Diagnostic approach to Anemia

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Outline

• Basic hematological parameters
• Discuss types of anemia and their clinical manifestations
• Talk about the relevant diagnostic tests
Basic hematologic lab tests

• Complete blood count (CBC)
  – Amount of hemoglobin
  – Number, size, and shape of red blood cells (RBCs)
  – Number of white blood cells (WBCs) and platelets
  – +/- automated WBC differential

• Manual differential/manual peripheral smear review
  • Abnormalities that fall outside of established parameters result in manual review
Complete blood count (CBC)

- Hemoglobin (g/dL) Amount of oxygen carrying protein
- Hematocrit (%) % of blood volume occupied by RBCs
- RBC count (M/uL) # of RBCs
- MCV (fL) mean cell volume
- MCH (pg) mean cell hemoglobin
- MCHC (g/dL) mean cell hemoglobin concentration
- RDW (%) red cell distribution width
- WBC (K/uL) # of WBCs
- Platelet count (K/uL) # of platelets
Measuring RBC parameters

- Hemoglobin (Hb)
  - Measured directly as absorbance of cyanomethemoglobin
- RBC count (RBC)
  - Measured directly by impedance
- Hematocrit (Hct)
  - Measured by centrifugation; ratio of volume of RBCs to volume of whole blood
  - Can also calculate (MCV x RBC)
- MCV
  - Measured by mean height of voltage pulses in an impedance counter
  - Can also calculate (Hct / RBC)
- MCH = Hb / RBC
- MCHC = Hb / Hct
Definition of anemia

- From Greek meaning “without blood”
- Condition where capacity of blood to transport oxygen to tissues is reduced
  - Decreased hemoglobin, RBC count, and hematocrit
- Anemia is not a disease but a manifestation of disease
- Treatment depends on discovering underlying cause
Evaluating anemia in the lab

Basic information

• Size of red blood cells: (small/ normal/ big)
• Abnormal cells on microscopic examination
• Status of leukocytes and platelets
• Reticulocyte count (ability of marrow to respond to anemia)
• Evidence of destruction (elevated LDH, indirect bilirubin)
A practical approach to anemia

Size of RBCs

- MCV (Mean Cell Volume)

  Microcytic  < 80 fl
  Normocytic  80-100 fl
  Macrocytic  > 100 fl
Differential diagnosis of microcytic anemia

- Iron deficiency
- Anemia of chronic disease
- Thalassemias
- Hemoglobinopathies
- Hereditary spherocytosis
- Hereditary X-linked sideroblastic anemia
- Lead poisoning (usually mild microcytosis)
Iron deficiency anemia

• Common nutritional deficiency
• Bleeding is a leading cause of iron deficiency anemia
• Iron facts
  – Body iron:
    • 80% functional (Hgb, myoglobin, cytochromes, etc.)
    • 20% storage
  – Absorption: primarily in the duodenum
  – Transferrin: transports iron in blood
  – Ferritin: storage form of iron
  – Hemosiderin: derived from ferritin, long-term storage of iron
Lab studies in iron deficiency anemia

• Microcytic, hypochromic anemia
  – Decreased MCV, MCH, & MCHC

• Iron studies
  – Low serum iron
  – High total iron binding capacity (TIBC, transferrin concentration)
  – Low % transferrin saturation
  – Low ferritin
  – Decreased bone marrow storage iron (hemosiderin)
Thalassemia

- Hemoglobin is a tetramer; with two alpha and two beta
- Due to abnormally low production of alpha or beta-globin chains: named for the chain which is decreased or absent
- + Indicates diminished, but some production of globin chain still happens: e.g. $\beta^+$
- 0 Indicates complete absence of production of globin chain by gene: e.g. $\beta^0$
Demographics: Thalassemia

- Found most frequently in the Mediterranean, Africa, Western and Southeast Asia, India and Burma
- Distribution parallels that of Plasmodium falciparum
Distinguishing features between iron def (IDA) and thalassemia

• Mentzer index: MCV/RBC < 13 favors thalassemia
• England and Fraser Index: MCV– (5 × Hemoglobin)
Distinguishing features between iron def and thalassemia

- The RBC count in thalassemia is more than $5.0 \times 10^6/\mu L$ ($5.0 \times 10^{12}/L$) and in IDA is less than $5.0 \times 10^6/\mu L$ ($5.0 \times 10^{12}/L$)
- MCV usually less than 70 in TT, more than 70 in IDA
- The red cell distribution width (RDW) in IDA is more than 17% and in TT is less than 17%.
Anemia of chronic disease

• Mild to moderate anemia due to increased hepcidin, leading to iron sequestration
  – Body unable to use iron stores
• Mildly microcytic or normocytic anemia
• Etiologies: chronic immune activation
  – Chronic infections
  – Collagen vascular disease
  – Malignancy
Macrocytic Anemia

- Non-megaloblastic
  - Liver disease
  - Myelodysplastic syndrome
  - Increased reticulocyte count
    - Hemorrhage

- Megaloblastic
  - Vitamin B12 deficiency
  - Folic acid deficiency
Anemia Due to Folate or Vitamin $B_{12}$ (Cobalamin) Deficiency

- Folate and cobalamin required for DNA synthesis
- Deficiency results in megaloblastic anemia due to impaired DNA replication
  - Impaired nuclear development but abundant cytoplasm (nuclear-cytoplasmic asynchrony)
  - Large marrow progenitors
- Similar clinical features* in peripheral blood and marrow morphology in folate and cobalamin deficiency

* Exception: Neurologic abnormalities in $B_{12}$ deficiency
Folate and Cobalamin Deficiency

**Clinical and Laboratory Findings**

- Non-specific signs and symptoms of anemia
- Macrocytic anemia
- Relatively low reticulocyte count
- Hypersegmentation of neutrophils
- Mild thrombocytopenia and/or neutropenia
- Megaloblastic changes in marrow
- Neurological findings (B₁₂ deficiency only): loss of position sense, ataxia, psychomotor retardation, seizures
Hypersegmented neutrophils

Peripheral smear of a Megaloblastic Anemia Patient

http://hematologypoint.com/atlas_topics/137.html
Bone Marrow Picture of Megaloblastic Anemia

Small black arrows (erythroid hyperplasia), Yellow arrow (megaloblastic change) and Arrowhead shows the dysplasia

http://www.pathpedia.com/education/eatlas/histopathology/bone_marrow/megaloblastic_anemia.aspx
Normocytic Anemia
### Differential Diagnosis of Normocytic Anemia

<table>
<thead>
<tr>
<th>Increased reticulocytes</th>
<th>Decreased reticulocytes</th>
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<td>- Anemia of chronic disorders</td>
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<td>- Post-hemorrhagic anemia</td>
<td>- Endocrine disease</td>
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<td>- Metastases</td>
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<td>- Myelodysplastic Syndrome</td>
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Evaluation of Normocytic Anemia

• PB smear, reticulocyte count
• Screen for liver, endocrine, renal disease
• Iron studies
• Bone marrow biopsy
Hemolytic Anemia

- Inherited hemolytic anemia
- Acquired hemolytic anemia
Hemolytic Anemia

- Inherited hemolytic anemia
  - Membrane defects (e.g., hereditary spherocytosis)
  - Globin defects (e.g., Sickle cell anemia)
  - Metabolic disorders
    - Glucose-6 phosphate deficiency
    - Pyrimidine 5’-nucleotidase deficiency (basophilic stippling).
Hereditary Spherocytosis

- Most common hereditary hemolytic anemia
- Spherocytes—microcytic, abnormal osmotic fragility
- May have broad RDW, but normal to low MCV and usually increased MCHC, depending on the degree of reticulocytosis
- Autosomal dominant inheritance (75%)
- Mutations in various structural membrane proteins
- “Cured” by splenectomy
Hereditary Spherocytosis
Diagnosis of HS

- Peripheral blood smear- Spherocytes
- Osmotic fragility
  - a laboratory test used in the diagnosis of HS, is sensitive but not specific. The test measures the *in vitro* lysis of RBCs suspended in solutions of decreasing osmolarity. Spherocytes are characterized by membrane loss and less redundancy to withstand
Osmotic Fragility Test

% Hemolysis vs. NaCl (% of normal saline)

- Normal
- HS
Diagnosis Discontinued!

- Flow cytometry
  - Greater than 95% sensitive and specific for HS
  - Labels patients RBCs with EMA (eosin-5-maleimide)
  - EMA binds specifically with band 3 protein
  - EMA binding is affected by all sorts of membrane protein abnormalities, not just band 3 deficiency
Glucose-6-Phosphate Deficiency (G6PD)

- Catalyzes the initial step in the pentose phosphate pathway
- X-linked; more than 300 variants identified
- 130,000,000 probably carry a mutant gene
  - Up to 20-30% of Africans
  - Up to 35% in Sardinia
  - Also seen in Asians
Glucose-6-Phosphate Deficiency (G6PD)

- Two types of hemolytic anemia
  - Acute, acquired hemolytic anemia
    - Associated with exposure to primaquine, sulfa drugs
  - Chronic mild hemolytic anemia (common in Africans, Caucasians, as compared to Mediterranean's)
G6PD—Heinz Bodies
G6PD—Bite Cells
Tests for G6PD deficiency

• Fluorescent spot test
  – Quick and cheap
  – Detects generation of NADPH from NADP+
  – G6P and NADP added to a drop of patient blood
  – Blood spot fluoresces at 340 nm if NADPH is generated
  – Only detects severe G6PD deficiency (enzyme levels below 30%)

• Enzyme activity assay (spectrophotometric assay)

• *Caveats for non-PCR-based tests:
  – don’t perform right after a hemolytic episode or blood transfusion!
  – May not detect heterozygous females or mild deficiencies

• Mutation testing by PCR
  – Useful in families with known mutation
  – Prenatal diagnosis
  – Also useful in targeted screening of populations with high frequency of common mutations, such as G6PD A− in Africans & African-Americans
Hemolytic Anemia

• Acquired hemolytic anemia
  – Immune mediated
  – Microangiopathic hemolytic anemia
  – Associated with infections
  – Paroxysmal nocturnal hemoglobinuria
Immune-mediated Hemolytic Anemia

- Premature destruction of red blood cells due to acquired antibodies directed against red cell antigens.
- Direct Coombs test: detection of immunoglobulin and/or complement molecules on the surface of red blood cells.
- Indirect Coombs test: incubates normal red blood cells with patient serum, searching for unbound red cell antibody in the patient serum.
Microangiopathic Hemolytic Anemia

• Characterized by red blood cell fragments in the peripheral blood (schistocytes)

• Differential Diagnosis:
  – Thrombotic thrombocytopenic purpura
  – HUS (renal, shiga toxin, E. coli O157)
  – Disseminated carcinoma (Mucinous adenoca)
  – DIC
  – Malignant hypertension
  – Giant hemangiomas
  – March hemoglobinuria
  – Drugs (mitomycin-C)
Microangiopathic Hemolytic Anemia

(www.dcss.cs.amedd.army.mil)
Microangiopathic Hemolytic Anemia

- March hemoglobinuria
  - Disorder of transient hemoglobinemia or hemoglobinuria due to forceful contact of body with hard surface
  - Reported in
    - Prolonged marches
    - Competitive running
    - Conga drumming
    - Karate
    - Jack hammer operators
Hemolytic Anemia Associated With Infection

- Clostridium sepsis: may be severe, overwhelming
  - Urgent identification and treatment is necessary
  - Lecithinase
- Malaria (Blackwater fever)
- Bartonella bacilliformis
- Babesia microcoti
- Trypanosomiasis
- Mycoplasma pneumonia (IgM against I antigen)
- Infectious mononucleosis (IgM against I antigen)
Paroxysmal Nocturnal Hemoglobinuria

- PNH characterized by pallor (anemia), dark urine at night, venous thrombi (especially large vessels)
- Acquired clonal disorder, mutation in the PIGA gene
- Defective synthesis of GPI-linked proteins
- Detected by flow cytometry done on both RBC and WBC initially
- Treatment with eculizumab—monoclonal Ab against C5
- Related to aplastic anemia and MDS
Questions!!!