Anemia

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Hematopoiesis

Hematopoietic stem cells (HSC) are capable of self-renewal & differentiation

Progenitors differentiate along a specific pathway

The Physiologic Basis of Red Cell Production

The physiologic regulation of red cell production by tissue oxygen tension, Hb, hemoglobin

A. Cytokine influence on hematopoiesis.

B. Regulation of erythropoiesis by hypoxia

Sources:

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http://blognyahana.wordpress.com/2010/05/17/hematopoiesis/
Physiology of RBC: Erythrocyte Production

- Pronormoblast
  - a large round nucleus, fine chromatin, and basophilic cytoplasm
- Basophilic normoblasts
  - chromatin condensation and deeply basophilic cytoplasm
- Polychromatophilic normoblast
  - light blue cytoplasm due to accumulation of hemoglobin
- Orthochromat normoblast
  - pyknotic nucleus and pink-gray cytoplasm
- Reticulocyte (polychromatophilic erythrocyte)
  - pink-gray cytoplasm due to residual RNA
- Erythrocytes
  - appear as circular, homogeneous disks of nearly uniform size, ranging from 6–8 μm in diameter, with central pallor not exceeding more than one third of the cell.
  - On average, the red cells are approximately the same size as the nucleus of a small lymphocyte

Erythrocyte

- The erythrocyte is a vehicle for the transport of hemoglobin.
- The function of hemoglobin is the transport of oxygen and carbon dioxide.
- The erythrocyte is also metabolically capable of keeping hemoglobin in a functional state.

Erythrocyte Production

- Red cell gradually undergoes metabolic changes in 120 days
- Senescent cell is removed from the circulation
- Aged red cells lose sialic acid from membranes
- Expose an asialoglycophorin, senescent antigen is recognized by autoantibody
- Senescent cell is removed from the circulation by the RE system (Spleen)
- Normally, 3 million red cells are removed per second
**Abnormal red blood cell in Blood smear**

- Abnormal in staining: Hypochromic, Hyperchromic
- Abnormal in size: Microcyte, Macrocyte
  - Microcyte: RBC < 6 µm or MCV < 80 fl
  - Macrocyte: RBC > 9 µm or MCV > 100 fl
- Variation in size: Anisocytosis
- Variation in shape: Poikilocytosis

**Abnormal red blood cell**

- Normal peripheral smear
- Erythrocytes appear as circular, homogeneous disks of nearly uniform size
- ranging from 6-8 µm in diameter, with central pallor ~ 1/3 of the cell.
- same size as the nucleus of a small lymphocyte

**Normal red blood cell**

- Normal peripheral smear
- Erythrocytes appear as circular, homogeneous disks of nearly uniform size
- ranging from 6-8 µm in diameter, with central pallor ~ 1/3 of the cell.
- same size as the nucleus of a small lymphocyte
Abnormal red blood cell

Microcyte

Found in:
- iron deficiency
- defective hemoglobin synthesis
- ex. Thalassemia
- imbalance of globin chains
- ex. Homozygous HB E
- defective porphyrin synthesis

Symptom: pica, anemic symptom
Finding:
- Hypochromic microcytic RBC
- Decreased serum ferritin & serum iron
Cause: ineffective erythropoiesis
- Increased Demand for Iron
  - Rapid growth in infancy or adolescence
  - Pregnancy
- Increased Iron Loss
  - Chronic or acute blood loss
- Decreased Iron Intake or Absorption
  - Inadequate diet/ Malabsorption
  - Acute or chronic inflammation

Example: Iron deficiency anemia

Hereditary spherocytosis: anisocytosis and several dark-appearing spherocytes with no central pallor

Abnormal red blood cell

Macrocyte

Found in:
- liver disease
- alcoholism
- aplastic anemia
- myelodysplasia
- Megaloblastic anemias (B12 and folate deficiencies)
- Reticulocytes are large immature red cells with polychromatophilia

Finding:
- Oval macrocytes (MCV is usually >100 fL)
- anisocytosis and poikilocytosis
- Some of the neutrophils are hypersegmented (≥ 5 nuclear lobes).
- May be leukopenia (>1.5 x 10⁹/L)
- May be reduced platelet count moderately

Cause: ineffective erythropoiesis
- deficiency of either cobalamin (vitamin B12) or folate
- defects in DNA synthesis (Drug: hydroxyurea, 6-mercaptopurine)

Example: Megaloblastic anemia

Definition of anemia

- ภาวะโลหิตจางหรือภาวะซีด หมายถึง การที่มี hemoglobin (Hb) เหรือ hematocrit (Hct) เหรือ red cell mass น้อยลง

- ตาม WHO classification ได้ตัดความถี่ของการซีดดังนี้

<table>
<thead>
<tr>
<th>เลือก/อายุ</th>
<th>ผู้ชาย</th>
<th>ผู้หญิง</th>
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<tbody>
<tr>
<td>&lt; 13 g/dL</td>
<td>&lt; 12 g/dL</td>
<td>&lt; 11 g/dL</td>
</tr>
<tr>
<td>&lt; 39 %</td>
<td>&lt; 36%</td>
<td>&lt; 33%</td>
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<tr>
<td>&lt; 33%</td>
<td>&lt; 33%</td>
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**Signs and symptoms**

- Fatigue
- Dizziness
- Pallor
- Cold, clammy skin
- Brittle or broken nails
- Reduced exercise tolerance
- Dyspnea
- Depression
- Headaches
- Impaired cognition
- Menstrual irregularities
- Loss of appetite
- Tachycardia
- Rales, peripheral edema, tachypnea

**Consequences of chronic anemia**

- Reduced function and quality of life
- Decreased survival (< 65 year-old)
- Increased risk of heart failure
- Changes in neurological function
- Increased risk of complications from surgery and anesthesia
- Increased risk of coronary death
- Decreased tolerance of chemotherapy

**Causes of anemia**

1. Blood loss
2. Hemolytic anemia
3. Impaired red cell formation

**Table 5-1: Anemia Grading Systems**

<table>
<thead>
<tr>
<th>Severity</th>
<th>WHO</th>
<th>NCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0 (WNL)*</td>
<td>≥11.0 g/dL</td>
<td>WNL</td>
</tr>
<tr>
<td>Grade 1 (mild)</td>
<td>9.5–10.9 g/dL</td>
<td>10.0 g/dL to WNL</td>
</tr>
<tr>
<td>Grade 2 (moderate)</td>
<td>8.0–9.4 g/dL</td>
<td>8.0–10.0 g/dL</td>
</tr>
<tr>
<td>Grade 3 (serious/severe)</td>
<td>6.5–7.9 g/dL</td>
<td>6.5–7.9 g/dL</td>
</tr>
<tr>
<td>Grade 4 (life threatening)</td>
<td>&lt;6.5 g/dL</td>
<td>&lt;6.5 g/dL</td>
</tr>
</tbody>
</table>

*WHO, World Health Organization; NCI, National Cancer Institute.

*Within normal limits: female 12.0–16.0 g/dL, male 14.0–18.0 g/dL.
Causes of anemia

1. Blood loss:
   • acute; GI hemorrhage, accident
   • chronic; hook worm, hypermenorrhea

2. Hemolytic anemia:
   • intracorpuscular
     1) membrane defects e.g. spherocytosis, elliptocytosis
     2) enzymatic defects e.g. pyruvate kinase deficiency, G6PD deficiency
     3) hemoglobin defects e.g. thalassemia
   • extracorpuscular
     1) immune
        - isoimmune
        - autoimmune e.g. autoimmune hemolytic anemia (AIHA)
     2) nonimmune (idiopathic, secondary)

3. Impaired red cell formation
   3.1 nutritional deficiency e.g.
      - iron
      - folic acid
      - vitamin B12
      - vitamin C
      - protein
      - vitamin B6

3.2 bone marrow failure
   1) failure of all cell lines
      - congenital e.g. Fanconi’s anemia, dyskeratosis congenital
      - acquired e.g. aplastic anemia
   2) failure of a single cell line e.g.
      - congenital pure red cell aplasia
      - acquired red cell aplasia

3.3 dyserythropoietic anemia
   (decreased erythropoiesis, decreased iron utilization)
   1) infection
   2) renal failure and hepatic disease

3.4 infiltration of bone marrow e.g. leukemia, lymphoma, disseminated carcinoma

Anemia

Morphologic classification

1. MCV (Mean corpuscular volume)
2. MCHC (Mean corpuscular hemoglobin concentration)
Microcytic (MCV < 80 fl)

Normochromic
- Iron deficiency; early
- Thalassemia trait
- Some hemoglobinopathies; Hb E
- Anemia of chronic disease*

Hypochromic
- Iron deficiency; late
- Thalassemia trait
- Sideroblastic anemia
- Anemia of chronic disease*

* most commonly normochromic/normocytic.

Macrocytic (MCV > 100 fl)

- B12 and folate deficiency
- Liver disease
- Alcoholism
- Myelodysplastic syndrome
- Blood loss #
- Hemolysis #
- Hypothyroidism
- Some drugs

Normochromic/Normocytic (MCV 80-100 fl)

- Anemia of chronic disease
- Anemia of renal failure
- Marrow infiltration
- Aplastic anemia
- Blood loss #
- Hemolysis #

* may be normocytic or macrocytic depending on severity.

Evaluation of Anemia

A. Hematologic
1. Complete blood cell count (CBC)
2. RBC indices: MCV, MCH, MCHC
3. Reticulocyte count
4. ESR (Erythrocyte sedimentation rate)
5. Stained blood smear: RBC morphology
Complete blood cell count

- White blood cell count; 5,000 -10,000/cu.mm
- Red blood cell count; 4.0-6.0 x10^12 /l
- Hematocrit (Hct) or pack cell volume (PVC); 35-45%
- Hemoglobin (Hb); 12 – 17.5 g/dl
- WBC differential; PMN, lymphocyte, monocyte,
- RBC morphology

Reticulocyte count

- เป็นค่าที่บอกถึงความสามารถในการสร้างเม็ดเลือดแดงของไขกระดูก
- Reticulocyte /1,000 RBC >>> %
- Automation >> absolute reticulocyte count
- Normal value of reticulocyte count
  1.65±0.82 % in male
  2.45±0.82 % in female
- Absolute reticulocyte count = 30-85 x 10^3 /ul

Reticulocyte production index (RPI)

- สามารถบอกว่า erythropoietic activity ใดวัดถึงกี่เท่าของภาวะปกติ
  RPI = (% reticulocytes x patient Hct/45) correction factor

โดยทั่วไป correction factor จะมีค่าเท่ากับ 2 ถึง 3 ค่าเท่ากับ

RPI > 2 - effective erythropoiesis
B. Urine analysis
1. Appearance: Color, pH, Clarity, specific gravity
2. Test for protein, Bence Jones protein
3. Bilirubin, Urobilinogen
4. Occult blood
5. Microscopic examination

C. Stool
1. Appearance: Color, consistency
2. Occult blood
3. Examination for ova, parasites

D. Serum or Plasma
1. BUN
2. Creatinine
3. Bilirubin: Direct, indirect
4. Protein
5. SI (Serum iron), TIBC (Total iron binding capacity)

E. Special tests in hematology
   - Hb typing / Ham acid test / Coombs’ test, G-6-PD, Ferritin, Sucrose test, Autohemolysis test, Haptoglobin, Flow cytometry, etc.

Diseases of Anemia
- Iron deficiency anemia
- Megaloblastic anemia
- Anemia of chronic disease
- Aplastic anemia
- Hemolytic anemia
- Thalassemia
IRON DEFICIENCY ANEMIA

Normal Hemoglobins

Hemoglobin
heme 4 molecules + globin chain 2 pairs
(α, β, γ, δ, ζ, ε)

The multiple forms of iron in the body

• Iron in food:
  – Heme sources: meat
  – Non heme sources: beans, clams, vegetables

• Iron in storage:
  – Ferritin: liver, spleen, skeletal muscle, BM
  – Hemosiderin: macrophages

• Iron in circulation:
  – Iron and globin are recycled as a result of red cell senescence
Enhancers of iron absorption
- Orange juice
- Vitamin C
- Pickles
- Soy sauce
- Vinegar
- Alcohol

Iron Transport across the intestinal epithelium

Inhibitors of iron absorption
- Tea
- Coffee
- Oregano
- Milk

Iron Metabolism
- Iron is Absorbed Primarily in Duodenum
  - 25% of Heme-Bound Iron (Red Meat)
  - 1-2% of Non-Heme Iron
- Body Losses of iron are Limited
  - 1-2 mg/Day By Epithelial Cell Shedding
- Mucosal Block - Maintains Balance
### Causes of Iron Deficiency

- External Blood Loss - Most Common
  - Female Genital Tract
  - Gastrointestinal Tract
- ↑ Demand - Infancy, Pregnancy
- Dietary Deficiency - Rare
  - (Vegetarian Diets)
- Intestinal Malabsorption Syndrome

### Iron Deficiency Clinical Manifestations

- Anemia - Non-Specific Findings
- Koilonychia
- Plummer-Vinson Syndrome
  - Hypochromic Microcytic Anemia
  - Atrophic Glossitis
  - Esophageal Webs (Dysphagia)

### Iron Deficiency Anemia Laboratory Findings

- Hypochromic Microcytic Anemia
  - ↓ RBC Count, ↓ MCV
- ↓ Serum Ferritin Levels
- ↓ Transferrin Saturation
  - ↓ Serum Fe, ↑ Transferrin
- ↑ TIBC (Total iron binding capacity)
**MEGALOBLASTIC ANEMIA**

**TREATMENT:**
- Correct causes
- Iron supplement
- Breast feeding

**MEGALOBLASTIC ANEMIA:**
- Impaired DNA Synthesis (Nucleus)
- Affects All Rapidly Dividing Cells
  - Mouth - Atrophic Glossitis
  - GI tract - Intestinal Malabsorption
Causes of megaloblastic anemia

- Vitamin B12 deficiency
- Folate deficiency
- Miscellaneous: orotic aciduria, liver disease, drugs e.g. purine analogues (6MP, 6TQ or 5FU)

VITAMIN B12 DEFICIENCY

Cobalamine

- Prevalence: 15-25% of population
- Functions of cobalamin: Coenzyme for 13 enzymes
- RDI = 2.4 ug/d
- Sources: Meat, liver, kidney, oyster, clams, fish, eggs, cheese and other dairy products
**Vitamin B\textsubscript{12} Deficiency**

**Causes:**
- Dietary deficiencies in vegans
- Malabsorption States: Gastric Atrophy, Pernicious anemia (absence of IF), Gastrectomy, ileal resection
- Food-cobalamin maldigestion: Achlorhydria, acid suppressive drugs
- Diphyllobothrium Latum - Fish Tapeworm

**Vitamin B\textsubscript{12} Deficiency - Cause**

Western World - Pernicious Anemia
- Autoimmune Disorder
  - Autoantibodies to IF and Parietal Cells
  - Chronic Atrophic Gastritis
  - Achlorhydria - Absent HCL

**Signs & Symptoms**

- Additional signs & symptoms
  - Sore, smooth, beefy red tongue
  - Numbness and paresthesia, weakness, ataxia
  - Cognitive disturbances (forgetfulness, dementia, psychosis)
  - Increased risk for venous and arterial thrombosis and cardiovascular disease
Glossitis with cobalamin deficiency. The smooth shiny tongue results from loss of papillae over the lingual surface. Thinning of the epithelium sometimes give the tongue a red "beefy" appearance.

**Vitamin B\textsubscript{12} Deficiency**

- Clinical - Similar to Folate Deficiency, but also include Demyelinating Neurologic Disorder
  - Affects Both Sensory and Motor Tracts (subacute combined degeneration)
  - Lack of Correlation With Anemia

**Laboratory Findings:**

- Low Serum Vitamin B\textsubscript{12} Levels
- Normal RBC Folate Levels
- Abnormal Schilling Test - Impaired Absorption of Radioactive Vitamin B\textsubscript{12} Correctable by Addition of IF
- Anti-Intrinsic Factor Antibodies (Anti-Parietal Antibodies Less Sensitive)
Treatment:
- Cobalamin
- Parenteral $B_{12}$ - Improves Anemia, +/- Resolution of Neurologic Symptoms
- Caution! Anemia of $B_{12}$ Deficiency Also Improves With Folate Supplementation

FOLATE DEFICIENCY ANEMIA

Folic acid
- Purposes of folic acid
  - Metabolism of serine, glycine, methionine, and histidine
  - Purine and pyrimidine synthesis

Folic acid
- RDI 400 ug/d
- Good sources: cereal, beef liver, cowpeas, spinach, asparagus, wheat germ, orange juice, baked beans, green peas, broccoli, egg noodles, white rice, avocado, peanuts, romaine lettuce, tomato juice, white bread, cantaloupe, papaya, banana, whole wheat bread
Metabolism of folate

Causes of folate deficiency
- Dietary: general malnutrition, alcoholism
- Impair absorption: Tropical sprue, Celiac disease
- Increased requirements: infancy, pregnancy, lactation, anticonvulsant drugs, folate antagonist, chronic exfoliative dermatitis

Signs and Symptoms
- Additional signs & symptoms
  - Diarrhea
  - Cheilosis
  - Glossitis

Folate Deficiency
Laboratory Findings:
- Macrocytic anemia (MCV >100 fl)
- Decreased folic acid
- Increased homocysteine level
  - Red Blood Cell Folate - Reflects Tissue Content of Folate Throughout Body
  - Serum Folate - Levels Fluctuate Based on Recent Intake, Do Not Reflect Stores
Megaloblastic Anemia
Peripheral Blood

- RBCs - Large Oval: Macroovalocytes
  - MCV > 100 fl
- Hypersegmented Neutrophils
- Thrombocytopenia, Neutropenia (Severe)

Megaloblastic Anemia - Bone Marrow

Nuclear- Cytoplasmic Asynchrony:

- Erythroid Series (Hallmark Changes)
  - Megaloblasts
  - Erythroid Hyperplasia
- Myeloid Series
- Megakaryocytic Series - (Infrequent)
Treatment

- Folic acid 1 mg daily
- Treatment for 1-2 months
- Indefinite treatment may be necessary for cases of malabsorption and chronic malnutrition

ANEMIA OF CHRONIC DISEASE

Anemia of Chronic Disease

- Normochromic Normocytic Anemia (or Hypochromic Microcytic)
- Chronic Disorders (Inflammation or Tissue Necrosis)
  - Chronic Microbial illnesses
  - Chronic Immune Disorders
  - Neoplasms
- Often ↓ TIBC, ↑ Ferritin

Diseases associated with anemia of chronic inflammation

- Acute infections
- Chronic infections: TB, infective endocarditis, chronic UTI, chronic fungal infection, HIV
- Chronic inflammatory disorders: Rheumatoid arthritis, collagen vascular diseases, hepatitis, decubitus ulcer
Diseases associated with anemia of chronic inflammation

- Chronic renal insufficiency
- Hypothyroidism
- Protein-energy malnutrition
- Malignancy: metastatic carcinoma, hematologic malignancy

Laboratory findings

- Normochromic, normocytic
- Normal or increased ferritin (indicates increased iron stores)
- Decreased serum iron
- Decreased TIBC

Treatment of ACI

- Correct or improve underlying abnormality
- Iron is not effective unless a true iron deficiency is also occurring
- Transfusions (for some indication)
- Erythropoietin (for some indication)
APLASTIC ANEMIA

Acquired BM failure syndromes
- Aplastic anemia
- Pure red cell aplasia
- Paroxysmal nocturnal hemoglobinuria
- Myelodysplasia

Etiology of aplastic anemia
- Inherited:
  - Fanconi anemia
  - Dyskeratosis congenita

Etiology of acquired aplastic anemia
- Idiopathic
- Radiation: cancer irradiation
- Chemicals: chemotherapy drugs, benzene
- Chemicals (idiosyncratic): chloramphenicol, gold, penicillamine, NSAIDs, sulfonamides, propylthiouracil
Etiology of acquired aplastic anemia

- Viruses: Hepatitis, EBV, HIV
- Immune disorders: SLE, thymoma, transfusion-associated graft-versus-host disease, pregnancy

Characteristic features of aplastic anemia

- Peripheral blood pancytopenia
- Reticulocytopenia
- Bone marrow hypocellularity
- Depletion of hematopoietic stem cells

Bone Marrow Aplasia (Lack of Cells)

- Failure of Multipotent Stem Cell
  - T-cell Mediated Suppression or
  - Genetic Damage
- Bone Marrow - Markedly Hypocellular
- Peripheral Blood - Pancytopenia
  - Normochromic Normocytic RBCs
Myelophthisic Anemia

BM Replacement >> BM failure:

- Metastatic Carcinoma Most Common
- Destruction By Non-Neoplastic Process is Less Common i.e. Fibrosis, Infection
- Peripheral Blood Cytopenias, Immature Circulating Cells

HEMOLYTIC ANEMIA
Definition of hemolytic anemia
- Short life span of RBC
- Defect in structure and metabolism
  >> destruction in RE system; spleen
- Hemolysis in RE system; extravascular hemolysis

Signs & Symptoms
- Pale, icteric
- Splenomegaly; prominent if chronic & Extravascular hemolysis
- Gall stone; esp in the young
- Hx of drug intake of underlying disease

? Hemolytic anemia
- ↑ rbc destruction & production at the same time
- Persistent anemia despite increased erythropoiesis with out blood loss
- Hb drop ≥ 1 g/dl per week
- Hemoglobinuria or signs of Intravascular hemolysis

Treatment
1. Splenectomy
2. Immunosuppressive agent
3. Prevent hemolytic reaction
4. Blood transfusion
THALASSEMIA AND HEMOGLOBINOPATHY

Normal Hemoglobins

Hemoglobin
heme 4 molecules + globin chain 2 pairs
(α, β, γ, δ, ζ, ε)

Hb A

Globin Gene

- α-globin gene cluster on chromosome 16
  - on each chromosome has two α-globin gene
- β-globin gene cluster on chromosome 11
  - on each chromosome has one β-globin gene

http://www.ironhealthalliance.com/disease-states/thalassemia/clinical-features.jsp

Chromosome 16
aa/αα

Chromosome 11
β/β

Harrisons Principles of Internal Medicine 18th

Henry's Clinical & Laboratory 22nd edition
**Hemoglobinopathy**

- A disease that results from genetic alterations of the globin gene, causing abnormal hemoglobin that cannot function properly or at all.
- Pathology of Thalassemia
  -领先的治病方。 lifelong average globin production is reduced or absent, leading to hemoglobin that cannot function properly or at all. It can be passed on to offspring, autosomal recessive.
  - Classified into two groups: α-thalassemia (α-thalassemia) and β-thalassemia (β-thalassemia).

**Pathology of Thalassemia**

- The disease that results from changes in the amino acid sequence of the globin chain, affecting the physical or chemical properties of the globin. This leads to abnormal globin structure.
- Most common abnormal hemoglobin found in Thailand is Hb E and Hb Constant Spring; Hb CS.

**Thalassemia**

- A disease that results from genetic alterations of the globin gene, causing abnormal hemoglobin that cannot function properly or at all, leading to hemoglobin that cannot function properly or at all. It can be passed on to offspring, autosomal recessive.
- Classified into two groups: α-thalassemia (α-thalassemia) and β-thalassemia (β-thalassemia).
Clinical manifestations:

β-Thalassemia

1. Thalassemia major; homozygous
   - Severe, transfusion-dependent anemia
   - Hb 3-6 g/dl
   - Without transfusions, death occurs at an early age from profound anemia
2. Thalassemia minor; heterozygous
   - Usually asymptomatic
   - More common than Thalassemia major
3. Thalassemia intermedia; heterogenous

α-Thalassemia

- severity is related to the number of α-globin genes deleted

1. Silent carrier state; asymptomatic
2. α-Thalassemia trait; clinical = β-thal minor
3. Hemoglobin H disease; deletion of three α-globin genes; clinical = β-thal intermedia
4. Hydrops fetalis; deletion of all four α-globin genes; Hb Barts, not compatible with life

Thalassemia facial bone abnormalities

Hemoglobin Bart's Hydrops Fetalis
Thank you for your attention