Red Blood Cell Disorders I:

RBC production and physiology
Laboratory investigation and classification of anemia

Elizabeth Rinker, M.D.

rinkere@musc.edu

July 2025

Objectives

- Determine which laboratory tests are appropriate for evaluation of anemia
- Understand how to interpret laboratory data during evaluation of anemia
- Know the clinical and pathologic findings associated with the types of anemia discussed
- Form a focused differential diagnosis for an anemic patient

Outline

Blood loss

I. Acute

II. Chronic

Increased destruction (hemolytic anemias)

I. Normal hemoglobin produced in normal quantities
   - A. Intravascular/non-autoimmune
     1. Mechanical damage
     2. Microangiopathic anemias
     3. Paroxysmal nocturnal hemoglobinuria (PNH)
     4. Glucose-6-phosphate dehydrogenase (G6PD) deficiency
     5. Malaria
   - B. Autoimmune
     1. Warm
     2. Cold
   - C. RBC membrane defects
     1. Hereditary spherocytosis

II. Abnormal hemoglobin production (hemoglobinopathies)
   - A. Hemoglobin S
   - B. Hemoglobin C
   - C. Hemoglobin SC

III. Normal hemoglobin produced in insufficient quantities
   - A. Thalassemia

Impaired production

I. Aplastic anemia
   - A. Fanconi anemia
   - B. Pure red cell aplasia

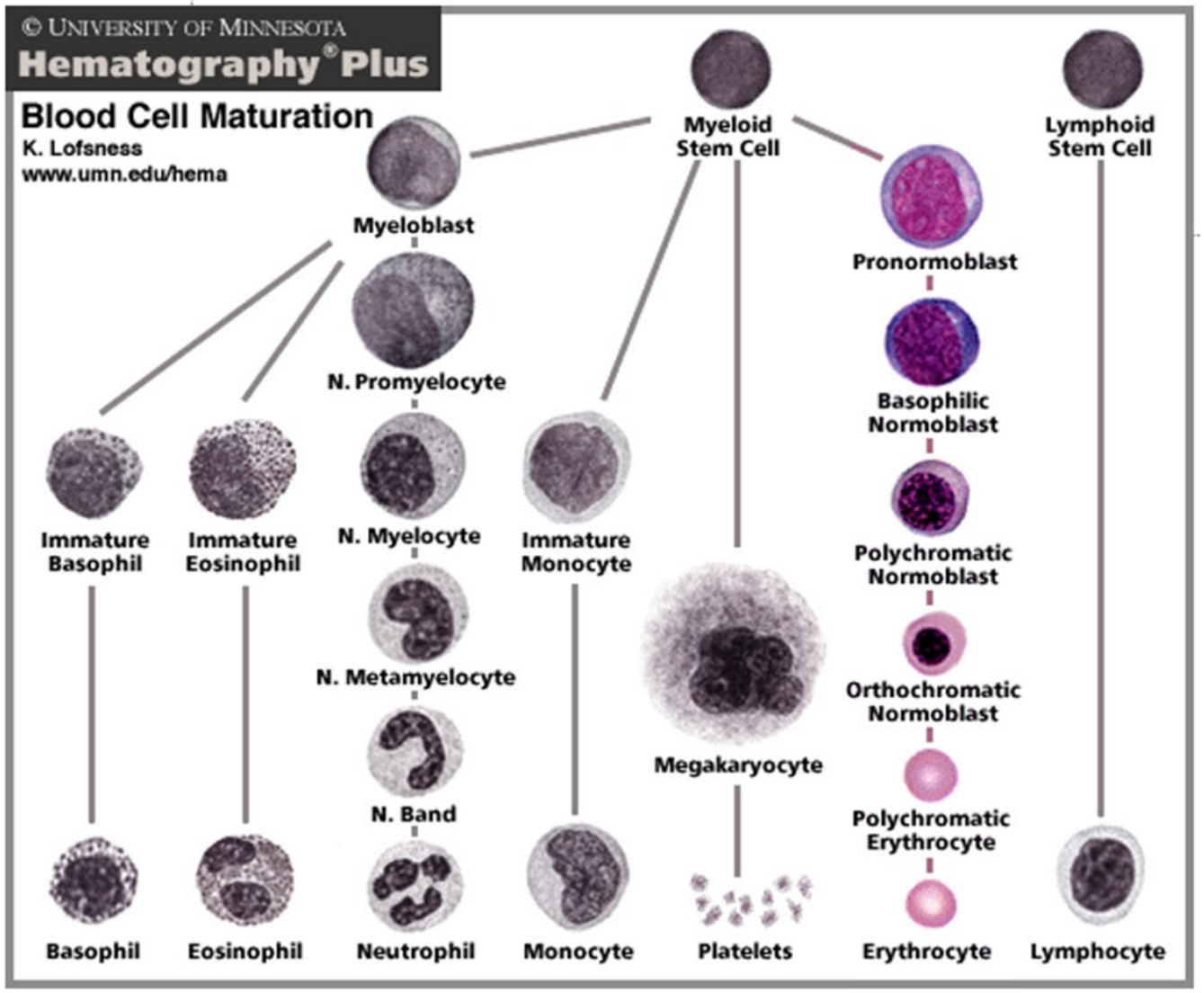
II. Macrocytic anemia
   - A. Folate deficiency
   - B. Vitamin B12 deficiency
     1. Pernicious anemia

III. Microcytic anemia
   - A. Iron deficiency
   - B. Anemia of chronic disease
   - C. Sideroblastic anemia
     1. Lead toxicity

IV. Myelophthisic anemia

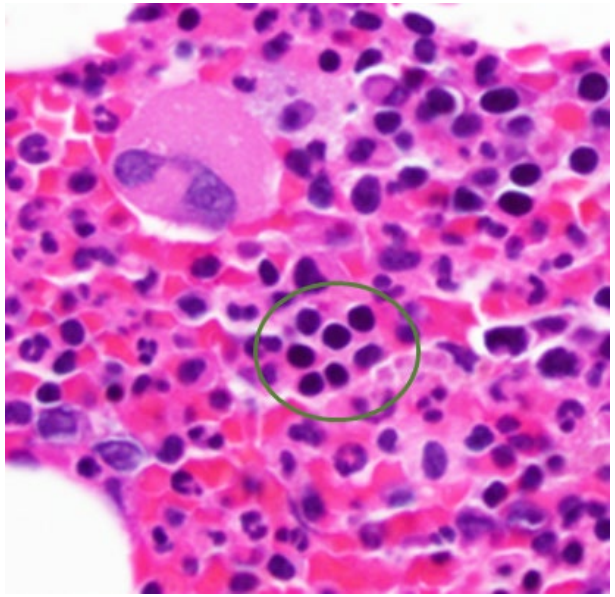
Red blood cell production: Bone marrow

- RBC precursors start large
- Become smaller with maturation
- Cytoplasm and nucleus mature synchronously



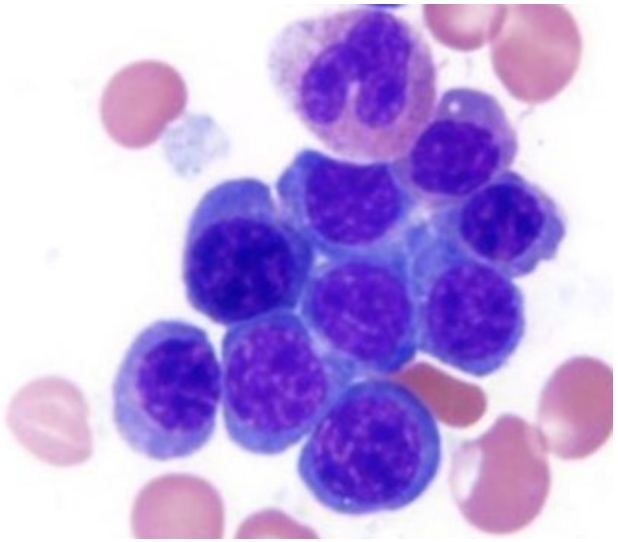
Red blood cell production: Bone marrow

Paraffin-embedded sections, H&E stain



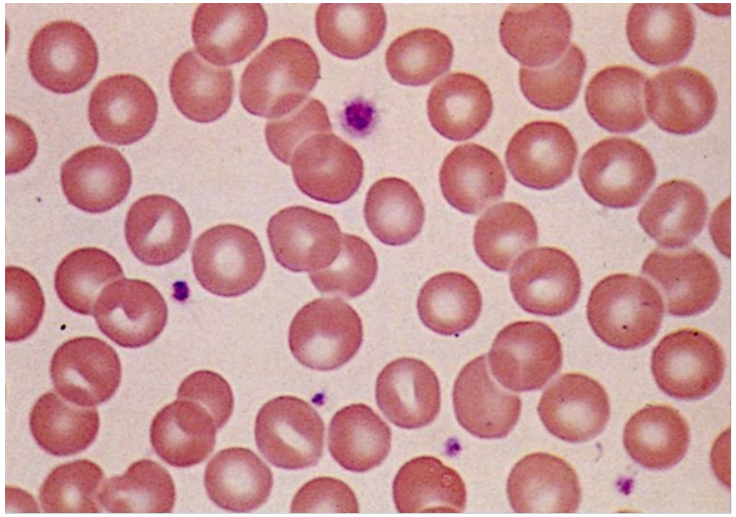
- Aggregates/islands of cells with round, very dark nuclei

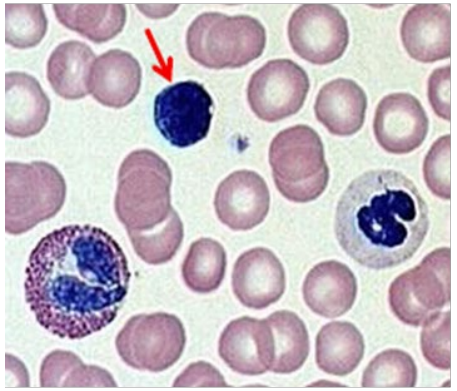
Smeared slides, Wright-Giemsa stain

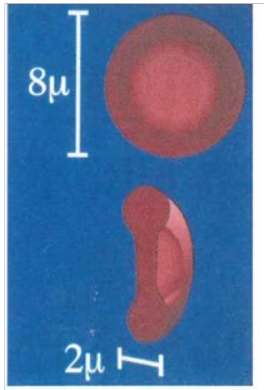


- Cells with round nuclei, metachromatic cytoplasm

RBC's: Peripheral blood smear



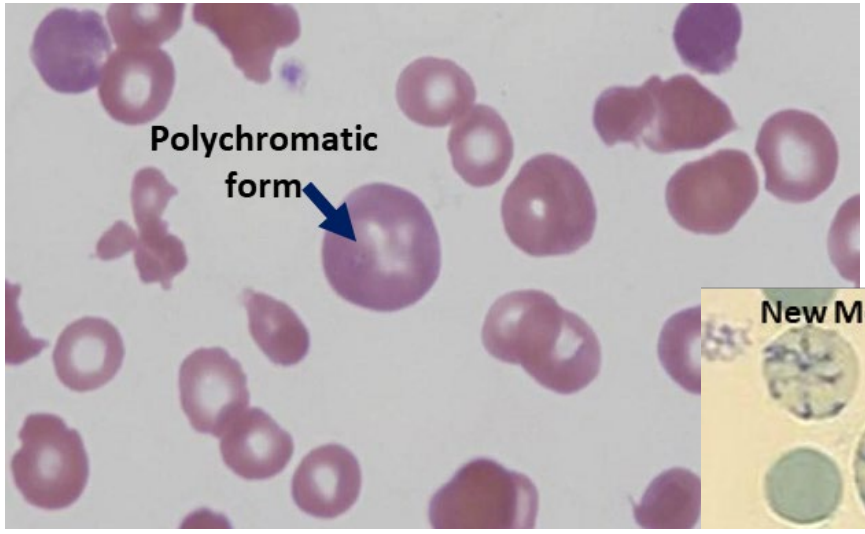


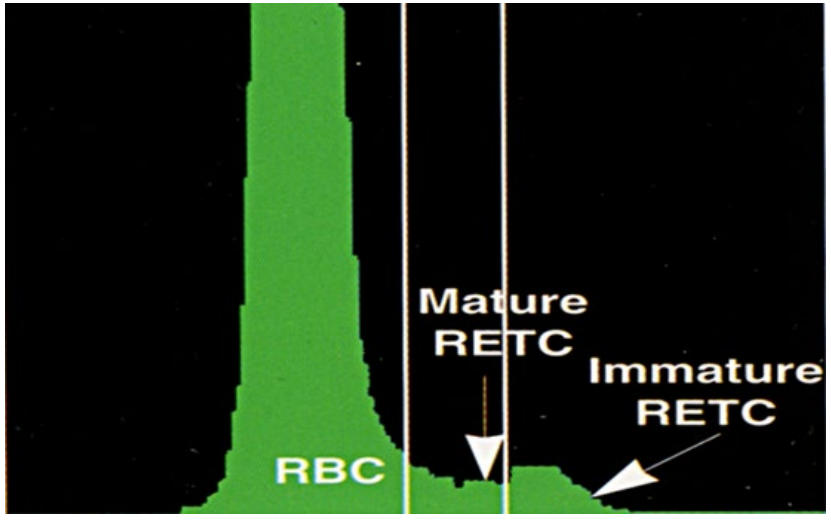


- Most common cell in blood
- Biconcave disc
  - Central pallor ~1/3 diameter
- Diameter of 6-8 μm
  - About the size of lymphocyte nucleus
- Specialized cell membrane
- "Little bags of hemoglobin"

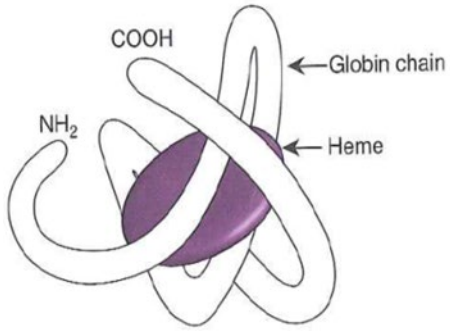
Early RBC's in peripheral blood smear

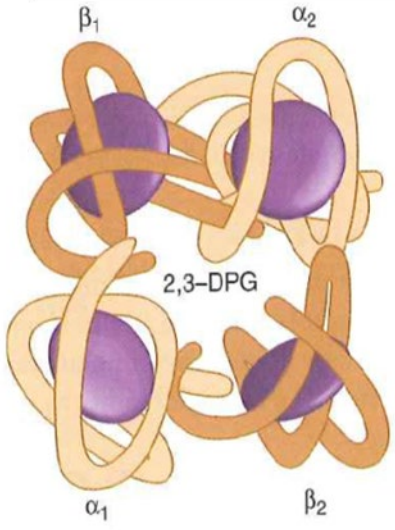
- Nucleated red blood cell (nRBC)
- Reticulocyte
  - Newly formed RBC that still has some RNA
  - Special stain is required to see RNA
- Polychromatic form = reticulocyte
  - Retained RNA → blue/purple
- Reticulocyte count
  - Adequate bone marrow compensation?





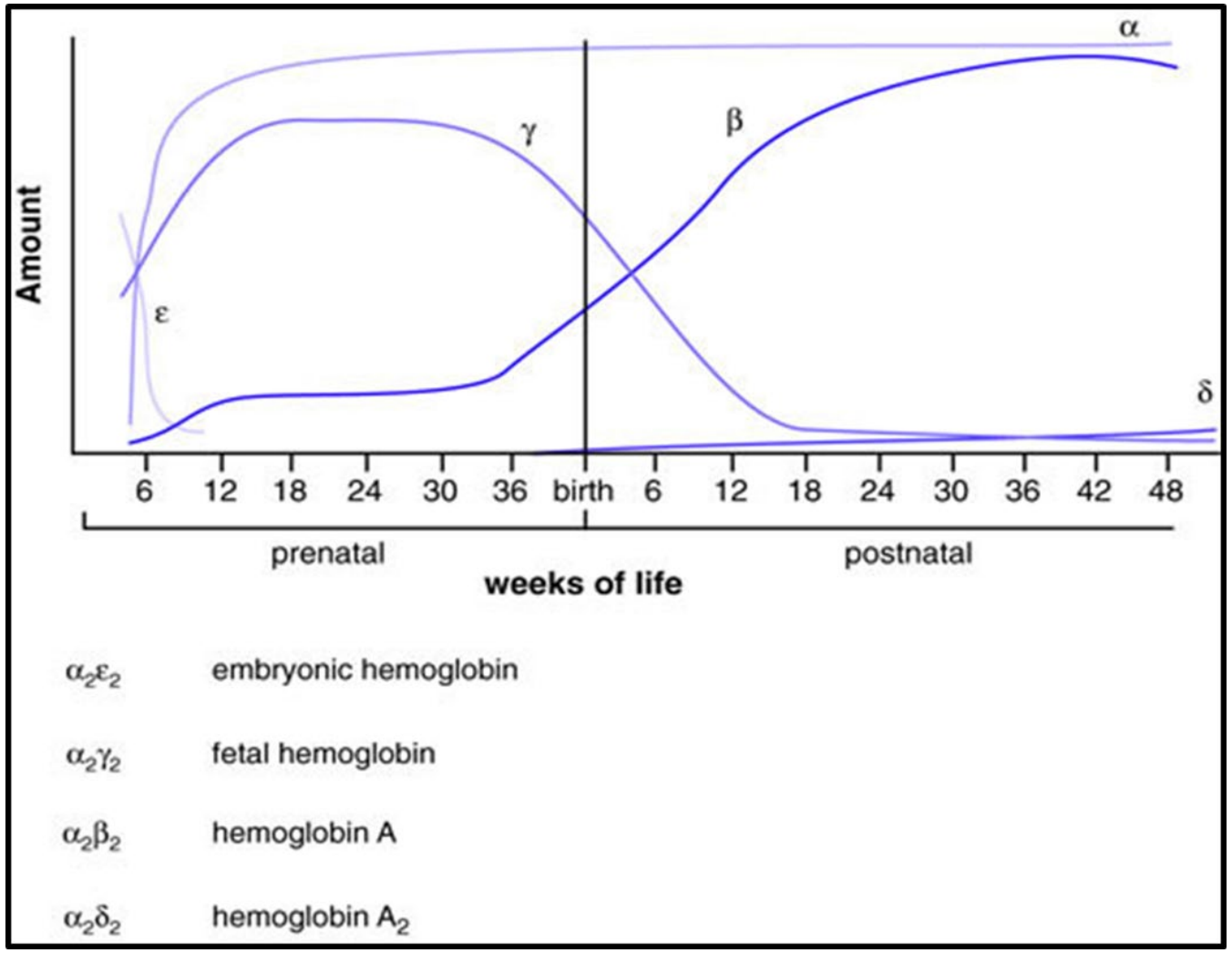
Hemoglobin



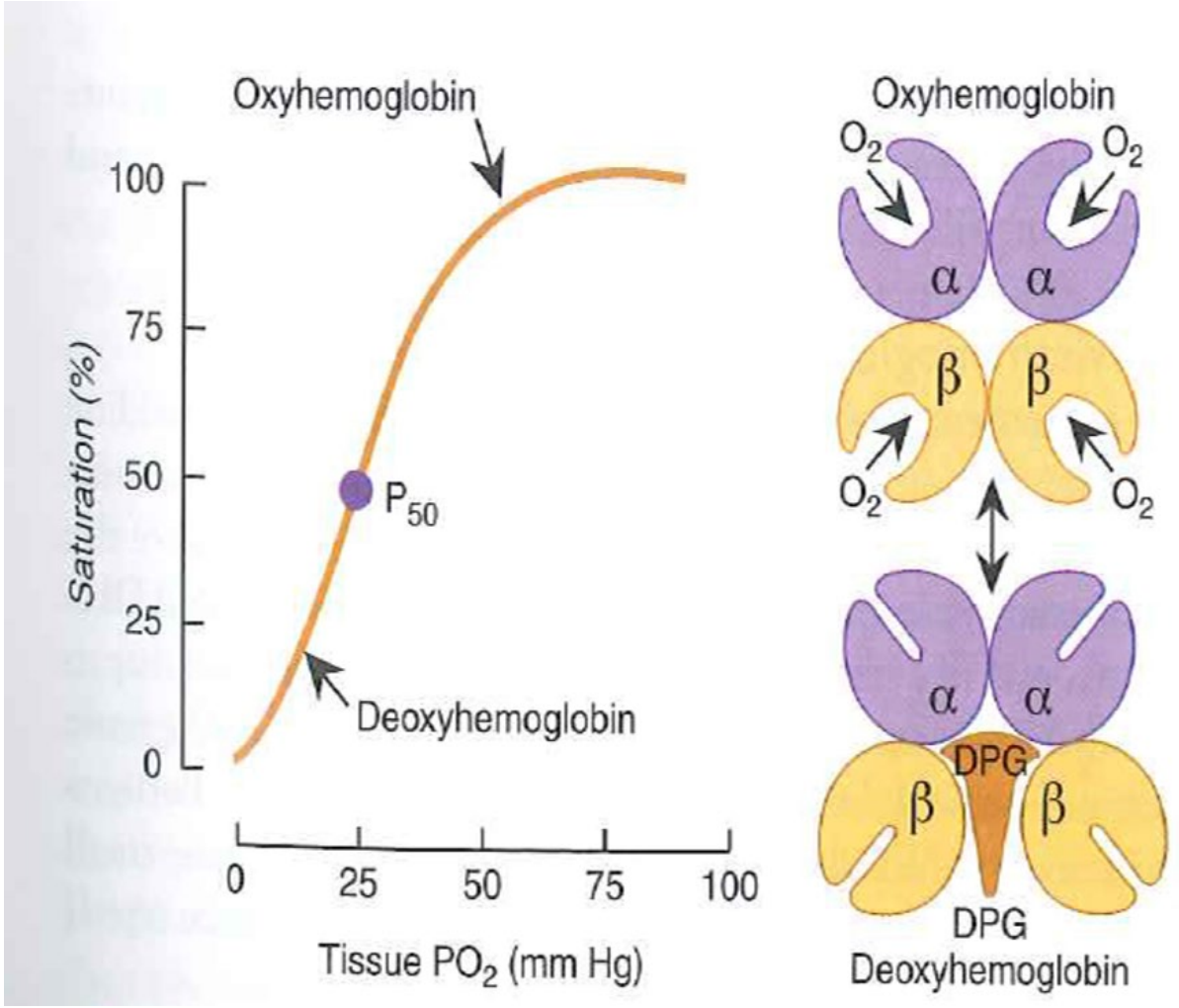


- Most of iron in body is associated with hemoglobin
- Large protein composed of 4 polypeptide chains each containing one heme group
- Each heme binds to one oxygen molecule
- Adult hemoglobin: HbA
  - 2 alpha globin + 2 beta globin chains

Hemoglobin development

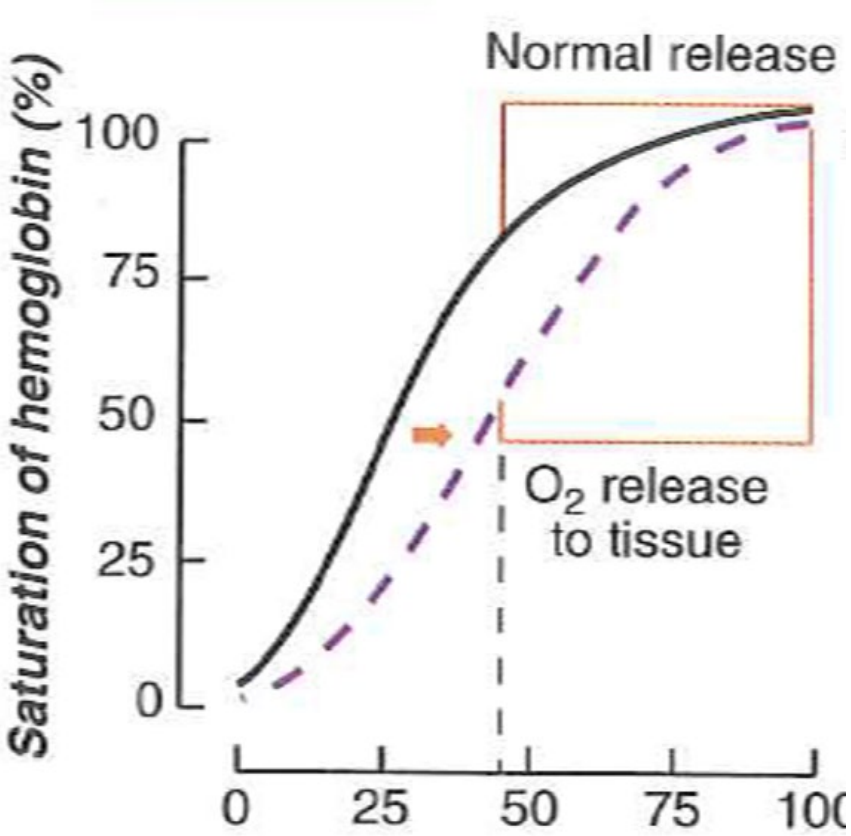


Oxygen dissociation curve

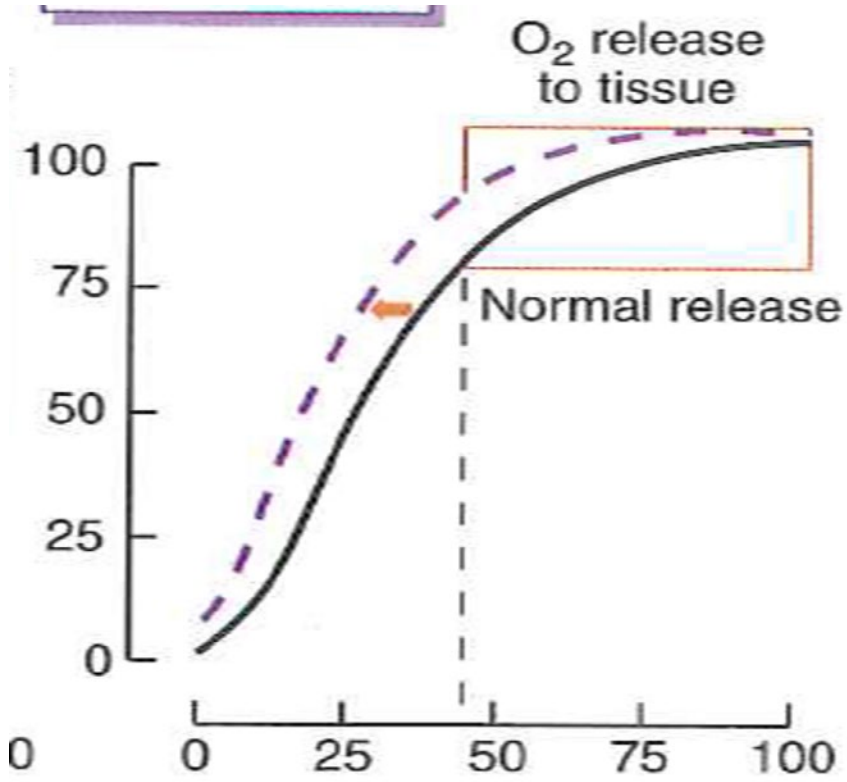


Oxygen dissociation curve

- Right Shift
  - Releases more oxygen to tissues & opening Hb to accept more CO_2
- Causes
  - ↓ pH/acidosis
  - ↑ 2,3-DPG
  - ↑ temperature



Oxygen dissociation curve

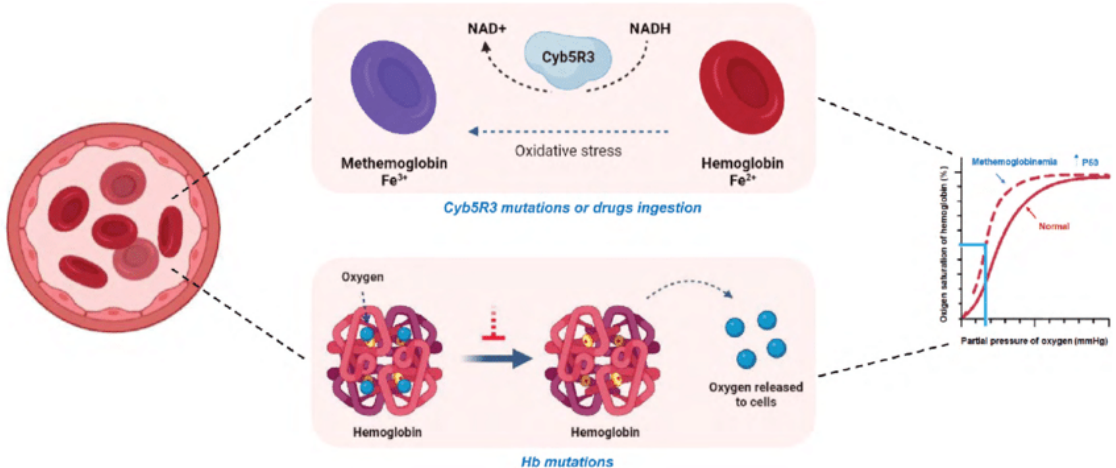


- Left shift
  - Hgb binds oxygen more tightly, reducing the amount of oxygen released
- Causes
  - ↑ pH/alkalosis
  - ↓ 2,3-DPG
  - ↓ temperature
  - Methemoglobinemia
  - Carboxyhemoglobinemia

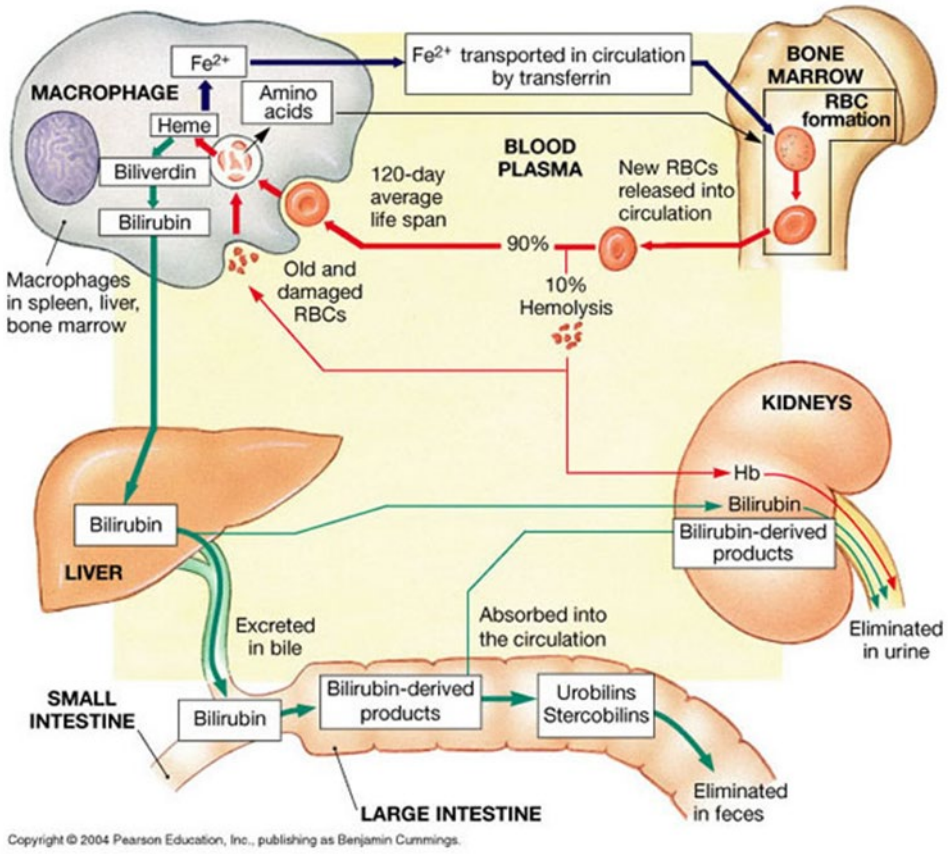
Methemoglobinemia

- Decreased oxygen-carrying capacity of hemoglobin
  - Conversion of iron from reduced ferrous (Fe2+) state to oxidized ferric (Fe3+) state
  - Ferric iron unable to bind/transport oxygen
- Etiology
  - Acquired
    - More common
    - Exposure to oxidizing agents
      - Benzocaine/prilocaine, nitrates
  - Congenital
    - Defects in cytochrome b5 reductase (CYB5R) enzyme
    - Mutations in globin protein genes

- Signs and symptoms
  - Hypoxemia refractory to supplemental oxygen
  - “Chocolate brown blood”
  - 10% methemoglobin: dyspnea, yanosis/pallor
  - 20%: anxiety, light-headedness, headache
  - 30-50%: tachypnea, confusion, loss of consciousness
    - Risk for metabolic acidosis, coma, death
- Treatment
  - Removal of causative agent
  - Methylene blue
    - Reduced to leukomethylene blue → acts as electron donor to reduce methemoglobin to hemoglobin
  - Supplemental oxygen



Clearance of hemoglobin



- Lab tests:
  - Haptoglobin
  - LDH
  - Bilirubin
    - Un-conjugated
    - Conjugated
  - Urine free hemoglobin

Anemia

What is anemia?

Reduction of the oxygen-transporting capacity of the blood, which usually stems from a reduction in the total circulating red cell mass below normal amounts.

Signs and symptoms of anemia

- Fatigue
- Weakness
- Dizziness
- Headache
- Shortness of breath
- Tachycardia
- Pallor
- Pale mucous membranes

- May be asymptomatic
  - Compensation

Laboratory investigation of anemia

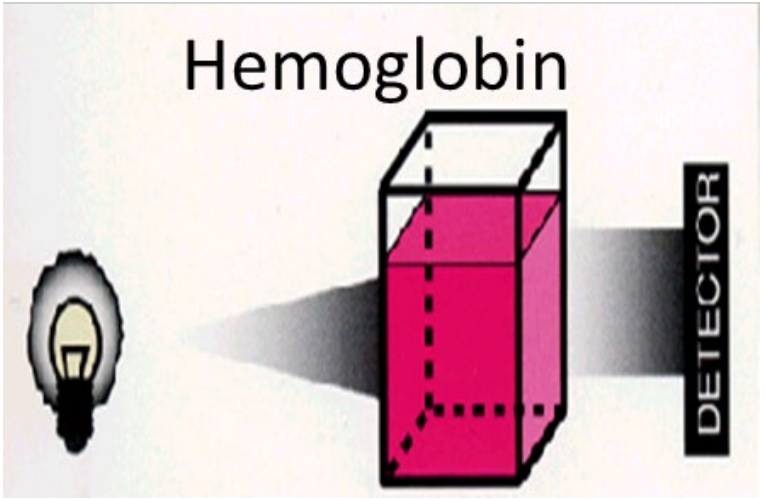
Complete blood count (CBC)

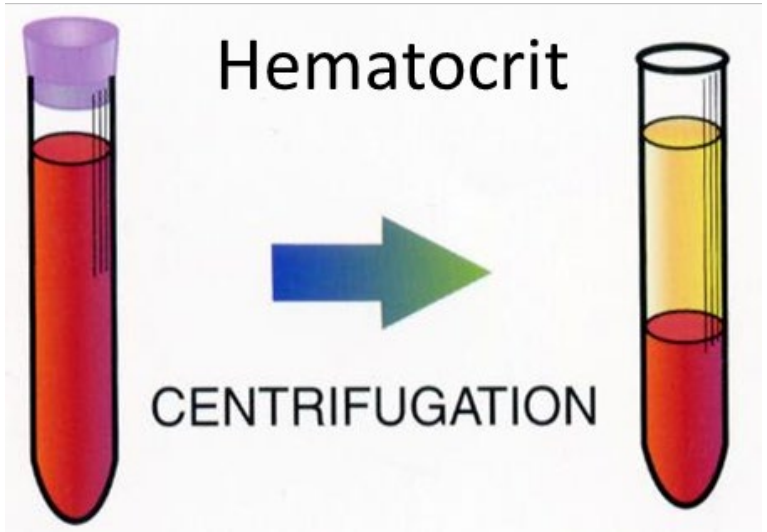
- One of the most common lab tests ordered
- Often ordered along with a white blood cell differential count
- Provides valuable information for benign/reactive and malignant disease processes

Component	Value	Range & Units
CBC PROFILE	RESULTS:	
WBC	8.7	4.5 - 11.0 10^3/UL
Red Blood Cell Count	4.81	4.5 - 5.9 10^6/UL
Hemoglobin	15.2	13.5 - 17.5 GM/DL
Hematocrit	44.1	40 - 51 %
MCV	91.6	80 - 100 FL
MCH	31.6	26 - 34 PG
MCHC	34.5	31 - 37 G/DL
RDW	13.3	11.5 - 14.5 %
Platelet Cnt	188	130 - 400 10^3/UL
MPV	9.2	7.4 - 10.4 FL
DIFFERENTAL	RESULTS:	
Differential Type	AUTO	
Neutrophils Absolute	4.4	1.8 - 8.0 10^3/UL
Lymphocytes Absolute	3.5	1.1 - 5.0 10^3/UL
Monocytes Absolute	0.7	0.2 - 1.1 10^3/UL
Eosinophils Absolute	0.1	0.0 - 0.6 10^3/UL
Basophils Absolute	0.0	0.0 - 0.2 10^3/UL
Neutrophils Relatives	51	%
Lymphocytes Relative	40	%
Monocytes Relative	8	%
Eosinophils Relative	1	%
Basophils Relative	0	%

CBC: Red blood cell indices

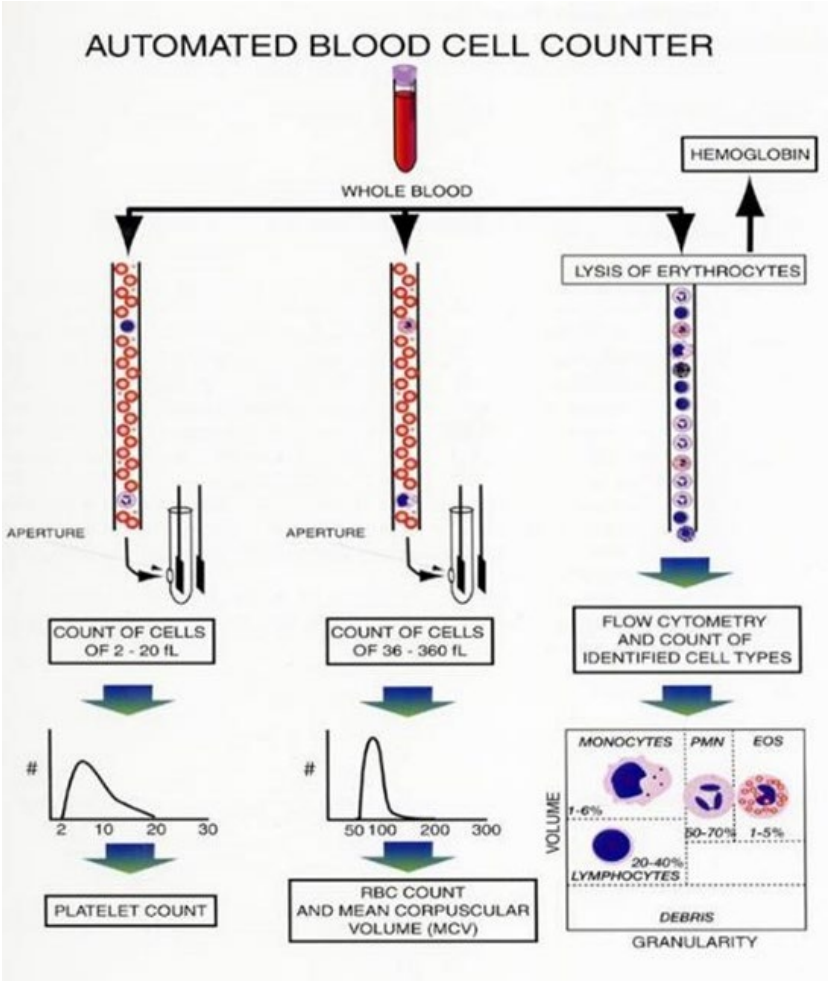
- Red blood cell count
  - Concentration of RBC's (million/uL)
- Hemoglobin
  - Concentration of Hgb (g/dL)
- Hematocrit
  - Relative volume of packed RBCs (%)
- Mean Cell Volume (MCV)
  - Average size of RBCs (fL)





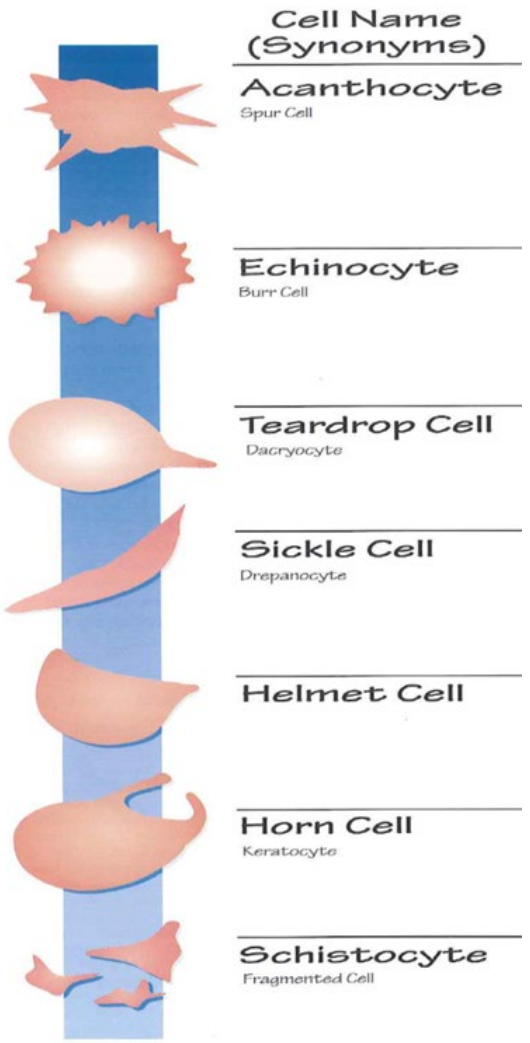
CBC: Red blood cell indices

- Mean Cell Hemoglobin (MCH)
  - Average amount of Hgb in each RBC (pg)
  - Calculated: Hb (g/dL) / RBC (M/uL) x10
- Mean Cell Hemoglobin Concentration (MCHC)
  - Average amount of Hgb per a certain concentration of RBC's (g/dL)
  - Calculated: Hb (g/dL) / hematocrit (%)
- Red Cell Distribution Width (RDW):
  - Amount of RBC size variability (%)
  - Higher = more variability



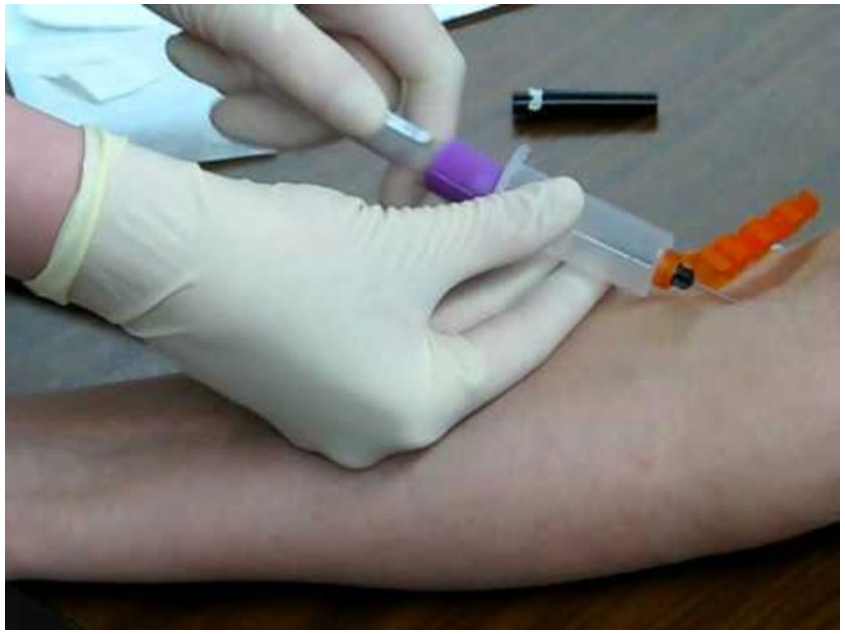
RBC vocabulary

- Microcytic – Small RBCs, ↓ MCV
- Normocytic - Normal MCV
- Macrocytic – Large RBCs, ↑ MCV
- Hypochromia - ↓ Decreased Hb in RBCs (↓ MCHC)
  - Central pallor >1/3 diameter
- Anisocytosis - Variation in cell size (↑ RDW)
- Poikilocytosis - Variation in cell shape
- Polychromasia - Bluish color in some cells (due to RNA in reticulocytes)



Other useful lab tests

- Complete Metabolic Panel
- Lactate dehydrogenase (LDH)
- Vitamin levels (B12, folate, etc.)
- Direct antiglobulin test (DAT)
- Erythropoietin level
- Haptoglobin
- Iron Studies

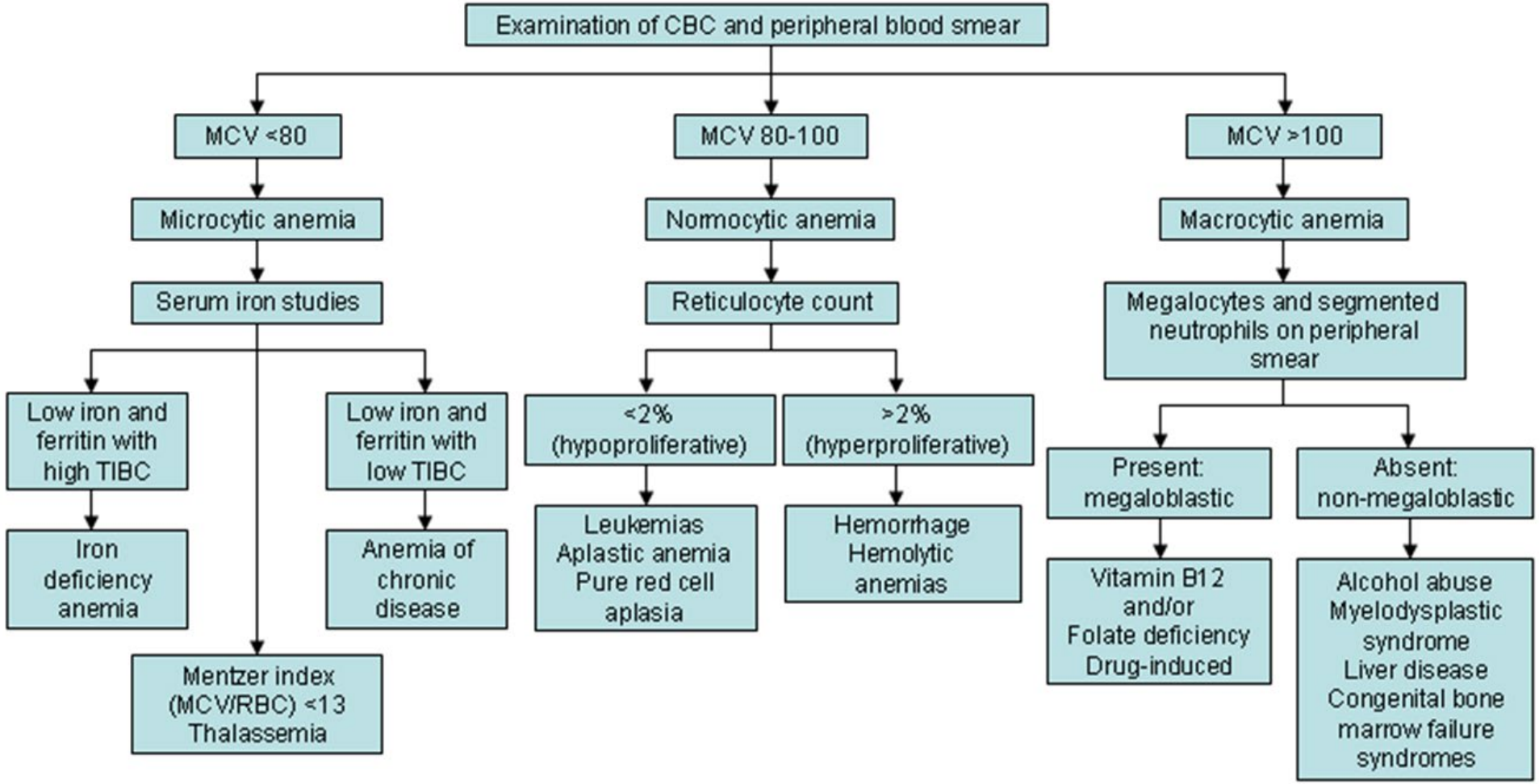


Classification of anemia

- MCV: microcytic vs. macrocytic
- Hemolytic vs. non-hemolytic
- Congenital vs. acquired
- Underlying mechanism

- Key for evaluation: CBC + peripheral blood smear review

Anemia classification based on MCV



Anemia classification based on mechanism

1. Blood loss
2. Increased destruction
3. Impaired production

Table 12–1. CLASSIFICATION OF ANEMIA ACCORDING TO MECHANISM OF PRODUCTION

I. Blood Loss

A. Acute: trauma
B. Chronic: lesions of gastrointestinal tract, gynecologic disturbances

II. Increased Rate of Destruction (Hemolytic Anemias)

A. Intrinsic (intracorpuscular) abnormalities of RBCs
  1. Hereditary
    a. Disorders of RBC membrane cytoskeleton (e.g., spherocytosis, elliptocytosis)
    b. RBC enzyme deficiencies
      1) Glycolytic enzymes: pyruvate kinase, hexokinase
      2) Enzymes of hexose monophosphate shunt: glucose-6-phosphate dehydrogenase, glutathione synthetase
    c. Disorders of hemoglobin synthesis
      1) Deficient globin synthesis: thalassemia syndromes
      2) Structurally abnormal globin synthesis (hemoglobinopathies): sickle cell anemia, unstable hemoglobins
  2. Acquired
    a. Membrane defect: paroxysmal nocturnal hemoglobinuria
B. Extrinsic (extracorpuscular) abnormalities
  1. Antibody mediated
    a. Isohemagglutinins: transfusion reactions, erythroblastosis fetalis (Rh disease of the newborn)
    b. Autoantibodies: idiopathic (primary), drug-associated, systemic lupus erythematosus
  2. Mechanical trauma to RBCs
    a. Microangiopathic hemolytic anemias: thrombotic thrombocytopenic purpura, disseminated intravascular coagulation
  3. Infections: malaria

III. Impaired Red Cell Production

A. Disturbance of proliferation and differentiation of stem cells: aplastic anemia, pure RBC aplasia, anemia of renal failure, anemia of endocrine disorders
B. Disturbance of proliferation and maturation of erythroblasts
  1. Defective DNA synthesis: deficiency or impaired utilization of vitamin B_{12} and folic acid (megaloblastic anemias)
  2. Defective hemoglobin synthesis
    a. Deficient heme synthesis: iron deficiency
    b. Deficient globin synthesis: thalassemias
  3. Unknown or multiple mechanisms: sideroblastic anemia, anemia of chronic inflammation, myelophthisic anemias due to marrow infiltrations

Blood loss

- Acute/traumatic
  1. Hypovolemia/shock → fluid shift
     - Loss of all blood elements (RBCs, WBCs, platelets)
  2. Hemodilution
     - Normochromic
     - Normocytic
  3. Reticulocytosis
     - ~5 days later
- Chronic
  - Often results in iron deficiency anemia
  - Common sources: GI, gynecologic tract

Questions?