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# Pathology HLS

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# SICKLE CELL ANEMIA

- Most common familial hemolytic anemia worldwide. It is more commmon than thalassemia
- Common in Africa, Middle East, Saudi Arabia, African Americans (in old world countries)
- Patients with abnormal hemoglobin are resistant to malaria falciparum infection, for unknown reasons
- Autosomal recessive, similar to thalassemia
- Caused by single amino acid substitution (glutamic acid ❼valine) in $\beta$-chain

Normally we have glutamic acid at codon number 6, which is a hydrophilic acid, it is mutated to give valine, which is a hydrophobic amino acid, so the characteristics of the beta chain will change.

- In sic kle c e ll disease (homozyg ous), Hg e le c trophore sis shows Hg S (abnormal) and absent HgA (normal)
- In sickle cell carrier (heterozygous), Hg electrophoresis shows both HgA and HgS bands

- Electrophoresis seperates the chains according to their charactersitics and chemical and physical properties, and since HgS is different from HgA, it gives different bands.

# PATHOGENESIS

- In de-oxygenated case (when oxygen is released), HgS tends to polymerize in a longitudinal pattern (builds up like a line), and it becomes more curved as it becomes longer, distorting cell shape and creating the sickle shape
- The change is reversible by re-oxygenation, however, with repeated sicklings (especially as the cell becomes aged over a longer time), the changed becomes irreversible, the cell membrane is damaged, and hemolysis occurs

The elongated hemoglobin will cause damage to the cell membrane and thus hemolysis, this hemolysis can occur inside the blood stream or in the spleen because the cell has an abnormal shape.

- The presence of normal HgA (carrier) and increased HgF (newborn) **inhibits** HgS polymerization
  - The HgS chain in order to cause sickilng of RBCs would have to be the only chain available (homozygous), and so carrier patients (heterozygous) do not have any symptoms because they do not have sickling or hemolysis.
  - Also, when HgF is high, as in newborns, patients with sickle cell anemia start to have symptoms after the age of six months, when the fetal hemoglobin levels drop, and the adult hemoglobin (mutated) appears).
  - In carrier SCA (presence of HgA) there is no sickling, carriers are completely asymptomatic.
  - In treatment, we can increase the amount of HgF in the blood of sickle cell anemia patients. Some drugs increase them and so they decrease the amount of hemolyisis and thus the symptoms.
- Increased HgS concentration inside RBC **promotes** sickling (and hemolysis) (dehydration, acidosis (these occur in infection)), while the presence of additional $\alpha$-thal decreases sickling, in this disease there is a decrease in the presence of the α chain and this will decrease the amount of HgS present. We call this a compound mutation, these patients have much less symptoms because they have less sickling due to the deficiency of the α chains.

# PATHOGENESIS

- Sickle-shaped RBCs take a longer time to pass through capillaries
- Removed by macrophages in spleen and they are destroyed (extravascular hemolysis)
- Also adhere to endothelial cells, may create a spontaneous thrombus

They can get stuck in the endothelial cells in any part of the body, once the capillary becomes small in diameter and form a thrombus.

# CLINICAL SYMPTOMS OF SSA

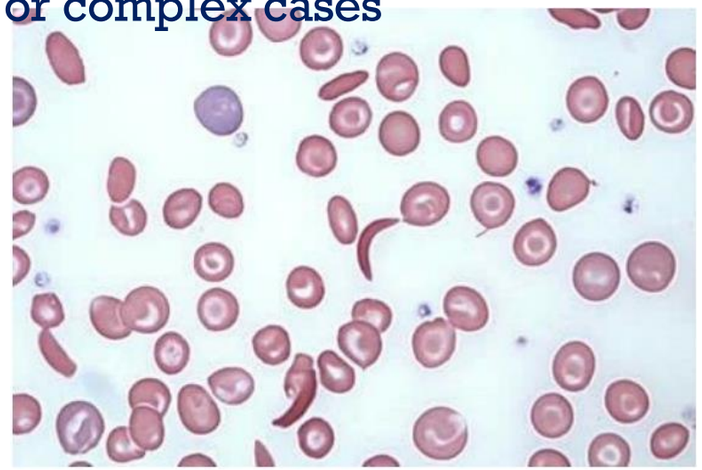
- Chronic persistant moderate-severe hemolytic anemia (life-long anemia), manifesting after the age of 6-months (after the fetal hemoglobin drops) (dependent on fraction of sickled cells). The chronic course is interrupted by repeated sudden attacks of worsening anemia and severe symptoms.
- Vaso-occlusive crisis (independent on fraction of sickled cells), results in organ infarction. Commonly associated with systemic infection, inflammation, dehydration and acidosis. This can be life-threatening, if it affects a vital organ it can be fatal. It can occur in any patient with sickle cell anemia even if they do have profound sickling. Patients have a lower life span.
- Hand-foot syndrome (if the crisis affects **(necrosis of)** the digits, most patients come in with severe pain in the digits because of ischemia, and the commonly have deformities because as a growing child, they have repeated infarctions in the bone and soft tissue of the digits and at the end they have abnormal growth of the fingers and toes.
- Acute chest syndrome, (can affect (cause necrosis of) the lung, the heart and the ribcage and the bones forming it, it will cause severe pain and shortness of breathe and this will worsen the hypoxia).
- Stroke, (it affects the cerebral circulation)

# CLINICAL SYMPTOMS OF SSA

- Myocardial infarc tion (notorious complcation of SCA, that's why these patients have a shorter life span than healthy individuals, they are a risk for a myocardial infarction even at a very young age.
- Retinopathy (it can directly cause thrombosis and ischemia in the blood vessels of the retina and sometimes the hypoxia there promotes other blood vessels to grow in order to compensate for the hypoxia in the retina and these new blood vessels will block the movement of the light and it will worsen the symptoms) *May cause blindness.*
- Autosplenectomy (during early life, the spleen of SCA patients is enlarged, splenomegaly, and with repeated infarctions the spleen will become fibrotic and disappear, so many patients will SCA do not have a spleen when they grow up, because it is infarcted and removed.
  That's why it's called autosplenectomy, because it doesn't need a surgery.
- Increases susceptibility to infection by encapsulated bacteria.
- ✦ Aplastic -crisis: this can occur secondary to bone infarction and it stops producing any cells, or secondary to infection by Parvovirus B19, causing worsening anemia, self-limited, mild pure red cell aplasia.
- ✦ Susceptibility for encapsulated bacteria (pneumococcus, salmonella), because of the disappearance of the spleen
- ✦ Sic kle c e ll c arrier: asymptomatic.
  It is very important in the premarital tests to do electrophoresis for the hemoglobin (HgA, HgS), because this is the only way that we will know if someone is a carrier, since no symptoms will appear.

# DIAGNOSIS

- Routine blood smear: presence of sickle cells in the blood film, target cells also form such as in IDA and thalassemia, it occurs secondary to abnormal hemoglobinization of the cells.
- Sickling test: (this test can detect both the carrier and the disease states), adding hypoxic agent to RBCs, promote sickling. In normal patients, it would not result in sickling, but in both carrier and patients with SCA, it would lead to the sickling of RBCs.
- Hemoglobin electrophoresis, it can discriminate between the carrier and the disease status
- DNA testing, in complicated or complex cases



# G6PD DEFICIENCY

- Glucose 6-phosphate dehydrogenase deficiency

This is an enzymatic deficiency

- X-linked inheritance, it affects males more than females

Normally, G6PD reduces nicotinamide dinucleotide phosphate dehydrogenase (NADP+) to NADPH, which maintains the level of glutathione that neutralizes reactive oxygen species (free radicals)

These free radicals are normally produced during the metabolism of the cell, and they carry high amounts of energy and can cause physical damage to the structures inside the cell.

- Recurrent, transient episodes of intravascular hemolysis

The RBC is very prone to this deficiency, unlike other eukaryotes, RBCs do no have a nucleus, its lifespan in relatively long (120 days) not like epithelial cells

- Most patients have deficiency in G6PD, if the level is zero then this is incompatible with life and the baby usually dies in utero, but most patients seen in clinical cases have a mutation which causes a very minimal amount, so once the RBCs are released from the bone marrow, they have only a small amount of G6PD, but they have a shorter life-span than normal RBCs.

# TRIGGERS OF HEMOLYSIS

- Infection, this is the most important cause because infection of WBCs produce the reactive oxygen species to kill the microorganism, the entire medium has a higher amount of ROS and the patient develops hemolysis.

Remember that the hemolysis occurs inside the blood stream.

- Certain drugs: sulfonamides, nitrofurantoin (these are both antibiotics), large dose of aspirin, vitamin K, primaquine (antimalarial drug), it is also used in the treatment of RA and these days there is a claim that it has a beneficial effect on the COVID-19 virus.

They produce metabolites that consume the glutathione.

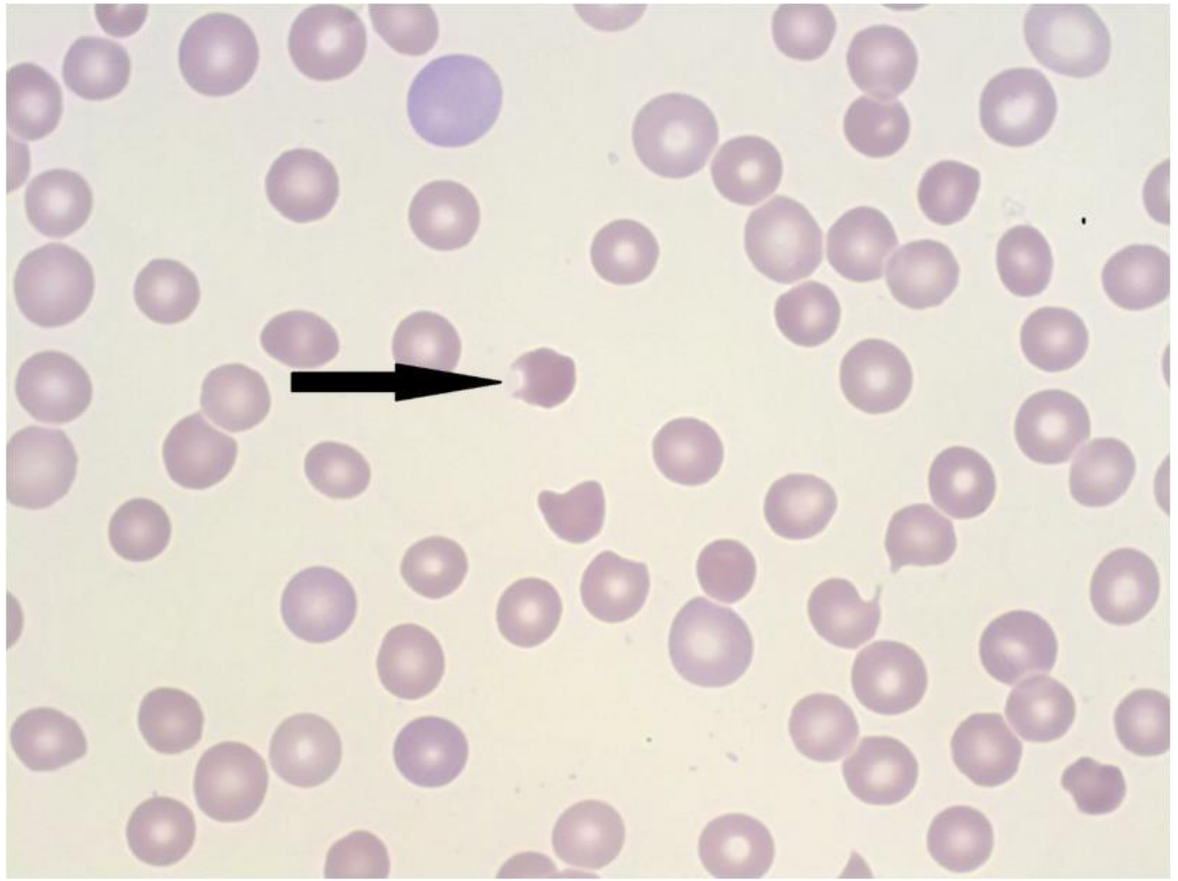
- Fava beans (contains vicine and convicine and both these are strong anti-oxidants so they can consume the glutathione and destroy the cells by acting as ROS. Causes favism.

- In all, large amount of oxidants are generated, G6PD cannot neutralize them, causing hemoglobin denaturation and precipitate forming insoluble solid particles (Heinz bodies), damaging cell membrane and massive hemolysis of RBCs, 2-3 days after trigger.

There is a special stain called the **supravital stain** which stains especially heinz bodies.

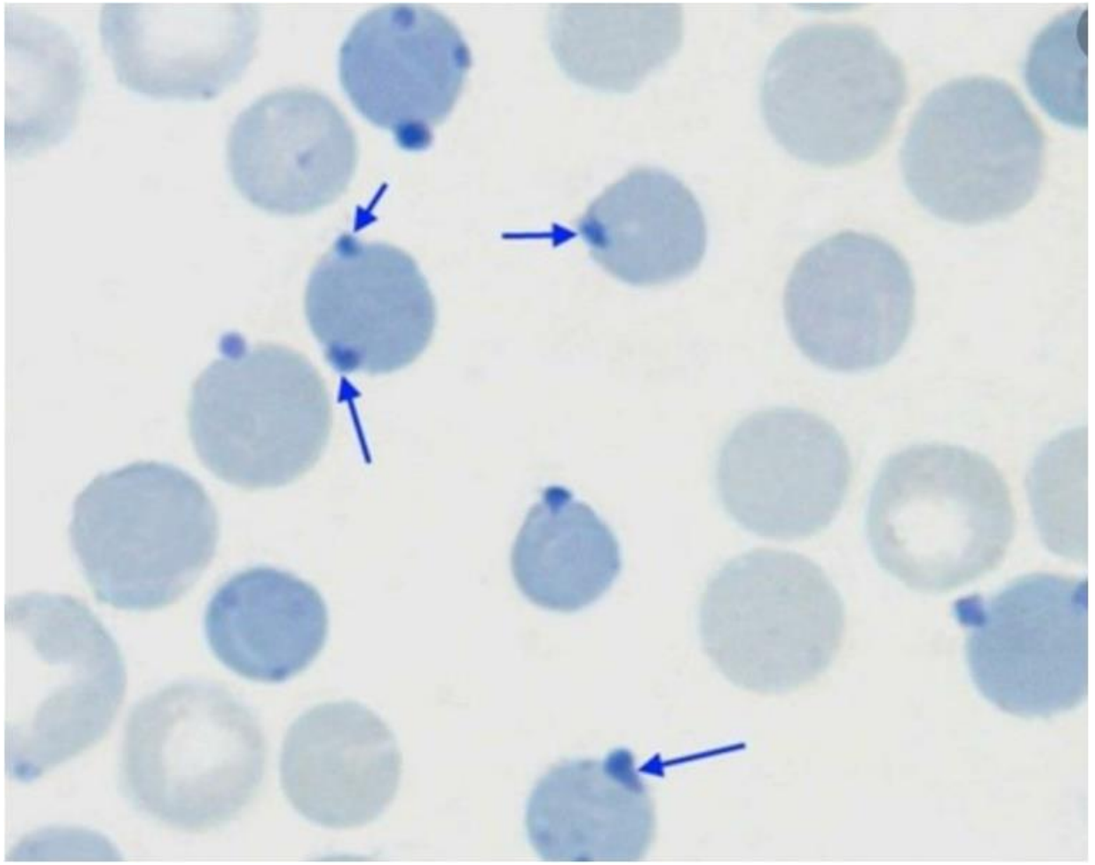
- Other cells lose demorfmability and partially phagocytosed inside spleen (bite cells)

The histiocytes in the spleen identify the heinz bodies, they phagoctose only that part of the RBC and the cell appears to be like a bitten cell



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

Patient with G6PD deficiency



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

# CLINICAL SYMPTOMS

- Symptoms of intravascular hemolysis: patients complain with symptoms of anemia and when it is severe they have pain in the bones of their entire body and they have dark urine, because the reduced hemoglobin goes directly into the urine.
Patients usually do not have anemia, it appears suddenly as recurrent attacks of hemolysis.
- G 6PD-A type : de c reased amount of G 6PD, b one marrow compensate by producing new RBCs, to compensate for the damaged and short-lived RBCs.
- G6PD-Mediterranian: qualitative defect of enzyme (low func tion), more severe symptoms, the amount of RBC is normal but that doesnt compensate for the decreased activity of the enzyme.
- Fe males: c an have symptoms if random inac tivation affe c ts the normal X-chromosome, (females can be affected by 10%)

# IMMUNE HEMOLYTIC ANEMIA (IHA)

- The presence of auto-antibody against RBC membrane protein

It coats the RBCs, it is attached to one of the cell membrane proteins.

- These antibodies are detected by Coombs test. It is the diagnostic test.
- Direct Coombs test: **RBCs** of patient are incubated with antibodies that target normal human antibodies (RBCs will agglutinate)

RBCs have antibodies on the cell surface so I few synthesize an antibody that can bind these antibodies, then at the end they will combine many of the cells them and cause agglutination and we can observe them as a clot.

If we added these antibodies to the RBCs of a normal human, they will not agglutinate because of the absence of these antibodies.

The target of these synthetic antibodies is the Fc fragment of the antibodies of humans.

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

These antibodies are previously known to be commonly targeted in autoimmune hemolytic anemia.

We add them to the serum and it contains the autoantibodies and if the patient has the disease they can agglutinate with these antibodies.

We perform both these tests and if the patient has the disease then there will be agglutination.

# WARM TYPE IHA

There are two types of IHA, the warm and the cold, and this is based on the site of agglutination

- High affinity auto-antibody (mostly **IgG** type)
- Binding occurs in core circulation (37₀C)
- Removed by macrophages in spleen, it pinches the RBC, it catches the antibody and pulls it away, and by removing multiple areas from the cell membrane the shape of the cell will change, it has a smaller surface area so it appears at the end as a ball (sphere).
- spherocytes develop, then destroyed by spleen (extravascular hemolysis), in their second circulation.
- 60% are idiopathic, 25% associated with systemic lupus e rythematosus (arthritic disease), 15% by drug s (*α*-methyldopa, pe nic illin)

Penicillin coats RBCs and it is deposited on the surface of the RBC and it can appear as a foreign body and antibodies will bind to it.

In *α*-methyldopa, this does not occur and it is not known exactly how it activates the immune system to attack the RBCs.

- Severity of anemia is variable, most patients have mild chronic anemia and splenomegaly, they also have reticulocytosis because it is extravascular hemolysis.

# COLD TYPE IHA

- Low-affinity autoantibody (**IgM**)

It is the largest one and it can bind to 5 RBCs at the same time

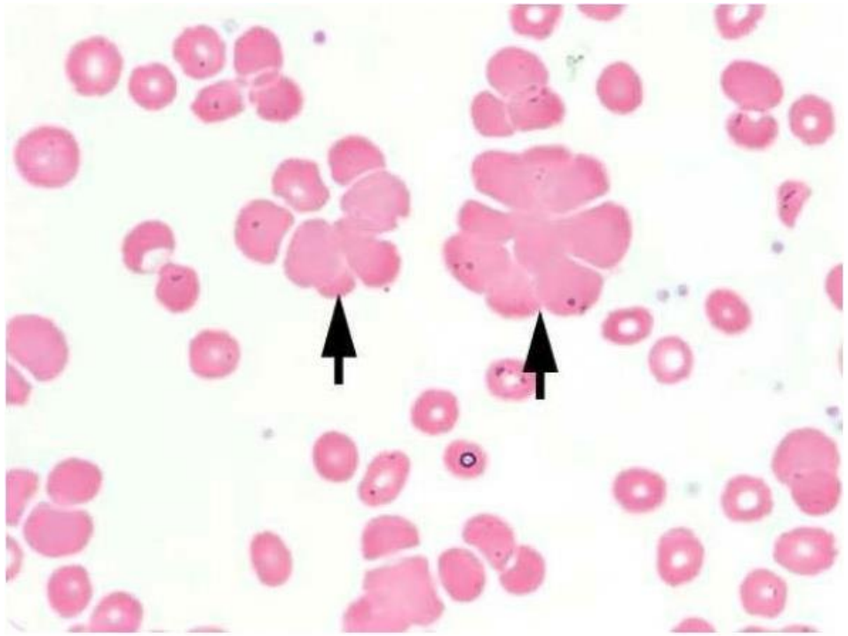
- Binding occur in peripheral areas of body (<30oC), this is where this antibody is more active, it occurs in the cold areas of the body and that is why it is called cold type IHA. This binding occurs mostly in the digits, nose, ears and the tip of the nose .

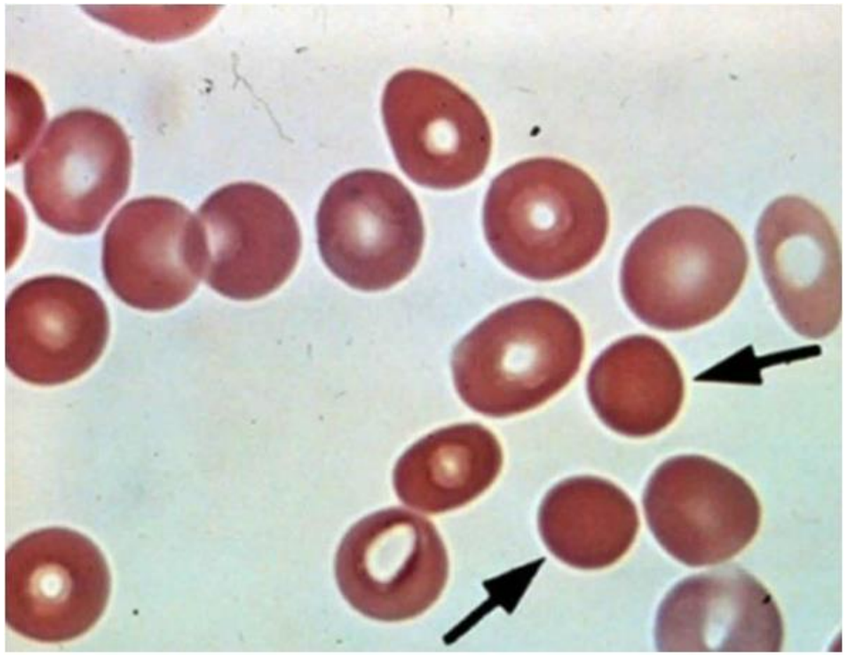
These are the coldest areas of the body where the anemia binds.

- After IgM binding, few C3b molecules bind RBCs, this protein (C3b) is part of the immune system and they are proteins that are important for the body, they are not cellular, they do not need cells and can act by themself and they cause damage to the cell membrane so they can kill microbes like bacteria.
- When RBCs return to core circulation, IgM dissociates, but C3b stays, identified by splenic macrophages and removed, so again here we have spherocytes similar to warm IHA.
- IgM binds 5 RBCs, thus creating in vivo agglutination, might block small capillaries in fingers and toes causing **Raynaud phenomenon** (pain, coldness, blue discoloration of the skin, this is caused by the hyperactivation of neurons and vasoconstriction of blood vessels in the digits.)
  this occurs secondary to decreased blood flow and vasoconstriction because of the cold.
- Sometimes we can see this agglutination on the blood film
- Transient (acute) forms of cold-IHA occur in recovery of infections by *mycoplasma pneumonia* and *infectious mononucleosis* caused by **Epstein barr virus** (mild, self-limited), can be treated by dealing with the underlying cause
- Chronic persistent form occur in **B-cell lymphoma** or idiopathic

These cells can produce a large amount of immunoglobulins and we will talk about the most important one, which is chronic lymphocytic leukemia.

In some research it was found out that malignant B lymphocytes activate normal B lymphocytes to produce a huge amount of IgM.





Left: RBC agglutination: RBC clumps in different directions, this is seen in cold IHA

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,

they affect the stability of the membrane.
The severity of the disease is based on the amount of protein and how many types are affected

- 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
- They take a longer time to pass through the capillaries of the spleen and so they are identified by macrophages and engulfed and destroyed
- Entrapped in small vessels in spleen, engulfed by histiocytes and destroyed (causing extravascular hemolysis)
- If spleen is removed, spherocytes persist in peripheral blood, thus, anemia is corrected.

The treatment of these patients is to remove the spleen, because the symptoms are due to its activity.

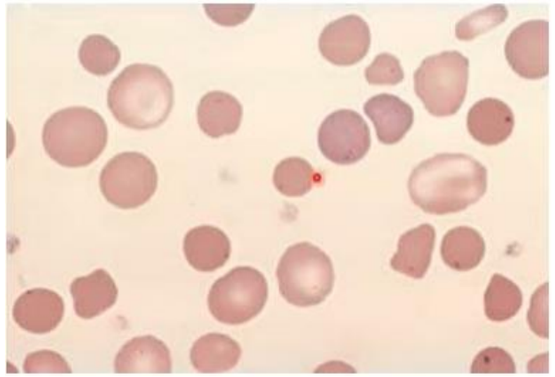
- The degree of anemia is variable (depends on the type of mutation)
- Some patients are asymptomatic (you only see it incidentally when you examine the blood smear), while others might have severe hemolysis (this can occur early in life)

# DIAGNOSIS

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

  The osmotic fragility test is not specific to spherocytosis.

  Family history is important



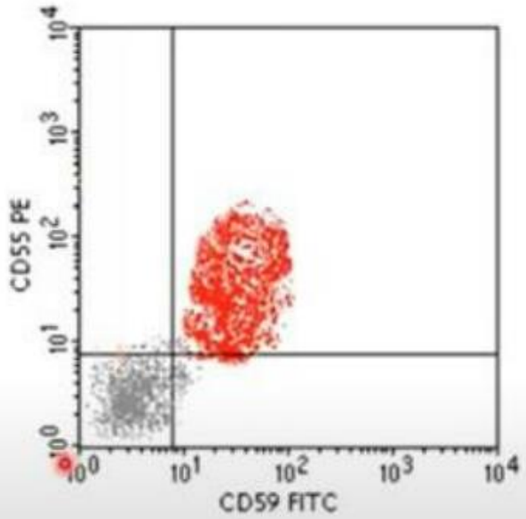
Small, do not have a central pallor.
(mentioned in 018 only) If the patient has splenectomy as therapy, we can see small black dots and they are called Howel-Jolley bodies, they are remnants of the DNA and they appear in RBCs, they are normally taken out by the spleen, but after the spleen is removed we will start to see them in the blood film

# PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

- Rare, ac quired dise ase
- Mutation in PIGA gene, results in deficiency in phosphatidylinositol glycan (PIG), a structural protein on cell membrane that anchors many other proteins
- This gene is located on the X chromosome, so when it is muted there is not production of PIG, so most of the anchoring proteins become absent and then the proteins that normally bind it will become absent.
- Mutation occurs in bone marrow stem cell, it happens in early unmature, undifferentiated cells and so all hematopoietic cells will be deficient of the PIG gene (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, but here PIG is absent so there is no way for cells to protect themselves and neutralize these proteins.
- In PNH: RBCs (they are the most effected), and to a lesser degree WBCs (causing leukopenia) and platelets, are spontaneously lysed inside blood
- During sleep, ↑CO2, ↓ blood PH, more active complement system, more hemolysis. It is called nocturnal because the chance of hemolysis is increased at night.
- Thrombosis is common; when platelets are lysed, they release their contents and cause thrombosis, so even if we have thrombocytopenia, instead of bleeding they have thrombosis.
- This is the most serious and common symptom and it can be fatal, because it can cause the formation of a thrombus in unexpected places such as the liver.

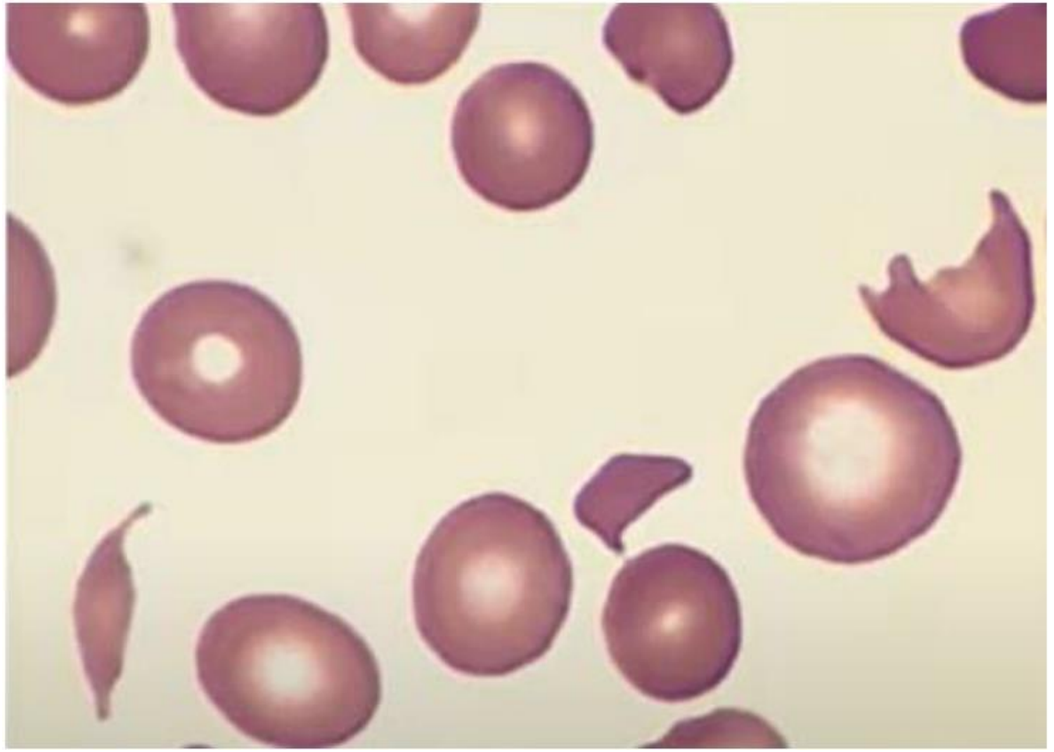



- Flow cytometry study: the red population shows expression of CD55 and CD59, while the gray one is negative for both (PNH clone)

- We take fresh blood from the patient, and we test for the markers or the antigens on the cell membrane, we test CD55 and CD59.
- The red spot (population of cells) moves to the right and that means that there is expression of CD59 and also moves up which means it expresses CD55.
- While the gray population stayed still and did not move direction and so it means it  is negative for both.

# TRAUMATIC HEMOLYSIS

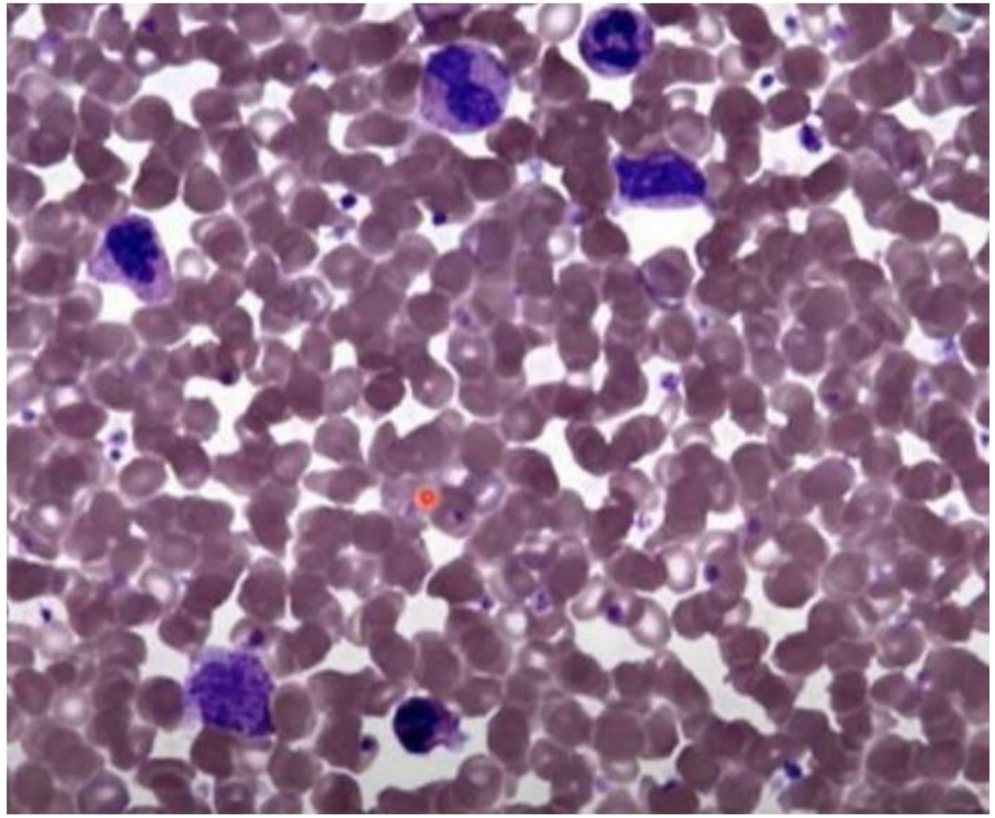
- Direct physical force, or turbulence causing lysis of RBCs
- Prosthetic heart valves
- Repetitive physical pounding (marathon, boxing, marching)
- Disseminated thrombi (microangiopathic hemolytic anemia)
- Hallmark of traumatic hemolysis: schistocytes



Schistocytes: torn and distorted RBCs

# POLYCYTHEMIA

- Increase in total RBC **mass**
- Erythrocytosis: increased RBCs number
- Relative polycythemia: secondary to decreased plasma volume (water deprivation, severe diarrhea, diuretics) the RCC is normal, the relative amount of RBCs increases.
- Absolute polycythemia: true increase in RBC mass, secondary to increased BM production (primary or secondary)
- Chronic hypoxia stimulates the bone marrow to produce a large amount of RBCs
- Primary: polycythemia vera (low erythropoietin, splenomegaly ) there is no obvious problem in the body, we do not have hypoxia, instead we have a neoplasm in the bone marrow.
- Secondary: there is hypoxia adaptive (high altitude, cyanotic heart disease congenital defective heart, with early-life hypoxia), paraneoplastc (renal cancer) renal cell carcinoma, or in pediatrics we have Wilm's tumor, and liver carcinoma and they all cause increased levels of erythropoietin, surreptitious (endurance athletes) these athletes take substances such as erythropoietin and sometimes even RBCs to enhance the delivery of oxygen to their muscles. Erythropoietin in high, no splenomegaly



Polycythemia: packed RBCs in peripheral blood