td>MCH</td>
<td>32.5</td>
</tr>
<tr>
<td>MCHC</td>
<td>34.9</td>
</tr>
<tr>
<td>RDW</td>
<td>14.1</td>
</tr>
<tr>
<td>Abs Retic</td>
<td>0.0572</td>
</tr>
<tr>
<td>% Retic</td>
<td>1.8</td>
</tr>
<tr>
<td>Im Retic Fraction</td>
<td>15.2</td>
</tr>
<tr>
<td>Polychrom</td>
<td>Slight</td>
</tr>
<tr>
<td>RBC Fragments</td>
<td>Few</td>
</tr>
<tr>
<td>Target Cells</td>
<td>Few</td>
</tr>
<tr>
<td>Teardrop Cells</td>
<td>Few</td>
</tr>
</tbody>
</table>
What do these numbers mean???

• RBC: Red Blood Cell number
  – Actual number of red blood cells

• MCH: mean corpuscular hemoglobin
  – Amount of hemoglobin within the cells

• MCV: mean corpuscular volume
  – How large the red cell is
What do these numbers mean???

• MCHC: mean corpuscular hemoglobin concentration
  – How much hemoglobin is packed in the RBC

• RDW: Red Cell Distribution Width
  – Size differential between the smallest and largest RBC
Reticulocytes

• Percentage
  – Number of reticulocytes compared to normal RBC

• Absolute
  – The total number of reticulocytes

• Immature Retic Fraction
  – Percentage of reticulocytes which are the newest
Which ones are the most helpful?

• MCV
  – Microcytic, macrocytic, normocytic

• RDW

• Reticulocytes
  – Production vs. destruction
Anemia: Definition

- Reduction in red cell mass or blood hemoglobin concentration
  - Hematocrit: fraction of whole blood made up of red blood cells. Expressed as a percentage
  - Hemoglobin: measure of the concentration in whole blood. Expressed as grams per deciliter
- Limit set at > two SD below mean for normal
  - 2.5% of normal population will be classified as anemic
Differentiating Anemia

Iron def
Lead
Thal
Chronic dz
Sideroblastic

Lo MCV

Hi MCV

NI MCV

Membrane Defects
- HS
- HE
Enzyme def
-G6PD
-PK
Hgbopathies

Autoimmune

Microangiopathic
-HUS
-TTP
-DIC
-spleen

CRF
-AA
Fanconi
TEC
Horm. Def
BM infil.

B12 def
Folate def
BM failure
DBA
Drugs
Trisomy 21
Case #1

• You receive a newborn screen in the mail from the state lab
• The results show Barts hemoglobin, which is

a. Four alpha chains
b. Four beta chains
c. Four gamma chains
d. 2 alpha and 2 delta chains
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

FIGURE 1. Number of states screening for the core bloodspot conditions in the Recommended Uniform Screening Panel (RUSP) — United States, 2004–2009

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>5,072</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>Hemoglobin SS (sickle cell anemia)</td>
<td>1,128</td>
</tr>
<tr>
<td>Hemoglobin SC (sickle C disease)</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</td>
<td>202</td>
</tr>
<tr>
<td>(excluding congenital hypothyroidism)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin 5/Δ thalassemia</td>
<td>162</td>
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<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>Methylmalonic acidemia (mimutase deficiency)</td>
<td>50</td>
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<tr>
<td>Glutaric acidemia type I</td>
<td>38</td>
</tr>
<tr>
<td>Isovaleric acidemia</td>
<td>32</td>
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<tr>
<td>Maple syrup urine disease</td>
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<tr>
<td>Citrullinemia type I</td>
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<tr>
<td>Propionic acidemia</td>
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<td>Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency</td>
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<td>Methylmalonic acidemia GluAB</td>
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<td>Homocystinuria</td>
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<td>Argininosuccinic acidemia</td>
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<td>Beta-ketothiolase deficiency</td>
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<tr>
<td>Hydroxymethylglutaric aciduria</td>
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<tr>
<td>Multiple carboxylase deficiency</td>
<td>3</td>
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<tr>
<td>Trifunctional protein deficiency</td>
<td>2</td>
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</tbody>
</table>

Total: 11,691

* One of the 29 disorders listed in the screening panel (tyrosinemia type I), and two recently approved additions (severe combined immunodeficiency and...
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>
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
The Thalassemias

Beta Globin Genes

Hemoglobin Protein

Alpha Globin Genes

Chromosome 11

Chromosome 16
Globins and Their Partners
Globins and Their Partners

Alpha Globin
Globins and Their Partners

Alpha Globin

\(\text{HbF}\)

Gamma Globin
Globins and Their Partners

Alpha Globin
Globins and Their Partners

Alpha Globin

HbA

Beta Globin
Globins and Their Partners

Alpha Globin
Globins and Their Partners

Alpha Globin

Hb A2

Delta Globin
Globins and Their Partners

Alpha Globin

Beta Thalassemia Trait or Disease

Hb A2

Delta Globin
If There’s Not Enough Alpha?

4 Gamma Chains

Barts Hemoglobin
If There’s Not Enough Alpha?

Alpha Thalassemia Trait = 2 genes missing

Barts Hemoglobin

4 Gamma Chains
If There’s Not Enough Alpha?

4 Beta Chains

Hemoglobin H
If There’s Not Enough Alpha?

Alpha Thalassemia Major or Hemoglobin H Disease = 3 genes missing

4 Beta Chains

Hemoglobin H
Case #1

• 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, most likely indicating alpha thalassemia trait
Case #2

- A 1 y/o infant is brought in by her mother for a routine checkup
- Routine hemoglobin = 7.8 gm/dl
- CBC: Hb 8 gm/dl, WBC 8 x 10e3, plt 425K
  - MCV 60 fl, RDW 19%
Peripheral Blood Smear

Wrights x1000
Case #2

• What is the diagnosis?
  a. vitamin B12 deficiency
  b. iron deficiency
  c. folate deficiency
  d. acute blood loss
  e. iPad™ deficiency
Case #2

What is the most common cause of this diagnosis?

a. Excessive intake of goat’s milk
b. Excessive intake of cow’s milk
c. Intake of chocolate milkshakes
d. Genetic abnormality leading to decreased globin synthesis
Case #3

How long should treatment continue?

a. Indefinitely

b. Until the cows come home

c. 3 months after the hemoglobin has normalized

d. Until resolution of anemia

e. No treatment is needed
Differential diagnosis of microcytic anemia

• Iron deficiency
• Thalassemia disease/trait
• Chronic lead poisoning
• Sideroblastic anemias
• Anemia of chronic inflammation
• Some unstable hemoglobins
Differential diagnosis of microcytic anemia

- Iron deficiency
- Thalassemia disease/trait
- Chronic lead poisoning
- Sideroblastic anemias
- Anemia of chronic inflammation
- Some unstable hemoglobins
Iron deficiency vs. Thalassemia

Iron Deficiency
- RBC number lower
- RDW high
- Higher Mentzer Index

Thalassemia
- High RBC number
- Normal RDW
- Normal Mentzer Index
Trick with Diagnosing Thalassemia w/ Fe deficiency

• Iron deficiency can mask thalassemia on hemoglobin electrophoresis
  – Electrophoresis will be falsely normal

• Patient MUST have iron replenished prior to electrophoresis being done
Case #3

- A 2 yo female presents to your clinic
  - History of jaundice in first week of life
- Mother reports that child occasionally looks yellow, and has been looking more pale over the past couple of days
- Exam: pallor, slight scleral icterus, spleen 2 cm below LCM
- CBC: Hgb 8.4 gm/dl
- MCV 85 fl, RDW 18%, MCHC 36
Case #3

What test will help define whether this patient’s anemia is from decreased production or increased destruction?

Reticulocyte count
Peripheral Blood Smear
Case #3

What test is most helpful to distinguish different causes of hemolytic anemia?

a. DAT
b. Osmotic fragility test
c. Hemoglobin electrophoresis
d. Peripheral blood smear
What other tests do you want?

- DAT: negative
- Osmotic fragility test:
Membrane abnormalities

• Spherocytosis
  – Autosomal dominant in most cases
  – Abnormality in proteins that anchor the membrane skeleton to the lipid bilayer
  – Hemolytic anemia with reticulocytosis
    • Severity extremely variable

• Elliptocytosis
  – Little hemolysis in most patients
Spherocytosis

• Treatment is mostly supportive care

• Splenectomy in rare cases
  – Needing lots of transfusions
  – Pain
  – Need PCN prophylaxis
  – Other cormorbidites (portal vein thrombosis)

• Total Splenectomy vs. partial Splenectomy
  – Total splenectomy more effective in raising hemoglobin and reducing reticulocytes
Case #3

The reticulocyte count of the patient is 0.4%. What is the most common cause of this phenomenon in this population?

a. Picornavirus
b. Adenovirus
c. Parvovirus B19
d. E. coli 0157:H7
e. Hepatitis B
Case #4

• A 15 yo female presents to the ED with complaint of fatigue and fever for 4 days
• Exam: pallor and jaundice, II/VI SEM, liver nonpalpable, spleen tip 2 cm below LCM
• CBC: Hgb 4.8 gm/dl WBC 10K plt 350K
Case #4

• A direct Coombs test is performed and it is strongly positive for both IgG and complement

• Type and screen: B+, positive Ab screen
Case #5

• What is the next appropriate course of action?
  a. IVIG 1 gm/kg
  b. Transfusion of best match blood
  c. Rituximab 375 mg/m²
  d. Corticosteroids 2-4 mg/kg
  e. B and D
Autoimmune hemolytic anemia

- Most young children have idiopathic disease
- Most antibody IgG
  - Can also have IgM
- Associated with underlying systemic diseases
  - Lymphoma
  - Lupus
  - Immunodeficiency
Coombs Test: Direct Antiglobulin Test

POSITIVE DIRECT COOMBS TEST

Anti-human antibody (Coombs Reagent)

Antibody/Antigen complex on Red cell surface

Anti-human antibody binds with Antibody attached to Red Cell Surface = Agglutination
Coombs Test: Indirect

1

2

3
Peripheral Blood Smear
Hemolysis

Etiology

Extrinsic

- Immune-mediated
- Infection
- DIC
- Macro/Microangiopathic anemia
- Galactosemia
- Acidosis

Intrinsic

- RBC Enzyme Deficiencies
- RBC Membrane Disorders
- Hemoglobinopathies
  - Hb SS, SC, etc.
  - Thalassemia
- Pyknocytosis
Conclusions/ Take Home Points

• Be aware of physiologic variability of both hematological values and parameters.

• Work through common causes of anemia in a stepwise manner.

• The MVPs of the anemia work-up
  – MCV
  – Reticulocyte count
  – DAT
Questions?