Lecture 4
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MACROPHAGES

Professor Tariq Aladily

Department of Pathology

The University of Jordan

tnaladily@ju.edu.jo

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PATHOPHYSIOLOGY

- RBC life span < 120 days immature destruction
- Hypoxia triggers release of erythropoietin
- Erythroid hyperplasia in bone marrow
- Peripheral blood reticulocytosis seen in all types of hemolytic anemia
- Extramedullary hematopoiesis in severe cases
- Hemoglobin is released in from damaged RBCs → e.g. red urine
- Serum haptoglobin: decreased (binds free Hg) in both intra and extravascular hemolysis → Hemolytic anemia: ↓ Haptoglobin ↓ Binds to iron and is released into urine

CLASSIFICATION

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

- *According to cause of hemolysis*
  - Extracorpuscular (extrinsic factor) vs intracorpuscular (e.g. Antibodies, Malaria)

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G6PD DEFICIENCY

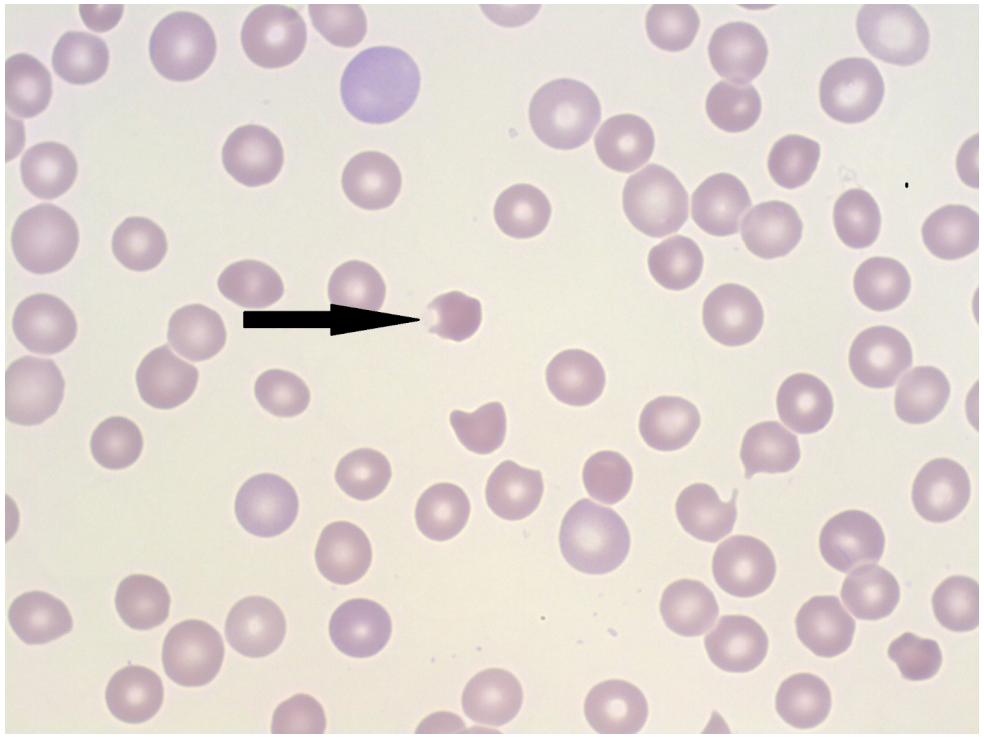
Found in all types of cells, but when it's deficient, RBCs are the most affected. (Most vulnerable) → What happens to the other cells? is 20% enough?
Other cells have a nucleus (They can produce more when needed)

- X-linked inheritance — males are the most affected, females are usually carriers
- Glucose 6-phosphate dehydrogenase deficiency
- Reduced production of glutathione, important for cell protection against harmful oxidants

TRIGGERS OF HEMOLYSIS

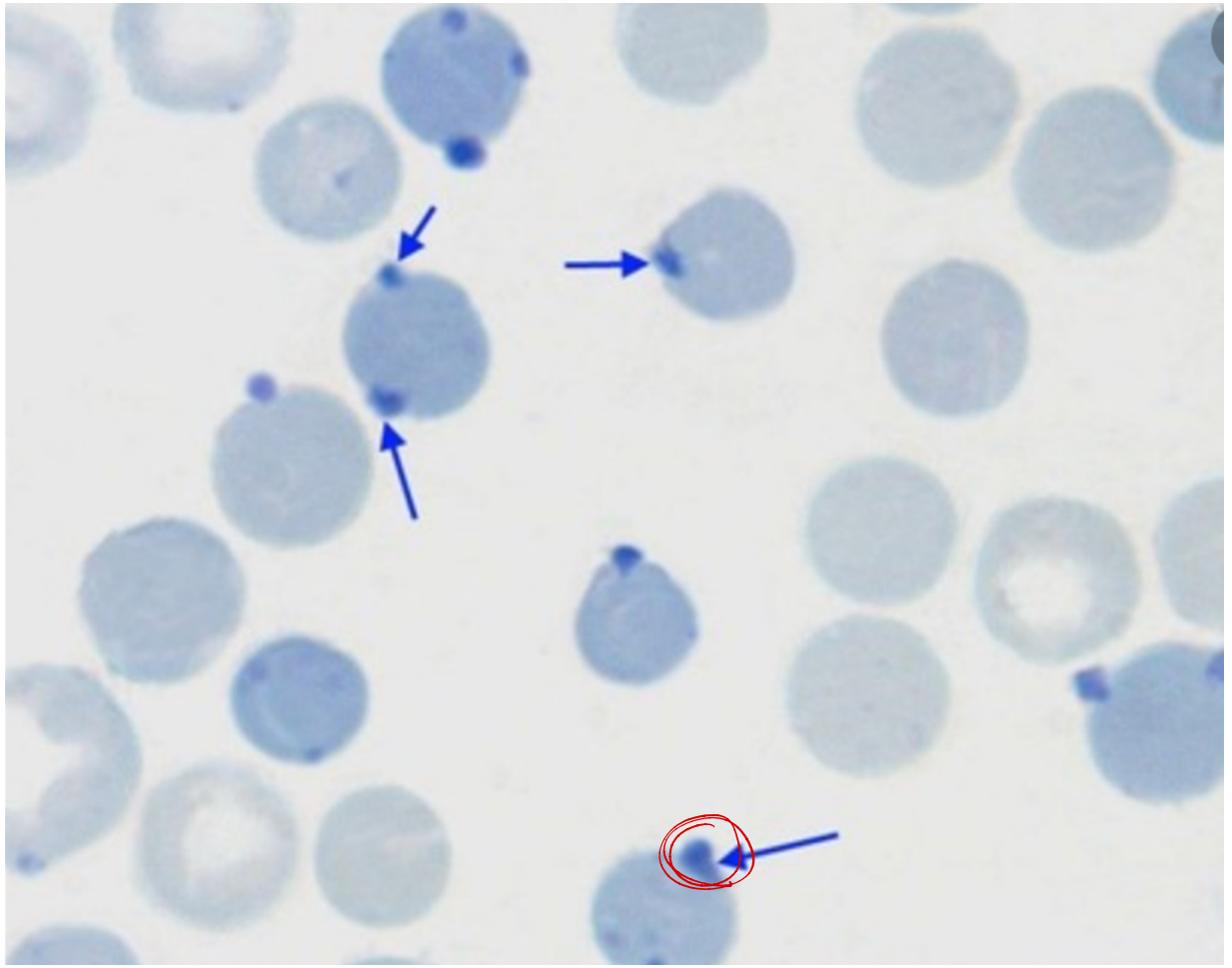
- Infection e.g. Rhinitis, Pharyngitis
  Release of oxidants → Massive hemolysis
- Certain drugs: sulfonamides, nitrofurantoin (used to treat UTIs), large dose of aspirin, vitamin K, primaquine (Anti-malaria / RA)
  Vitamin K-dependent clotting factors? Only in large doses
- Fava beans (Favism) Their metabolism leads to the production of oxidants
- In all, large numbers of oxidants are generated, G6PD cannot neutralize them, causing hemoglobin denaturation and precipitate (Heinz bodies), damaging cell membrane and massive hemolysis of RBCs, 2-3 days after trigger (Glutathione depletion; Not instant)
- Other cells lose demorfmability and partially phagocytosed inside spleen (bite cells)

Normal RBC turnover also occurs due to accumulation of free radicals.



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

Bind Heinz bodies
↓
Forming bite cells



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

CLINICAL TYPES

- Extravascular and intravascular hemolysis processes develop (phagocytosis of bite cells and cell membrane damage by Heinz bodies)
- G6PD-A type: modest decrease in amount of G6PD, bone marrow compensate by producing new RBCs
- G6PD-Mediterranian: qualitative defect of enzyme (low function), more severe symptoms
- Females: can have symptoms if random inactivation affects the normal X-chromosome

We need to test for both the amount and function of G6PD

Not common

② IMMUNE HEMOLYTIC ANEMIA

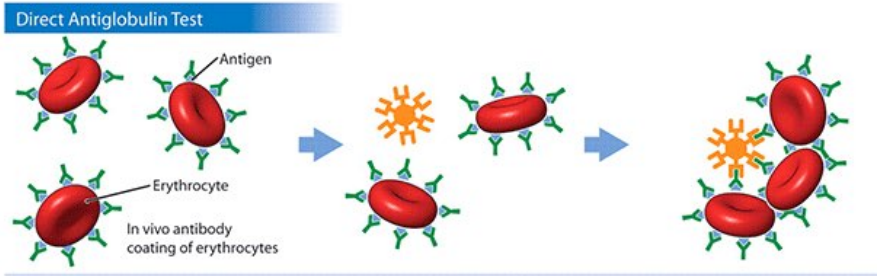
Common in clinical practice

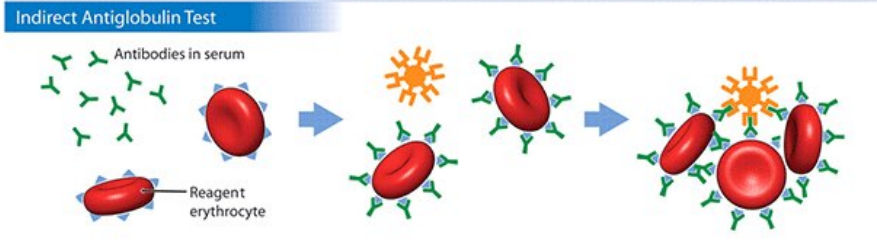
- The presence of auto-antibody against RBC membrane protein
- These antibodies are detected by Coombs test
- Direct Coombs test: RBCs of patient are incubated with antibodies that target normal human antibodies (RBCs will agglutinate) Becomes turbid
- Indirect Coombs test: patients' serum is added to "test RBCs" that have certain surface proteins (identify the type of antigen)

Direct Coomb's Test

vs

Indirect Coomb's Test





WARM TYPE

- High affinity auto-antibody (mostly IgG type)
- Binding occurs in core circulation (37oC)
- Removed by macrophages in spleen
- spherocytes develop, then destroyed by spleen (extravascular hemolysis)
- 60% are idiopathic, 25% associated with systemic lupus erythematosus, 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)
- Binding occur in peripheral areas of body (<30oC)
- After IgM binding, few C3b and C3d molecules bind RBCs
- When RBCs return to core circulation, IgM dissociates, but C3b stays, identified by splenic macrophages and removed
- IgM binds 5 RBCs, thus creating in vivo agglutination, might block small capillaries in fingers and toes causing Raynaud phenomenon
- Transient forms of cold-IHA occur in recovery of infections by mycoplasma pneumonia and infectious mononucleosis (mild, self-limited)
- Chronic persistent form occur in B-cell lymphoma or idiopathic

Complement system (Acellular)

Different from the warm type

Big AB

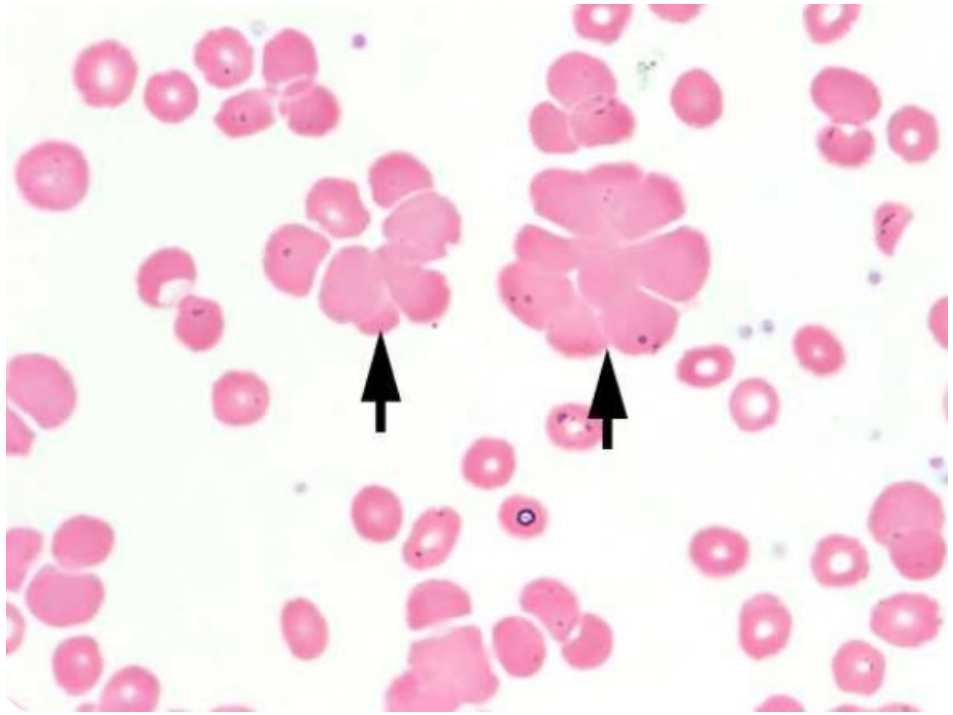
IgG only binds two

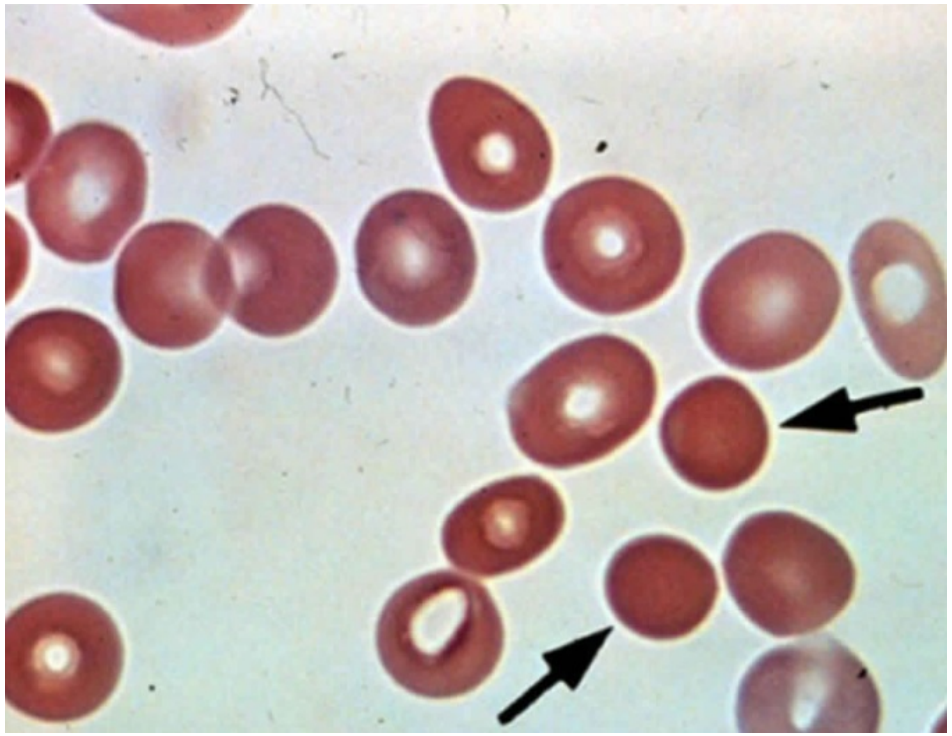
Forming a thrombus

Ischemia, painful, white (Reversible)

EBV

Causes (Common exam question)





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

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HEREDITARY SPHEROCYTOSIS

- Present in family history
  Autosomal Dominant, sometimes recessive
- Mutation is RBC cell membrane skeleton
- Most commonly affects ankyrin, band 3 or spectrin
- Cell membrane becomes unstable, keeps losing parts of it as the RBC age
- Little amount of cytoplasm is lost
- With decreasing surface area, the RBC loses it normal biconcave morphology and becomes a smaller sphere

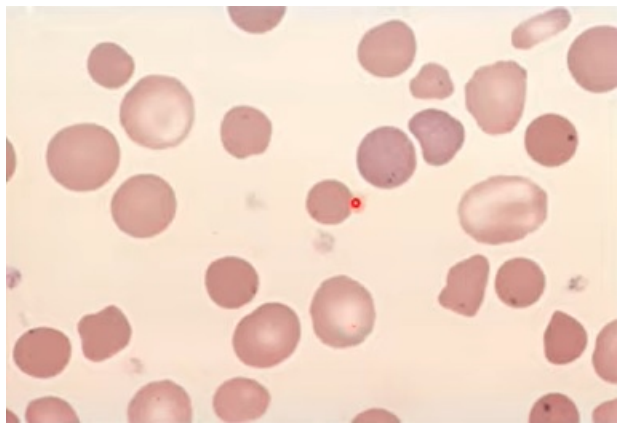
PATHOGENESIS

- Spherocytes are nondeformable
- Entrapped in small vessels in spleen, engulged by histiocytes and destroyed (extravascular hemolysis)
- If spleen is removed, spherocytes persist in peripheral blood, thus, anemia is corrected
  Optimal treatment
- The degree of anemia is variable (depends on the type of mutation)
- Some patients are asymptomatic, while others might have severe hemolysis

LABORATORY FINDINGS

- Appearance of spherocytes in peripheral blood
- Spherocytes have a smaller size (low MCV)
- Little cytoplasm is lost, normal amount of Hg (normal MCH)
- MCHC is increased
- Spherocytes show increased fragility when put in hypotonic solution (increased osmotic fragility)

Cells will burst → lysis earlier than normal → Spherocytes (Not indicative) ↓ Morphology check is necessary



Smaller MCV → Lost cell membrane not cytoplasm + looks more red

MCH is normal

MCH/MCV ratio ↑

4 PAROXYSMAL NOCTURNAL HEMOGLOBINUREA

- Occurs late in life (Not congenital)
  Rare, acquired disease
- Mutation in PIGA gene, results in deficiency in phosphatidylinositol glycan (PIG), a structural protein on cell membrane that anchors many other proteins
- 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
- In PNH: RBCs, and to a lesser degree WBCs and platelets, are spontaneously lysed inside blood
- During sleep, ↑CO2, ↓ blood PH, more active complement system, more hemolysis
- Thrombosis is common

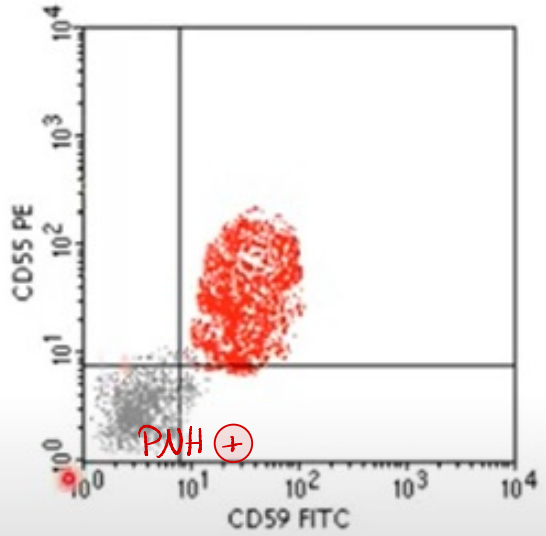
Pts taking Aspirin are usually given ABs against complementary system from the complement system

Suppress the complement system

Patients usually come with anemia, leukopenia & thrombocytopenia to a lesser degree

increased in infections as well

Due to cell lysis
↳ Prothrombotic agent
↓
Thrombosis instead of bleeding



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

5 TRAUMATIC HEMOLYSIS

- Direct physical force, or turbulence causing lysis of RBCs → Intravascular Hemolysis
- Prosthetic heart valves
- Repetitive physical pounding (marathon, boxing, marching)
- Disseminated thrombi (microangiopathic hemolytic anemia) 3 diseases → widespread thrombosis
- Hallmark of traumatic hemolysis: schistocytes "Torn apart"