Iron and Heme

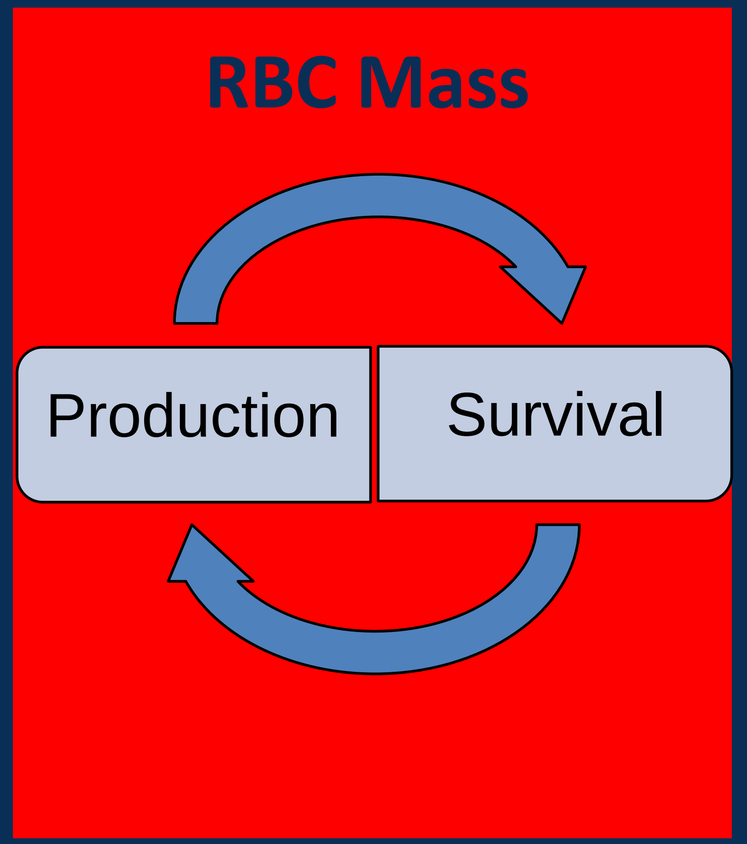
Intro to Anemia

Objectives

- Etiologies of anemia
- Clinical presentations
- Methods of laboratory assessment
- Define classifications of anemias

Anemia

- Decreased ability of oxygen delivery
  - ↓ RBC and/or ↓ HGB
- High prevalence in clinical medicine
- Not a disease but expression of underlying disorder
- RBC Kinetics: RBC Mass = Production * Survival
- Anemia = RBC loss/destruction > production
  - Or = production impaired
- Classified as functional or morphological anemia



Screening for Anemia

- Relative HGB/HCT values to plasma, consider:
  - Hypervolemia (↑ plasma) vs Hypovolemia
- Physician evaluates
  - Patient history, physical examination, symptoms
  - Laboratory results
- Acute blood loss
  - HGB normal → decreased
- High altitudes, smoking

Screening for Anemia

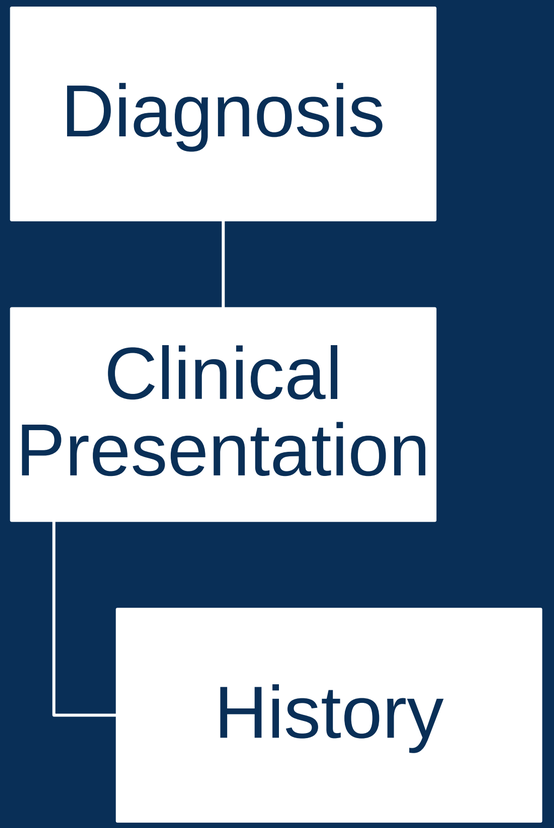
- Symptom presentations
  - Slight fatigue → life threatening reaction
- Acute hemorrhage (related to hypovolemia)
  - < 20% = normal at rest, tachycardia with exercise
  - 30 – 40% = circulatory collapse and shock
  - > 50% = death
- Slow developing
  - HGB ↓ to 50% normal = body compensates

Body Compensation

- ↑ oxygenated blood flow
  - ↑ cardiac output and circulation rate
  - ↑ pulmonary O2 uptake
  - ↓ blood viscosity → ↑ blood flow
  - ↑ blood flow to vital organs
- ↑ oxygen utilization (ODC shifts right)
  - ↑ 2,3-BPG in RBCs
  - Bohr effect → tissues create acidic environment

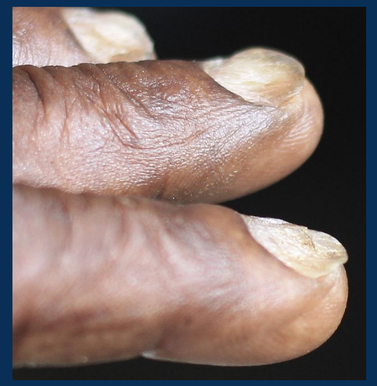
Diagnosis of Anemia

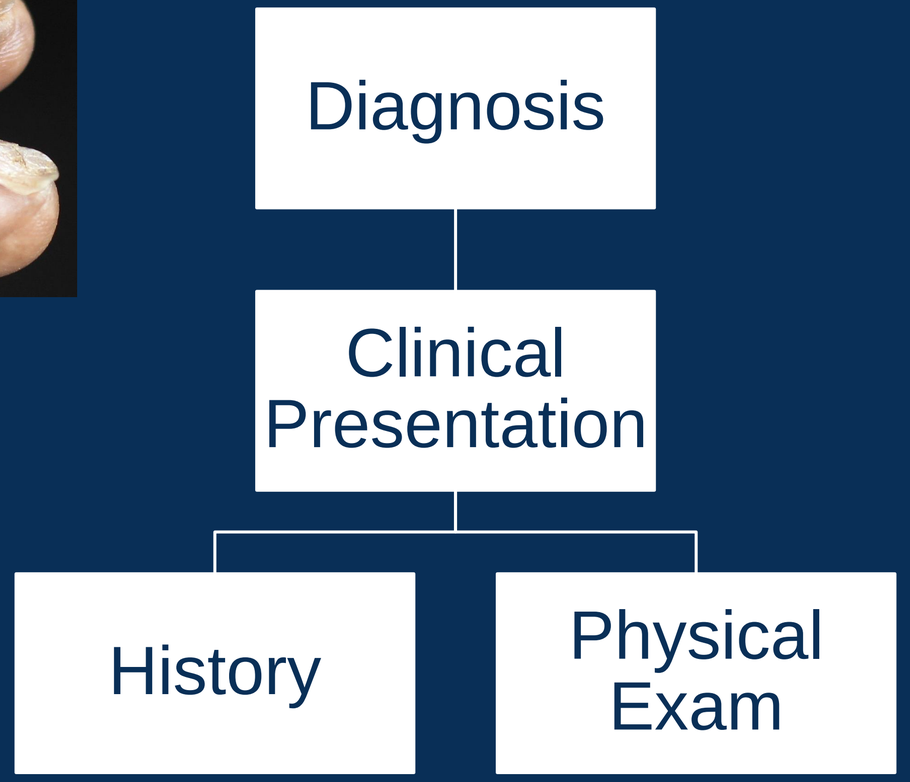
- Patient history and presentation
  - Symptom description/duration
  - Weakness/fatigue vs headache/vertigo/dyspnea
    - Slow vs severe HGB drop
    - Hematuria/emesis, bloody/black stools
    - Any blood loss
  - Dietary/medication changes
  - Chemical/toxin exposure



Diagnosis of Anemia

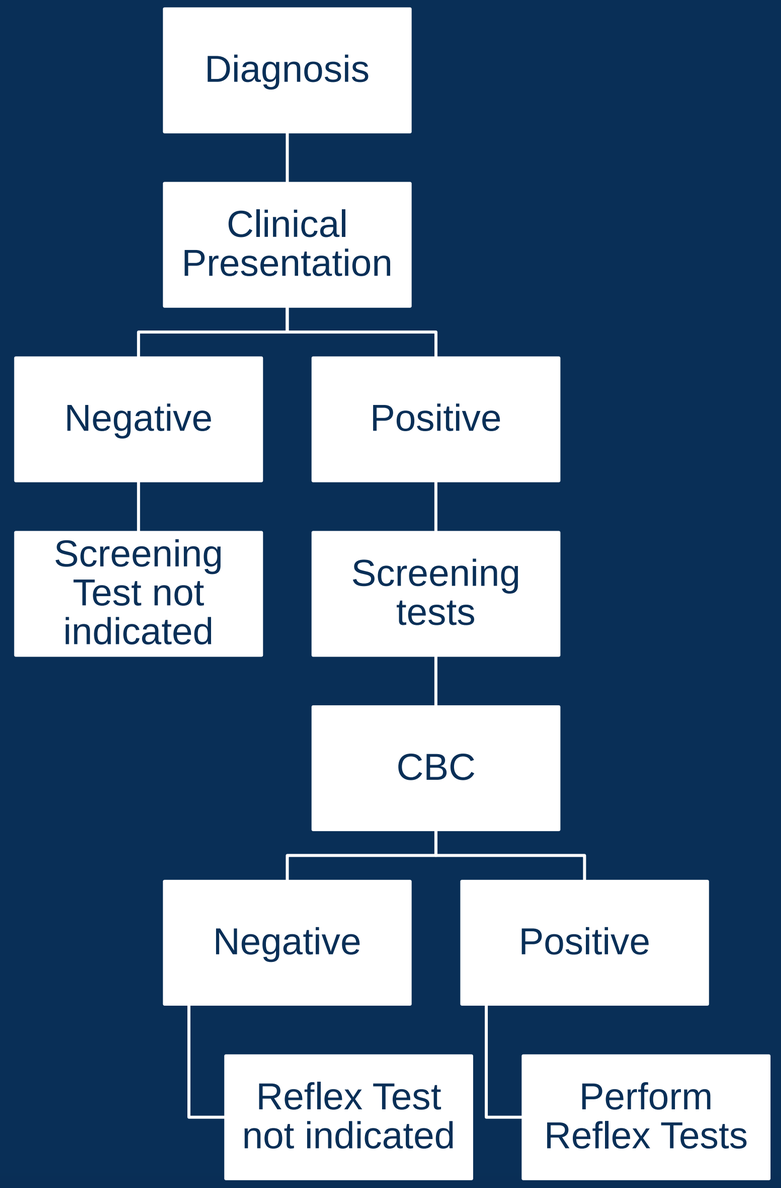
- Physical examination
  - Pale skin, hypotension, organomegaly
  - Koilonychia – iron-deficiency anemia
  - Smooth tongue – megaloblastic anemia
  - Jaundice, dark urine – hemolytic anemias
  - Bone deformities, hepato-/spleno-megaly – hereditary anemias or autoimmune anemias
  - Neurologic dysfunction – pernicious anemia





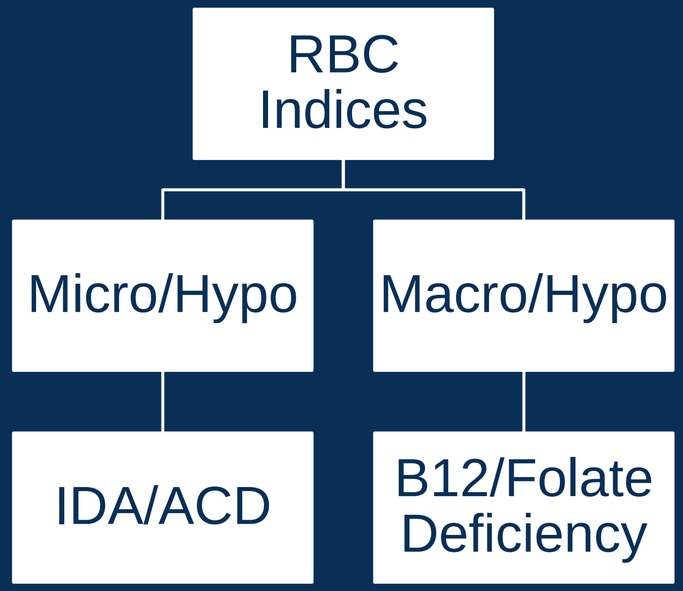
Laboratory Diagnosis

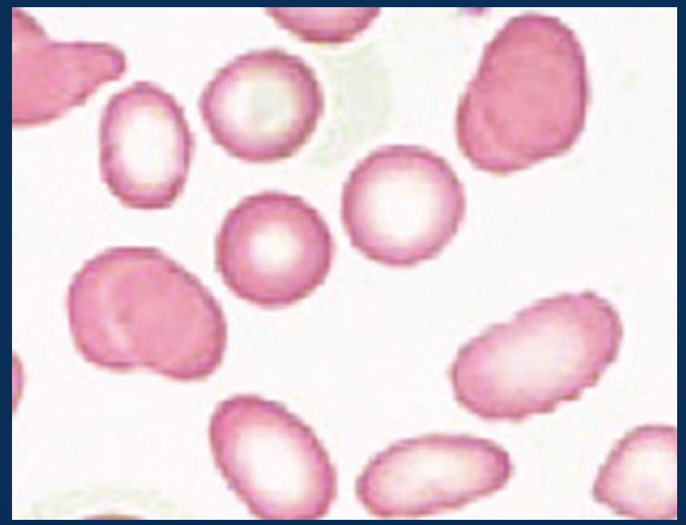
- Screening tests
  - CBC: RBC indices, PLT, WBC
- Reflex tests
  - Retic, bilirubin, smear evaluation
  - Urine, stool evaluation



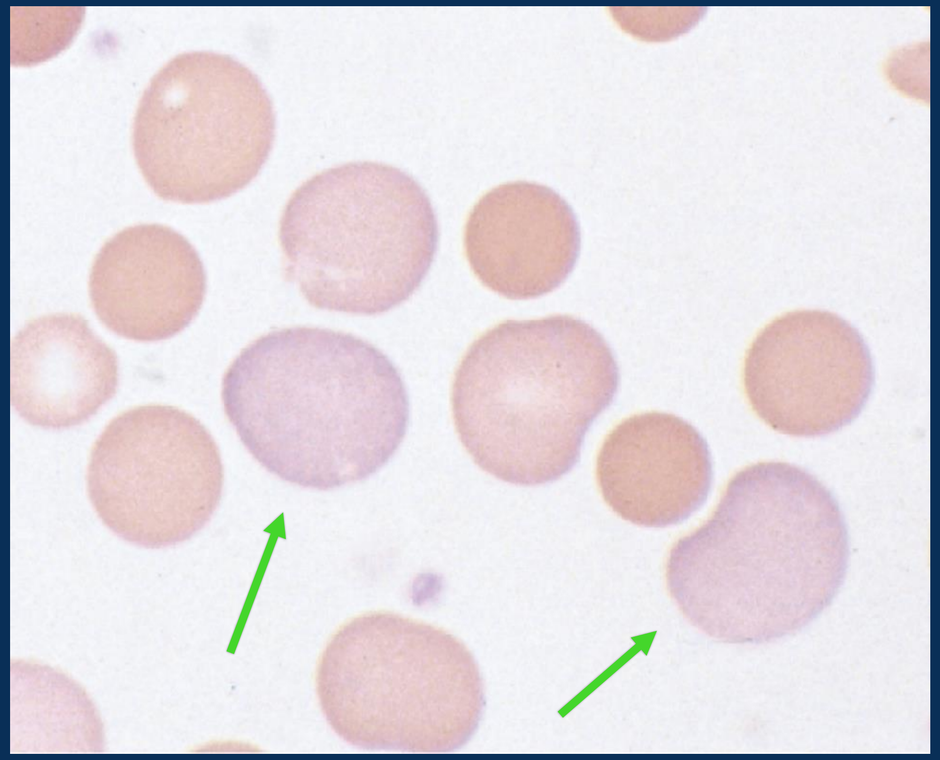
RBC Indices

- MCV, MCH, MCHC, RDW
  - Used in morphologic evaluation
  - Guide reflex testing
    - Micro/hypo
      - IDA
    - Macro/hypo
      - Vitamin B12/folate deficiency



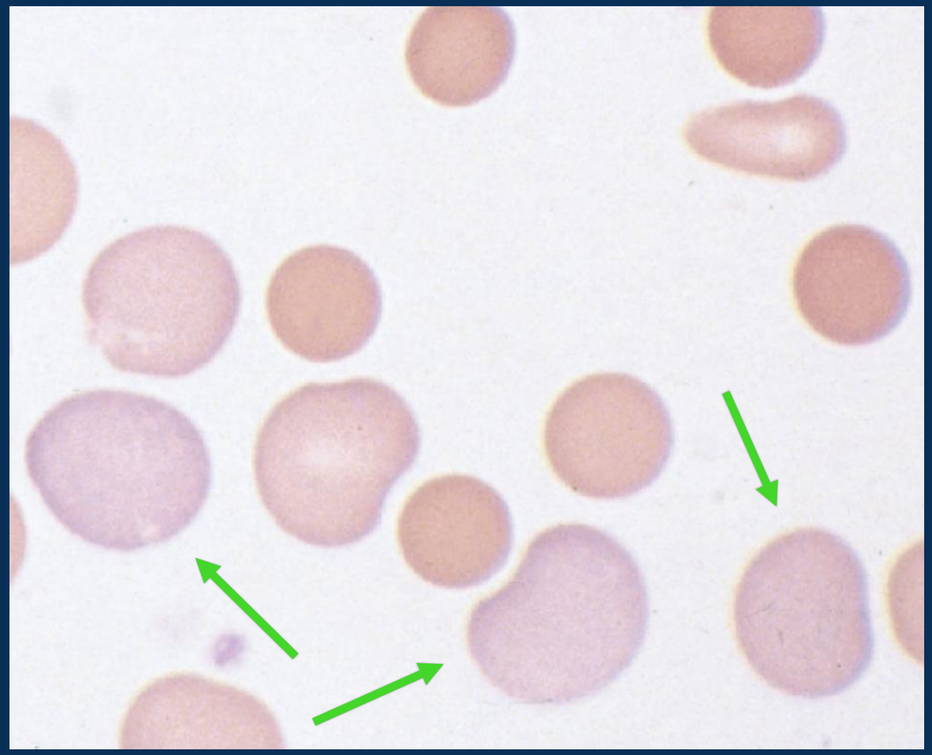


Reticulocyte



- Reference: 0.5-2.5% or 25-75 x10^3/µL)
- Indicates degree of BM compensation
- Manual and automated methods
  - Methylene blue and light scatter
  - Fluorescent flow cytometry
- Consider with severity of anemia

Reticulocyte



- Corrected Reticulocyte
  - Adjusts for severity of anemia

$$Corrected\ Retic\ = \left(\frac{\mathrm{HCT_{Pt}}}{\mathrm{HCT_{Norm}}}\right) \times \%\ \mathrm{Retic}$$

  - < 2% or 25-75 x10^3/µL

    → hypoproliferative anemias
  - > 2% or 25-75 x10^3/µL

    → blood loss, hemolytic anemia

Reticulocyte

- Reticulocyte Production Index (RPI)
  - Account for stress retics (or shift retics)

$$RPI = \left(\frac{\text{HCT}_{\text{Pt}}}{\text{HCT}_{\text{Norm}}}\right) \times \left(\frac{\%\ \text{Retic}}{\text{Retic Maturation}}\right)$$

  - RPI > 2 = appropriate BM response
  - RPI < 2 = inadequate BM response

HCT Levels	Retic Maturation
35%	1.5 days
25%	2.0 days
15%	2.5 days

Reticulocyte

- Automated Reticulocyte Indices
  - Assessment of maturity levels
    - BM response to anemia, response to therapy
  - Immature Reticulocyte Fraction (IRF)
    - Based on RNA concentration
  - CHr = Reticulocyte Mean [Hemoglobin]
    - Based on HGB concentration

Blood Smear Evaluation

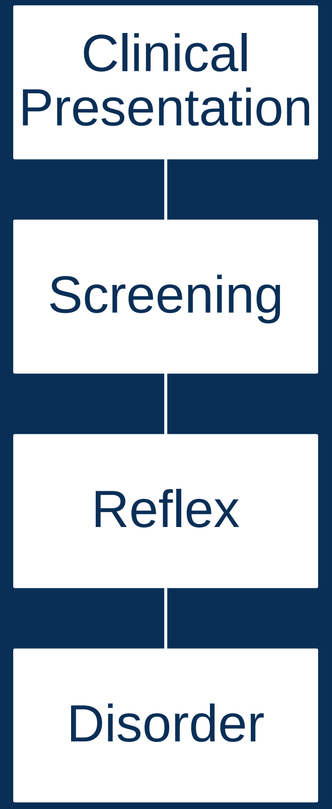
- RBC Size
  - Micro/Normo/Macrocytes, Anisocytosis
- RBC Color
  - Hypo/Normochromia
- RBC Shape and Inclusions
  - Poikilocytosis

Tests for RBC Destruction

- Used to evaluate RBC survival due to extravascular hemolysis
  - ↑ hemosiderinuria
  - ↓ plasma haptoglobin and hemopexin
  - ↑ methemalbumin
  - ↑ serum unconjugated bilirubin
  - ↑ uric acid
  - ↑ LDH = lactate dehydrogenase

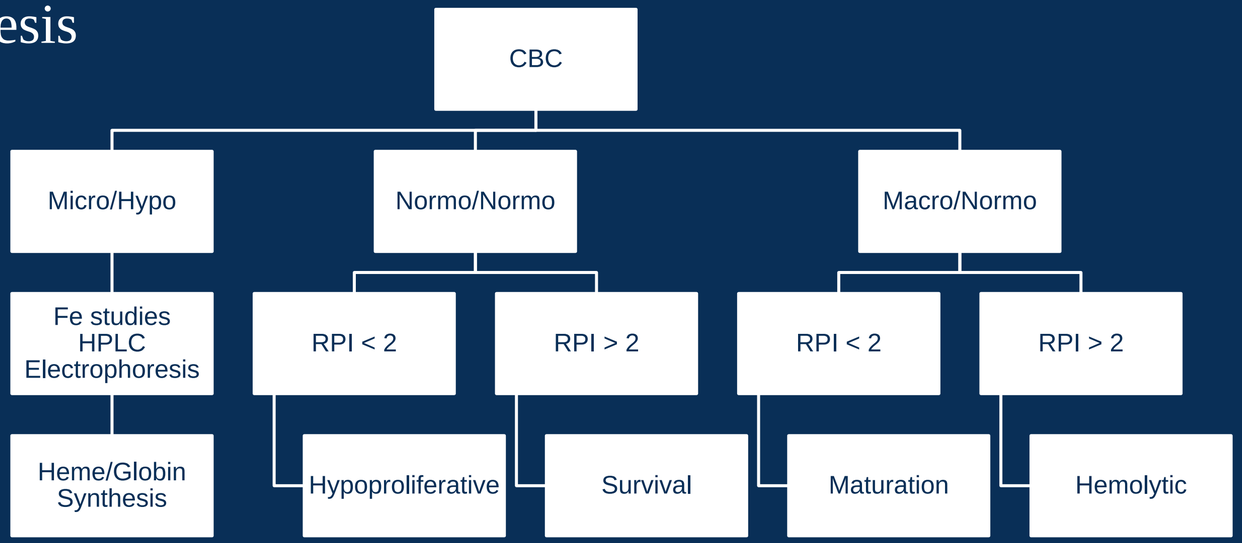
Classification of Anemia

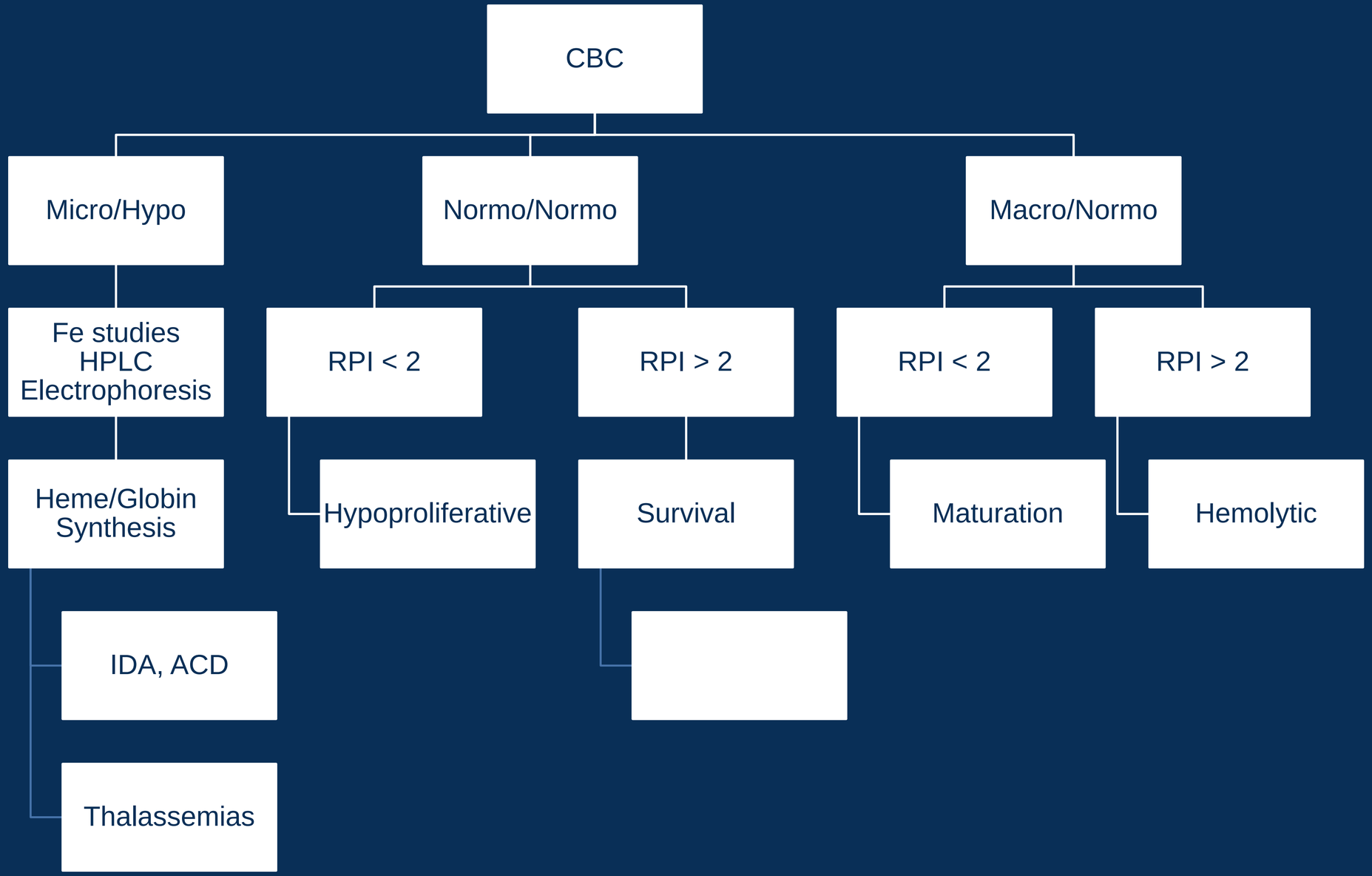
- Morphology
  - RBC size and HGB concentration
- Pathophysiology (functional)
  - Proliferation defect (decreased production)
  - Maturation defect
  - Survival defect (increased destruction)
  - Use Retic count, IRF or RPI, serum iron studies



Morphologic Classification

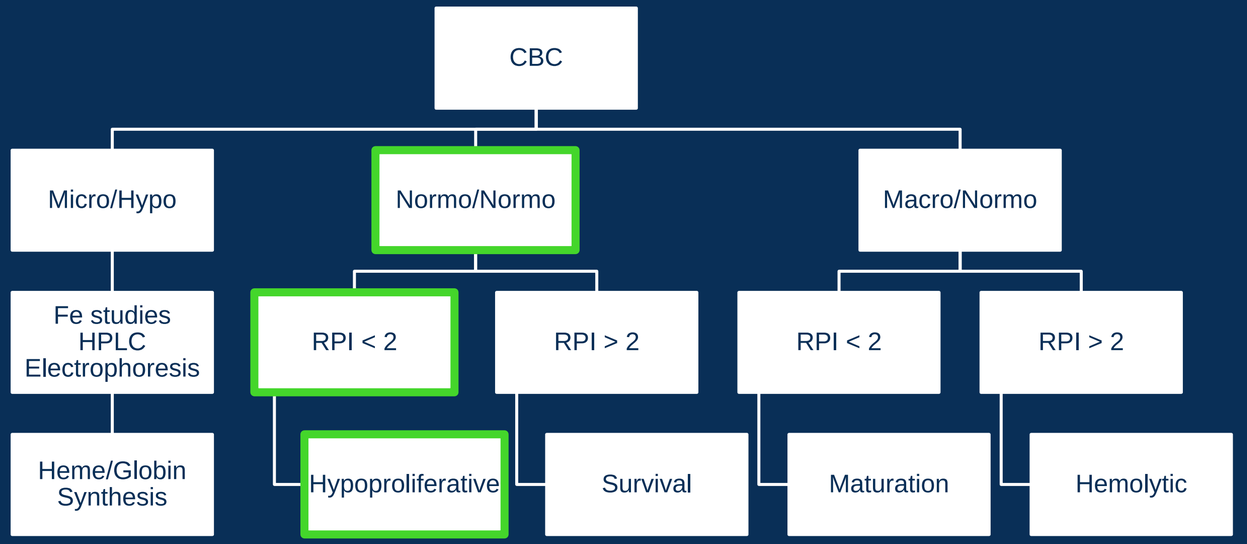
- Microcytic hypochromic
  - Defective HGB synthesis
  - Serum iron studies, HGB HPLC/electrophoresis
- Normocytic normochromic
  - Hypoproliferative anemias (RPI < 2)
  - Survival defects (RPI > 2)
- Macrocytic normochromic
  - Hemolytic anemia (RPI > 2)
  - Nuclear maturation defects (RPI < 2)




CBC
Micro/Hypo
Normo/Normo
Macro/Normo
Fe studies
HPLC
Electrophoresis
RPI < 2
RPI > 2
RPI < 2
RPI > 2
Heme/Globin
Synthesis
Hypoproliferative
Survival
Maturation
Hemolytic
IDA, ACD
Thalassemias

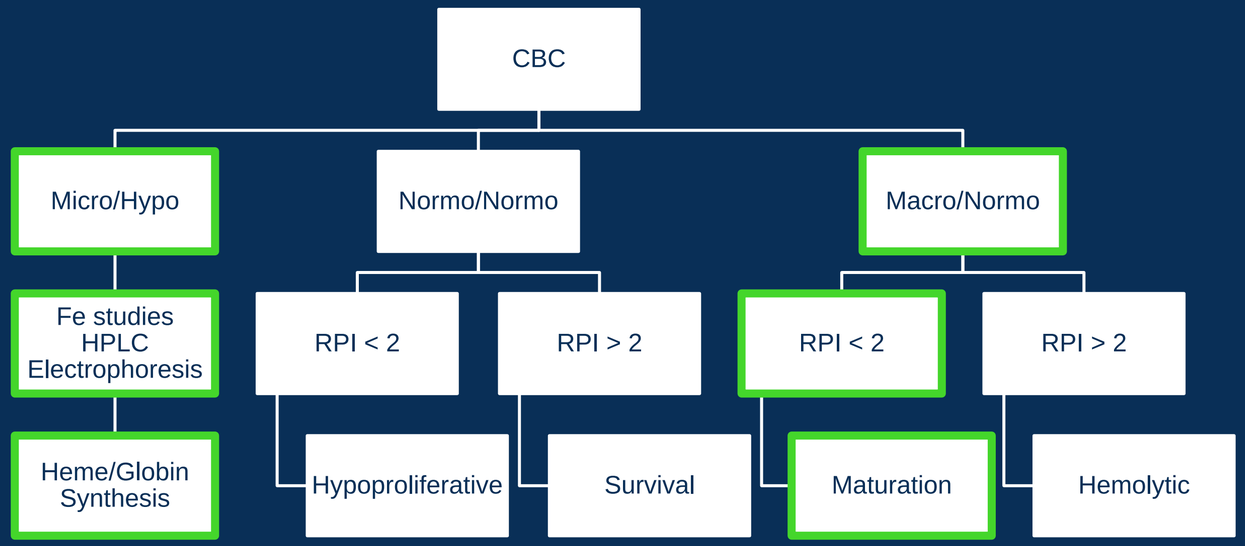
Functional Classification

- Proliferation defect (decreased production)
  - Normal RBCs, retic, IRF, RPI, serum iron studies
  - Inappropriate EPO or cytokine regulator production
  - BM infiltrated fibrous/neoplastic tissue
  - BM damaged by chemicals, drugs, radiation
  - Hypoplasia



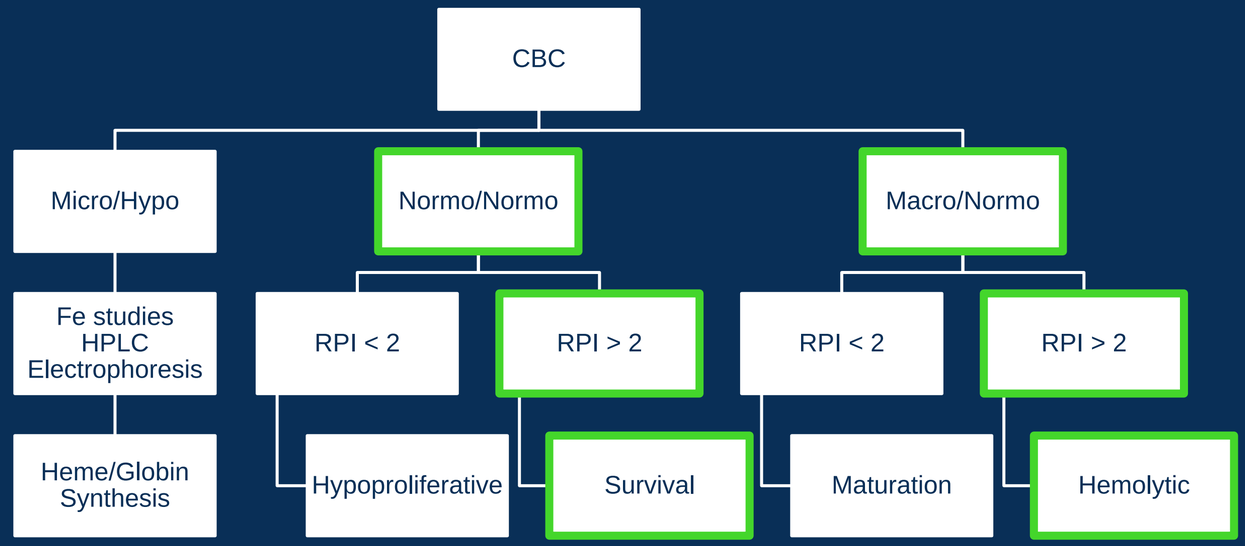
Functional Classification

- Maturation defect
  - Disruption in development (BM erythroid hyperplasia)
    - Normal retic, IRF, RPI
    - Nuclear (macrocytic)
      - Affects all cell lines (megaloblastic)
    - Cytoplasmic (microcytic)
      - Only RBC lineage
      - Abnormal HGB production



Functional Classification

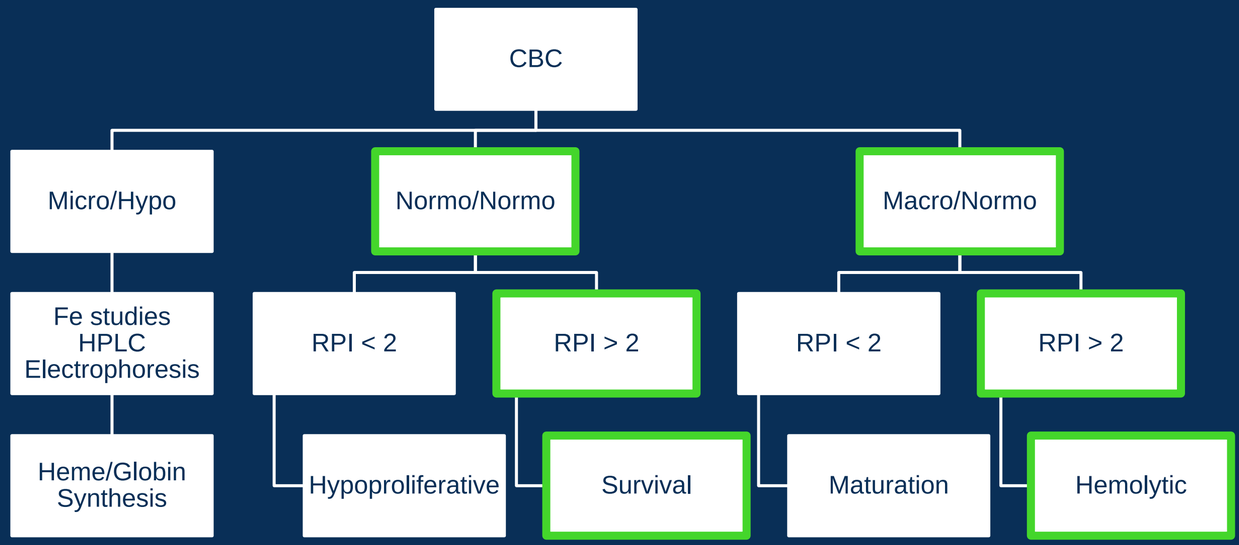
- Survival defect (increased destruction)
  - RBC normo/macrocytic normochromic
  - ↑ retic, IRF, RPI, abnormal serum iron studies
  - Loss of circulating RBCs
    - Compensated hemolytic anemia
    - Hemolytic crisis
    - Aplastic crisis



Functional Classification

- Survival defect (increased destruction)
  - Schistocytes and spherocytes most common
  - Intravascular hemolysis (extrinsic anemias)
    - Complement activation, physical/mechanical trauma
    - ↓ haptoglobin, ↓ hemopexin, ↑ LDH (early indicator)
  - Extravascular hemolysis (extrinsic/intrinsic anemias)
    - ↑ serum bilirubin, ↓ haptoglobin, and hemopexin if severe or chronic, +DAT

- Intrinsic anemias
  - Hereditary abnormality
- Extrinsic anemias
  - Antagonist to RBC



Anemias of Disordered Iron Metabolism and Heme Synthesis

Objectives

- *Review hemoglobin production lecture*
- Distribution of iron in the body
- Systemic iron kinetics/balance
- Laboratory assessment of iron
- Pathophysiology, clinical, and laboratory features
  - IDA, ACD, Hemochromatosis
  - Sideroblastic Anemia, Porphyrias

Hemoglobin Production

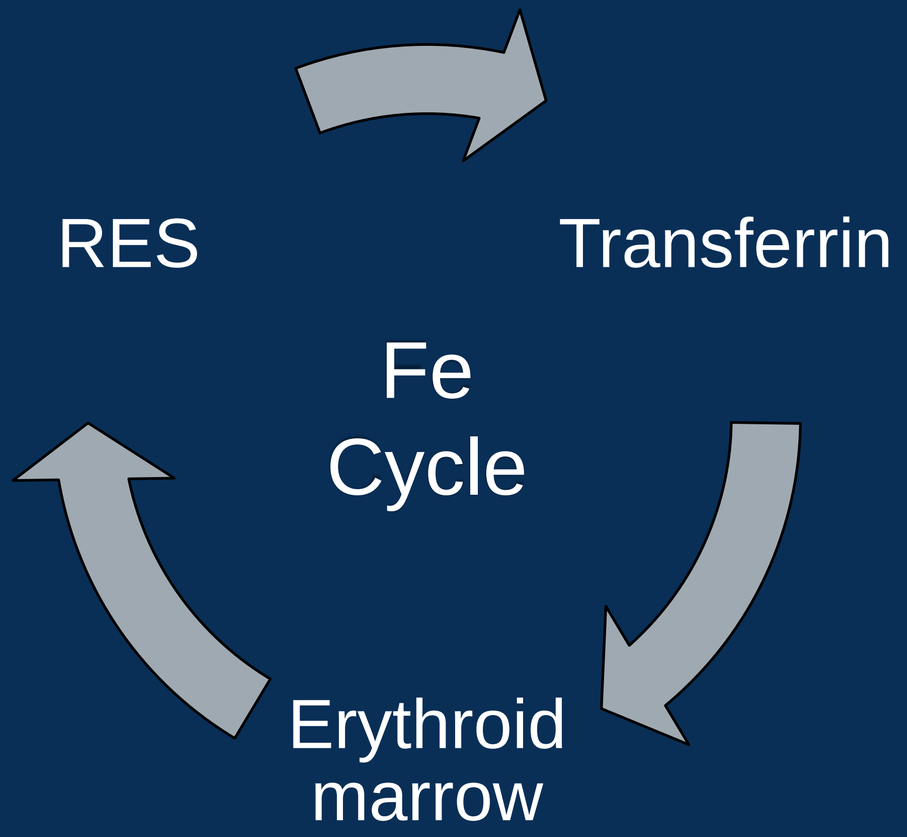
- Disruption related to:
  - Iron => Iron Deficiency Anemia, Anemia of Chronic Disease
  - Cytokines + EPO
    - Anemia of Chronic Disease
  - Heme Enzymes
    - Sideroblastic Anemias, Porphyrias

Iron

- Metabolism : required by every cell
- Distribution : two types of iron-containing compounds
  - Transport (transferrin) and storage (ferritin)
  - Metabolic (HGB) and enzymatic
- [Re]Absorption : macrophages, hepato/enterocytes (ferritin)
  - [Ferritin] directly related to [total iron storage]
- Transport : transferrin (binds ferric iron, Fe^{+++})

Iron

- Transport : transferrin (not APR/APP)
  - Total iron-binding capacity (TIBC)
    - 250–450 µg of iron/dL of serum
  - 95% serum iron complexed with transferrin
  - TIBC ↔ % transferrin saturation (normal ~33%)
    - Unsaturated iron-binding capacity (UIBC)
- Storage : ferritin (APR/APP)
  - Hemosiderin in macrophages of RES



APR = Acute Phase Reactant

APP = Acute Phase Protein

Iron Requirements

- Normally, body iron concentration is constant
- Normal factors that ↑ iron requirements
  - Menstruation
  - Pregnancy
  - Growth in infants and children
- Pathologic factors that ↑ iron requirements
  - Blood loss, malabsorption

Iron Imbalances

- Iron deficiency
  - Insufficient dietary intake
  - Impaired absorption
  - Bleeding
- Iron overload
  - Abnormal increase in absorption
  - Multiple transfusions
  - Iron injections

Systemic Iron Balance

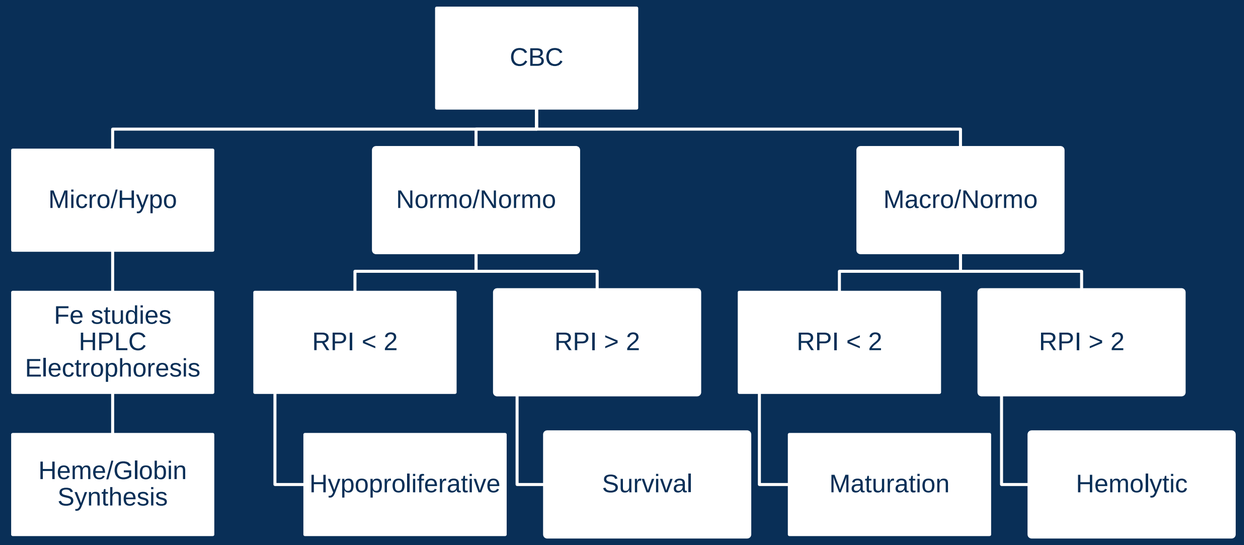
- Hepcidin (APP)
  - Master iron-regulator
    - Inhibits iron absorption and/or transport
      - Degrades ferroportin (cellular iron exporter)
- HFE
  - Binds cellular transferrin receptors triggering cellular uptake/absorption
    - Regulating cellular absorption of iron
    - Modulates hepcidin levels

Lab Assessment of Iron

- Iron studies
  - Transferrin (measured as TIBC)
    - ↑ storage iron = ↑ serum iron = ↓ TIBC
    - ↓ storage iron = ↓ serum iron = ↑ TIBC
  - Ferritin = direct relation to total storage iron
  - sTfR = inverse relation to total body iron
  - ZPP = zinc incorporated into heme
    - Measure fluorescence of blood

CBC

- RBC indices
  - Microcytic hypochromic vs normocytic, normochromic
  - RDW
- CHr (mean cell hemoglobin of reticulocytes)
  - Early indicator of iron-restricted erythropoiesis



Iron Deficiency Anemia (IDA)

- Most common nutritional deficiency in the world
- Etiology = dietary, blood loss, hemodialysis, malabsorption
- Pathophysiology = sequential stage development
  - Iron depletion
  - Iron deficient erythropoiesis
  - Iron deficiency anemia

IDA – Pathophysiology

- Iron depletion
  - No anemia
  - ↓ serum ferritin
    - Hospitalized patients normal to ↑
  - RBCs normal (SL ↑ RDW)
- Iron deficient erythropoiesis
- Iron deficiency anemia

IDA – Pathophysiology

- Iron depletion
- Iron deficient erythropoiesis
  - ZPP develops and remains throughout RBC life
  - RBC SL microcytic
  - Develop ↑ RDW
  - ↓ CHr
- Iron deficiency anemia

IDA – Pathophysiology

- Iron depletion
- Iron deficient erythropoiesis
- Iron deficiency anemia
  - Iron studies abnormal
  - RBC microcytic, hypochromic anemia

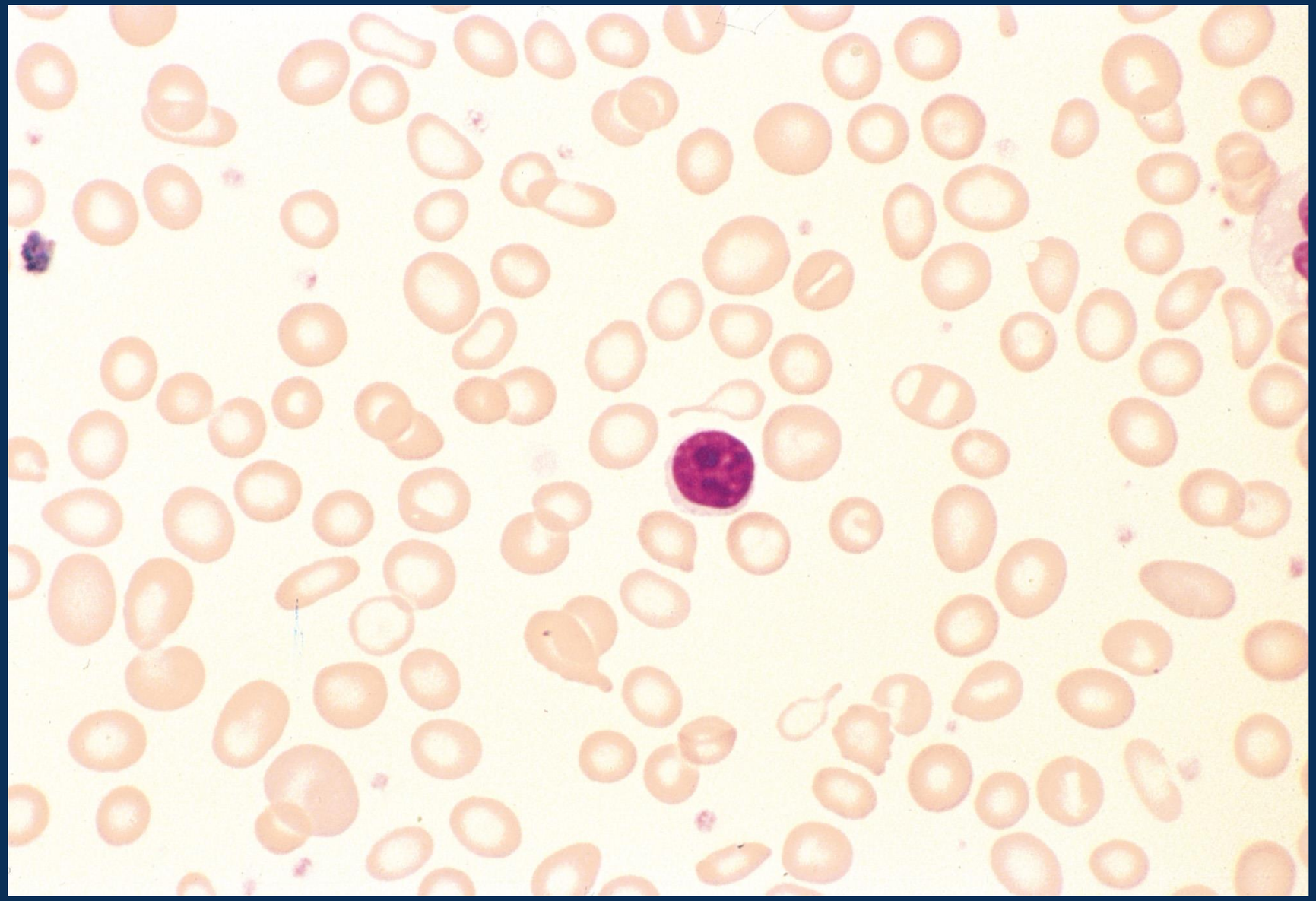
IDA – Clinical Features

- Insidious onset
- Variety of abnormalities
  - Koilonychia, glossitis, muscle dysfunction
- Pica syndrome (unusual cravings)
  - Phagophagia (ice-eating), Geophagia (dirt/clay-eating), Amylophagia (starch-eating)
- Iron deficient infants = poor mental/motor development
- Iron deficient children = irritability, loss of memory

IDA – Laboratory Features

- CBC
  - MCV 55 – 74 fL (microcytic)
  - MCH 14 – 26 pg
  - MCHC 22 – 31 g/dL (hypochromic)
  - ↑ RDW
  - Target cells, ellipt/ovalocytes, teardrops
  - %/# retic normal to ↑, RPI < 2
  - WBC normal to ↑, PLT variable

IDA – Laboratory Features



IDA – Laboratory Features

- Iron studies
  - ↓ = Serum Fe, % transferrin saturation (<16%), ferritin
  - ↑ = TIBC, sTfR, ZPP (later stages)
- Bone marrow
  - ↑ erythroid hyperplasia with ineffective erythropoiesis
  - ↓ Iron stores (macrophages, sideroblasts)
- Therapy
  - Change in blood picture

Anemia of Chronic Disease (ACD)

- Prevalence second to IDA
- aka Anemia of inflammation or infection (1/3 hospitalized)
  - Chronic infections, Chronic inflammatory disorders, Trauma, Organ failure, Neoplastic disorders
- Low serum iron
- Normal iron stores

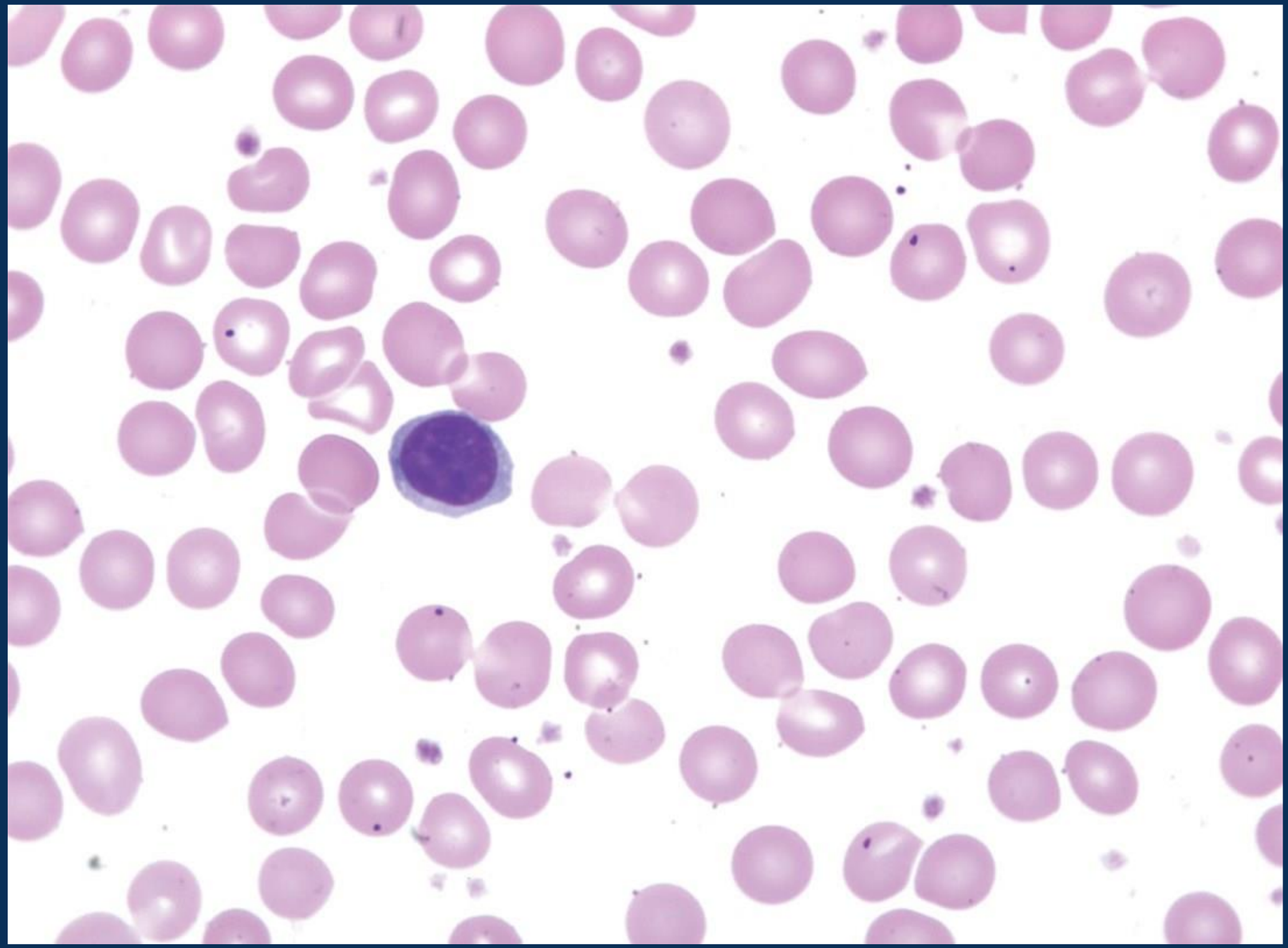
ACD – Pathophysiology/Clinical

- Pathophysiology
  - Multifactorial, mostly linked to inflammatory cytokines
  - ↑ hepcidin (APP), serum ferritin, BM macrophage iron stores
  - ↓ transferrin/TIBC/saturation
- Clinical
  - Signs/symptoms of underlying disorder

ACD – Laboratory Features

- Nonspecific
- CBC
  - Mild anemia
    - HGB > 9 g/dL
    - HCT > 27%
  - RBC normocytic, normochromic
  - RPI < 2
  - WBC and platelet normal

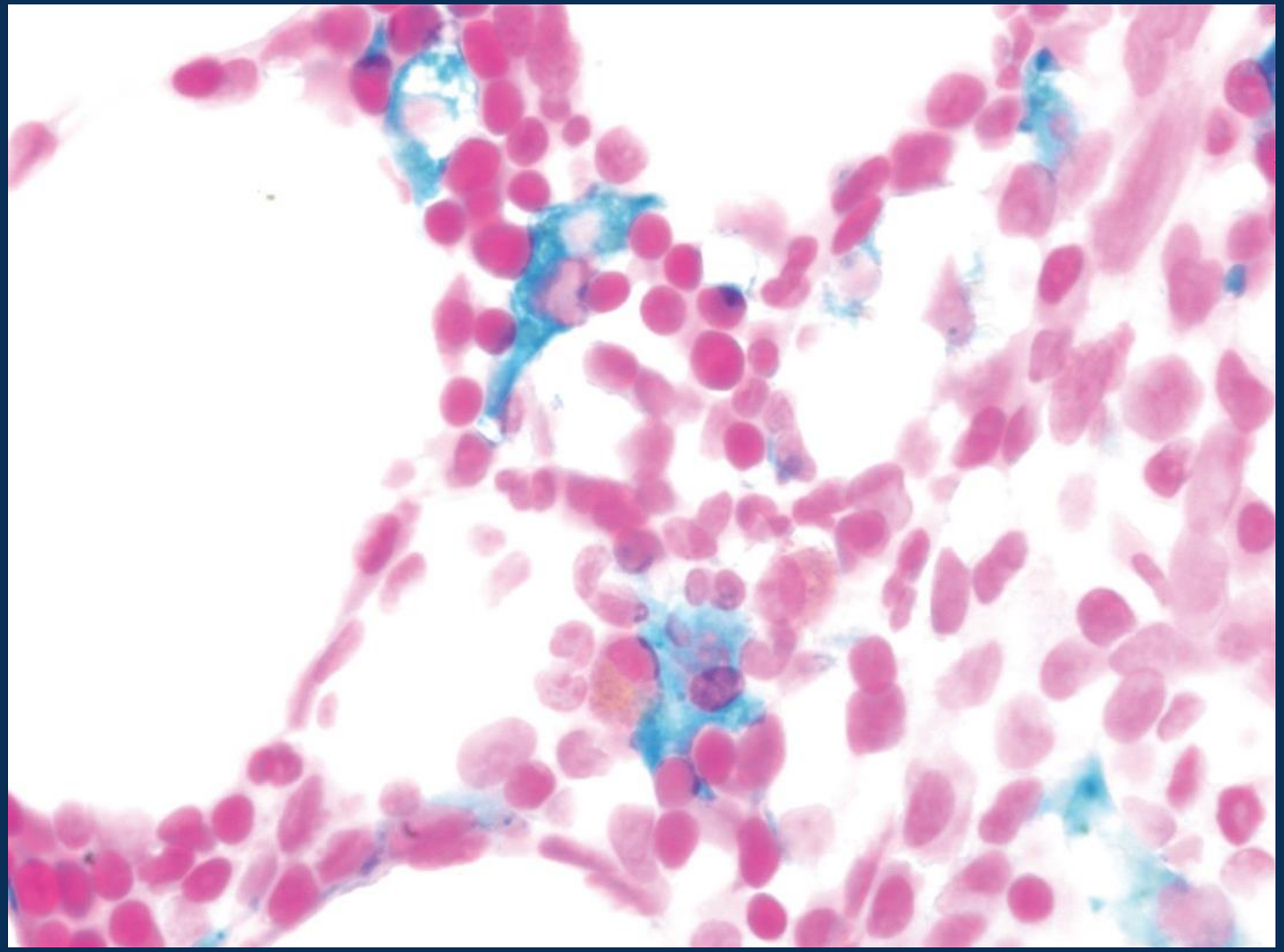
ACD – Laboratory Features



ACD – Laboratory Features

- Iron studies
  - ↓ serum iron,
  - ↓ to normal TIBC, % transferrin saturation
  - Normal to ↑ serum ferritin (different from IDA), ZPP
  - Normal sTfR
- Bone marrow
  - ↑ M:E
  - ↑ BM macrophage iron stores (hemosiderin)

ACD – Laboratory Features



Hemochromatosis

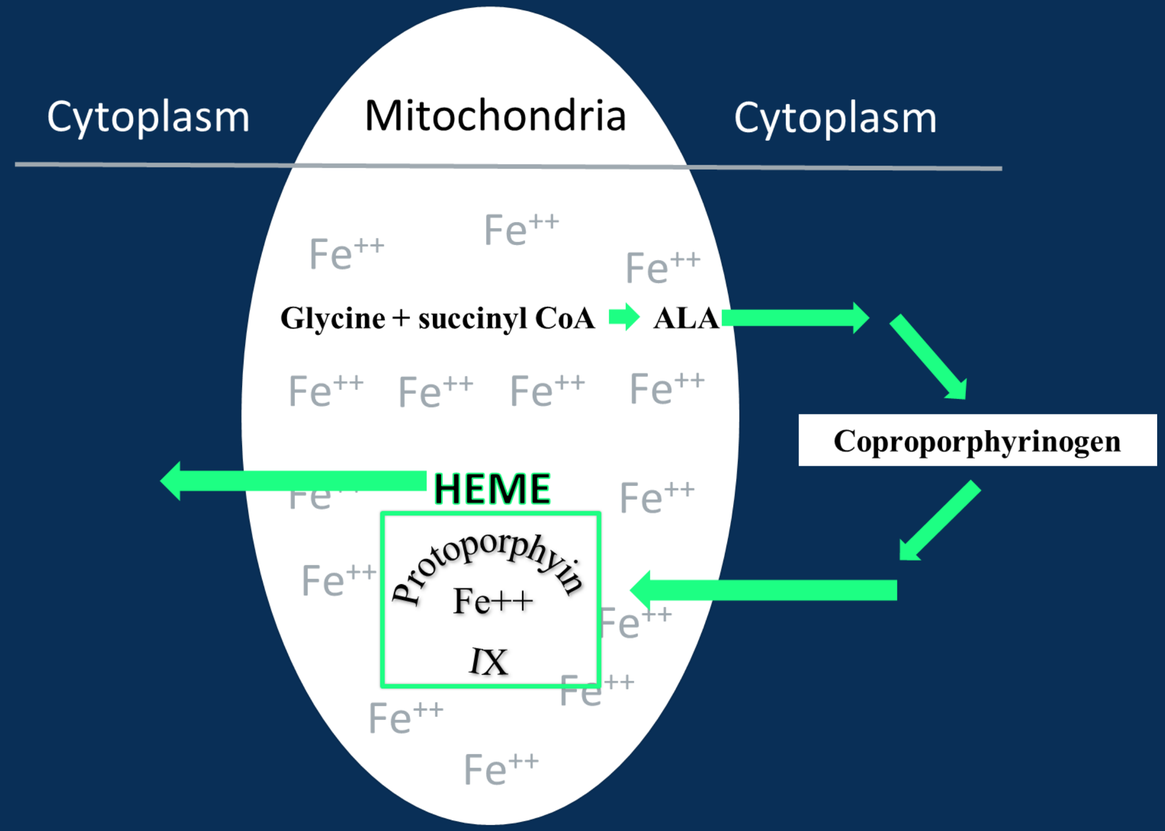
- Progressive iron overload
  - ↑ serum ferritin, % transferrin saturation
  - Excess iron deposits (stores)
- Hereditary (genetic mutations)
  - ↑ iron absorption in the gut + uncontrolled release to plasma
- Secondary (other hematology diseases)
  - Ineffective erythropoiesis + ↑ iron absorption in the gut

Abnormal Heme Synthesis Anemias

- Enzyme defects of heme production pathway
  - Abnormal heme sythesis
- Include
  - Sideroblastic anemias
  - Porphyrias
- ↑ iron stores, serum ferritin

Sideroblastic Anemias

- Mutation affecting formation of ALA
- Classified as hereditary or acquired
- All types characterized by:
  - ↑ total body iron
  - Ring sideroblasts in the BM
  - Hypochromic anemia



Sideroblastic Anemias

- Hereditary
  - Defective X-linked recessive gene, abnormal ALAS2
    - Carrier females: anemia rare, possible dimorphic RBCs
    - Males show signs/symptoms of SA
- Acquired (more common, idiopathic)
  - RARS (refractory anemia with ring sideroblasts)
    - Either sex affected, may terminate to leukemia
    - Secondary to drugs/toxins

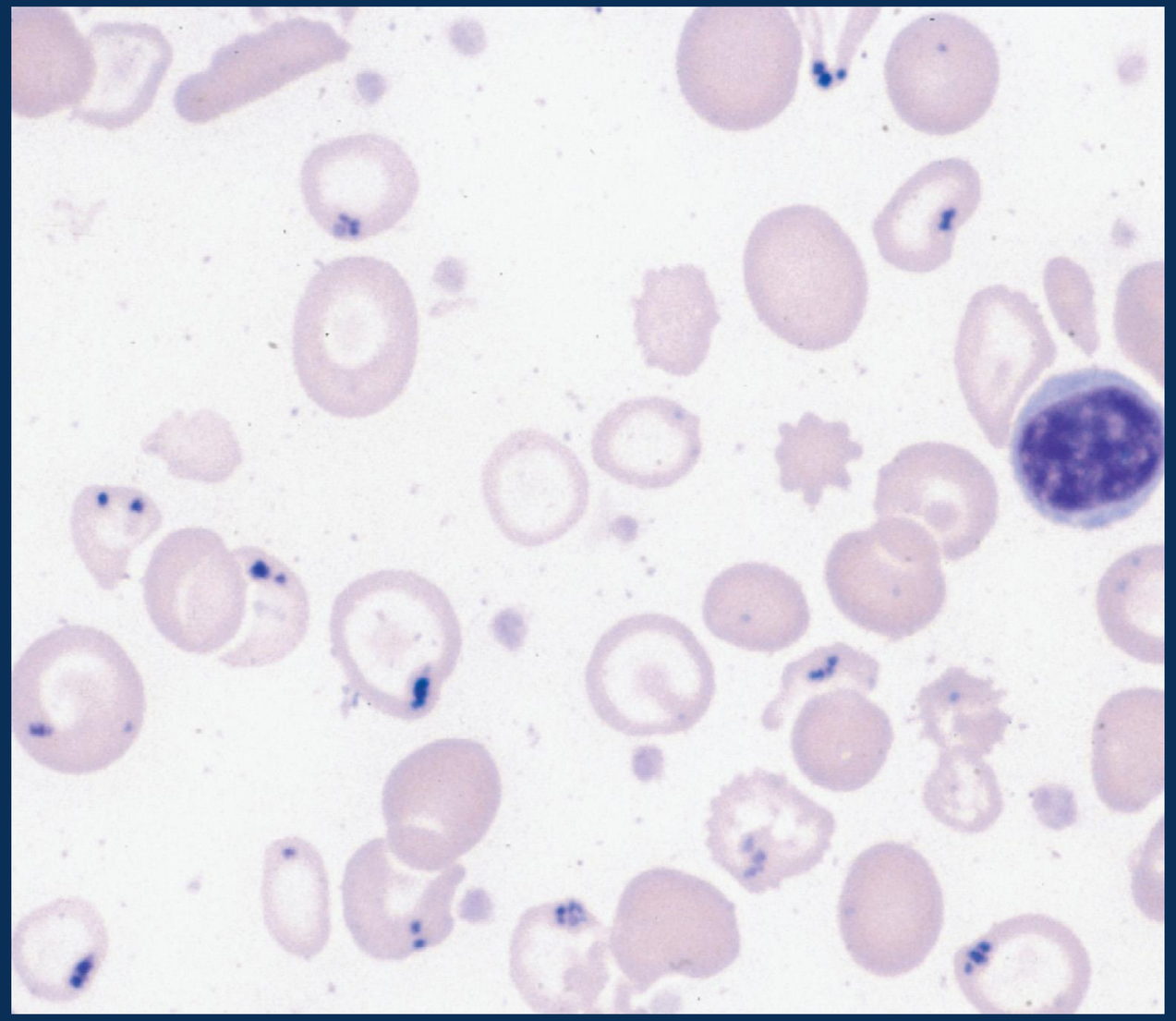
Sideroblastic Anemias

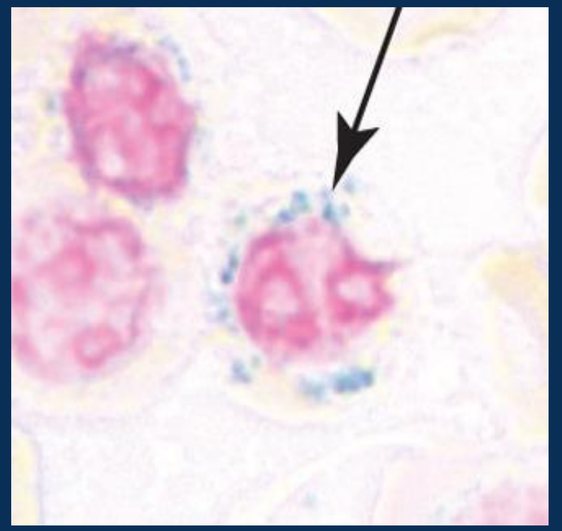
- Other Acquired (more common, idiopathic)
  - Lead poisoning (plumbism)
    - Children – lead paint ingestion
    - Adults – lead compounds inhalation
  - Alcoholism
  - Ringed sideroblasts associated with malignancy (not SA)

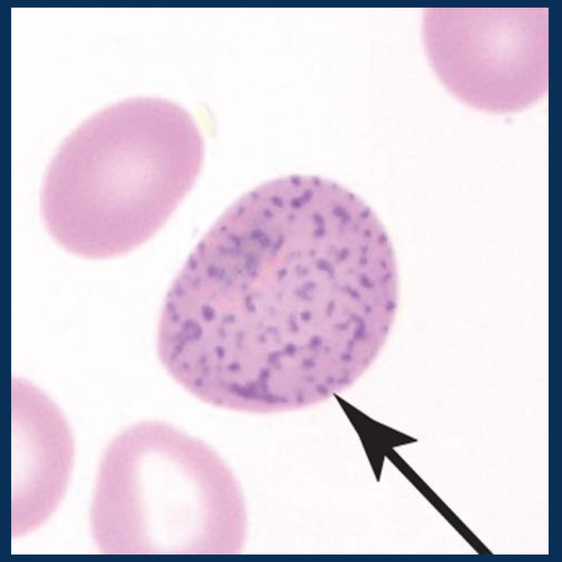
SA – Laboratory Features

- CBC
  - Moderate/severe anemia (with dimorphic RBC)
  - RPI < 2
  - Target cells, pappenheimer bodies, basophilic stippling
- Iron studies
  - ↑ serum iron, serum ferritin, % transferrin saturation
  - Normal to ↓ TIBC
- Bone marrow
  - Erythroid hyperplasia, ringed sideroblasts, ↑ macrophage iron stores

Sideroblastic Anemias

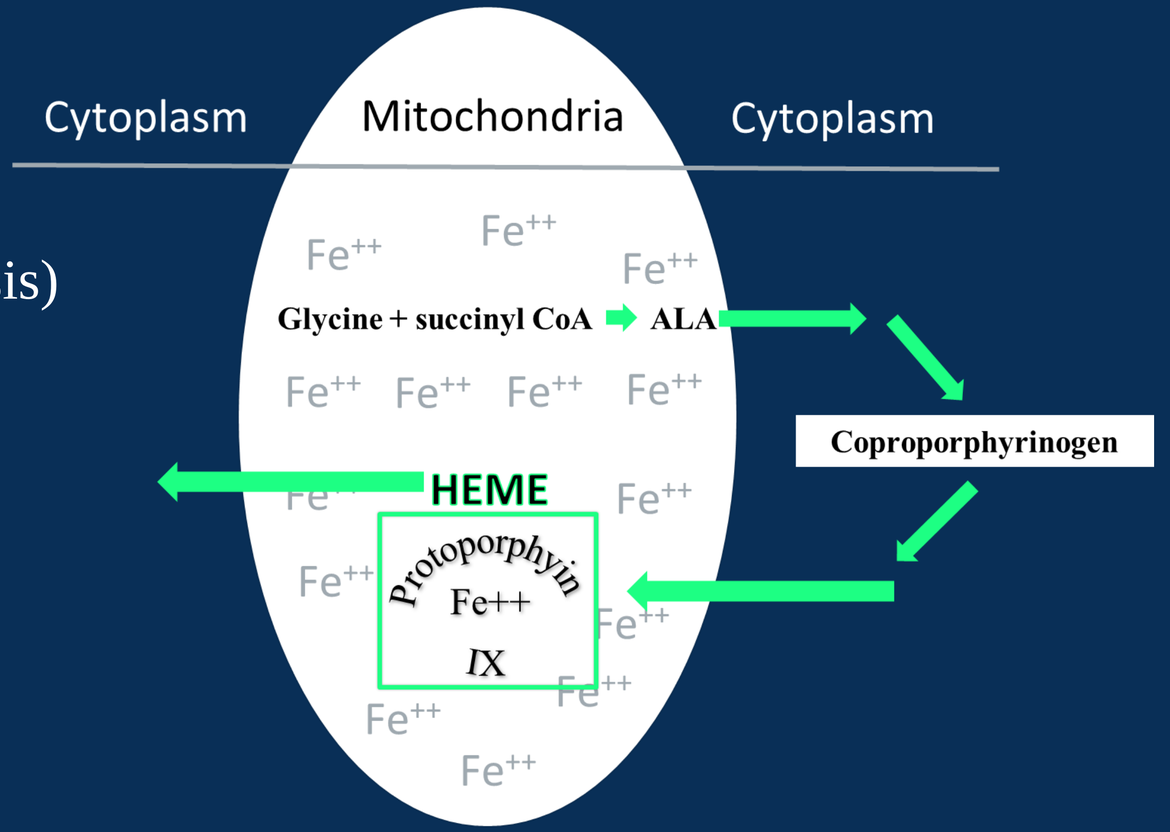






Porphyrias

- Block in porphyrin synthesis (review heme synthesis)
  - Accumulation of porphyrin heme precursors
    - Porphyrins are functionless
      - Fluoresce red with UV light
- Two erythropoietic porphyrias
  - Congenital erythropoietic porphyria (CEP)
  - Erythropoietic protoporphyria (EPP)



Congenital Erythropoietic Porphyria

- Excess of Type I porphyrins
- Urine pink to reddish brown
- Extreme sunlight photosensitivity
- Mild to severe hemolytic [normocytic] anemia
  - RBC lifespan ~ 18 days (↓ haptoglobin)
- ↑ anisopoikilocytosis, polychromasia + nRBCs
- BM erythroid hyperplasia
  - Erythroblasts fluoresce under UV light

Erythropoietic Protoporphyria

- Overproduction of protoporphyrin
- Photosensitivity not severe
- RBCs fluoresce intensely
- No hemolytic anemia
- Blood/BM ~ normal

References

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