Pathology of Anemia I

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Objectives

• Review of hematopoiesis
• Structure and function of normal RBCs
• Definition of anemia
• Classification of anemia
  • Hereditary Spherocytosis
  • G-6PD Deficiency
  • Sickle cell anemia

Hematopoiesis

• Series of events in which pluripotent stem cells mature into functional blood cells

• Pluripotent stem cell is precursor of all blood cell lines: red blood cells (RBCs), white blood cells (WBCs), platelets
Red Blood Cells (erythrocytes)

- Bulk of the formed elements in blood; anucleate biconcave discs that carry oxygen to the tissues and return to the lungs carrying carbon dioxide
Formed blood elements are visualized by means of the peripheral blood smear
- Drop of blood is smeared on a glass slide
- Dried smear is stained

Evaluation of hematopoiesis is accomplished by a bone marrow aspirate and biopsy

Peripheral Blood Smear (High Power)

Red cell maturation sequence
- Reticulocyte is usually first stage of red blood cell released from marrow into peripheral blood; cytoplasm may be slightly bluish-pink due to residual RNA (polychromasia)
- Majority of RBCs in peripheral blood are mature biconcave discs with a normal life-span of about 120 days
RBC Membrane

- Phospholipid bilayer combined with glycolipids and cholesterol; integral proteins span the bilayer
- Membrane skeleton controls biconcave shape and cell deformability (qualities essential for travel through microcirculation)

Hemoglobin

- Hemoglobin synthesis involves 2 biosynthetic pathways
  - Synthesis of heme
  - Synthesis of globin chains
Heme
- Heme consists of 4 pyrrole groups joined into large ring with ferrous ion incorporated into center

Globin Chains
- Globin chains contain approximately 150 amino acids and each chain is arranged in a “knotted sausage” fashion; heme binds to a specific region of each globin chain
- Two globin dimers form hemoglobin. Most important hemoglobin is hemoglobin A (α2β2), comprising more than 95% of the hemoglobin in the normal adult red cell

Hemoglobin Structure
RBC Catabolism
- Aging RBCs are largely removed by mononuclear phagocytic engulfment in the spleen
- Heme and globin chains are separated; heme is divided into iron (which is recycled) and porphyrin rings (eliminated as bilirubin), and globin is dismantled into amino acids
- Accelerated RBC destruction is called "hemolysis"

Anemia-Definition
- Reduced oxygen carrying capacity of blood
- Anemia may be defined as reduction below normal limits of the total circulating red cell mass
  - Reduction below normal in volume of packed cells (hematocrit)
  - Reduction in hemoglobin concentration of blood

Anemia
- Many classification schemes
- Underlying mechanism (Robbins)
- Basis of appearance on peripheral smear and red cell size
Anemia

- Blood loss – acute and chronic
- Increased rate of destruction (hemolytic anemias)
- Impaired red cell production (diminished erythropoiesis)

Classification of Anemia

Increased Rate of Destruction (Hemolytic Anemias)
- Intrinsic (intracorpuscular) abnormalities of RBCs
- Extrinsic (extracorporeal) abnormalities of RBCs

Intrinsic abnormalities of RBCs
- Hereditary
  - Red cell membrane disorders
  - Red cell enzyme deficiencies
  - Disorders of hemoglobin synthesis
    - Structurally abnormal globin synthesis
    - Deficient globin synthesis
- Acquired (PNH)

Extrinsic abnormalities of RBCs
Classification of Anemia

Increased Rate of Destruction

Intrinsic abnormalities of RBCs

Extrinsic abnormalities of RBCs

• Antibody-mediated
• Mechanical trauma
• Infections
• Chemical injury
• Sequestration

Classification of Anemia

1) Blood loss – acute and chronic

2) Increased rate of destruction (hemolytic anemias)

a) Intrinsic abnormalities of RBCs

Hereditary
- Red cell membrane disorders (hereditary spherocytosis)
- Red cell enzyme deficiencies (G-6-PD deficiency)
- Disorders of hemoglobin synthesis
- Defective mitochondrial function (porphyria)
- Defective globin production (sickle cell disease)

Acquired
- Paroxysmal nocturnal hemoglobinuria (PNH)

b) Extrinsic abnormalities

Mechanical trauma (microangiopathic hemolytic anemia)

3) Impaired red cell production (diminished erythropoiesis)

a) Disturbances of proliferation / differentiation of erythroblasts

Deficient heme synthesis (iron deficiency anemia)
Deficient DNA synthesis (megaloblastic anemia: B12/folate)

Multiple mechanisms (anemia of chronic disease)

b) Disturbances of proliferation / maturation of stem cells (aplastic anemia)

Red cell indices

Mean cell volume (MCV)

Mean cell hemoglobin (MCH)

Mean cell hemoglobin concentration (MCHC)

RBC distribution width (RDW): coefficient of variation of red cell volume, measures anisocytosis
Classification based on MCV

1. Microcytic Anemia (MCV <80 fL): iron deficiency, thalassemias, anemia of chronic disease

2. Macrocytic Anemia (MCV >100 fL): Megaloblastic anemia (Vitamin B12/folate deficiency)

3. Normocytic Anemia (MCV 80-100 fL)

Classification of Anemia

1) Blood loss – acute and chronic

2) Increased rate of destruction (hemolytic anemias)
   a) Intrinsic abnormalities of RBCs
      - Red cell membrane disorders (Hereditary spherocytosis)
      - Red cell enzyme deficiencies (G-6-PD deficiency)
      - Disorders of heme synthesis
      - Deficient globin production
      - Acquired — Paroxysmal nocturnal hemoglobinuria (PNH)
   b) Extrinsic abnormalities
      - Mechanical trauma

3) Impaired red cell production (diminished erythropoiesis)
   a) Disturbances of proliferation / differentiation of erythroblasts
      - Deficient heme synthesis
      - Deficient DNA synthesis
      - Multiple mechanisms (anemia of chronic disease)
   b) Disturbances of proliferation / maturation of stem cells

“Sideroblastic anemia”

- Not a specific entity—outdated term
- Presence of abnormal erythroid precursors in the bone marrow: “ring sideroblasts”
- Seen in anemias due to many different causes
  - Hereditary ALAS2 mutation (rare)
  - Lead poisoning
  - Vitamin B6 (pyridoxine) deficiency
  - Drugs (e.g. Isoniazid)
  - Chronic alcoholism
  - Myelodysplastic syndrome (malignant)
- Microcytic, normocytic or macrocytic depending on cause
Classification of Anemia

1) Blood loss – acute and chronic

2) Increased rate of destruction (hemolytic anemias)
   a) Intrinsic abnormalities of RBCs
      Hemolysis
      - Red cell membrane disorders (hereditary spherocytosis)
      - Red cell enzyme deficiencies (G-6-PD deficiency)
      - Disorders of hemoglobin synthesis
        - Abnormal globin production (β-thalassemia)
        - Abnormal heme production (α-thalassemia)
      - Acquired e.g. Paroxysmal nocturnal hemoglobinuria (PNH)
   b) Extrinsic abnormalities
      - Mechanical trauma (microangiopathic hemolytic anemia)

3) Impaired red cell production (diminished erythropoiesis)
   a) Disturbances of proliferation / differentiation of erythroblasts
      - Deficient heme synthesis (iron deficiency anemia)
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      - Multiple mechanisms (anemia of chronic disease)
   b) Disturbances of proliferation / maturation of stem cells (aplastic anemia)

Hemolysis (Intrinsic or Extrinsic)

- Hemolytic anemias are characterized by:
  - Shortening of normal RBC life span (premature destruction of RBCs)
  - Accumulation of products of hemoglobin catabolism
  - Marked compensatory increase in erythropoiesis within the bone marrow

Hemolysis

- Intravascular
- Extravascular
Hemolysis

- **Intravascular**
  - RBCs are destroyed within the vascular compartment resulting in hemoglobinemia, hemoglobinuria, and hemosiderinuria
  - Free hemoglobin forms complexes mostly with haptoglobin (levels usually become low) and is removed by the liver

- **Extravascular**
  - RBCs are destroyed primarily by tissue macrophages (as in normal RBC catabolism, but exaggerated)
  - Does not usually result in significant hemoglobinemia or hemoglobinuria, but often the reticuloendothelial system is hyperactive, resulting in splenomegaly
  - More common than intravascular hemolysis

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Classification of Anemia

### Increased Rate of Destruction

- **Intrinsic abnormalities of RBCs**
  - **Hereditary**
    - Red cell membrane disorders (hereditary spherocytosis)
    - Red cell enzyme deficiencies
    - Disorders of hemoglobin synthesis
      - structurally abnormal globin synthesis
      - deficient globin synthesis
  - **Acquired (PNH)**

- **Extrinsic abnormalities of RBCs**
Hereditary Spherocytosis

General

- Intrinsic defect in RBC membrane
- RBCs are spheroidal, less deformable, vulnerable to splenic sequestration and destruction
- Autosomal dominant in ~75%

Pathophysiology

- Deficiency of any of the membrane skeletal proteins may adversely affect RBCs
- Deficiency of spectrin seems to be the most common biochemical abnormality in HS
- Spectrin content varies from 60-90% – correlates with severity of disease
Hereditary Spherocytosis
Pathophysiology

- **Autosomal dominant form**
  - most frequent is a mutation in the ankyrin gene, resulting in reduced synthesis of ankyrin, and a secondary reduction in spectrin assembly
  - mutations in band 3 in ~20%

- **Autosomal recessive form**
  - mutation in α-spectrin gene

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While the molecular basis of spectrin deficiency is varied, all of the mutations in HS diminish interactions between the membrane cytoskeleton and the overlying lipid bilayer

- This results in reduced membrane stability and loss of membrane fragments

- Reduction in cell surface to volume ratio causes the cells to assume the smallest possible diameter for a given volume: a sphere
Hereditary Spherocytosis

Pathophysiology

- Crucial role of the spleen is proved by the beneficial effect of splenectomy
  - spherocytes persist, anemia improved

Hereditary Spherocytosis

Clinical Features

- Peripheral blood smear shows spheroidal red cells, abnormally small cells without central zone of pallor
Hereditary Spherocytosis
Clinical Features

- Anemia
- Splenomegaly
- Jaundice

Largely asymptomatic in 20-30%
- Mild hemolysis with compensatory erythropoiesis

Most patients have a chronic mild to moderate hemolytic anemia

Aplastic crisis may occur
Hereditary Spherocytosis

Diagnosis

- Family history
- Hematologic findings
- Increased mean cell hemoglobin concentration
- Laboratory evidence of increased osmotic fragility

Classification of Anemia

Increased Rate of Destruction

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Extrinsic abnormalities of RBCs

G-6-PD Deficiency

- Most important of the enzyme abnormalities
- Considerable polymorphism exists and there are more than 250 variants
- Inheritance is X-linked
  - all red cells of affected males are deficient in enzyme activity, so men with G-6-PD deficiency are more susceptible to oxidant injury than women
G-6-PD Deficiency

- Usually no symptoms unless red cells are subjected to oxidant injury following exposure to offending drugs or toxins:
  - drugs, such as antimalarials, sulfonamides, nitrofurantoin, and others
  - infections, probably acting by generation of oxidant free radicals in macrophages

G-6-PD Deficiency

Pathophysiology

- The red cell deals with oxidizing species through a variety of mechanisms
  - most important is the glutathione system
G-6-PD Deficiency
Pathophysiology

- If enough NADPH is not available to regenerate reduced glutathione, H₂O₂ accumulates, oxidizing sulphydryl groups of globin chains
- Hemoglobin denatures, forming precipitates known as Heinz bodies
  - attach to the RBC membrane, causing membrane damage and decreasing deformability

Membrane damage results in intravascular hemolysis
- RBCs with Heinz bodies pass through splenic cords
  - macrophages bite out Heinz bodies, with ensuing loss of membrane ("bite cells") and formation of spherocytes
- Extravascular hemolysis results when red cells are trapped in the spleen
Drug-induced hemolysis is acute with variable severity
- intravascular hemolysis, characterized by hemoglobinemia, hemoglobinuria and decreased hematocrit, typically develops after two or three days
- Older red cells are more susceptible to lysis
- The bone marrow begins to produce new RBCs to compensate

Classification of Anemia
Increased Rate of Destruction

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Extrinsic abnormalities of RBCs
**Sickle Cell Disease**

**General**
- Disorder of hemoglobin synthesis, producing a structurally abnormal hemoglobin
- Most common form of familial hemolytic anemia worldwide
- About 8% of African-Americans are heterozygous for HbS (sickle cell trait) and about 1 in 600 is homozygous

**Sickle cell disease**

**Pathophysiology**
- Results from point mutation leading to substitution of valine for glutamic acid at the sixth position of the β-globin gene, resulting in Hgb S
- Upon deoxygenation, HbS molecules aggregate and polymerize (gelation or crystallization)
- The red cell becomes distorted into the sickle shape

**Sickle cell anemia**
Sickle cell anemia (EM)

Pathophysiology

- Sickling of the red cells is influenced by many factors, importantly:
  - Amount of HbS and its interaction with other hemoglobin chains in the red cell
  - Mean corpuscular hemoglobin concentration
  - Fall in pH

Sickle cell disease

Clinical

- Major 2 clinical consequences of sickling are chronic hemolytic anemia and occlusion of small blood vessels
Sickle cell disease

Clinical

- Intravascular hemolysis may occur due to increased mechanical fragility of severely damaged RBCs
- Extravascular hemolysis occurs from rigid sickled RBCs becoming sequestered in the spleen

- Children may have moderate splenomegaly caused by the red pulp congestion
- Over time, hypoxic tissue damage occurs and the spleen becomes small and fibrotic: “auto- or functional splenectomy”

- Widespread microvascular obstruction from the rigid red cells and other abnormalities may result in ischemic damage
- Intermittent sudden episodes are called vasoocclusive, or painful, crises
- Aplastic crises may occur occasionally
- Increased susceptibility to infections
Sickle cell disease

Clinical

- An ordinary peripheral blood smear can show sickled cells or a blood sample can be treated in such a way as to induce sickling in susceptible cells, to suggest diagnosis
- Usually diagnosed definitively by hemoglobin electrophoresis
- Patients with sickle cell trait usually have an uneventful clinical course, but with rare cases of exertional rhabdomyolysis, splenic infarction, renal medullary carcinoma

Sickle cell trait

Is universal screening justified?

- NCAA: Mandatory sickle cell trait screening
  2010 Division 1
  2012 Division 2
  2013 Division 3

- American Society of Hematology (ASH) Statement

  “ASH does not support testing or disclosure of sickle cell trait status as a prerequisite for participation in athletic activities. ASH believes that current scientific evidence does not justify this requirement. It is also not consistent with good medical practice or established principles of public health ethics.”
  (http://www.hematology.org/Advocacy/Statements/2650.aspx)
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Extrinsic abnormalities of RBCs

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Thank you!