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Pathology

MID | Lecture 4

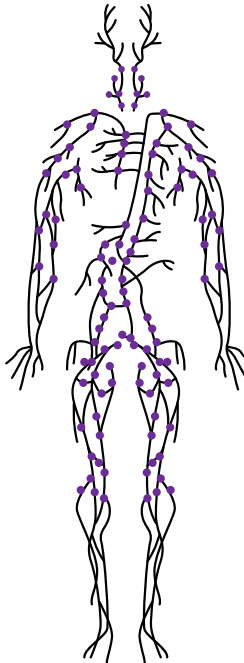
Hemolytic Anemias

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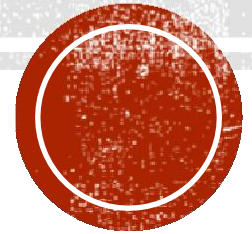
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HEMOLYTIC ANEMIAS

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PATHOPHYSIOLOGY of Hemolytic Anemia

- RBC life span < 120 days (in Anemia, this number is reduced)
- The Anemia causes Hypoxia, which in turn triggers the release of erythropoietin, leading to Erythroid hyperplasia in bone marrow (Activation of bone marrow to produce more RBCs)
- Peripheral blood reticulocytosis occurs, indicating that the bone marrow is normal, and the problem is in the RBCs.
- Extramedullary hematopoiesis in severe cases
- Hemoglobin is released from damaged/destroyed RBCs, and from here it follows two main pathways of excretion:
 - 1) It may be **metabolized into bilirubin**, potentially causing **jaundice**.
 - 2) It may **enter the blood and get excreted in the urine**, leading to **red-colored urine**.
- Serum haptoglobin: decreased (binds free Hg) in both intra and extravascular hemolysis

Haptoglobin is a plasma protein that binds and neutralizes free hemoglobin released into the blood after red blood cell destruction. The resulting haptoglobin-hemoglobin complex is then removed from the circulation and excreted in the urine. Therefore, **haptoglobin levels fall** because it becomes bound (to Hb) and is cleared, making serum haptoglobin a useful laboratory marker for hemolytic anemia.

CLASSIFICATION

- Main site of hemolysis:
- 1) **Extravascular Hemolysis** (Outside the bloodstream, primarily in the spleen)
Occurs when RBCs have abnormal shape or are coated with antibodies or are slower than normal → removed by macrophages (histiocytes). Patients have Jaundice, Pigmented gallbladder stones, Splenomegaly (enlarged spleen due to hyperfunctioning).
- 2) **Intravascular Hemolysis** (Inside the bloodstream)
Occurs when RBCs are destroyed inside blood vessels → causes sudden release of hemoglobin into the bloodstream, so here's no time for hemoglobin to be converted into bilirubin and the majority of hemoglobin will be excreted in the urine. Patient have Hemoglobinemia (which will cause depletion of haptoglobin), Hemoglobinuria (red-colored urine), Hemosiderinuria (iron in the urine, leading to iron deficiency and precipitation of iron in kidneys).
- According to cause of hemolysis (less important classification)
- Extracorpuscular (extrinsic factor) vs intracorpuscular
 outside RBC, like antibody-mediated inside RBC, such as enzyme defects
 destruction or malarial attacks

G6PD DEFICIENCY

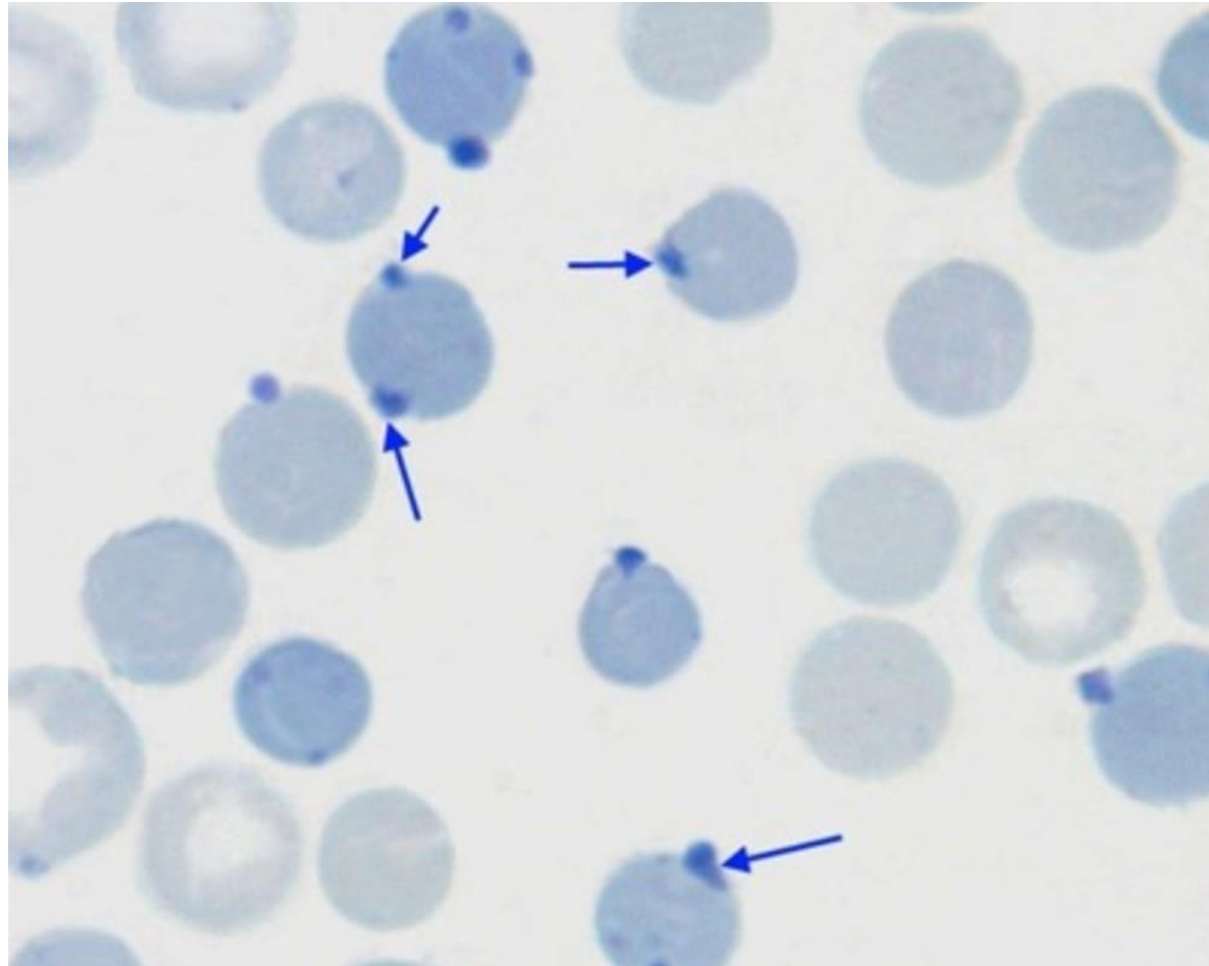
- X-linked inheritance, so it is more common in young males, while females are usually carriers.
- Glucose 6-phosphate dehydrogenase deficiency, the enzyme amount is reduced (up to about 20%) but not completely.
- Reduced production of glutathione, important for cell protection against harmful oxidants.
- G6PD enzyme is responsible for recycling glutathione, which neutralizes reactive oxygen species – harmful products of metabolic reactions. In cases of **G6PD deficiency**, the body has **reduced levels of glutathione**, which impairs its ability to handle oxidative stress.
- Since RBCs produce a small amount of G6PD and **lack a nucleus**, they cannot synthesize more G6PD once mature. As a result, G6PD is consumed early in the RBC lifespan, and its depletion leads to **premature RBCs destruction**.
- Under normal conditions, individuals with G6PD deficiency may not show any symptoms, as the RBCs function relatively well. However, during a crisis, such as exposure to oxidative stress (infections, certain drugs, or food triggers), the rate of RBC destruction increases, leading to severe hemolysis and more noticeable symptoms

TRIGGERS OF HEMOLYSIS

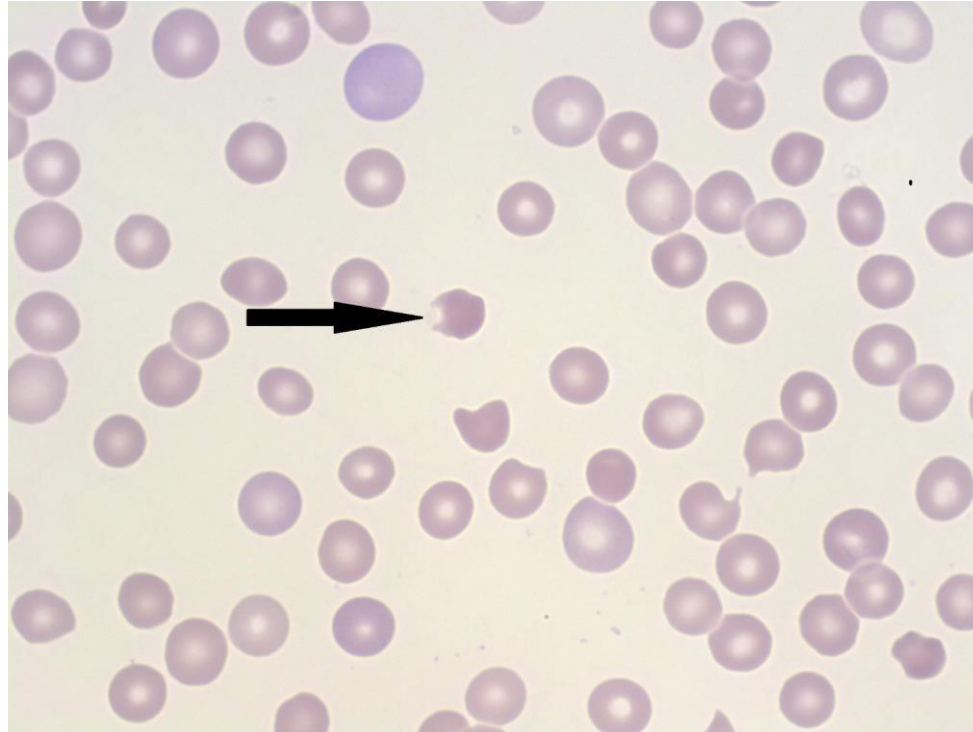
- Infection, since the WBCs use oxidants to kill microbes.
- Certain drugs: (1) sulfonamides, (2) nitrofurantoin, (3) large dose of aspirin, (4) vitamin K, (5) primaquine (anti-malaria drug), their metabolism increase the oxidative stress.
- Fava beans (most famous cause), their digestion increase the oxidative stress.
- In all, large numbers of oxidants are generated, G6PD cannot neutralize them, causing hemoglobin denaturation and precipitate in the membrane (Heinz bodies), damaging cell membrane and massive hemolysis of RBCs, 2-3 days after trigger.
- Other cells lose deformability and partially phagocytosed inside spleen (bite cells)

Both are antibiotics

Hemolysis occurs (1) when **Heinz bodies** directly cause rupture of the RBC membrane (intravascular hemolysis), or (2) when they deform the affected cells, making them recognized and removed by splenic macrophages (extravascular hemolysis).



- **Supravital special stain** highlights Heinz bodies as membrane-bound, dark spots representing condensed and denatured Hg (hemoglobin).
- Heinz bodies make RBCs less flexible and harder to circulate, leading macrophages to remove these inclusions and form characteristic '**bite cells**'. (See the following slide)



- **Bite cells:** appears as indented defect in part of cell membrane of RBCs
- After these cells are bitten by splenic macrophages during their first circulation, they undergo complete hemolysis (either intravascular or extravascular) in subsequent circulations.

CLINICAL TYPES

- Extravascular and intravascular hemolysis processes develop (phagocytosis of bite cells and cell membrane damage by Heinz bodies)
- G 6PD-A type (African type): modest decrease in amount of G6PD, bone marrow compensate by producing new RBCs
- G6PD-Mediterranean: qualitative defect of enzyme (low function), more severe symptoms.
 - Here, the amount of the enzyme is normal, but its activity is reduced; therefore, laboratory evaluation should include tests for both the quantity and the quality (Activity) of G6PD.
- Females: can have symptoms if random inactivation affects the normal X-chromosome.
 - It is a rare condition in which the normal X chromosome in a carrier female becomes inactivated, while the mutated one remains active, leading to the development of G6PD deficiency.
 - Females, unsurprisingly, can also contract the disease if they are homozygous for the mutated allele.

IMMUNE HEMOLYTIC ANEMIA

It is an autoimmune disease, also known as **Autoimmune Hemolytic Anemia**.

- The presence of auto-antibody against RBC membrane protein coat the red blood cells, which are then recognized and destroyed when they reach the spleen.
- These antibodies are detected by **Coombs test**
- **Direct** Coombs test: RBCs of patient are incubated with antibodies that target normal human antibodies (RBCs will agglutinate)
- **Indirect** Coombs test: patients' serum is added to "test RBCs" that have certain surface proteins (identify the type of antigen)

Direct Coombs Test:

The patient's RBCs are already coated with autoantibodies. When an external (anti-human) antibody is added, it binds to these autoantibodies on multiple RBCs at once, causing **agglutination** (the RBCs clump together like a small thrombus).

- ✓ **Positive test:** Autoantibodies are attached to RBCs → agglutination occurs → indicates autoimmune hemolytic anemia.
- ✓ **Negative test:** No autoantibodies on RBCs → no agglutination → normal.

Indirect Coombs Test:

Used to **identify the specific antigen** being targeted by the antibodies.

The patient's **serum (containing antibodies)** is mixed with **synthetic RBCs** that each express only one known antigen.

- ✓ **If agglutination occurs:** The antibodies in the serum are reacting with that specific antigen → identifies the target antigen.
- ✓ **If no agglutination:** Try another set of RBCs with different antigens until the specific target is found.

WARM TYPE

Immune hemolytic anemia is classified into two types:

- ❖ **Warm type:** Mediated by IgG antibodies, active at body temperature ($\sim 37^{\circ}\text{C}$), typically affecting central warm areas like the neck and trunk (discussed in this slide).
- ❖ **Cold type:** Mediated by IgM antibodies, active at lower temperatures, usually affecting cooler peripheral areas (discussed in next slide).

- High affinity auto-antibody (mostly Ig G type)
- Binding occurs in core circulation (37°C)
- The antibodies are removed by splenic macrophages, and after repeated removals, the RBCs lose their normal shape and become spherocytes.
- spherocytes develop, then destroyed by spleen (extravascular hemolysis)
- Causes: 60% are idiopathic, 25% associated with systemic lupus erythematosus (Autoimmune disease), 15% by drugs (α -methyldopa, penicillin)
- Severity of anemia is variable, most patients have mild chronic anemia and splenomegaly

COLD TYPE

- Low-affinity autoantibody (**IgM**).
 - IgM is a **large molecule** that can bind 5 RBCs at a time, resulting in RBCs agglutination.
- **Binding occurs in peripheral areas of body** like the digits, nose and the ear, where low temperatures ($<30^{\circ}\text{C}$) are present, in which IgM performs best.
- After IgM binding, few **C3b and C3d molecules bind RBCs**
- When RBCs bound to IgM and C3b and C3d return to core circulation ($\sim 37^{\circ}\text{C}$), **IgM dissociates, but C3b stays**, which are then identified by **splenic macrophages by specific receptors** and removed with the appearance of **spherocytes**, which are again destroyed in subsequent circulations.

❖ The Complement System: A Brief Explanation

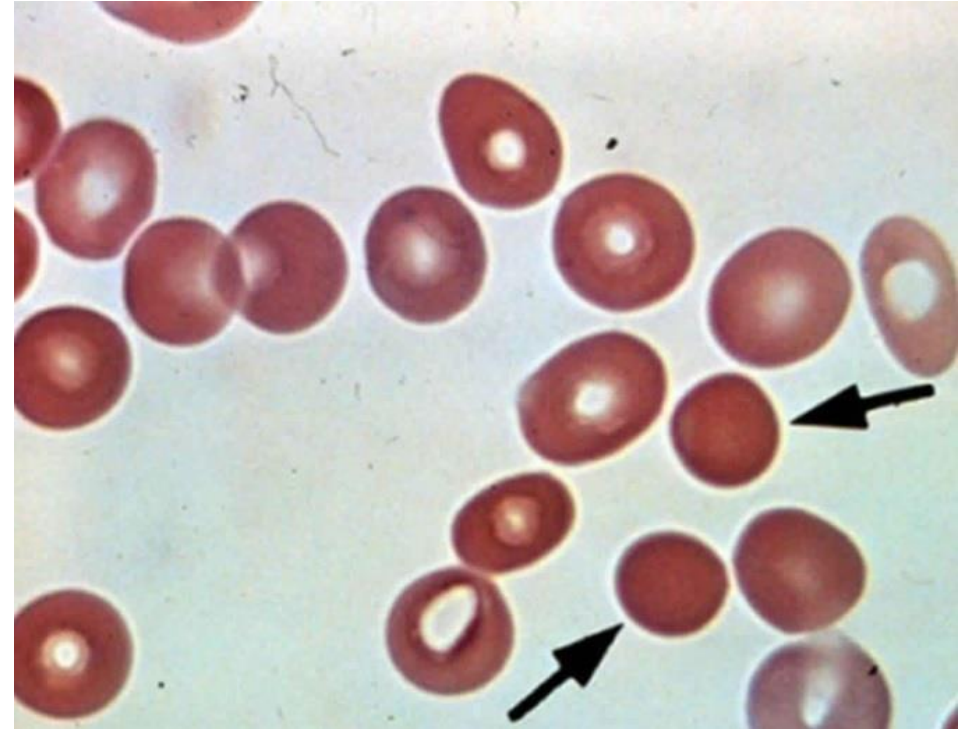
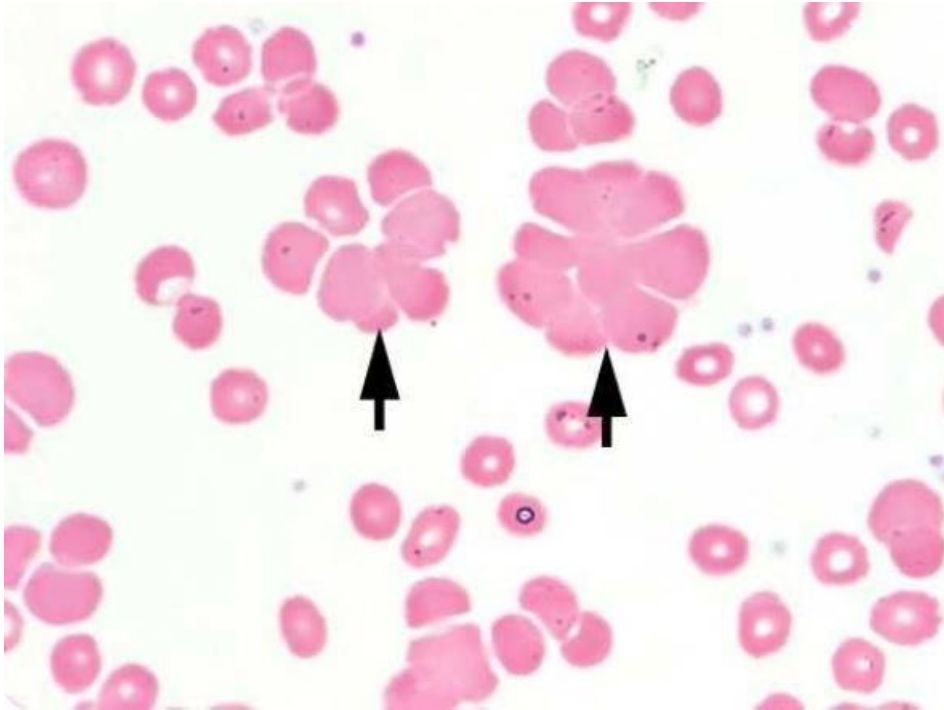
- The complement system consists of multiple proteins that are activated in a cascade, ultimately forming membrane attack complexes (MACs) that pierce cell membranes.

COLD TYPE

- IgM binds 5 RBCs, thus creating in vivo **agglutination**, which might **block small capillaries** resulting in symptoms of **ischemia** in fingers and toes causing **Raynaud phenomenon**.
 - A reversible condition, no necrosis nor infarction, where digits are cold and painful.

Clinically

- Transient forms, **resolve without treatment**, of cold-IHA occur in recovery of infections by **mycoplasma pneumonia** and infectious mononucleosis **caused by Epstein–Barr virus (EBV)**.
 - After recovery from these infections, transient cold-type anemia may occur due to immune system dysregulation.
 - These forms are **mild**, and **self-limited** that can affect children and young adults.
- **Chronic and persistent** form occurs **in individuals** with **B-cell lymphoma** or idiopathic.
 - Neoplastic B cells produce excessive and abnormal immunoglobulins, which can trigger hemolysis.



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

➤ So, Findings in immune hemolytic anemia:

1. Presence of **spherocytes** on peripheral blood smear (seen in warm and cold types).
2. **Agglutinated RBCs** are seen only in cold IHA (IgM-mediated).

Immune Hemolytic Anemia: A Comparison

Feature	Warm IHA	Cold IHA
Antibody Type	High-affinity IgG	Low-affinity IgM
Temperature / Site	Acts at 37°C in core circulation	Acts at <30°C in peripheral areas (digits, nose, ears)
Mechanism	RBCs coated with IgG are removed by splenic macrophages → spherocytes → destroyed in spleen (extravascular hemolysis)	IgM binds 5 RBCs → agglutination → binds C3b/C3d → after warming, IgM detaches, complement remains → RBCs destroyed by macrophages
Causes	60% idiopathic, 25% SLE , 15% drug-induced (α-methyldopa, penicillin)	Transient: after Mycoplasma pneumoniae or EBV infection Chronic: with B-cell lymphoma or idiopathic
Clinical Features	Variable anemia, often mild and chronic with splenomegaly	Raynaud phenomenon , cold-induced pain and pallor, reversible, no necrosis
Complement System	–	Complement activation (C3b, C3d).
Course	Chronic, variable	Transient in infections (mild, self-limited) or chronic in neoplastic conditions
Spherocytes	present	present
Agglutination	absent	present

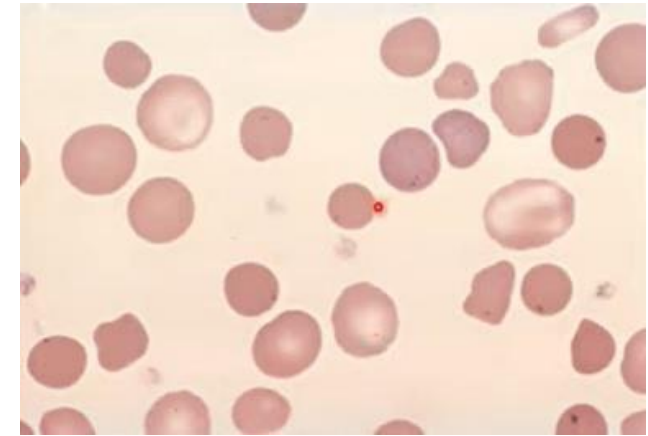
HEREDITARY SPHEROCYTOSIS

- A **genetic (mostly)** Autosomal Dominant, sometimes recessive) condition in which RBCs are inherently spherocytes without antibody involvement, unlike IHA.
- Caused by a **mutation** that is in the RBC cell membrane skeleton.
 - Most commonly affects **ankyrin, band 3 or spectrin**, these normally give the RBC stability. In this case, mutations cause membrane instability and progressive loss of cell membrane.
- Cell membrane becomes unstable, keeps losing parts of it as the RBC age, and eventually becomes a spherocyte.
- **Little amount of cytoplasm is lost.**
- With **decreasing surface area**, the RBC loses its normal biconcave morphology and becomes a smaller sphere.

PATHOGENESIS

- Spherocytes are **rigid, nondeformable** and slower than normal RBCs.
- Entrapped in small vessels in **spleen**, engulfed by histiocytes (macrophages) and destroyed (**extravascular hemolysis**).
- The only curative treatment is **splenectomy**, which corrects anemia and hypoxia.
 - If spleen is removed, spherocytes persist in peripheral blood, thus, **anemia is corrected**, since spherocytes usually function adequately.
- The degree of anemia is variable (depends on the type of mutation).
 - Some patients are **asymptomatic**, while others might have **severe hemolysis** depending on the extent of protein abnormality.
- **A positive family history is typical.**

LABORATORY FINDINGS



- ✓ MCH: Mean Cell Hemoglobin
 - ✓ MCV: Mean Cell Volume
 - ✓ MCHC (MCH/MCV): Mean Cell Hemoglobin Concentration
- Appearance of **spherocytes** in peripheral blood
 - Spherocytes have a **smaller size** (low MCV)
 - Little cytoplasm is lost, **normal amount of Hg** (normal MCH)
 - **A defining characteristic is that** MCHC is increased, as (**MCH/MCV**) ratio rises.
 - Spherocytes show **increased fragility** when put in hypotonic solution (increased osmotic fragility).
 - ✳ **So, diseases with spherocytosis:**
 - A. Autoimmune hemolytic anemia (**positive** Coombs test).
 - B. Hereditary spherocytosis (**negative** Coombs test).

PAROXYSMAL NOCTURNAL HEMOGLOBINUREA

Sudden-onset

Occurs at night

Intravascular hemolysis (red urine)

- **Rare**, and acquired disease.
- An **acquired Mutation in PIGA gene**, affecting older individuals, results in deficiency in **phosphatidylinositol glycan (PIG)**, a structural protein on cell membrane that anchors many other proteins.
 - ✓ The absence of PIG **prevents binding of protective antigens CD55 and CD59** on cell membranes.
- Mutation occurs in **bone marrow stem cell** (leukocytes, RBCs and platelets are all affected).

PATHOGENESIS

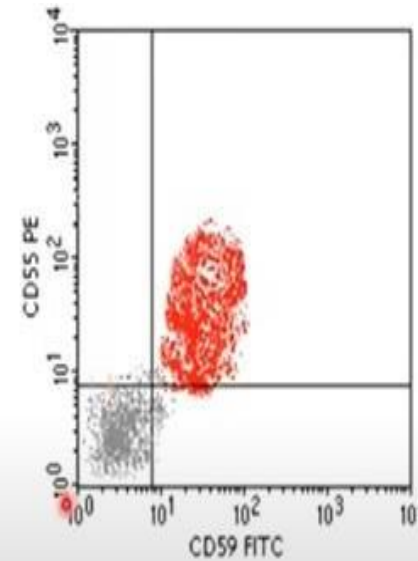
- Complement system: circulating proteins that are part of immune system. They are activated (C5b-C9) and attack cell membrane to create pores, causing lysis
- Blood cells protect themselves by membrane proteins **CD55** and **CD59**, that are normally attached to PIG.
 - Normally, these proteins **stop** the complement system from attacking blood cells, including erythrocytes.
- In PNH: RBCs, and to a lesser degree WBCs and platelets, are spontaneously lysed inside blood. **Therefore, Hemolytic anemia, leukopenia, and thrombocytopenia** are observed.
- During sleep, $\uparrow\text{CO}_2$, $\downarrow$ blood PH, more active complement system, more hemolysis – next slide.
- Thrombosis is common – next slide.

PNH – Features

- **During sleep, $\uparrow$ CO₂ (hypercapnia), $\downarrow$ blood PH, more active complement system, more hemolysis.**
 - Hemolysis occurs mainly at night (nocturnal) due to mild acidosis from reduced respiration during sleep, which activates complement.
- **Thrombosis is common.**
 - Despite low platelet counts, patients develop thrombosis due to release of prothrombotic substances (e.g., ADP, thromboxane) from lysed platelets.
 - Thrombosis is the most serious and potentially fatal complication.

Diagnosis of PNH

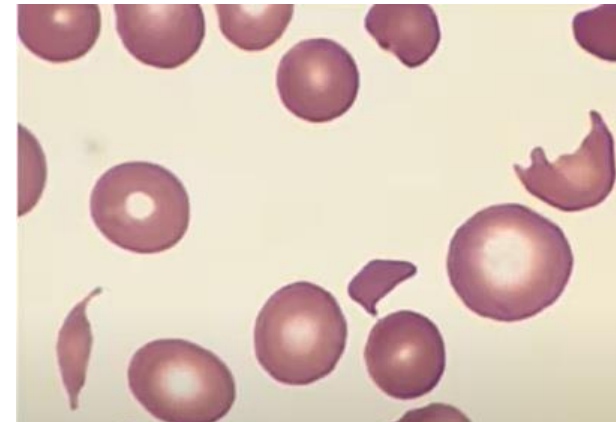
- Done using **flow cytometry** to detect the presence (or absence) of CD55 and CD59 on RBCs.
- **Normal cells** express these markers, observed by their diagonal movement; deficient cells (lacking CD55/CD59) are detected as a separate population on flow cytometry plots.



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

TRAUMATIC HEMOLYSIS

- **Direct physical force, or turbulence**, unorganized flow of blood, causing lysis of RBCs, resulting in fragments of RBCs.
- ❖ Seen in patients with:
 1. **Prosthetic heart valves**, made from metal, and when an RBC hits the valve, it's lysed.
 2. **Repetitive physical pounding** (marathon, boxing, marching)
 3. Disseminated thrombi (**microangiopathic hemolytic anemia**)
 - Associated with thrombotic disorders and is caused by widespread (**disseminated**) microthrombi in small blood vessels.
 - Thrombi fragment RBCs as they pass through narrow capillaries, producing schistocytes.
- Hallmark of traumatic hemolysis: **schistocytes** (“split cells”).



Pathology Quiz 4



For any feedback, scan the code or click on it.



Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			