**Phil Grimley, M.D.**
**Office: B3088, Phone: 295-3458**
**pgrimley@usuhs.mil**

# ANEMIA I

## I. INTRODUCTION: RED CELL DISORDERS & ANEMIAS

**A. What is Polycythemia ?** An increase in the concentration of RBCs in the circulation (Robbins p. 415). This may be relative (secondary) due to a decrease in plasma fluid volume or absolute (primary or true=p.vera) due to a real increase in the total body mass of RBCs. P.vera is a neoplastic condition and will be discussed with the myeloid leukemias. ***Erythroleukemia is rare.***

**B. How do we define anemia ?** In anemia the circulating hemoglobin (Hb) carried by erythrocytes (RBCs) is not sufficient for optimal oxygenation of vital tissues.

**C. How is the diagnosis determined ?** A general diagnosis of anemia is made based upon measurements of peripheral blood samples: RBC count, hematocrit and hemoglobin. Age and sex of the patient determine the normal ranges.

**D. As a physician what questions must I answer before I can treat a patient for anemia ?**

1. Is the anemia due to **recent hemorrhage**
   = acute and potentially self-correcting
2. Is the anemia due to a **nutritional deficiency**
   = dietary insufficiency or malabsorption of vitamins or iron
3. Is there a **failure of bone marrow function**
   = may be primary or secondary
4. Is this anemia due to an acute or chronic **hemolysis**
   **=** premature death of red cells

Anemia can be a sign of serious systemic disease or it may be the result of an easily corrected nutritional deficiency. This distinction requires more specific information to explain the pathophysiologic cause. This is the fundamental basis for treatment decisions.

**E. How will I determine the root of the problem ?** Information from the history, physical examination, and selected laboratory tests will lead to a correct diagnosis. The answer is patho~LOGIC.

1. **History:** Age & gender. Is there a source of blood loss: trauma, GI, Gyn, urine? Is there a family history of anemia (congenital anemia)

2. **Physical exam:** Is there a rapid pulse, heart murmur, large spleen or lymph nodes ? Is there conjunctival pallor or jaundice = icterus ?

**3. Complete Blood Count (CBC):** While much can be inferred from a positive history or physical findings, the pathophysiologic mechanism of an anemia must be verified by laboratory tests. A peripheral blood (PB) sample and CBC provides essential information. In most hospitals and clinics, an automated CBC provides the concentration of circulating Hb (gm/dL) , the packed cell volume (PCV) = hematocrit (HCT), the absolute cell counts of RBCs, white blood cells (WBCs) and platelets in a unit volume of blood (per μL = $mm^3$ ; or per L), and the relative percentages of different WBC types (= differential count). A reticulocyte count may be requested. RBC indices provide a critical interpretation of RBC data.

**2. Microscopy:** A PB film microscopic examination can yield valuable clues to the cause of anemia. For example, automated equipment will not detect RBC infestation by parasites or RBC shape changes associated with certain types of hemolytic anemia. A bone marrow (BM) examination can be important to evaluate BM failure.

**3. Other Relevant Lab Tests:** serum iron, transferrin (= iron transport protein), serum ferritin, serum $B_{12}$ , and serum erythropoietin (EPO).

## II. FUNCTIONAL ABNORMALITIES AND COMPLICATIONS OF CHRONIC ANEMIA

A. **Decreased $O_2$-Carrying Capacity Initiates Homeostatic Responses**

↑ pulse rate, ↑ cardiac output (↑ stroke volume) ensure oxygenation of vital organs

B. **Major Symptoms & Physical Signs** (in typical order of appearance)

*pallor, fatigue, rapid pulse* (hemodynamic change)
shortness of breath (SOB)
headache, tinnitus (ear ringing), dizziness
hemic heart murmur

angina pectoris (chest pain with exertion)
heart failure -- dependent edema (*depends on age and physical status*)

***heart failure in anemia is of high output type***

C. **Tissue Effects of Sustained Hypoxemia**

1. hydropic or fatty change: cells of renal proximal convoluted tubules, myocardium, liver
2. ischemic necrosis / apoptosis: cerebral cortex, hippocampus, basal ganglia, myocardium

## III. ESSENTIAL LABORATORY DATA [PB SAMPLES]

A. **Circulating Hb** = gm of Hb / dL [gm %]
measured by Hb conversion to cyanmet-Hb and spectrophotometry

B. **HCT** = volume of packed RBCs [ml/dL = %] - can be measured manually or electronically.

***dehydration, iatrogenic fluid overload or vasoconstriction following acute blood loss can give misleading HCT results.***

**Blood volume = 7% of body weight.**

**Total RBC mass = the blood volume x HCT.**

| | **Reference Ranges** | | **Anemia** | |
|---|---|---|---|---|
| **Age** | Hb (g/dL) | HCT (%) | Hb (g/dL) | HCT% |
| cord blood | 14-20 | 42-60 | <15 | <42 |
| neonate | 10-18 | 31-55 | <10 | <31 |
| pre-adolescent | 11-14 | 35-45 | <11 | <35 |
| adolescent ♂ | 13-16 | 37-49 | <13 | <37 |
| ♂ **> 18 y/o** | **13.5-17.5** | **41-53** | **<13.5** | **<41** |
| ♂ conditioned | 13.5 * | 41 * | | |
| ♀ > **12 y/o** | **12-16** | **36-46** | **<12** | **<36** |
| pregnancy (2nd & 3rd trimester) | 10-12 * | 32-34 * | <10 | <30 |

* decrease is due to body fluid redistribution

**C. Reticulocyte Count = count of RBCs newly released into the PB**

Polyribosomes persist for 3-4 days after nuclear extrusion from normoblasts. and stain blue with a supravital dye (see lab demo). In Wright-Giemsa stains, the reticulocytes appear as "polychromatophilic macrocytes" (= polychromasia).

Counting retics: manual = % per $10^3$ mature RBC's,
reference range = 1 ± 0.6 %.
flow cytometry reference range = 25-85 x $10^3$ / µL

↑ **retics** = active replacement of RBCs (sign of EPO activity)

↓ **retics** = deficient RBC production **= diminished erythropoiesis**

## IV. ERYTHROCYTE INDICES HELP TO DETERMINE THE CAUSE OF DIMINSHED ERYTHROPOIESIS

**A. Erythrocyte Indices** are determined from the Hb, HCT and RBC count
Reference range o f RBCs = 4-6 x $10^{12}$ / L (age and gender dependent)

**B. Mean RBC size ( MCV) and mean RBC Hb concentration (MCHC)** are major clues to the mechanism of anemia.

**RBC Size** = Mean Corpuscular Volume **[MCV]**
[HCT / RBC count x $10^{-6}$] X 10 = $\mu m^3$ (fl)
**reference range 76 to 100 fl** (normal = normocytic)

**Hb Concentration** = Mean Corpuscular Hb Concentration **[MCHC]**
[Hb / HCT X 100] = gm/dL or %
**reference range 32 to 36 gm/dL**
(normal = normochromic)

☞ ↓ **MCV or ↓ MCHC indicates deficient Hb biosynthesis**

**C. RBC Distribution Width [RDW]** = standard deviation of RBC size
RDW >14 % = abnormal size variation = anisocytosis

☞ **an ↑ RDW is typical of advanced iron deficiency anemia**

## V. DIMINISHED ERYTHROPOIESIS = RBC PRODUCTION IMPAIRMENT

### A. ANEMIAS DUE TO BONE MARROW FAILURE
**typically normocytic (normal MCV)**
**normochromic (normal MCHC)**

1. CD34 multiipotent progenitor cells
   CD 34 cells can form xenograft colonies in spleens of irradiated mice or in tissue culture
   ~30 cells are required to repopulate the entire BM of a radiated mouse
   ☛ **CD = Cluster Designation antigens**. Antigenic proteins in the surface membranes of hematopoietic **stem cells and lymphocyte** progeny have been assigned numbers to replace a variety of original names. Historically, most surface antigens of RBCs were described a half century earlier and retain alphabetical designations or eponyms used in transfusion medicine: e.g. A, B, O, Rh, Duffy, Kell etc.

2. Trilineage myeloid stem cells (CFU-S)
   These are **self-renewing** and vital for continuous replacement of deciduous RBC's

3. Committed erythroid precursors (CFU-E) *"the workaholics"*
   **EPO** stimulates maturation
4. intramedullary RBC maturation from the CFU-E requires 3-5 days
5. reticulocytes enter the PB: mature in 3-4 days

## B. PRIMARY BM FAILURE = FAILURE OF STEM CELL or PRECURSOR CELL FUNCTIONS or PHYSICAL LOSS OF STEM CELLS

. **1. Aplastic Anemia**:= suppression of CD34 stem cell activity

a. Spontaneous: up to 50% of cases with uncertain cause = **idiopathic**
   A tragic problem in children, adolescents or young adults

b. Direct damage to stem cells
   1). B-19 parvovirus infection in children - other DNA viruses
   2). whole body ionizing radiation or systemic chemotherapy **= iatrogenic**
   3). toxic chemicals (environmental) or drugs (iatrogenic).

c. T cell-mediated cytotoxic damage to stem cells
   1). reaction to DNA virus infections, hepatitis C
   2). hapten effect of drugs or intermediate metabolites

**Hypothesis of immune pathogenesis:** Activated T suppressor cells eliminate pathologically altered stem cells, but normal stem cells are killed as "innocent bystanders". IFN-γ or TNF-α released by the T cells play a role in apoptosis of the BM stem cells. This hypothesis is the basis for treatment with anti-lymphocyte globulins or immunosuppressive drugs (reviewed in NEJM 336:1365, 1997).

**Pure red cell aplasia =** a rare condition. Antibodies to EPO prevent maturation of red cell precursors, incidence increasing in patients receiving EPO therapy

**Congenital aplastic anemias** = rare congenital stem cell defects
Fanconi anemia = a DNA repair defect with apoptosis of RBC precursors

**2. Diagnostic features of an aplastic anemia**

1. CBC: persistent decline of HCT with ↓↓ reticulocytes ( < 25 x $10^3$ / μL),
   **WBC's (< 200 μL), and platelets (<20,000/ μL)**
   **normochromic, normocytic anemia**
2. BM aspirate → "dry tap"
   trephine biopsy: **hypocellular** (excessive fat),
   normal or ↑ iron stores
   **pancytopenia** = all precursors ↓↓**:** erythroid, myeloid, platelet
3. serum: ↑↑ **EPO** (measured by immunoassay)

4. clinical signs:

**petechia** = superficial capillary bleeding due to platelet deficiency

opportunistic infections due to WBC deficiency

**spleen should be normal or small size !!**

**3. Rx and Prognosis:** Immunosuppression, growth factors to stimulate stem cells or BM transplantation. Disease may resolve, persist for long periods, or transform to leukemia due to evolution and selective survival of an abnormal stem cell clone (**Fig. 14-26 in large Robbins**). Strict isolation procedure often necessary.

## C. SECONDARY BM FAILURE = MALFUNCTION DUE TO A SYSTEMIC DISEASE

**1**. Replacement Anemia = "Myelophthisic" or"Leukoerythroblastic"

causes **space occupation** by abnormal tissue

and / or a **microenvironmental perturbation**

a. Examples:

1) fibrosis or granulomatous inflammation in BM stroma
2) neoplasia in BM: metastatic carcinoma, lymphoma
3) genetic lipid storage disease (e.g. Gaucher's)

b. Compensatory pathophysiology

= **extramedullary hematopoiesis**

= recapitulation of fetal hematopoiesis

☞ **results in hepatosplenomegaly**

c. Diagnosis:

1) CBC: **normochromic normocytic anemia**

"shift to left"

**many "tear drop" RBCs =** dacryocytes

2). BM aspiration or biopsy: to determine cause

3). radiographic bone scan for lesions

**2. Anemia of Chronic Systemic Disease = a chronic depression of BM function** ☞ ***very common in clinical practice***

a. Examples of clinical settings:

1) chronic **renal failure**

2) chronic inflammatory process: **rheumatoid arthritis**, **inflammatory bowel diseases**

3) **neoplasia** such as breast or lung cancer but **not** due to metastasis

b. Multifactorial pathogenesis

1). ↓ **EPO** production due to renal failure

2). CD34 cytotoxic damage: ↑BUN, ethanol

3). **failure of normal iron transfer to normoblasts**

4) ↓ serum transferrin in chronic liver disease

5). **autoantibodies** cross-react with CD34 cells

6). **TNF-α or IFN inhibition of stem cells** in cancer or chronic inflammation

c. Diagnosis: exclude all else

1) BM: **cellularity often normal**, iron stores may be excessive@

2). PB: ↓ **reticulocytes**, RBC's often thin (reduced protein)-- appear hypochromic

3) Serum: ↓ **EPO**, ↓ transferrin, ↓ Fe,
@ ↑ **serum ferritin**

d. Rx:

1) treat the underlying systemic disease

2). **recombinant EPO in chronic renal disease**

**D. MEDICAL TERMS RELATED TO DIMINISHED RBC PRODUCTION**

1. **Ineffective Erythropoiesis**
   = Stem cell maturation is delayed or arrested
2. **Intramedullary hemolysis** - maturing RBCs are destroyed before release into the PB due to apoptosis or shape distortions
   ☛ anemia of chronic disease, megaloblastic anemia, Pb poison
3. **"shift to the left"**
   = **premature release of RBCs or WBCs** from the BM
   a. Excess nucleated RBCs in PB indicates severe BM stress
   b. Excess nucleated RBCs + immature WBCs in PB
   **= Leukoerythroblastosis** characteristic response to replacement of the BM by space occupying tissues such as cancer, infectious processes or fibrosis

## VI. BONE MARROW [BM] EXAMINATION FOR EVALUATION OF ANEMIA

**Aspiration or trephine biopsy**

used to search for microscopic evidence of neoplasia, fibrosis or infection (including bacteriologic cultures)

**A. Cellularity:** normally 80-30% in axial marrow (decreases with age)

**B. Cytology**: normal = progressive maturation of each committed lineage

Myeloid series: ~ 75% Erythroid series: ~ 25%

***M::E ratio*** *in range of 3:1*

Megakaryocytes < 0.1% but large and very obvious

**C. Iron stores:** may need to evaluate for diagnosis of deficiency

**Phil Grimley, M.D.**
**Office: B3088, Phone: 295-3458**
**pgrimley@usuhs.mil**

# ANEMIA II

## I. HEMOLYTIC MECHANISMS IN ANEMIAS

### A. Lifespan and Catabolism of Circulating RBCs

1. RBC half-life is 60 days (4 month life-span)
2. membrane phospholipid changes signal RBC catabolism
3. catabolism is predominantly extravascular
   [RE system of mononuclear phagocytes]
   bilirubin and iron are important catabolic products

### B. Definition of hemolytic anemia:

an accelerated (premature) RBC destruction

1. ↓↓ RBC survival van be shown with $^{51}$Cr-tagged RBC's
   ***the mean ½ life of circulating RBC's may decrease to < 20 days before anemia becomes manifest.***
2. Healthy BM can expand up to 8 X

**Destruction of senescent RBCs normally occurs in the splenic or hepatic sinusoids. This extravascular (EV) hemolysis accounts for ~90% of blood cell destruction. RBC lysis within blood vessel lumina or intravascular (IV) hemolysis normally is minimal.**

### C. Pathophysiology

1. Intravascular hemolysis (IVH): premature RBC lysis occurs within the blood vessels: much of the free Hb binds to haptoglobin and is catabolized in hepatocytes. Excess free Hb dimers filter through the renal glomeruli.

A major danger is acute renal failure: ***glomerular damage*** results from trapped RBC stroma, while ***renal tubules*** fill with Hb or bile casts. There may be a rapid onset of jaundice due to ***unconjugated bilirubinemia.*** In hemolytic disease of the newbom ***kernicterus*** is a grave complication. IRON STORES IN the BM ARE NORMAL OR EXCESSIVE

2. Extravascular hemolysis (EVH): accelerated RBC destruction by the RE system: increased biliary excretion of ***conjugated bilirubin***. The urine ***urobilinogen*** may increase***.*** There is a risk of cholecystolithiasis with formation of bilirubinate "pigment" stones. Secondary splenomegaly exacerbates hemolysis (a "catch 22").
   *an enormous "big-bad spleen" can occur in.*
   a. Kala-azar (leishmaniasis)
   b. malaria
   c. chronic myelogenous leukemia (CML)

d. chronic auto-immune disease: rheumatoid arthritis

The increasing mass of RE cells accelerates hemolysis "bureaucracy" effect

3. Intrasplenic "sequestration" or "pooling" of abnormal RBC's can exacerbate anemia.

## D. RELEVANT NORMAL PHYSIOLOGY OF RBCs

1. **The mature RBC is a deciduous wafer**. Extrusion of the nucleus helps it to travel light on its 300 mile daily flight through the microcirculation.
2. **A cytoskeleton of spectrin and actin** sustains the flexible biconcave shape required for laminar flow in capillaries. Shape abnormalities (= poikilocytosis) accelerate RBC destruction
3. **RBC energetics require ATP**. Absent a nucleus and organelles, the mature RBC is stripped down metabolically. It no longer synthesizes new proteins, and survives using free energy released by enzymatic degradation of phosphorylated glucose in the Embden-Meyerhof fermentative pathway. ATP maintains critical levels of 2,3 DPG (diphosphoglycerate) as well as integrity of the RBC membrane and cytoskeleton. These are important factors in sustaining RBCs during storage for transfusion.
4. **Survival from oxygen toxicity.** RBC's are HAZMAT carriers: they must transport $O_2$ without being burned alive. The hexose monophosphate alternate pathway of glycolysis (HMP shunt) generates reduced glutathione. This is the fire extinguisher which maintains Hb in a reduced state and as an effective $O_2$ carrier. ***Defects in the HMP shunt are the most common cause of hemolytic anemia worldwide.***
5. **RBC catabolism occurs in reticuloendothelial cells (RE cells)**
   a) Spleen has a pitting function - removal of RBC nuclear remnants
      RE cells probe RBCs as they traverse cords of red pulp to exit via thin-walled sinusoids. They clean out denatured Hb; and remove DNA remnants in young circulating RBCs
   b) Trapping and culling of RBCs occurs in the spleen and liver:
      senescent RBCs - membrane cholesterol or phospholipid changes are a signal for RBC trapping by RE cells.
      abnormal RBCs - cells with deformed shape: e.g. sickle cells, codocytes, RBCs with surface bound autoantibodies

# II. General Diagnostic Features

A. CBC: ↑↑ **reticulocytes**, nucleated RBCs

RBC microscopic examination may detect abnormal shapes

***spherocytosis*** = hyperchromic RBCs (↑ MCHC) due to membrane damage or loss

B. BM: **erythroid hyperplasia**

C. Coombs (antiglobulin) test: **++ in immunohemolysis**

D. Serum biochemical abnormalities:

***in acute IVH***

1. ↑ **unconjugated bilirubin** (total >1 mg/dl)
2. ↓ **haptoglobin ($\alpha_2$-globulin)**
3. ↑ plasma Hb  d. ↑ LDH (isoenzymes 3 & 5)

***in chronic EVH***

4. ↑ **conjugated bilirubin** (direct >0.2 mg/dL) due to overload
5. Urine:
   a) in ***acute IVH***
      i. free Hb, + bilirubin
      ii. **urine hemosiderin** is a delayed sign
   b) in ***chronic EVH***:
      i ↑ **urobilinogen**

E. **Examples of Disease Mechanisms of Hemolysis**

1. Intracorpuscular = intrinsic RBC defects --- mostly *genetic*
   a. metabolic enzyme defects (*genetic*) - most common
   b. hemoglobinopathies → shape defects (*genetic*)
   c. cytoskeletal disorders: spherocytosis or elliptocytosis (*genetic*)
   d. paroxysmal nocturnal hemoglobinuria (PNH) → membrane receptor defect (*acquired*)
   e. alcoholic cirrhosis → membrane defect and macrocytosis (*acquired*)
2. Extracorpuscular - extrinsic RBC damage --- usually *acquired*
   a. non-immune RBC damage
      i. circulatory trauma
      ii. certain infections: malaria
      iii. poisons or toxins
   b. antibody-mediated (Coomb's test = DAT positive)
      i. immune complex reaction (type III hypersensitivity)
      ii. drug-related haptens or immune complexes
      iii. response to infections
      iv. transfusion incompatibility (type II hypersensitivity)
         maternal reaction to fetal Rh factor = ***erythroblastosis fetalis***

E. **Complications:** BM overactivity may deplete folate and cause an ***aplastic crisis***.

***Cutaneous leg ulcers*** may develop due to microcirculatory

disturbance by spherocytes, target cells or sickle cells***. Iatrogenic iron overload*** from repeated blood transfusions can cause cardiac problems.

## II. SPECIFIC EXAMPLES OF EXTRACORPUSCULAR HEMOLYTIC ANEMIAS

A. **Physical or Circulatory Trauma** *mainly IV H*

1. Thermal, impact or crush injuries
   examples: third degree burns, march hemoglobinuria
2. Cardiac turbulence
   example:. calcified aortic valve or prosthetic valve
3. Peripheral vascular turbulence = **microangiopathic hemolytic anemia:** DIC, polyarteritis

B. **Infectious Agents**

1. Acute IVH
   a. bacterial septicemia (often combined with DIC)
   b. C. perfringens (acute RBC membrane damage by lecithinase-toxins)
   c. **Falciparum malaria ("blackwater" fever)**
   d. Bartonella (Oroya fever) - sandfly vector in S. America
2. Chronic EVH
   a. all types of malaria
   b. other infections with splenomegaly

C. **Toxic Hemolysis**: e.g. reptile venoms (acute IVH), Pb (chronic EVH)

D. **Immunohemolysis - *usually chronic EVH***

1. Hemolysis is proportional to cell-bound γ-globulin or complement (β-globulin).
   a. **specific = type II hypersensitivity - opsonization by Ab and complement increases phagocytosis of RBCs**
   b. **non-specific = type III hypersensitivity - immune complex binding to RBCs**
2. Types of autoantibodies
   a. **optimal @ 37°C = IgG warm agglutinins = 70% of cases**
      40% are against RBC Rh system antigens
      the IgG coated RBCs bind to $F_c\gamma$ receptors on splenic macrophages and are removed from circulation
   b**. optimal @ < 30°C = cold agglutinins = 30% of cases**
      ***typically IgM -*** cause RBC agglutination + C-fixation
      ***1/3 - due to m. pneumoniae infection (anti-I)***

1/10 - due to ***infectious mononucleosis*** (anti-i)

1/6 - due to an ***IgG anti-P*** (= Donath-Landsteiner antibody)

☞ *clinical signs of acrocyanosis, Raynaud's phenomenon*

3. Drug-induced hemolysis

a. IgM immune complex and complement interaction near RBC surface = Type III hypersensitivity, RBC is an innocent bystander.

☞ *examples: quinidine, phenacetin, sulfonamides*

b. drug absorbed on RBC membrane acts as a hapten

stimulates IgG antibody →opsonization & phagocytosis

☞ *examples*: *penicillin, cephalosporin antibiotics*

## III. EXAMPLES OF INTRACORPUSCULAR HEMOLYTIC ANEMIAS

A. **Hereditary Spherocytosis** - ***chronic EVH***

1. Mechanism: spleen traps spherocytic RBCs
2. Specific cause: absent or defective spectrin causes membrane instability

   autosomal dominant mutation (220 cases / $10^6$ humans)
3. Diagnosis:

   a. family history

   b. CBC: ***spherocytes*** (10-30%), MCHC ↑

   c. ↑ ***RBC fragility*** **in hypotonic salt** - due to lack of reserve surface area

B. **G-6-PD Deficiency ("Heinz Body Anemia")** - ***acute IVH***

1. Mechanism: Abnormal Hb oxidation due to a deficiency of reduced glutathione (GSH)

   a. RBCs are vulnerable to stress produced by oxidant drugs, infections, acidosis

   ☞ *sulfonamides, phenacetin, primaquine, nitroglycerine*

   b. sudden episodic hemolysis occurs within 24 h after stress

   c. normal reduced Hb ($Fe^{++}$ oxyhemoglobin) becomes methemoglobin ( $Fe^{+++}$)
2. Specific cause: X-linked mutation of glucose-6-phosphate dehydrogenase (G-6-PD)

   expressed in the hemizygous ♂ :

   G-6-PD is abnormally folded: enzyme function is lost.

   a. A - mutation ⟶ only 7-15% of normal enzyme

remains in aged RBCs

**8-20% of West Africans, 10 % of Americans with African ancestry**

b. Mediterranean variant*:* mutation produces ↓↓ G-6-PD both in young and old RBC's. **Clinically more severe**, including cases of ***favism*** from eating fava beans

3. Diagnosis:
   a. family history or testing of an RBC sample for G-6-PD activity: [immunofluorescence with a specific antibody or enzyme quantitation by electrophoresis is used as a military induction screen]
   b. *Heinz bodies* in RBCs (= accumulated metHb) seen with crystal violet stain
   c. spherocytosis and "bite cells" due to splenic pitting of Heinz bodies (**Fig. 14-8 large Robbins**)

C. **Paroxysmal Nocturnal Hemoglobinuria (PNH)** - *IVH*

1. Mechanism
   a. defect of protein anchoring to RBC surface membrane (**Fig. 14-16 large Robbins**)
      ↑↑ sensitivity to C3 lysis (alternate complement pathway)
   b. lysis initiated by circadian ↓ in pH.
2. Specific cause: somatic mutation of *PIGA* in pluripotent stem cell
3. Diagnosis:
   a. **intermittent hemoglobinuria, hemosiderinuria**
      **–** often after waking in AM
   b. activation of RBC lysis by acid serum or sucrose solution
4. Complications
   a. risk of thrombosis/thromboembolism
   b. iron deficiency due to urinary loss
   c. aplastic anemia: 25% of adults, >50% of adolescents/children (NEJM 325:991, 1991).
   d. ↑ **risk of myelogenous leukemia = preleukemic condition**

D. **Chronic Liver Disease (alcoholic cirrhosis) -** *EVHs*

1. Hemolytic Mechanism: spleen trapping
2. Specific cause: lecithin-acyl transferase deficiency → ↑ RBC membrane cholesterol

E. **Hemoglobinopathies -** *EVH*

1. Hemolytic Mechanism: spleen trapping due to RBC deformity / size changes

2. Primary Causes

a. ↓ biosynthesis of globin chains (= ***thalassemias***)

b. mutant globin chain polypeptides (*e.g.* ***sickle cell disease***)

## IV. MAJOR HEMOGLOBINOPATHIES

A. **Thalassemia Syndromes** - gene mutations originated in malarious seacoast regions of Eurasia

*affect US populations of Mediterranean or southeast Asian ancestries*

☛ ***all thalassemias are globin chain deficiency diseases, there is no structural abnormality of the globin chains***

1. β-thalassemias: there is **1 pair** of β-globin genes. ***Mutations in non-coding regions alter β-globin gene transcription or mRNA processing***

β-globin mRNA is ↓ in $\beta^+$ mutations

**absent** in **β°** mutations

2. α-thalassemia syndromes: there are 2 pairs of α-globin genes.

gene **deletions** account for most cases of reduced or absent α-globin chain

B. **β-Thalassemias - *severity is variable due to unique expression of each β-globin gene mutation.*** > 50 mutations are known

fetal diagnosis is possible if the β-globin gene mutation is linked to a familial DNA polymorphism. Some point mutations involve a restriction site which can be mapped directly.

1. Heterozygous mutations

e.g. thalassemia minor (trait): β **/** β**+**

"intermedia": β**+ / β+**

PB: ***target cells***, anisocytosis [RDW > 12]

Hb electrophoresis: ***Hb $A_2$ > 5 % of total***, ↑ ***Hb F***

☛ **δ-globin or γ-globin substitute for the ↓ β-globin**

2. Homozygous mutations = Cooley's anemia = thalassemia major

**β+/β° or β°/β° (lethal)**

a. manifests in infancy/early childhood: ***jaundice, abnormal bone growth***

b. BM: expansion causes ***skull "hair-on-end"*** (**x-ray in Fig. 14-14**)

c. PB: excess α-globin chains precipitate ➜ RBC crystals and exacerbate EVH

d. Hb electrophoresis: ***Hb $A_2$ >> Hb A***, Hb F ↑↑↑

C. **α-Thalassemia Syndromes (self-study)**

Two pairs of α-globin genes (4 alleles) regulate α-globin synthesis

. ☛ ***Mutations delete one or more genes.***

1. Heterozygous (= trait) in Americans of African or Asian ancestry
   a. 2/4 α-globin genes deleted (*in "trans" positions*)
   b. anemia minimal, but can mask folate or $B_{12}$ deficiencies
2. Hb H disease
   a. 3/4 α-globin genes deleted
   b. hemolysis and microcytic anemia
   c. Hb H ($\beta_4$) = an abnormal complex of 4 β-globin polypeptides
      RBC inclusions exacerbate hemolysis
3. Hydrops fetalis - lethal *in utero*
   a. no α-globin genes → absent mRNA for α-globin
   b. 80% show **Hb Bart's** in cord blood = *abnormal complex of 4 γ-globins)*

D. **Sickling Hemoglobinopathies** most important = homozygous Hb S

***first described by Herrick (1861-1954) in Chicago***

1. Hb S point mutation: A → T at position 6 in gene
   glutamic acid → valine = Hb S (*discovery by Linus Pauling)*
   → lysine = Hb C

***There is a survival advantage of HbS trait in regions of high falciparum malaria and mutations arose independently at least 3 times in equatorial Africa during prehistory. Sickle mutations are widespread in Africa, the Mid-East and Southern Mediterranean regions.***

2. Variable gene combinations: allelic Hb genes on paired chromosomes are *autosomal codominants*, each RBC thus contains an equal mixture of Hb molecules.

**β-chain with HbS mutation may combine with**:

a. normal β-chain of Hb A (sickle trait)
b. mutant β-chain of Hb S (sickle disease)
c. mutant β-chain of Hb C (SC disease)
d. a β-thalassemia mutation (= S thal)

E. **Molecular Pathophysiology of RBCs containing Hb S:**

1. Sickle cell trait: ***Hb S & Hb A are equal on electrophoresis.*** The 50% Hb A in each RBC inhibits sickling under normal $O_2$ tension. ***Thus, carriers should not be anemic, do not hemolyze, and normally are usually asymptomatic***. There is danger of exertional collapse in conditions of excess heat and dehydration (➢ Am Fam Physician 54:237, 1996), hypoxemia during anesthesia, or prolonged low pressure in aircraft: temporary sickling may cause splenic infarcts.
2. Sickle cell anemia (Hb SS)

   **Abnormal cyclization of β-globin chains follows deoxygenation**: pairs of deoxy-HbS molecules interlock to form insoluble polymers. **Hb gelation and elongation causes sickle deformation of RBC.** Initially this can be reversed by $O_2$, but **repeated membrane damage leads to irreversible sickling and enhanced adhesion of RBCs to microcapillary endothelium** impedes laminar blood flow. Metabolic acidosis and capillary sludging intensifies local hypoxemia, perpetuates sickling. **Result is vaso-occlusion**.

   . Anemia begins from the time of birth. **BM expansions reflect compensatory erythroid hyperplasia. Painful swelling of hands or feet in children = "hand-foot" syndrome.**. Repeated infarcts of the spleen reduce its size such that there is **no functional spleen in adults**. Femoral heads are highly sensitive to **aseptic necrosis** due to blood sludging in small vessels and bone infarcts may be followed by **salmonella osteomyelitis**. Repeated infarcts of kidney and glomeruli reduce renal function and **ability to concentrate urine diminishes**. CNS infarcts = strokes are major risks. **Acute chest syndrome** = fat embolism to lung from marrow infarct +/- infection. **S. Pneumonia infections are common.**

3. Hb S / β-thalassemia (Hb S > A by electrophoresis). Patient inherits two mutant alleles, one for Hb S and one for β-thalassemia. With up to 35% Hb A, the clinical course is much milder than in Hb SS anemia. **PB smear may show both sickle and target cells**.

4. Hb SC Co-dominant alleles (Hb S = Hb C by electrophoresis). More compatible with long life than homozygous SS anemia.. Infarcts are fewer. In women, SC disease is often silent until parturition when pulmonary or placental infarcts develop.

**F. Diagnostic tests for discussion in laboratory**

1. Identification of a sickling hemoglobin
   a. morphology: sickled cells on smear
   b. metabisulfite prep: sickling is provoked by sealing blood under a cover slip and adding 2% *sodium metabisulfite* which chemically deoxygenates Hb and causes RBC's with Hb S or Hb C to assume the abnormal shape.
      ☛ ***this test detects any sickling trait or disease***
   c. differential solubility (*reduced Hb S insoluble*)
      ➢ ***does not distinguish between sickle disease (SS) and trait (AS)***
   d. electrophoresis (Hb dimer migrations due to molecular charge)
      ➢ ***diagnostic - discriminates Hb AS or AC from SS or SC***.
2. Prenatal diagnosis: DNA is extracted from chorionic villi. Part of the β-globin gene is amplified by PCR, A specific cDNA probe detects the normal locus.

G. **Therapies**

1. Vaccination of infants to prevent S. pneumonia infections which exacerbate disease
2. Low dose hydroxyurea increases Hb F productions
3. Allogeneic stem-cell transplantation: prior to age 16
4. Patients can survive several decades with supportive medical care (**reviewed in NEJM 340:1021-1030, 1999**)

H. **Hemoglobinopathies in Americans of African Ancestry**

| Condition | Genotype(s) | Incidence |
|---|---|---|
| α-Thal trait | α-thal / -- | 30% |
| Sickle cell trait | AS | 8-14% |
| Hb C trait | AC | 2-3% |
| β-Thal trait | $\beta^+$-thal / -- | 1% |
| Sickle cell anemia | SS or SC | 0.4-1.4% |

☞ **sickle or thalassemia traits also may be combined with G6PD deficiency**

I. **Hemoglobinopathies in Americans of Asian ancestry**

1. hemoglobin E - homozygous or combined with other mutations
2. α-thal trait - found in 5% of Hong Kong residents

**Phil Grimley, M.D.**
**Office: B3088, Phone: 295-3458**
**pgrimley@usuhs.mil**

# ANEMIA III

## II. DIMINISHED ERYTHROPOIESIS: RESTRICTED DNA REPLICATION = *megaloblastic anemias , macrocytic RBC's*

A**. Major Causes:** ↓↓ availability of folic acid <u>or</u> vitamin $B_{12}$

B. **Biochemical Pathogenesis: deficient regeneration of tetrahydrofolate** (Fig. 14-21 large Robbins**)**,
also DNA antimetabolites in cancer Rx

C. **Effect on Cell Division Cycle:** S phase is impaired but RNA transcription proceeds. A delay in cell division results from an ↑ratio of RNA / DNA

D. **Pathophysiology: ineffective erythropoiesis and extravascular hemolysis** result of apoptosis in BM, macrocytosis and abnormal shapes of RBCs

E. **General Diagnostic Features:**

1. <u>BM:</u> **hyperplasia**: with ↑ volume and hypercellularity
   **megaloblastic erythropoiesis** = Nuclear / Cytoplasmic size dissociation
   **giant white cell precursors**
2. <u>CBC:</u> **macrocytosis** (MCV >100 $\mu m^3$), **normal MCHC**
   ovoid macrocytes are typical in $B_{12}$ deficiency
   ↓ reticulocytes (this is an exception to other hemolytic anemias, see Anemia III)
   ☛ mild leukopenia, thrombocytopenia
   **hypersegmented PMNs** [ **> 5 lobes**] **= macropolycytes** (**Fig 14-18**)
3. <u>Serum:</u> **folate < 2 ng/ml** or **$B_{12}$ < 200 pg/ml**
   hyperbilirubinemia and ↑ urinary urobilinogen due to **excess RBC catabolism**
   ↑ LDH isoenzymes 3 & 5 reflect the intramedullary hemolysis

F. **Primary Folic Acid (*Pteroylmonoglutamic Acid*) Deficiency**

1. <u>Folate sources:</u> leafy vegetables, liver, kidney, dairy products
   *thermolabile folylpolyglutamates destroyed by excess cooking*
2. <u>Absorption:</u> ingested folylpolyglutamates are split into monoglutamates by small bowel conjugases. The

monoglutamates are absorbed best in the proximal jejunum.

3. Causes:
   a poor diet, ethanol effect, intestinal malabsorption
   b. ↑ metabolic demand during pregnancy or chronic hemolytic anemia
   c. anti-folate cancer chemotherapies
4. Rapid pathophysiologic progression: ***folate storage pool is relatively small***

   3 weeks: ↓ **serum folate**

   7 weeks: **hypersegmented PMNs**

   12-18 weeks: megaloblastic BM, ↓ RBC folate

   18-20 weeks: obvious macrocytic anemia.

G. **Vitamin $B_{12}$ (Cobalamin) Deficiency**

1. $B_{12}$ sources: product of microbial action in animal tissues
2. $B_{12}$ Absorption: couples to "R binding protein" in gastric juice, then released in upper small bowel by pancreatic enzymes → **complex with intrinsic factor (IF)**
3. IF is a glycoprotein secreted by gastric body parietal cells.

   **IF-$B_{12}$ binds to ileal receptors**

   transcobalamin II (serum β-globulin) transports $B_{12}$ to liver & BM

   **$B_{12}$ ~2 yr half-life** in normal liver storage (depleted in chronic liver diseases)
4. Causes of deficiency:
   a. *relatively common*

      total/subtotal gastrectomy (↓ IF & ↓ acid)

      chronic autoimmune (type A) gastritis (↓ acid & ↓ IF)

      malabsorption/sprue; ileal fistulas or resection

   b. *other classical causes*

      fish tapeworm (diphyllobothrium latum)

      vegan diet

      congenital ↓↓ β-globulin (transcobalamin II)
5. Schilling test enables differential diagnosis of pathogenesis:

6. Pernicious anemia (PA) = an uncommon but specific type of $B_{12}$ deficiency

   a. **familial predisposition** - Scandinavia, African-Americans

   b. cause: **cytotoxic autoantibodies to parietal canicular**

**cells of stomach** ☛ can be detected in gastric biopsy by immunofluorescence

c. cause: $B_{12}$-IF blocking antibodies or IF-receptor-binding antibodies

d. complications of cytotoxic autoantibodies:

thyroiditis due to cross-reaction with thyroid epithelium

**chronic type A gastritis with increased risk of gastric cancer**

7. Neuropathies: **impaired homocysteine → → methionine metabolism *( Fig. 14-21)***

excess methylmalonate → → abnormal myelin lipids

**Vitamin $B_{12}$ deficiency is now corrected with oral recombinant IF and $B_{12}$ ☛ Neurologic problems in PA are not prevented by folate alone**

## III. ANEMIAS DUE TO REDUCED HB BIOSYNTHESIS

**hypochromic, often microcytic RBC's**

A. **Iron Deficiency**: $Fe^{++}$ is critical for enzyme activity in erythroblasts and for heme biosynthesis

1. Iron sources: meats, eggs, fish, beans, grapes, some greens
2. Predisposing causes:
   a. malnutrition during periods of rapid growth
      a major cause worldwide
   b. chronic bleeding with inadequate iron replacement
   --- **menses, GI cancers**, hookworm (in barefoot populations)
   c. malabsorption due to small bowel disease
3. Socioeconomic consequences:
   a. fetus / infants / children: intellectual impairments.
   b. adults: chronic fatigue limits work capacity
4. Pathophysiology: **latent iron deficiency progresses stepwise**
   a. macrophages in BM and liver or spleen become depleted of storage iron
   b. plasma ferritin declines to minimal levels
   c. serum iron ↓, serum transferrin ↑ in effort to compensate
   d. RBC free protoporphyrin ↑ because heme does not form
   e. ectodermal signs occur in prolonged deficiency

5. Diagnosis:

*BM:* absence of stainable iron ☛ **serum ferritin is a substitute test**

*CBC:* MCHC < 32%

**MCV < 80 fl, anisocytosis with *RDW* > *14***

**shape variation = poikilocytosis**

↓↓ reticulocytes (rapid ↑↑ in response to iron Rx)

*Serum*: ↓ ferritin [< 10 μg/L], ↑ **transferrin (β-globulin),**

↑ **sTfR [> 28 nM]**

↓ serum iron [< 30 μg/dL],

↑ **total iron binding capacity (TIBC μg/dL)**

B. **Heme (Protoporphyrin) Deficiency** (iron-loading or "sideroblastic" anemia).

1. Pathogenesis:

a. **acquired plumbism**: children's environmental hazard, industrial toxic waste

b. associated with **"myelodysplastic syndrome"** (see Neoplasia I)

2. Diagnostic features:

*CBC:* **hypochromia**

**basophilic RBC stippling in plumbism**

*BM:* erythroid hyperplasia with "ineffective erythropoiesis"

intramedullary hemolysis, ↑ iron stores

↑↑ $Fe^{++}$ in mitochondria → → "ringed sideroblasts"

*Serum:* ↑ iron, ↑ ferritin

C. **Impaired Globin Chain Production (= *Thalassemia*)**

1. Genetic pathogenesis: . **abnormal RBC shapes or Hb inclusions predispose to EVH**

2. Diagnostic features: see above

## IV. SUMMARY OF ANEMIAS BY CAUSE AND PATHOPHYSIOLOGIC MECHANISM

A. **Decreased Erythropoiesis** = impaired production = ↓↓ retics; normo- or hypocellular BM

1. BM failure ➜ **limited normocytic, normoblastic erythroid maturation**
2. Restricted DNA replication ➜ **megaloblastic erythroid and WBC maturation**
   *major examples: folic acid and cobalamin (vitamin $B_{12}$) deficiencies*
3. Reduced biosynthesis of Hb ➜ **hypochromic and microcytic anemias**
   *major examples: iron deficiency and thalassemias*

B. **RBC Loss with Compensated Production** = ↑↑ retics and typically hypercellular BM

1. initial stage of chronic bleeding (without iron deficiency)
2. due to chronic hemolysis

**CHANGES OF RBC PRODUCTION IN ANEMIAS**

| Type of Anemia<br>**RBC production** | Examples of Common Causes |
|---|---|
| BM Failure<br>– primary aplastic<br>**production** ↓ despite EPO ↑↑<br><br>-- secondary<br>**production** ↓ **EPO can be** ↓ | Idiopathic<br>Drug reaction or virus infection<br>Congenital (rare)<br><br>Renal failure / uremia<br>Chronic inflammation or infection<br>Malignancy |
| ↓ DNA Synthesis<br>**production** ↓ | Folate or $B_{12}$ nutritional deficiency<br>Vitamin malabsorption<br>Chemotherapy |
| ↓ Hb Synthesis<br>**production** ↓ | Iron deficiency or malabsorption<br>Plumbism<br>Thalassemia |
| Hemolytic<br>**production** ↑ **=** ↑↑ **retics** | G-6PD enzyme deficiency<br>Autoimmune reaction<br>Drug-immune reaction<br>Hereditary spherocytosis |
| Hemoglobinopathy<br>**production** ↑ **=** ↑↑ **retics**<br>↓ **production of globin chains** | Mutated hemoglobin (Hb S, C)<br>Thalassemia |