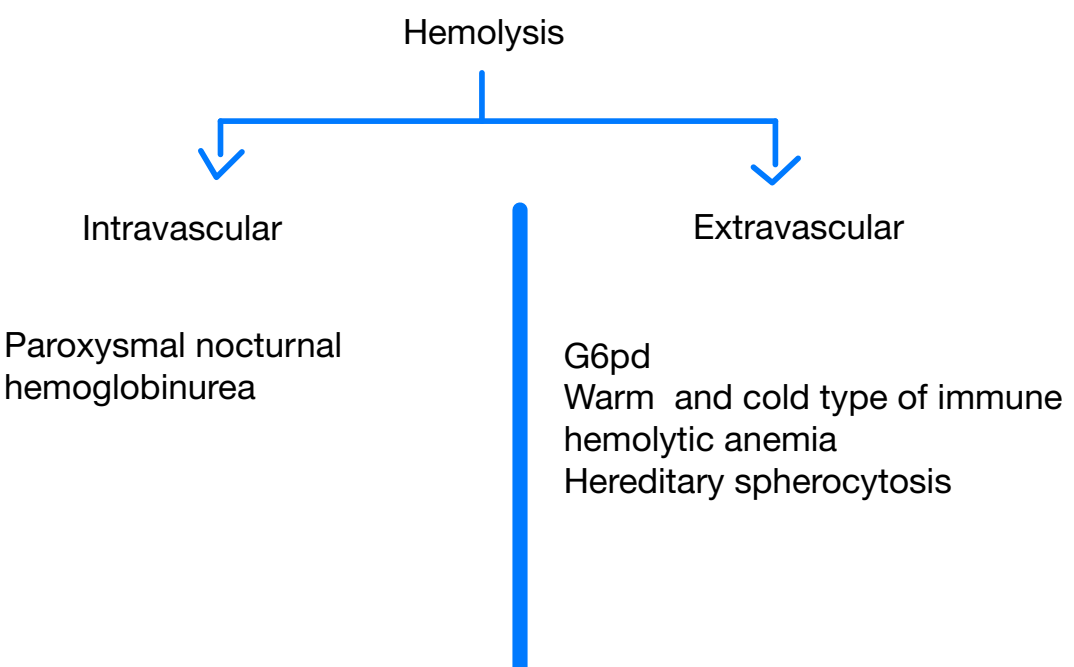
- RBC life span < 120 days
- Hypoxia triggers release of erythropoietin
- Erythroid hyperplasia in bone marrow
- Peripheral blood reticulocytosis
- Extramedullary hematopoiesis in severe cases
- Hemoglobin is released in from damaged RBCs
- Serum haptoglobin: decreased (binds free Hg) in both intra and extravascular hemolysis

CLASSIFICATION

- *Main site of hemolysis:*
- 1) Extravascular: occurs primarily in spleen (RBCs have abnormal shape or coated with antibodies, removed by macrophages, patients have jaundice, pigmented gall bladder stones, splenomegaly)
- 2) Intravascular: inside blood stream (sudden release of Hg, patients have hemoglobinemia, hemoglobinurea, hemosiderinurea, iron deficiency)

- *According to cause of hemolysis*
- Extracorpuscular (extrinsic factor) vs intracorpuscular



G6PD Deficiency Overview

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked inherited disorder affecting the enzyme responsible for producing glutathione, which protects red blood cells (RBCs) from oxidative damage. Without sufficient G6PD, RBCs are more susceptible to destruction under certain stress conditions.

Genetics

- Inheritance: X-linked recessive.
- Enzyme Deficiency: G6PD deficiency reduces the production of glutathione, impairing the cell's ability to neutralize harmful oxidants.

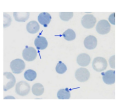
Triggers of Hemolysis

Hemolysis in G6PD-deficient individuals is typically triggered by factors that increase oxidative stress, leading to the destruction of RBCs:

1. Infections.
2. Certain Drugs: Sulfonamides, nitrofurantoin, large doses of aspirin, vitamin K, and primaquine.
3. Fava Beans: Consumption can lead to a large generation of oxidants.

In all these situations, excess oxidants cause hemoglobin denaturation, leading to the formation of Heinz bodies (precipitated hemoglobin), which damage the RBC membrane and result in massive hemolysis 2-3 days after exposure to the trigger.

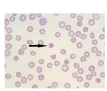
Pathophysiology



• Supravital special stain highlights Heinz bodies as membrane-bound, dark spots representing condensed and denatured Hg

1. Heinz Bodies: Formed by denatured hemoglobin due to oxidative stress. They appear as membrane-bound, dark spots in RBCs and are highlighted using supravital stains.
2. Bite Cells: When Heinz body-laden cells lose deformability, they are partially phagocytosed in the spleen, leaving an indented defect in the RBC membrane, creating "bite cells."

Clinical Types



• Bite cells: appears are indented defect in part of cell membrane of RBCs

1. G6PD-A Type:
   - Common in African populations, associated with a modest decrease in G6PD activity. Symptoms are typically milder as the bone marrow compensates by producing new RBCs.
2. G6PD-Mediterranean:
   - More severe form, common in Mediterranean populations, caused by a qualitative defect in G6PD function, leading to more severe oxidative stress and hemolysis.
3. Females:
   - Although the disorder is X-linked, females may exhibit symptoms if random inactivation of the normal X chromosome occurs, affecting the production of G6PD.

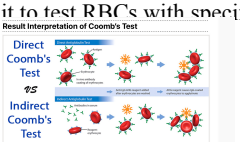
Clinical Presentation

- Symptoms of Extravascular hemolysis, such as jaundice, dark urine, and fatigue, usually occur after exposure to triggers like infection, drugs, or fava beans.

Immune Hemolytic Anemia Overview

Immune hemolytic anemia (IHA) occurs when auto-antibodies target red blood cell (RBC) membrane proteins, leading to RBC destruction. These antibodies are detected using the Coombs test:

1. Direct Coombs Test: Detects antibodies directly bound to RBCs by causing agglutination when anti-human antibodies are added.
2. Indirect Coombs Test: Detects antibodies in the patient's serum by adding it to test RBCs with specific antigens to identify the type of antigen involved.

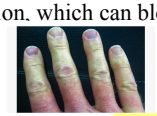


Warm Type IHA

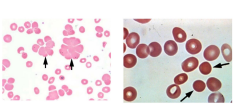
- Antibody Type: IgG auto-antibodies, which bind with high affinity to RBCs in the core circulation at 37°C.
- Pathophysiology:
  - Bound RBCs are removed by macrophages in the spleen (extravascular hemolysis), leading to the formation of spherocytes that are eventually destroyed by the spleen.
- Causes:
  - 60% idiopathic
  - 25% associated with systemic lupus erythematosus (SLE)
  - 15% caused by drugs (e.g., α-methyldopa, penicillin)
- Clinical Features: Most patients present with mild chronic anemia and splenomegaly, but the severity can vary.

Cold Type IHA

- Antibody Type: IgM auto-antibodies, which bind with low affinity at temperatures below 30°C in the peripheral body regions.
- Pathophysiology:
  - After IgM binds, complement components (C3b and C3d) attach to RBCs. When RBCs return to core circulation, IgM dissociates, but C3b remains, marking the RBCs for removal by splenic macrophages.
  - IgM can bind multiple RBCs at once, causing in vivo agglutination, which can block small capillaries, leading to Raynaud phenomenon (restricted blood flow in extremities).



- Causes:
  - Transient cold-IHA may follow infections like Mycoplasma pneumonia and infectious mononucleosis (self-limited).
  - Chronic cold-IHA is associated with B-cell lymphoma or occurs idiopathically.
- Clinical Features:
  - Agglutination of RBCs leads to clumps in various directions on a blood smear.
  - Spherocytes (small, round, hyperchromatic RBCs) are seen due to partial phagocytosis.



• Left: RBC agglutination: RBC clumps in different directions
• Right: spherocytes appear as small, round hyperchromatic RBC

Hereditary Spherocytosis Overview

Hereditary spherocytosis is a genetic disorder affecting the red blood cell (RBC) membrane, most commonly inherited in an autosomal dominant manner, though autosomal recessive cases also occur. It results from mutations in proteins that make up the RBC membrane skeleton, primarily **ankyrin**, **band 3**, or **spectrin**.

Pathogenesis

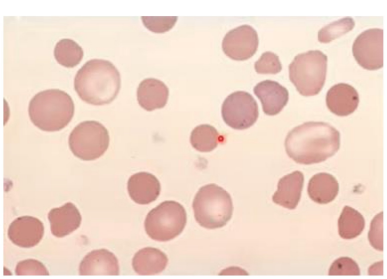
Little amount of cytoplasm is lost

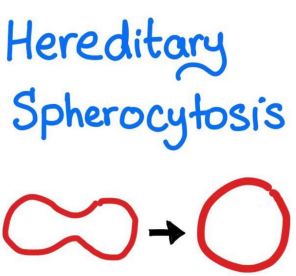
- **Membrane Instability**: Mutations in membrane proteins lead to instability, causing the RBC membrane to lose small fragments as the cell ages.
- **Loss of Biconcave Shape**: As the surface area decreases, RBCs lose their normal biconcave shape and become smaller, more spherical (spherocytes).
- **Extravascular Hemolysis**: Spherocytes, being nondeformable, get trapped in the spleen's small vessels and are engulfed and destroyed by splenic macrophages.
  - **Splenectomy**: Removing the spleen stops the destruction of spherocytes, correcting the anemia.
- **Variable Anemia**: The severity of anemia varies depending on the type of mutation, ranging from asymptomatic cases to severe hemolysis.

↳ histiocyte

Laboratory Findings

- **Spherocytes in Blood**: Peripheral blood smear shows spherocytes, which are smaller than normal RBCs (low MCV).
- **Normal Hemoglobin Content**: Despite the smaller size, spherocytes retain a normal amount of hemoglobin (normal MCH), but their mean corpuscular hemoglobin concentration (MCHC) is increased.
- **Osmotic Fragility**: Spherocytes are more fragile in hypotonic solutions, leading to increased osmotic fragility.



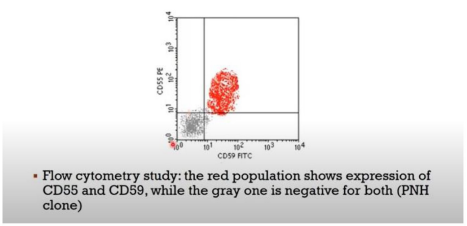


Paroxysmal Nocturnal Hemoglobinuria (PNH)

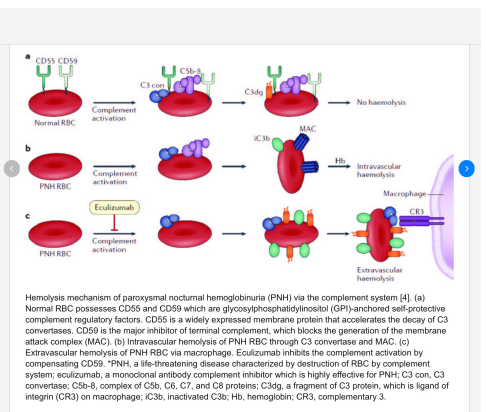
PNH is a rare acquired disorder caused by a mutation in the **PIGA gene**, leading to a deficiency in **phosphatidylinositol glycan (PIG)**, a membrane protein that anchors protective proteins to blood cells. This mutation affects bone marrow stem cells, impacting RBCs, WBCs, and platelets.

Pathogenesis

- **Complement System**: The complement system attacks cell membranes by creating pores, leading to cell lysis (C5b-C9).
- **Protective Proteins**: Normally, RBCs, WBCs, and platelets are protected from complement attack by **CD55** and **CD59**, which are attached to the cell membrane by PIG.
- In **PNH**, due to the lack of PIG, blood cells (mainly RBCs) are vulnerable to spontaneous lysis.
- **Nocturnal Hemolysis**: Hemolysis occurs more during sleep due to increased CO_2 and decreased blood pH, which activates the complement system.
- **Thrombosis**: Thrombosis is common in PNH.



Flow cytometry study: the red population shows expression of CD55 and CD59, while the gray one is negative for both (PNH clone)



Hemolysis mechanism of paroxysmal nocturnal hemoglobinuria (PNH) via the complement system [4]. (a) Normal RBC possesses CD55 and CD59 which are glycosylphosphatidylinositol (GPI)-anchored self-protective complement regulatory factors. CD55 is a widely expressed membrane protein that accelerates the decay of C3 convertases. CD59 is the major inhibitor of terminal complement, which blocks the generation of the membrane attack complex (MAC). (b) Intravascular hemolysis of PNH RBC through C3 convertase and MAC. (c) Extravascular hemolysis of PNH RBC via macrophage. Eculizumab inhibits the complement activation by compensating CD59. *PNH, a life-threatening disease characterized by destruction of RBC by complement system; eculizumab, a monoclonal antibody complement inhibitor which is highly effective for PNH; C3 con, C3 convertase; C5b-8, complex of C5b, C6, C7, and C8 proteins; C3dg, a fragment of C3 protein, which is ligand of integrin (CR3) on macrophage; iC3b, inactivated C3b; Hb, hemoglobin; CR3, complementary 3.

Traumatic Hemolysis

Traumatic hemolysis occurs when physical forces or turbulence cause the destruction of RBCs. Common causes include:

- **Prosthetic heart valves**.
- **Repetitive physical activities**: such as marathon running, boxing, or marching.
- **Disseminated Thrombi**: in microangiopathic hemolytic anemia.

Schistocytes (fragmented RBCs) are the hallmark of traumatic hemolysis.