HEMOLYTIC ANEMIAS

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PATHOPHYSIOLOGY

- RBC life span < 120 days
- Hypoxia triggers release of erythropoietin
- Erythroid hyperplasia in bone marrow
- Peripheral blood reticulocytosis Indicates active BM in Hemolytic Anemia for Compensation
- Extramedullary hematopoiesis in severe cases
- Hemoglobin is released in from damaged RBCs And it will be either metabolized to bilirubin or leak into the urine causing red urine
- Serum haptoglobin: decreased (binds free Hg) in both intra and extravascular hemolysis Haptoglobin Binds free Hg that leaks out of the dead RBCs and go together to the urine to protect the body from toxic Hg . Decreased (consumed) haptoglobin at the test indicates hemolytic anemia

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) Splenomegaly is due to hyper-functioning MACs inside the spleen .
- 2) Intravascular: inside blood stream (sudden release of Hg, patients have hemoglobinemia, hemoglobinurea, hemosiderinurea, iron deficiency)
  Spleen is normal . Theres no time for the Hg to be converted into bilirubin . The majority of Hg will go to the urine . Still they will have hemoglobinemia (free Hg in the blood) because haptoglobin have been consumed . And the iron could precipitate in the kidney .
- *According to cause of hemolysis*
- Extracorpuscular (extrinsic factor) vs intracorpuscular

Outsides the RBC such as
antibodies and malaria .

G6PD DEFICIENCY مرض التفوّل

- X-linked inheritance Mostly affect young boys .
- Glucose 6-phosphate dehydrogenase deficiency Not total absence
- Reduced production of glutathione, important for cell protection against harmful oxidants Causing damage to the cells .

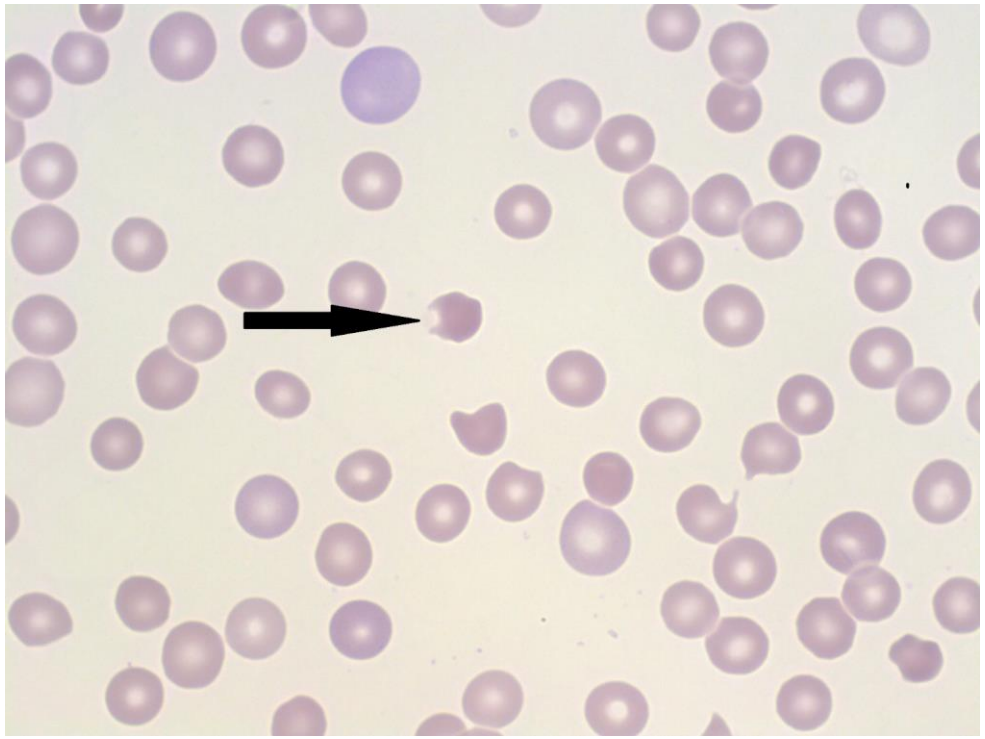
RBCs produces a little amount of G6PD that will be consumed early leading to destruction of RBCs . Because the don't have a nucleus and can't synthesize more G6PD after maturation . The will die prematurely

Asymptomatic in normal situations . Crisis (severe anemia) after being exposed to triggers

TRIGGERS OF HEMOLYSIS

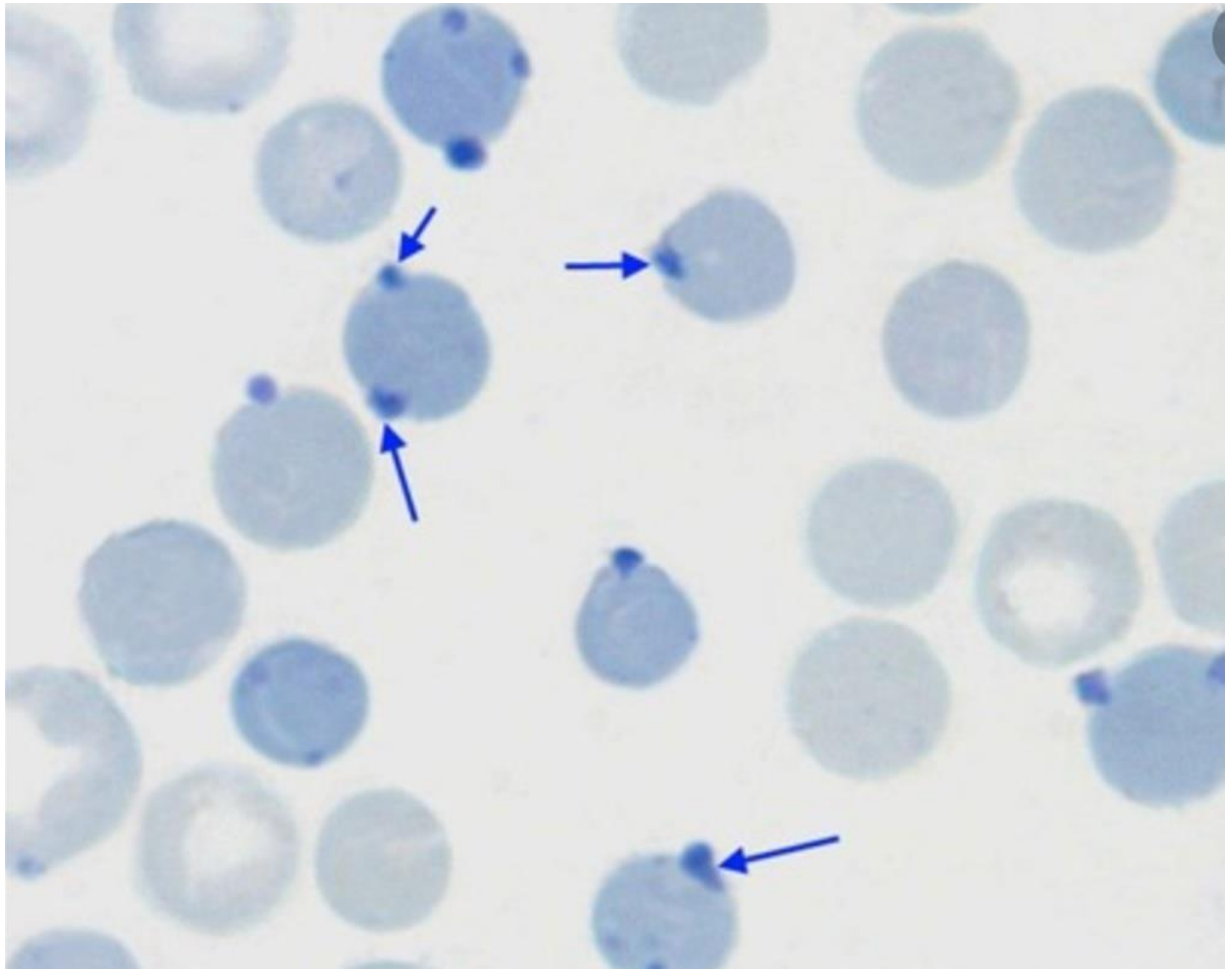
- Infection
- C ertain drugs: sulfonamides, nitrofurantoin, large dose of aspirin, vitamin K, primaquine
- Fava beans
- In all, large numbers of oxidants (ROS) are generated, G6PD cannot neutralize them, causing hemoglobin denaturation and precipitate (Heinz bodies), damaging cell membrane and massive hemolysis of RBCs, 2-3 days after trigger
- Other cells lose demorfmability and partially phagocytosed inside spleen (bite cells)

Hemolysis happens because Heinz bodies causes rupture of the membrane (intravascular) , or they will be sensed & killed by macrophages in the spleen (Extravascular)



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

  Because the Macrophages in the spleen bite the abnormal RBCs . (بتنتشها)



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

  You can't see them with normal stain

CLINICAL TYPES

- Extravascular and intravascular hemolysis processes develop (phagocytosis of bite cells and cell membrane damage by Heinz bodies)
- G 6PD-A type: modest decrease in amount of G 6PD, 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

African

You need to test the amount and the (function) of the enzyme .

Its a rare genetic phenomena

IMMUNE HEMOLYTIC ANEMIA

- 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)
- Indirect Coombs test: patients' serum is added to "test RBCs" that have certain surface proteins (identify the type of antigen)

The majority of RBCs in this patients is coated with Antibodies .

Direct Coombs : The RBCs are coated with Auto-Antibodies . We will add an external Antibody that targets the Auto-antibodies at more than one RBCs at the same time causing (agglutinates) : RBCs become close to each other forming like a thrombus If the Auto-Ab are attached to the RBCs indicating(+ disease) . If Not (normal person) no Auto-Ab No agglutination.

Indirect Coombs : I can Identify what antigen on the RBCs are being attacked . By taking serum from the patient and adding a synthetic RBCs (that have only one antigen) . If agglutination happens then this Antigen is being targeted . If Not then we need to try another Synthetic RBCs with another antigen until we know .

WARM TYPE

Because IgG perfect temperature is 37 .
IgG can only binds two RBCs

- High affinity auto-antibody (mostly IgG type)
- Binding occurs in core circulation (37°C)
- 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)
  Antihypertensive
  Attach to surface of the RBCs and it will be targeted by the immune systen
- Severity of anemia is variable, most patients have mild chronic anemia and splenomegaly

IgG is bound to RBCs so when they reach the spleen . The Antibody will be removed By macrophages (and it will take part of the cell membrane) the RBC will become smaller (spherocyte) .it will go outside of the spleen circulate and go back to the spleen again then it will be destroyed.

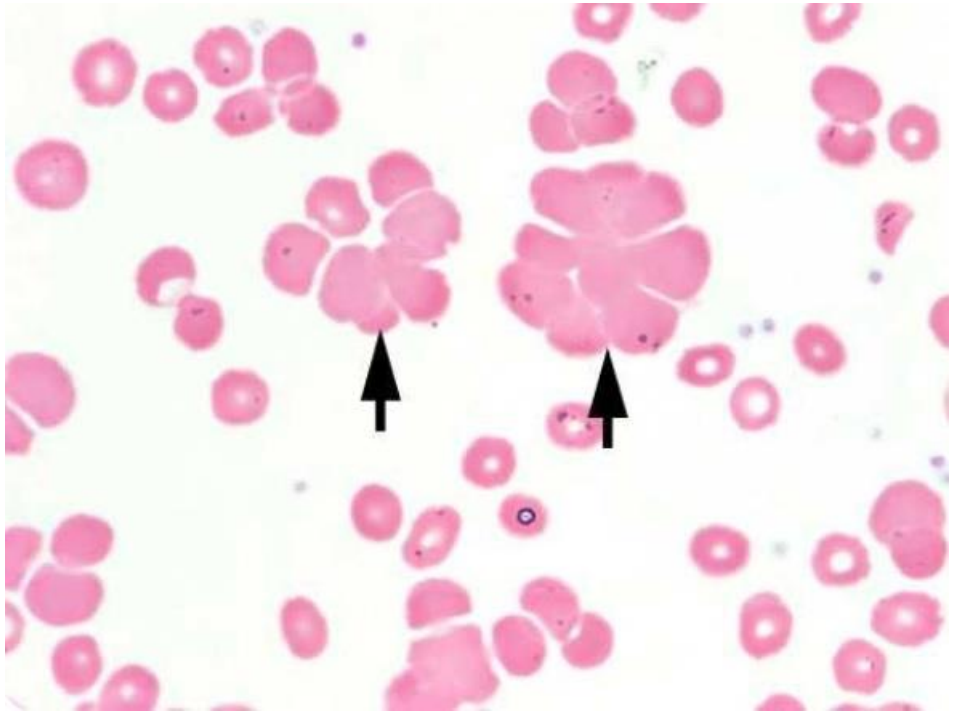
Because IgM perfect temperature is less than 30 . Digits , nose , ear .

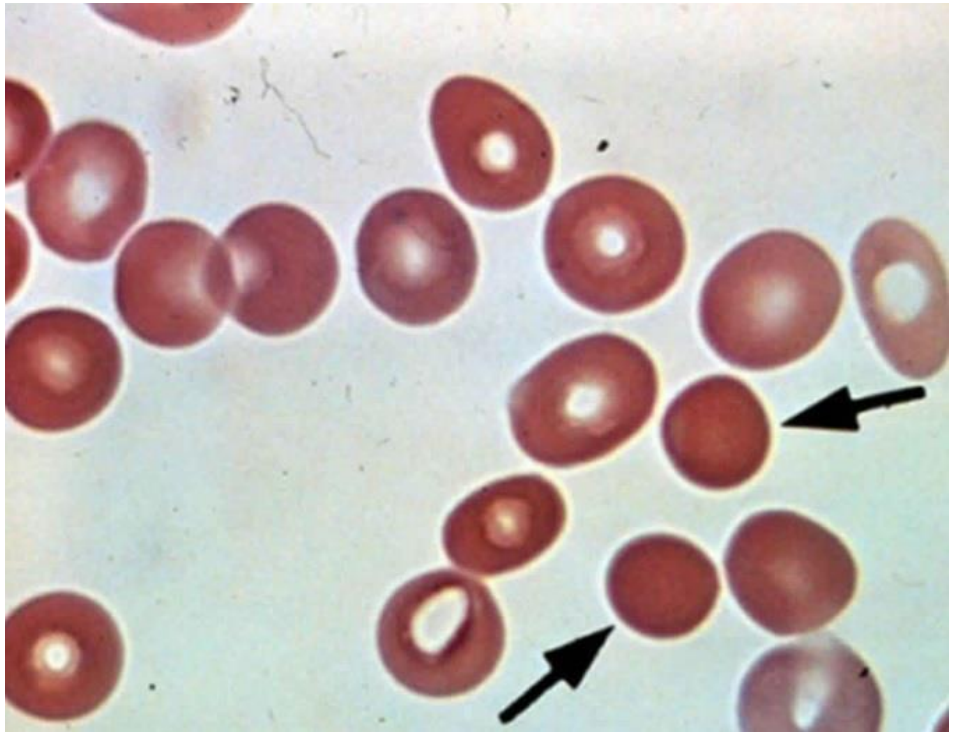
COLD TYPE

In the cold type when IgM binds RBCs it will be followed by C3b & C3d all of them will binds the RBCs in the cold areas .
Now when The RBCs circulate back to the warm areas (37*) circulation . The IgM will Detach from the RBC BUT the C3b & C3d are still attached. And they will be removed by Macrophages in the spleen causing spherocyte that will be destroyed in the second circulation .

- Low-affinity autoantibody (IgM)
- Binding occur in peripheral areas of body (<30°C)
- 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 Reversible very cold very painful digits
- Transient forms of cold-IHA occur in recovery of infections by mycoplasma pneumonia and infectious mononucleosis (mild, self-limited)
  Epstein-barr virus
- Chronic persistent form occur in B-cell lymphoma or idiopathic

Those microbes will make the immune system go crazy (بعجقوه)
making it attaking our own RBCs temporarily





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

HEREDITARY SPHEROCYTOSIS

- Autosomal Dominant, sometimes recessive
- Mutation is RBC cell membrane skeleton
- Most commonly affects ankyrin, band 3 or spectrin Which stabilizes the cell membrane
- 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 Which will be destroyed by spleen because its slower than then normal RBCs

The only treatment is splenectomy .

PATHOGENESIS

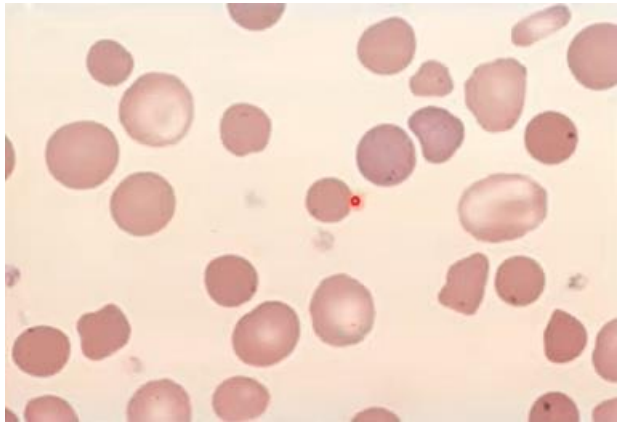
Family history is important

- 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
- 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)
- Important • MCHC is increased MCHC is MCH over MCV .
- Spherocytes show increased fragility when put in hypotonic solution (increased osmotic fragility)

Osmotic fragility test : when we add a hypotonic solution to force water to enter inside the cells . Normal RBCs will Swell and resist lysis to a higher degree than these spherocytes . They will lyse quickly in hypotonic solution . The same finding for Autoimmune hemolytic anemia . So we need coombs test to differentiate between these two diseases .



Coombs Test :
Autoimmune hemolytic anemia : +
Hereditary spherocytosis : —

Remember ; water follows salt

Sudden

At night

PAROXYSMAL NOCTURNAL HEMOGLOBINUREA

Red urine

- Rare, acquired disease Late in life
- 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

The patient usually comes with
Thrombocytopenia
Leukopenia and
Hemolytic Anemia . (Intravascular)

- 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

They stops the complement system

- In PNH: RBCs, and to a lesser degree WBCs and platelets, are spontaneously lysed inside blood

Because When PIG is absent CD55 And CD59 can't bind the RBC .
And thus the complement system will Destroys RBCs , WBCs and platelets .

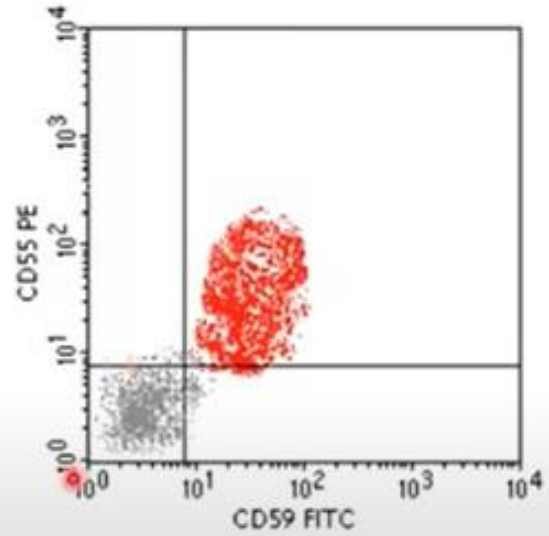
- During sleep, ↑CO2, ↓ blood PH, more active complement system, more hemolysis

Relative Hypercapnia and acidosis

- Thrombosis is common

Platelets are low but still we have thrombosis . Why ??
Because when platelets lyse they release prothrombotic agents
Thrombosis here is more important than anemia cause it can be fatal .

Red population is normal cells carrying CD55 & CD59 . But we still have abnormal gray population that is sufficient to cause (PNH)



- 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 causing lysis of RBCs
- Prosthetic heart valves Because its metal . Can break the RBCs can cause anemia .
- Repetitive physical pounding (marathon, boxing, marching)
- Disseminated thrombi (microangiopathic hemolytic anemia)Wide spread small thrombi all over the body
- Hallmark of traumatic hemolysis: schistocytes Broken