Doctor.021

no. 5

HLS PATHOLOGY

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HEMOLYTIC ANEMIA

*In all types of hemolytic anemia there is:

- reduced **RBC life span < 120 days,** which causes hypoxia.
- **Hypoxia triggers release of erythropoietin**
- **Erythroid hyperplasia in bone marrow**
- **Peripheral blood reticulocytosis** in response to increased EPO.
- **Hemoglobin is released in from damaged RBCs** which is then called free hemoglobin.
- **Serum haptoglobin: decreased (binds free Hg) in both intra and extravascular hemolysis**

Hemoglobin is toxic to the tissues, so when it's released from the RBCs, haptoglobin, which is a protein synthesized normally in the body and present in the blood, binds the free Hg molecules to neutralize it.
Low levels of haptoglobin in the serum indicate the presence of hemolysis, because more haptoglobin will be bound to hemoglobin.

*But only in severe cases:

- **Extramedullary hematopoiesis in severe cases**

CLASSIFICATION based on:

- **Main site of hemolysis:**
  1) **Extravascular: occurs primarily in spleen** (most common site) **(RBCs have abnormal shape or coated with antibodies, removed by macrophages, patients have jaundice, pigmented gall bladder stones, splenomegaly** (because it increase their activity) **)**
  2) **Intravascular: inside blood stream (sudden release of Hg, patients have hemoglobinemia, hemoglobinurea** (dark urine)**, hemosiderinurea** (some released Fe molecules go out with urine, others precipitate in kidneys)**, iron deficiency** (severe and recurrent)**)**
- **According to cause of hemolysis:**
  1) **Extracorpuscular (extrinsic factor):** here the factor that causes hemolysis is outside the RBC.
  2) **intracorpuscular** (factor inside the RBC)

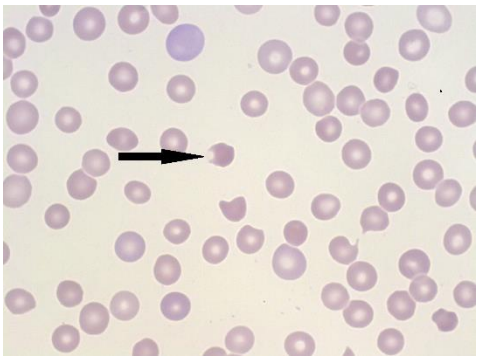
G6PD DEFICIENCY

- **X-linked inheritance** (affected Boys > Girls , mostly affecting boys early in life).
- **Glucose 6-phosphate dehydrogenase deficiency** (RBCs is the most affected in G6PD deficiency because they don't have nucleus).
- **Reduced production of glutathione, important for cell protection against harmful oxidants** (the oxidants cause damage to RBCs more than other cells because they're anucleated).

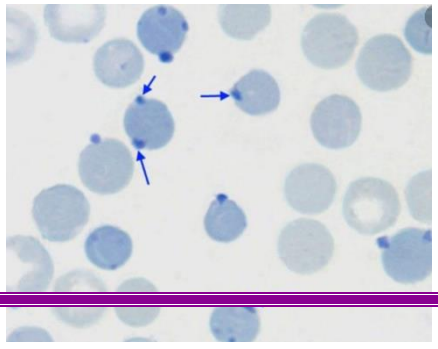
Usually this disease causes mild hemolysis and mild anemia, but patients may develop severe sudden hemolysis if exposed to triggers that increase the levels of oxidants:

TRIGGERS OF HEMOLYSIS

- **Infection**
- **Certain drugs:** antibiotics:(**sulfonamides, nitrofurantoin**), **large dose of aspirin, vitamin K, primaquine** (antimalarial)
- **Fava beans** (they have high amount of oxidants).
- **In all, large amount of oxidants are generated, G6PD cannot neutralize them, causing hemoglobin denaturation** (the oxidants change the characteristics of Hg ,so it become less soluble) **and precipitate (Heinz bodies), damaging cell membrane and massive hemolysis of RBCs, 2-3 days after trigger**
- **Other cells lose demorfmability** (due to the Heinz bodies) **and are partially phagocytosed inside spleen (bite cells):** macrophages in spleen target the solid part of RBCs (Heinz bodies) and bite them off the side of the cells, producing partially phagocytosed cells which are called (bite cells).
- **Bite cells: appears are indented defect in part of cell membrane of RBCs** (on the right)



- **Supravital special stain highlights Heinz bodies as membrane-bound, dark** blue **spots representing condensed and denatured Hg.**(⬇)



CLINICAL TYPES

- **Symptoms of intravascular hemolysis** and sudden attacks.

There are two types of G6PD defeciency:

1. **G6PD-A type: modest decrease in amount of G6PD, bone marrow compensate by producing new RBCs** (low amount, normal function).
2. **G6PD-Mediterranian: qualitative defect of enzyme (low function), more severe symptoms** (low function, normal amount)

- **Females: can have symptoms if random inactivation affects the normal X chromosome:** sometimes normal inactivation happens to one of the X chromosomes in females, which might cause the disease to occur in carrier females since it's an X-linked disease, but that rarely happens.

IMMUNE HEMOLYTIC ANEMIA

An acquired autoimmune disease.

- **The presence of auto-antibody against RBC membrane protein** in the serum, and some are already coating the RBCs.
- **These antibodies are detected by <u>Coombs test</u>:**

-Direct Coombs test: RBCs of patient are incubated with synthesized **antibodies that target normal human antibodies** (specifically target the Fc portion of the autoantibodies coating the RBCs) **(**if the test is positive the antibodies combined together that mean the RBCs combined together ,so **RBCs will agglutinate)**, if it's negative then the sample will remain fluid.

-Indirect Coombs test: patients serum is added to "test RBCs (synthsized RBCs)**" that have certain surface proteins (identify the type of antigen)** Here we do the opposite, instead of taking the RBCs of the patient we take their serum and incubate it with RBCs containing targeted antigens, to test if the serum of the patient contains antibodies for it. The advantage of this test is that we can target the antigen specifically, like in Rh tests.

There are two types of immune hemolytic anemia:

1) WARM TYPE

- **High affinity auto-antibody (mostly IgG type,** sometimes IgA**)**
- **Binding occurs in core circulation (37oC),** the temperature at which binding occurs at its best.
- **Removed by macrophages in spleen**

▪ **spherocytes develop, then destroyed by spleen (extravascular hemolysis)**

Macrophages in the spleen have receptors for Fc portions of antibodies, so they detect the antibodies that coat the RBCs and phagocytose them alone, taking with them small amounts of RBC cell membranes. That reduces the size of the RBC because the surface area decreases, resulting in spherocytes formation. The cells then leave the spleen as spherocytes and recirculate in the blood, then get detected by the spleen as abnormal cells and are destroyed (extravascular hemolysis).

▪ **60% are idiopathic, 25% associated with** other autoimmune diseases such as **systemic lupus erythematosus** which targets all body tissues**, 15% by drugs in** certain individuals only **(α-methyldopa:** anti-hypertensive, unknown how it induces this disease, **penicillin** attaches to antigens of RBCS that get targeted**)**

▪ **Severity of anemia is variable, most patients have mild chronic anemia and splenomegaly**

2) COLD TYPE

▪ **Low-affinity autoantibody (IgM)**

▪ **Binding occurs in peripheral areas of body** and tips of nose and ears (areas where the temperature is **(<30^{o}C)**).

▪ **After IgM binding, few C3b and C3d molecules bind RBCs.**

IgM doesn't bind to the RBC alone, proteins from the complement system bind to it too, they normally circulate the blood but when they get activated they bind to cell membranes and cause their lysis.

▪ **When RBCs return to core circulation** where the temperature is 37^{0} C**, IgM dissociates** (remember it binds with low affinity which makes it easy to detach), **but C3b stays, identified by splenic macrophages and removed.** The same concept happens here, macrophages recognize complement proteins and phagocytose them, pinching off pieces of the membrane as they do so, turning the RBCs into spherocytes. The RBCs leave the spleen as spherocytes and get destroyed in the spleen in second circulation.

▪ **IgM binds 5 RBCs** because it's big**, thus creating in vivo agglutination** (RBC clumps formation, acts like a thrombus)**, might block small capillaries** (ischemia) **in fingers and toes causing Raynaud phenomenon.**

*Cold IHA can be acute or chronic.

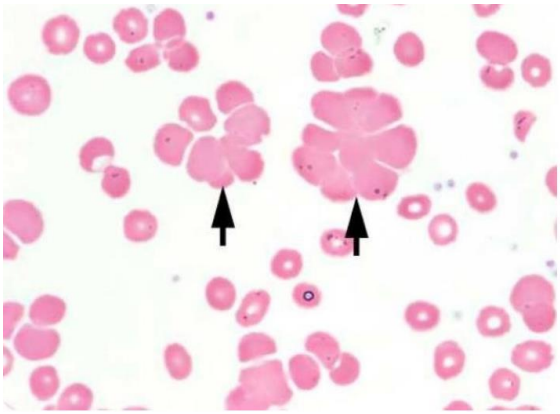
1- Acute: follows infection:

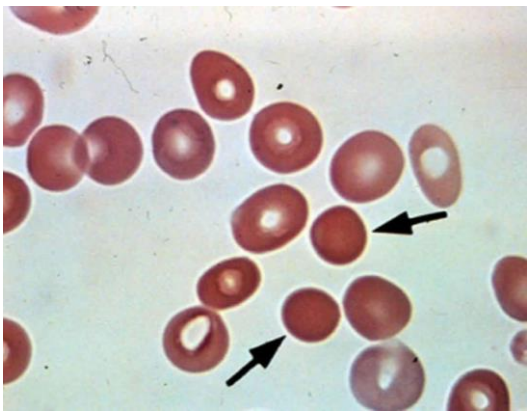
Transient forms of cold-IHA occur in recovery of infections by:

(1) mycoplasma pneumonia and (2) Epstein Barr virus (EBV): causes **infectious mononucleosis** which is a systemic disease. Both cases are **mild, self-limited.**

2-Chronic: persistent form occur in B-cell lymphoma (it's a cancer in which neoplastic cells produce different types of antibodies) **or idiopathic**

in blood smear they appear:





- **Left: RBC agglutination: RBC clumps in different directions**
- **Right: spherocytes appear as small, round** (there's no central pallor) **hyperchromatic RBC.**

HEREDITARY SPHEROCYTOSIS

This hemolytic disease is caused by a genetic mutation (intrinsic) that results in the production of RBCs that are spherical in shape

- In most of the cases, the disease is transmitted as an **autosomal dominant** trait (results in mild hemolysis), but sometimes it is **autosomal recessive** (results in severe hemolysis). So, different mutations can cause this disease, with the recessive ones usually causing more severe hemolysis.

- The **mutation is in RBC skeleton membrane**'s genes that encode for the proteins providing structural support of the membrane.

- **Most commonly affects ankyrin, band 3 or spectrin**

**As you remember from histology, the RBCs plasma membrane's integrity is important for the biconcave shape of the erythrocyte and its stability, maintained by the underlying cytoskeletal elements forming a meshwork, and all the proteins facilitating structural support of the RBC's membrane (ankyrin, spectrin, band 3 are all examples)

So the mutation can change the structure of one of these proteins causing mild hemolysis, or it cause deletion of one or more, so we have a spectrum of severity in the same disease, and each patient is different

▪When the proteins are abnormal or absent → RBC loses part of cytoskeleton→ **cell membrane becomes unstable→ keeps losing parts of it as the RBCs age** (as it is circulating).

▪ **little amount of the cytoplasm is lost** (hemoglobin stays inside).

▪With decreasing surface area, the RBC loses it normal biconcave morphology and becomes a smaller sphere

*More explanation: the membrane is shedding (surface area decreases), but the cytoplasm stays the same (volume is constant) and the ratio between them decreases until cells become spherical.

PATHOGENESIS:

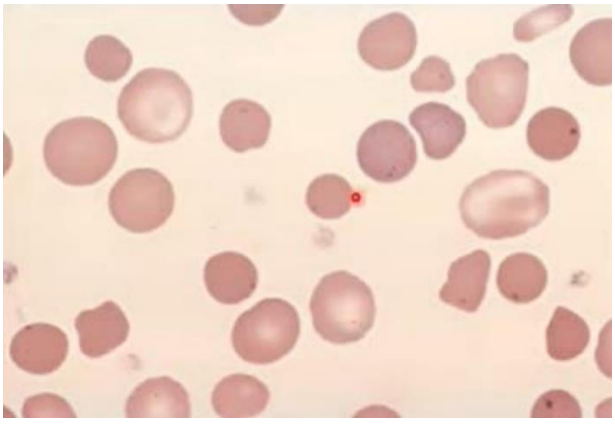
▪ **Spherocytes are non deformable** (take a longer time when they pass through capillaries) **→Entrapped in small vessels in the spleen, engulfed by histiocytes and destroyed (extravascular hemolysis).**

▪ Patients are treated by a splenectomy. **If the spleen is removed, spherocytes persist in peripheral blood, thus, anemia is corrected,** and the cells continue their movement in the circulation to deliver oxygen normally even though the cell defect persists.

As mentioned above; **the degree of anemia is variable (depends on the type of mutation), some patients are asymptomatic, while others might have severe hemolysis.**

LABORATORY FINDINGS

In the blood film, we are obviously going to see **the appearance of spherocytes in peripheral blood** (morphology similar to warm type hemolytic anemia, except coombs test is negative, and there is family history)



*Changes in RBC indices:

▪ Large number of spherocytes causes a change in the mean cell volume (**spherocytes have a smaller size → low MCV**)

▪ volume of the cell is constant**, Little cytoplasm is lost, normal amount of Hg (normal MCH).**

Hence, **MCHC is increased** (we divide MCH which stays the same by MCV which is decreased).

So spherocytosis can be predicted by CBC through the combination of having anemia + high MCHC.

▪ Spherocytes show increased fragility when put in hypotonic solution (increased osmotic fragility)

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

This one has a long name, so let's analyze it: Paroxysmal: sudden hemolytic attack. Nocturnal: more active at night. Hemoglobinuria: intravascular hemolysis

▪ It is a **Rare, acquired disease,** in which a **Mutation in PIGA gene results in deficiency in phosphatidylinositol glycan (PIG), a structural protein on cell membrane that anchors many other proteins,** and carries the normal antigens on RBCs as we will discuss later.

▪ Mutation occurs in bone marrow stem cell, leukocytes, RBCs and platelets are all affected.

PATHOGENESIS

**Immunology revision: As you remember from immunology, the complement system is a group of circulating proteins that, when activated through multiple pathways, serve multiple in the immune system, with the relevant one here is that in the shared terminal step, they are able to form a membrane attack complex (MCC) by the C5b-6-7-8-9 that induces lysis of a cell through pore formation, without differentiating the type of cell, which is why each step of complement activation is regulated by soluble and cell surface proteins (like CD55 and CD59 antigens on blood cells mentioned below)

▪ **Complement system: circulating proteins that are part of the 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** (and since the PIG is deficient in this disease, the RBCs cannot negatively regulate the complement effect and get lysed through pore forming complexes).

▪ **In PNH: RBCs** (most prominent)**, and to a lesser degree WBCs and platelets, are spontaneously lysed inside blood** (so sometimes it could also lead to pancytopenia not just anemia)

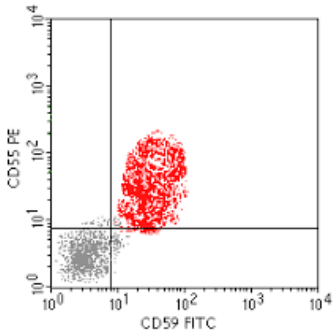
Why nocturnal? **During sleep,** we have relative hypercapnia **(↑CO2),** leading to relative acidosis **(↓ blood PH), more active complement system, more hemolysis.**

▪**Thrombosis is common,** which can be life-threatening and even the main symptom that the patient presents with, and upon further investigation we find out its PNH. So these patients have both thrombocytopenia (low platelets) and paradoxically thrombosis; as when the platelets get lysed they also release their internal molecules that lead to thrombi formation.

DIAGNOSIS:

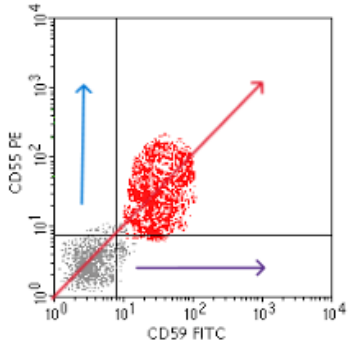
flow cytometry study

PNH is diagnosed by a special flow cytometry test by using it to detect the presence of CD55 and CD59 molecules on the surface of cells, which fail to be detected when PIG isn't holding them like in PNH. We take a fluid sample from the body (blood) and examine cell surface antigens and then it creates a chart that represents whether these cells' antigens have reacted with the test's previously added antibodies or not.



So, this photo on the left shows the end output of the test, for a person that has PNH. as you can see the X-axis represents CD59 detection and Y-axis represents CD55 expression, so as the dots move along the X-axis (purple arrow on bottom picture) it means more CD59 is being detected, and as they move along the Y-axis (blue arrow) more CD55 is detected, and the diagonal movement (red arrow) means both are being detected.

So basically, the red population have moved diagonally, meaning they have the antigens as normal, while the gray population hasn't moved, meaning the antigens were not detected, so they lack PIG (remember only stem cells which acquired mutations are producing abnormal colonies, so you can find normal colonies in an NPH patient).

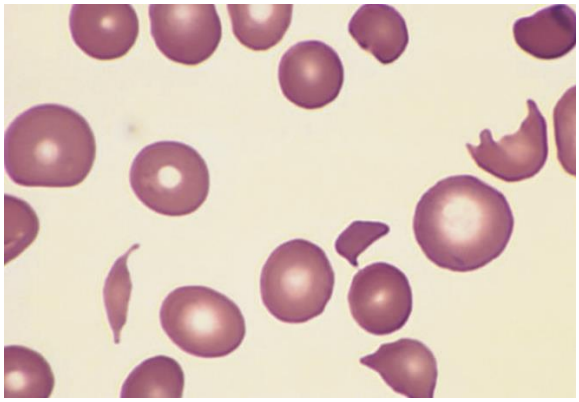


Again: **the red population shows expression of CD55 and CD59, while the gray one is negative for both (PNH clone)**

TRAUMATIC HEMOLYSIS.

Is caused by:

- **Direct physical force, or turbulence causing lysis of RBCs (intravascular hemolysis)**
- Patients who have **Prosthetic heart valves** (traumatic hemolysis is seen in patients who have heart valve problems, for example stenosis which restricts the RBCs causing their lysis. And also in those who have prosthetic valves).
- Vigorous sport exercise: **Repetitive physical pounding (marathon, boxing, marching)**
- **Disseminated thrombi (microangiopathic hemolytic anemia:** these are an entity of medical disease in which hemolysis happens in small blood vessels + thrombosis in multiple distributed vessels of the body, the doctor said that we will touch on this topic more in the following lectures.)
- **Hallmark of traumatic hemolysis: schistocytes** (broken, torn, fragmented RBCs)



GOOD LUCK ♡