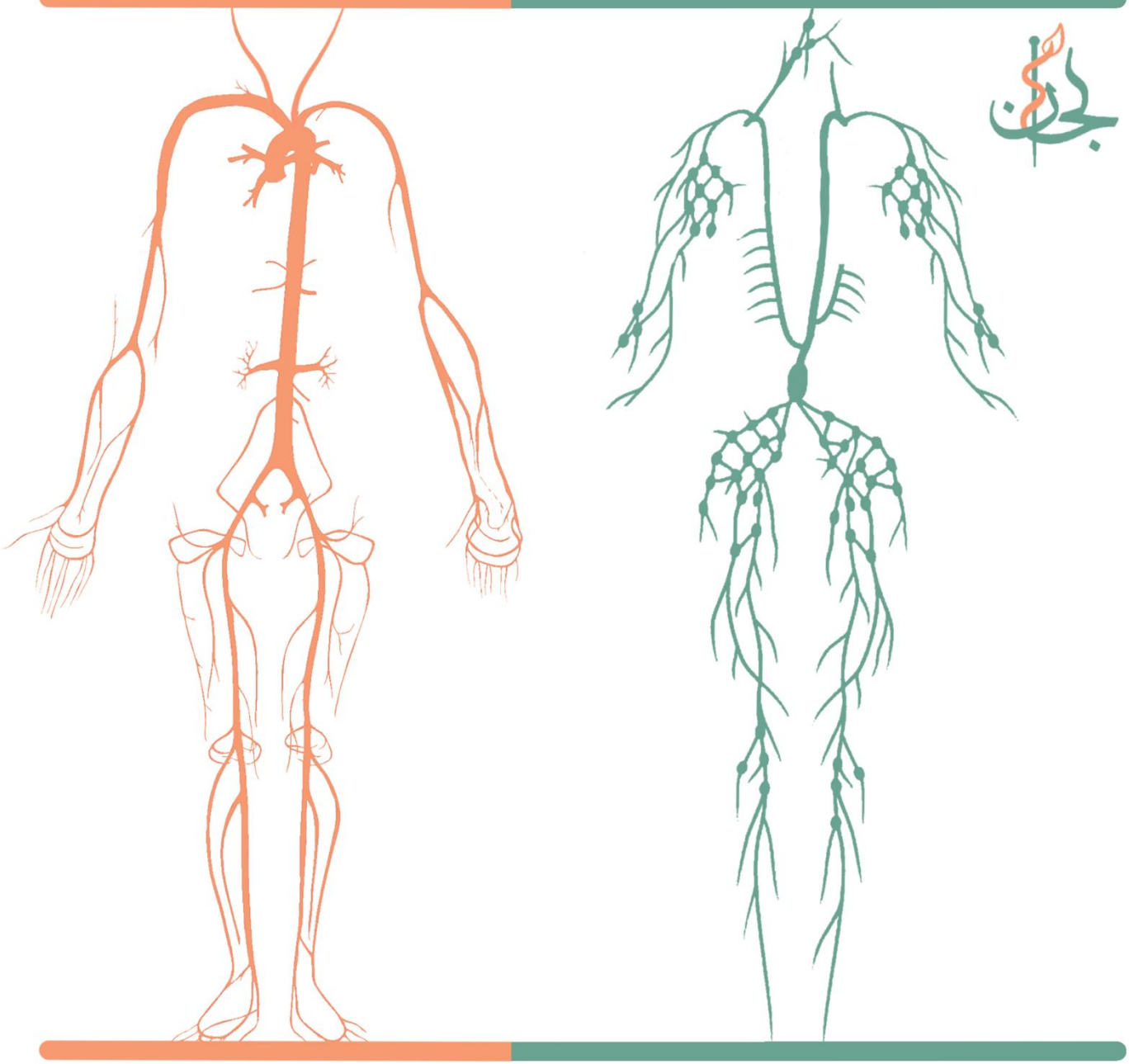
# Pathology
# HematoLymphatic



Title: Sheet 4 020 correction: إيثار ناصر

Writer: Raghad Tayseer

Scientific Correction: Ameen Alsaras

Final Correction: لينا عبد الهادي

Doctor: Tariq Al-Adaily

# *SICKLE CELL ANEMIA*

Sickle cell anemia is an **autosomal recessive genetic** disease caused by single amino acid substitution in the Beta - chain of hemoglobin at codon 6 where the hydrophilic **glutamic acid** is replaced by the hydrophobic **valine** amino acid.

It's the most common familial hemolytic anemia worldwide, especially in old world countries like Africa, Middle East (e.g. Saudi Arabia) and African Americans population.

For unknown reason, patients with inherited abnormal hemoglobin like in thalassemia and sickle cell anemia are resistant to infection by malaria falciparum.

In electrophoresis :

If the patient is **homozygous** (both genes are mutant) → **sickle cell disease.** Identical bands

If the patient is **heterozygous** (one gene is mutant) → **sickle cell carrier.** Two different bands

You will see the pics soon be patient

**Pathogenesis:**

When the oxygen is released (**deoxygenated state**), **HgS** tends to polymerize in a longitudinal pattern, distorting and changing the RBCs shape. It becomes elongated and curved creating sickle shape and that's why it's called sickle cell disease.

This change is reversible by re-oxygenation → when the oxygen comes back; the RBCs come back to its normal shape. However, with repeated episodes of sickling, especially when the red blood cells become aged, this change becomes irreversible and causes damage to the cell membrane then eventually **hemolysis - which can occur inside the blood stream or in the spleen-.**

When the RBCs become sickled in shape, they take longer time to pass through capillaries, especially in the spleen, so they are taken out by macrophages (histiocyte) then destroyed causing **extravascular hemolysis**.

Also, the sickle-shaped RBCs can adhere to the endothelial cells in any part of the body when the circulation becomes too tiny and thus can from spontaneous thrombus (blood clot).

**In carrier patients**, we have **Hgs** and the normal **HgA** which interacts weakly with the deoxygenated HbS and thus inhibit polymerization.

**In newborn babies**, the **fetal hemoglobin** is high. Because of that, patients with sickle cell anemia start to have symptoms after the age of six months when the fetal hemoglobin drops, and the adult hemoglobin appears. And this is the basis of some modality of treatment.

We can treat patients with sickle cell anemia by increasing the fetal hemoglobin in their bloods by using some drugs. So, the degree of hemolysis and symptoms decreases.

**Any increase in the HgS concentration** inside RBC promotes sickling. This occurs commonly in dehydration and acidosis which appears in infection.

In some rare cases**, patients with additional α-thalassemia** mutation have lesser amount of HgS than the patients with ordinary sickle cell anemia. So, sickling would decrease.

So, much less symptoms

Deficiency in alpha chain + sickle cell anemia = **compound mutation**

| Clinical symptoms of SSA | notes |
|---|---|
| **1) Chronic and persistent moderate-severe hemolytic anemia** | -Life long anemia.<br>-It manifests after the age of six months when the fetal hemoglobin drops **(dependent on the fraction of sickled cells).**<br>- During life, the chronic course is interrupted by repeated crisis or sudden attacks of worsening anemia. |
| **2) Vaso-occlusive crisis** | - Life threatening condition.<br>-Painful compilation.<br>-Sickled cells can form thrombus without the need of platelets and the coagulation system.<br>-It occurs in any patient with sickle cell anemia even if the number of sickled cells in the blood stream is low **(independent on fraction of sickled cells).**<br>- It results in organ infarction. Commonly associated with systemic infection, inflammation, dehydration and acidosis.<br>-If it affects vital organ, it could be fatal. |
| **3) Hand-foot syndrome** | -Patients come with severe pain in their digits because of ischemia and commonly they have deformities because during the children's growth, they have repeated infarction in bones and soft tissue of the digits.<br>-At the end they have abnormal growth of the fingers and toes. |
| **4) acute chest syndrome** | -If it affects the **lungs** and the **ribcage.**<br>-Causes severe pain, shortness of breath and this will worsen the hypoxia. |
| **5) stroke** | -If it affects **the cerebral circulation**. |
| **6) myocardial infarction (heart attack)** | - Known complication of sickle cell anemia.<br>- Because of this complication, patients with sickle cell anemia usually have **shorter life expectancy** than normal adults.<br>-Patients can have myocardial infarction even at very young ages and during childhood. |

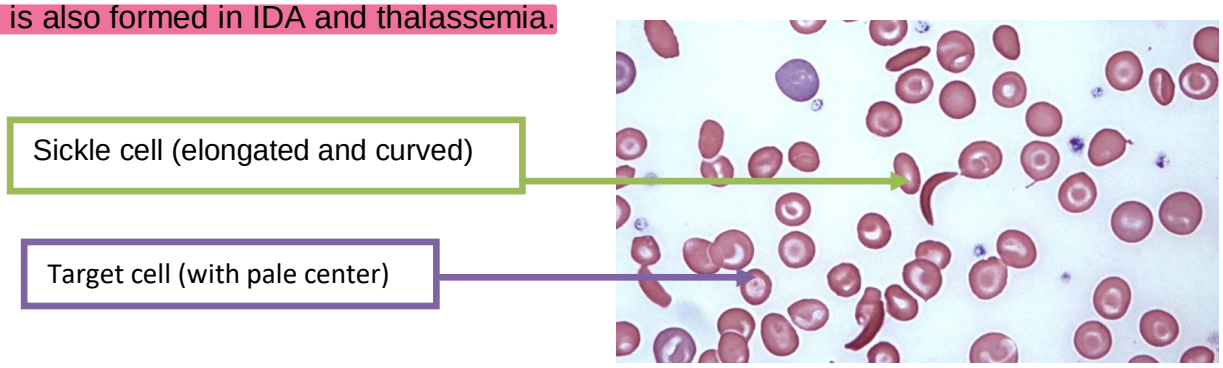
| | |
|---|---|
| **7) Retinopathy** | -The **blood vessels of the retina** can directly cause thrombosis; ischemia and sometimes the hypoxia promote other blood vessels to grow in order to compensate for the loss in retina.<br>-The new blood vessels will block the movement of the light, so it worsens the symptoms. |
| **8) Autosplenectomy** | -The spleen has a special situation in sickle cell anemia during the early life. When the hemolytic anemia takes place, it enlarges, and the patients will have splenomegaly.<br>- But with repeated infarction, the spleen becomes fibrotic and it disappears. As a result. Many patients with sickle cell anemia don't have spleen when they grow up because it's infarcted and removed **(Self removal without surgery).** |
| **9) Aplastic-crisis** | -It can occur **secondary to infarction by vaso crisis,** so the bone marrow stops producing any cell lineages or it can occurs **secondary to infection by parvovirus B19.**<br><br>- B19 virus cause symptoms mostly in patients with chronic anemia like in thalassemia , sickle cell anemia and other chronic hemolytic anemia while in normal adults it can't form major symptoms.<br><br>- Patients with sickle cell anemia can develop pure red aplasia secondary to infection of B19, usually it worsens the anemia but it's mild and self-limited. While the infarction in the bone marrow secondary to vaso crisis will cause aplastic anemia which means that all the cell lineages disappear. |
| **10) Susceptibility for encapsulated bacteria** | After the spleen is dead, the patient can be susceptible for more bacterial infection, especially for **encapsulated bacteria like pneumococcus and salmonella.** That's why the patients take vaccine of pneumococcus or in the old days used to take routine antibiotics. |

- Remember that **the sickle cell carrier is completely asymptomatic**, so we can't diagnose this case by physical examination. However, we can examine it by doing hemoglobin electrophoresis and it's important in the premarital state.

## Diagnosis:

**- Routine blood smear**: presence of sickle cells and target cells (secondary to abnormal hemoglobinization).

-Target cell is also formed in IDA and thalassemia.



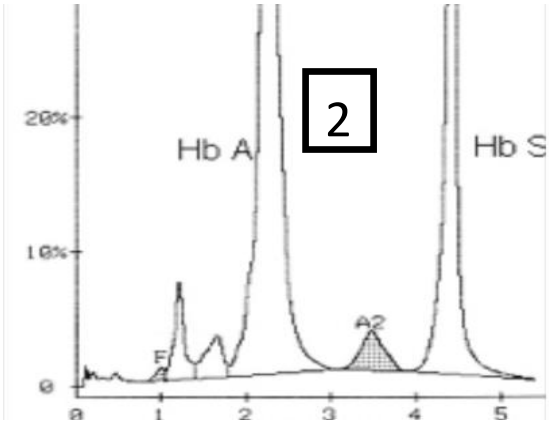

**- Sickling test:** in this test we can detect both the carrier and the disease state. We add **hypoxic agent (promote sickling)** to the blood of the patient then we do blood films. As a result RBCs in both carrier and disease state are sickled.However, in normal humans they don't.

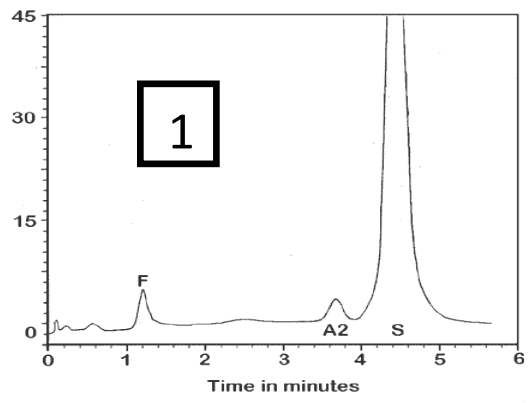
**- Hemoglobin electrophoresis**: a device that separates chains according to the physical and chemical properties; it can discriminate between the carrier and the diseases state.

**In picture 1**, sickle cell disease (**homozygous**), shows HgS band and absent HgA.

In this case, the HgA2 and HgF can be increased because we don't have beta chain → so alpha chain can bind to more of these bands.

**In picture 2**, sickle cell carrier (**heterozygous**), showsboth HgA and HgS bands.




-**DNA testing** is also useful.

*مرض التفول*

# *Glucose 6-phosphate dehydrogenase deficiency*

It's an **X-linked recessive disorder** that results in defective glucose-6-phosphate dehydrogenase enzyme and it affects males more than females.

This enzyme is important for the reduction of **$NADP^+$** (Nicotinamide adenine dinucleotidephosphate) **to NADPH**. And **NADPH** is important for the production of glutathione which protects the cell from **reactive oxygen species (free radicals)** which are normally produced during the metabolism of the cell, they carry high amount of energy and can cause a physical damage to the structures inside cells. So, these cells protect themselves from reactive oxygen species by a group of molecules called **antioxidants** like glutathione, vitamin E and vitamin C.

**In normal state**: ↑ G6PD enzyme, ↑ production of NADPH, ↑ production of reduced glutathione

Production of reduced glutathione protects cells from damage by neutralizing (reducing) the reactive oxygen species and the glutathione becomes oxidized in this process.

When **there is G6PD deficiency**: ↓ G6PD enzyme, ↓ Production of NADPH, ↓ Production of reduced glutathione, ↑ oxidative stress caused by ROS, ↑ with recurrent, patients at the end will have transient episodes of **intravascular hemolysis**.

-**RBCs** are very prone for this deficiency, unlike other eukaryocyte because they don't have a nucleus and their half life is relatively long (120 days) not like the epithelial cells.

Most patients have a decreased amount of G6PD enzyme. So, if the production is zero, this is incompatible with life and patients will die in utero. However, most patients that are seen in the clinical life have reduced amount of G6PD enzyme. So they have a mutation which produces a very minimal amount of the enzyme, so RBCs which are released from bone marrow have small amount of G6PD enzyme which can give them some life time but they can't survive for the normal life-time (120 days).

## Triggers of hemolysis:

**-Infection:** (the most important one) in infection, the WBCs produce ROS to kill microorganisms.So, the entire medium has more amount of ROS and the patient will develop hemolysis**. (Remember, hemolysis occurs in the bloodstream).**

**-Certain drugs:** they produce metabolites that consume the glutathione then severe hemolysis occurs.

Examples of these drugs:

-Antibiotic drugs like sulfonamides and nitrofurantoin.

- Large doses of aspirin, vitamin K, Anti- malaria drugs like primaquine.

*Primaquine is used to kill malaria, used in the treatment of rheumatoid arthritis and in current days it's claimed to have a beneficial role against COVID-19*

*commonly used in the medical practice*

**-Fava beans**: it contains 2 molecules (vicine and convicine).Both of them are strong oxidizing agents, so they consume glutathione and destroy the cells by acting as ROS. **(the doctor said that they are antioxidant agents).**

- This disease is known for a long time in human history and we call it Favism (patients become sick after eating fava beans).

-**Certain food coloring called aniline dye**: it's known to cause hemolysis in patients with G6PD deficiency.

**-Naphthalene**: it's deodorant, used in toilets and as a repellent for insects.

-It can be accidentally ingested by kids.

- It has a strong oxidative property.So it can cause hemolysis in kids, if they swallow the naphthalene balls or even breathe enough beside it (inhale an enough amount of naphthalene).

**In all previous situations**, large amount **of oxidants** are generated, glutathione can't neutralize them, because it's removed and its level becomes zero, causing hemoglobin denaturation and precipitation as solid particles forming what we call **(Heinz bodies**). These bodies are stained by a special stain called supravital stain. So, we can see them as dots inside the cytoplasm. These dots make the RBCs less deformable and thus causing damage to the cell membranes and massive **hemolysis** (usually takes **2 to 3 days** after the trigger)

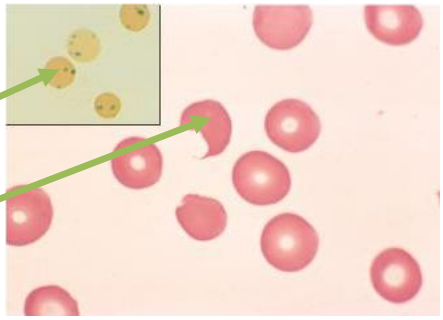
Other cells lose deformability and partially phagocytosed if the histiocyte in spleen identify Heinz bodies, they can sense them and they only take that part of the RBCs. So, cells will appear **as bitten cells**.

## -Diagnosis:

Blood films,supravital stain (Heinz bodies), enzyme assays.

If we do supravital stain, we can see the solid dots (Heinz bodies) inside cytoplasm.

Blood film from a patient with G6PD deficiency: we can see a bitten part of the RBC.



## Clinical symptoms:

-Patient in normal days don't have anemia.

- Anemia appears suddenly as recurrent attack of **intravascular hemolysis**.Patients will mainly complain from symptoms of anemia and when it's severe, they'll have pain in the entire body (e.g in the bone) and dark urine because the released hemoglobin goes directly into urine.

- G6PD deficiency has **two major types**:

**G6PD-A type:** decreased amount of G6PD. Bone marrow compensate by producing new RBCs (to compensate for the damaged and shortened survival ones).

**G6PD-Mediterranian:** qualitative defect of enzyme (low function) .The enzyme is present (produced amount is normal) but it can't function efficiently like the normal one. → This one has more severe symptoms and even if the bone marrow produces new ones, they can't correct the anemia.

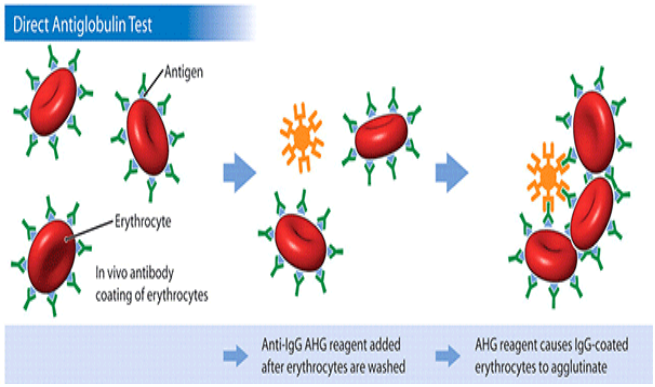
- Females still have the chance to develop this disease and, in some studies, they form around 10% of the entire patients → this is a big percentage.(Remember that sometimes one X chromosome is randomly inactivated in females. So, if a carrier female was unlucky enough, the normal X chromosome is inactivated, and she's left with the mutated one, then symptoms will present).

## *Immune Hemolytic Anemia*

**An autoimmune hemolytic anemia** caused by the presence of **auto-antibodies** against RBC membrane protein.

We can detect these auto-antibodies by **a test called coombs**.There are two ways to diagnosis this disease:

**Direct Coombs test:** we take patient's RBCs and incubate them with antibodies (**synthetic antibodies**) that target normal human antibodies (**auto-antibodies**).

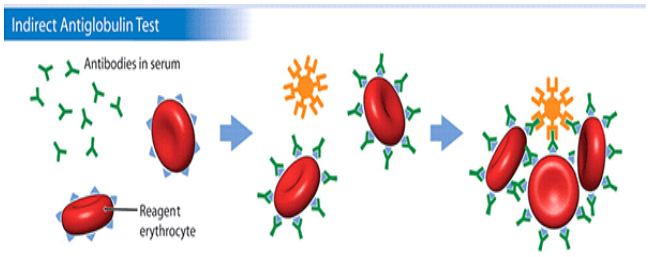



Patients have RBCs that are coated with auto-antibodies.So, if we add the synthetic antibodies that target the normal human ones which are attached to the RBC membrane protein, they will combine and cause agglutination that can be seen by the naked eye.

However, in normal human, they don't bind. So, there is no agglutination.

**Indirect Coombs test**:Patient's serum is added to "**test RBCs**" which are manufactured RBCs that have certain surface proteins (antigens). These antigens are previously known to be commonly targeted by auto-antibodiesin IHA.

If the patient's serum contains auto-antibodies, agglutinations will occur → positive result.

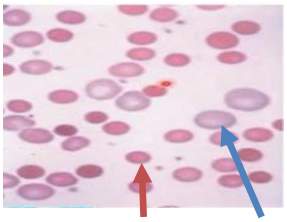


## There **are two types of IHA** based on the **site of agglutination**

| Warm type IHA | Cold type IHA |
|---|---|
| **-**Auto-antibody (mostly **IgG** type)<br>-It has high affinity | - Autoantibody (**IgM**), it's the biggest one, it can bind 5 RBCs at the same time<br>-It has low-affinity |
| -Binding occurs **inside the core** of the body where the temperature is (37℃) | - Binding occurs **in peripheral areas** of body (<30℃), occurs mostly in digits, ears, and in the tip of the nose; these are the coldest area of the body where the anemia starts. |
| -When RBCs reach the **spleen**, the macrophages in spleen pinch the RBCs and remove the auto-antibodies. | -After IgM binding, **few C3b molecules** follow IgM and bind RBCs.<br>C3b is protein in the complement system, which is important for the body's immunity.<br>Proteins in complement system aren't cellular, they don't need cells, and act by themselves causing damage to the cell membrane to kill microbes.So C3b joins the IgM because it has a normal function to follow it. |
| -Normally, the shape of RBC is biconcave but by removing multiple areas from the cell membrane, the shape changes (less surface area, appears like a ball).<br>- **Spherocyte develops** and it can be destroyed by the spleen because they have abnormal shape, **extravascular hemolysis.** | -Remember,IgM has low affinity. Because of that, when RBCs return to core circulation**, IgM dissociates, but C3b remains** and when it reaches to the **spleen,** it can identified by splenic macrophages and removed and again we have **spherocytes.**<br><br>-IgM binds **5 RBCs**, thus creating in vivo (inside our bodies) agglutination. This might block small capillaries (worsening symptoms) in fingers and toes causing **Raynaud phenomenon** which appears in digits, especially in the toes in cold weather. They become very cold, painful and blue in color secondary to decreased blood volume. Sometimes this agglutination is seen in the blood film. |
| **Causes** of anemia:<br>-**60%** are idiopathic (we don't know the cause).<br>-In **25%** of patientsis associated with another autoimmune disease and the most important one is systemic lupus erythematosus.<br>-**15%** (least common): secondary to drugs such as Penicillin which coats the RBCs. So in large amounts of penicillin, it deposits on the surface of the RBCs and can appear as a foreign body, so the antibodies will bind to penicillin.<br>The second drug is α-methyldopa: binding doesn't occur and it's unknown how it activates the | -Clinically the cold type IHA, can be chronic or acute:<br>-**Transient forms of cold-IHA (acute)** occur after the infection by mycoplasma pneumonia (atypical pneumonia) and sometimes by Epstein Barr virus that causes infectious mononucleosis.<br>Patients can have this type of anemia after recovery and usually it's mild and self-limited.<br>- **Chronic persistent form** occurs in B-cell lymphoma, they are neoplastic (cancer cells) and these cells can produce large amount of immunoglobulin. |

| | |
|---|---|
| immune system to attack RBCs.<br><br>-**Severity of anemia** is variable; most patients have mild chronic anemia, reticulocytosis and splenomegaly.<br>Reticulocytosis because there is an extravascular hemolysis. | The most important one is the chronic lymphocytic leukemia,and it is found that the malignant B lymphocytes activate the normal B lymphocytes to produce this huge amount of IgM.<br>-Sometimes it's idiopathic as the patients don't have B cell lymphoma |

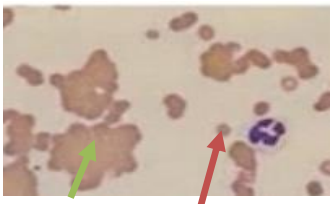
**-Worm type IHA:**



-**Spherocyte** (very small without pale center).

- **Reticulocyte** is the big one; it appears in large amount, Andit's more bluish in color than RBCs.

-Polychromasia: This means high amount of reticulocyte, so we can see both colors.



**- Cold type IHA:**

We can see **large clumps of RBCs** because there is an IgM that binds to these in large amount and we have also **spherocyte** (very small).

## *HEREDITARY SPHEROCYTOSIS*

**An inherited defect of RBC cytoskeleton-membrane tethering proteins**, most commonly affects **ankyrin**, **band 3** or **spectrin**. So, the cell membrane becomes unstable and keeps losing parts of it as the RBC age, thus little cytoplasm is lost and the cell's surface area is decreased. As a result, the RBC loses its biconcave morphology and becomes a smaller sphere.

**Pathogenesis:**

Spherocytes are nondeformable as the normal RBCs, they take longer time to pass through capillaries in the spleen (entrapped in small vessels in spleen) and thus they are identified by **histiocytes** and engulfed and destroyed → the patient will have **extravascular hemolysis**.

Spherocytes can function normally; they can deliver oxygen to the tissue and take $CO_2$ to the lungs. So, if we remove the spleen, spherocytes will persist in peripheral blood functioning normally then the symptoms will be corrected so the **treatment** of this patient is just **to remove the spleen.**

Clinically, there are many mutations, so the **anemia is variable** depending on the type of mutation (not the same in all patients).

Some patients are completely asymptomatic, and you only see it incidentally when you examine the blood smear. While others have severe hemolysis in early life.

**Diagnosis:**

**-Demonstration of spherocytes in peripheral blood:** when patients come with hemolysis,we do the blood film, so we can see the spherocyte in the peripheral blood.This is one diagnostic point.

-**Spherocytes** are small in size (**low MCV**): **important**

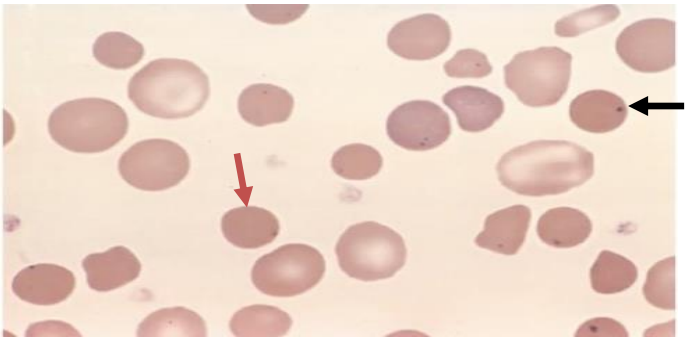
-**Little cytoplasm is lost, but normal MCH** because cells didn't lose parts of hemoglobin. So, when we divide the mean cell volume by mean cell hemoglobin, the MCHC is increased and this is a characteristic of spherocytes and also in sickle cell change in the shape of cell while the hemoglobin content didn't change.

-**Increased MCHC** indicates that we have either spherocytes or sickle cell.

-Spherocytes show increased fragility.So,when put in a hypotonic solution. It will burst easily not like the normal RBCs which can resist until a certain degree. And this test is called **osmotic fragility test.**

This test is **positive** in hereditary spherocytosis but it's not specific because other forms of abnormal shapes other than spherocyte can also cause change to this test.

Don't forget **the family history (important).**



**Blood smear** is taken out from a patient with hereditary spherocytosis. The **spherocytes** are small and don't have central pallor.

When the patient's spleen taken out as a therapy, we can see small **black dots** inside RBCs which are called (**Howel-jolly bodies**),they are remnants of the DNA (splenectomy).

## *Paroxysmal nocturnal hemoglobinuria*

Paroxysmal: suddenNocturnal: at night

**An Acquired, rare disease caused by Mutation in PIGA gene which is located on the X chromosome**, results in deficiency in phosphatidylinositol glycan (PIG), a structural protein on cell membrane that anchors many other proteins.

-**In normal situation**, PIGA gene normally produces the PIG protein.

-**Mutation occurs in bone marrow stem cell**.Remember that the RBCs don't have nucleus. So the mutation occurs in early immature cells (stem cells)→ all hematopoietic cells will become deficient of PIGA gene including **RBCs, leukocytes and platelets.**

**Pathogenesis:**

In normal situations, Blood cells protect themselves by membrane proteins **CD55** and **CD59**, that are normally attached to PIG and these proteins protect the cells against the complement system → when PIG protein is absent, these proteins will be absent too.

**Complement system**: When the system is activated, the most activated proteins (C5b-C9) form a complex that create pores in the cell membrane and destroy cells.

Patients in this case can't neutralize it causing a lysis in the cell membrane of RBCs and other cells.

**RBCs** are the mostly affected ones,so patients will have sudden hemolysis. **WBCs** also can lyse, so the patient will have leukopenia. **Platelets** can lyse and and thrombocytopenia is seen. However, when they lyse they release their content they cause thrombosis and not bleeding. This is **the most common symptom and the most serious one.**

-This disease can fatal because it can cause sudden thrombosis in unexpected places like **the liver.**

-Why is it called **nocturnal**? Because the chance of **hemolysis is high at night** because during sleep, there's more $CO_2$ thus the blood becomes more acidic and this will activate the complement system.

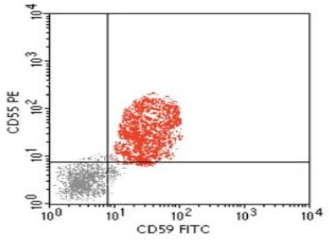
**Diagnosis**:

We use a test called **Flow cytometry study**: we take fresh blood from the patient and then we test the markers or antigens on the cell membrane (**test the CD55 and CD59**).

**In red population**, it moves to the right which means there is expression of CD59 and also moves up which means there is expression of CD55→ co-expression (both of them).

**In gray population**, it remains still, it doesn't move to any direction, which means they are negative for both→ the problem is in the stem cells (when one of the stem cells become mutant, the entire progeny will become mutant).

-Sometimes it can occur in all entire cells but more commonly partially.




CD55 and CD59 work as inhibitors against the immune response mediated by the complement system.

## *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) **we will talk about this later**

-Hallmark of traumatic hemolysis: schistocytes

## *Polycythemia*

-Increase in thetotal RBC mass.

**Erythrocytosis doesn't always = Polycythemia**

-Erythrocytosis: increased RBCs number

-Remember in thalassemia, we have erythrocytosis, but they are deficient in hemoglobin, so it doesn't produce polycythemia.

-Sometimes the RBC count is normal but still **they can produce polycythemia**.

-In **Relative polycythemia**: secondary to decreased plasma volume (we lose water and plasma), this occur in severe dehydration (water deprivation, severe diarrhea, diuretics) → We lose the plasma the RBCs become concentrated producing polycythemia.

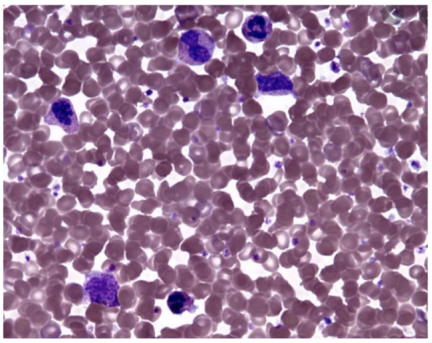
- **Absolute polycythemia**: True increase in RBC mass, caused by increased BM production (It can be primary or secondary), as in chronic hypoxia that stimulates the bone marrow to produce large amount of RBCs.

**Absoulte polycythemia**

**Primary: polycythemia vera**

In this situation there is no obvious problem in the body and we don't have hypoxia. Instead we have a **neoplasm** in the bone marrow which produces large amount of RBCs and this disease is characterized by low erythropoietin (negative feedback), it suppresses the secretion of erythropoietin andthe RBCs countstays high, so the patients will have splenomegaly

- **(low erythropoietin, splenomegaly)**



**Secondary: erythropoietin is high, no splenomegaly**

More common

- Remember we have hypoxia and the patient doesn't have splenomegaly because of **adaptive process** that occurs in people who live in hypoxic areas like **in high altitude** and in patients with **cyanotic heart disease**, they have hypoxia in early life, so they become polycythemic.

-**In alcoholism**: it's notorious to cause polycythemia because these people commonly have hypoxia and acidosis in the blood, they sleep a lot, urinate a lot. And all of these situations will create state of hypoxia.

- **In paraneoplastic syndrome**, this occurs in patients with renal cancer (kidney cancer) like in the renal cell carcinoma or Wilm's tumor in pediatrics. Both are cancers of the kidney, so there is production of more erythropoietin. Also, the liver carcinoma can make this.

-**In surreptitious (hidden) cases**. this occur in in athlete (endurance athletes) who takes hormone to enhance their performance and one of these hormones is erythropoietin. They take it or sometimes even take more RBCs to enhance the delivery of oxygen to their muscles and we can easily test them, thenwe can find high hemoglobin or erythropoietin levels.

Heavy one I know, if you feel a bit sleepy take a nap and أجّل عمل اليوم لغد عادي