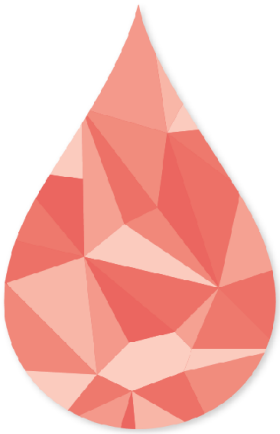


10-approach to hemolysis and hemoglobinopathies.

= Haematology 435 =

إن أحسنا فمن الله عزوجل, وإن أخطأنا فمن أنفسنا والشيطان.
نظراً لشمولية العمل لمحاضرات الطلاب والطالبات بإمكانكم اعتماده كمصدر للمذاكرة

-To be able to define hemolysis and hemolytic anemia.
-To be able to classify hemolytic anemia's into congenital and acquired types, and to know the etiological factors in each division.
-To understand the difference between intravascular and extravascular hemolysis and to recognize the laboratory features of each.
-To appreciate that disorders of globin function such as sickle cell disease are subtypes of hemolytic anemia.
-To understand the role of autoantibodies in the production of hemolytic and to know the types of disease with which they are associated.
-To understand some causes of non-immune acquired hemolytic anemias.

➢ **Color Codes:**

- Pink: Girls' notes. Blue: Boys' notes. Red: Important Notes. Gray: Extra notes.
- Purple: Lecture notes & Pathoma notes.

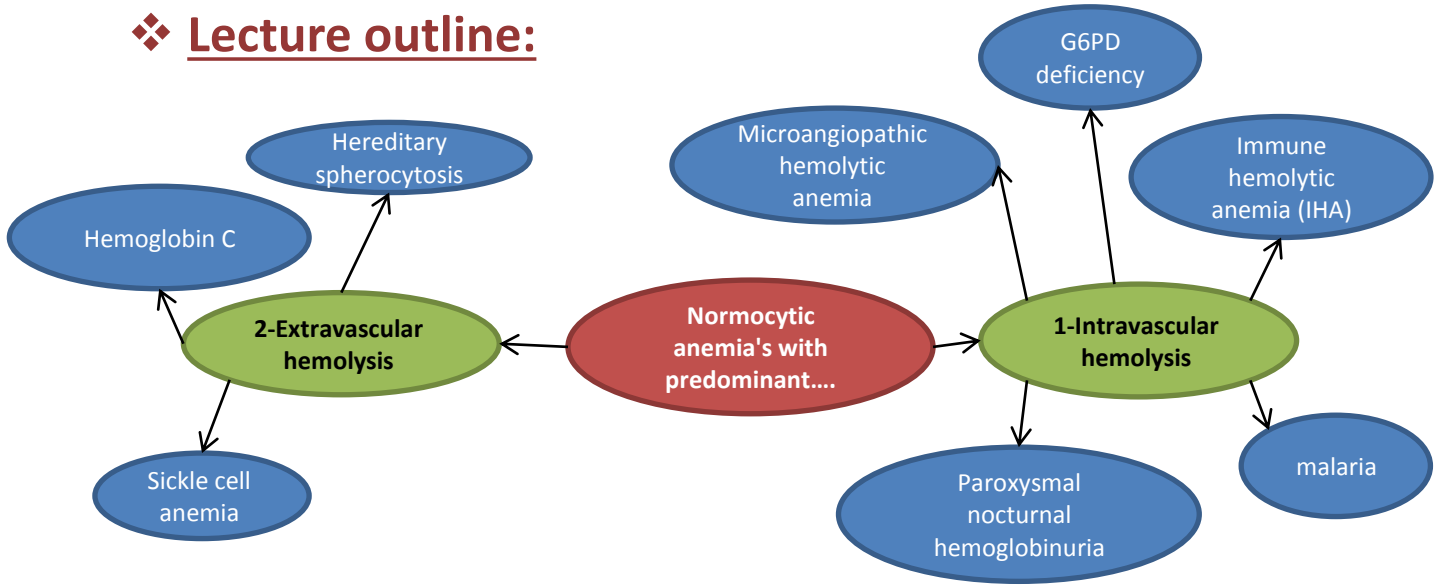
➢ **References:**

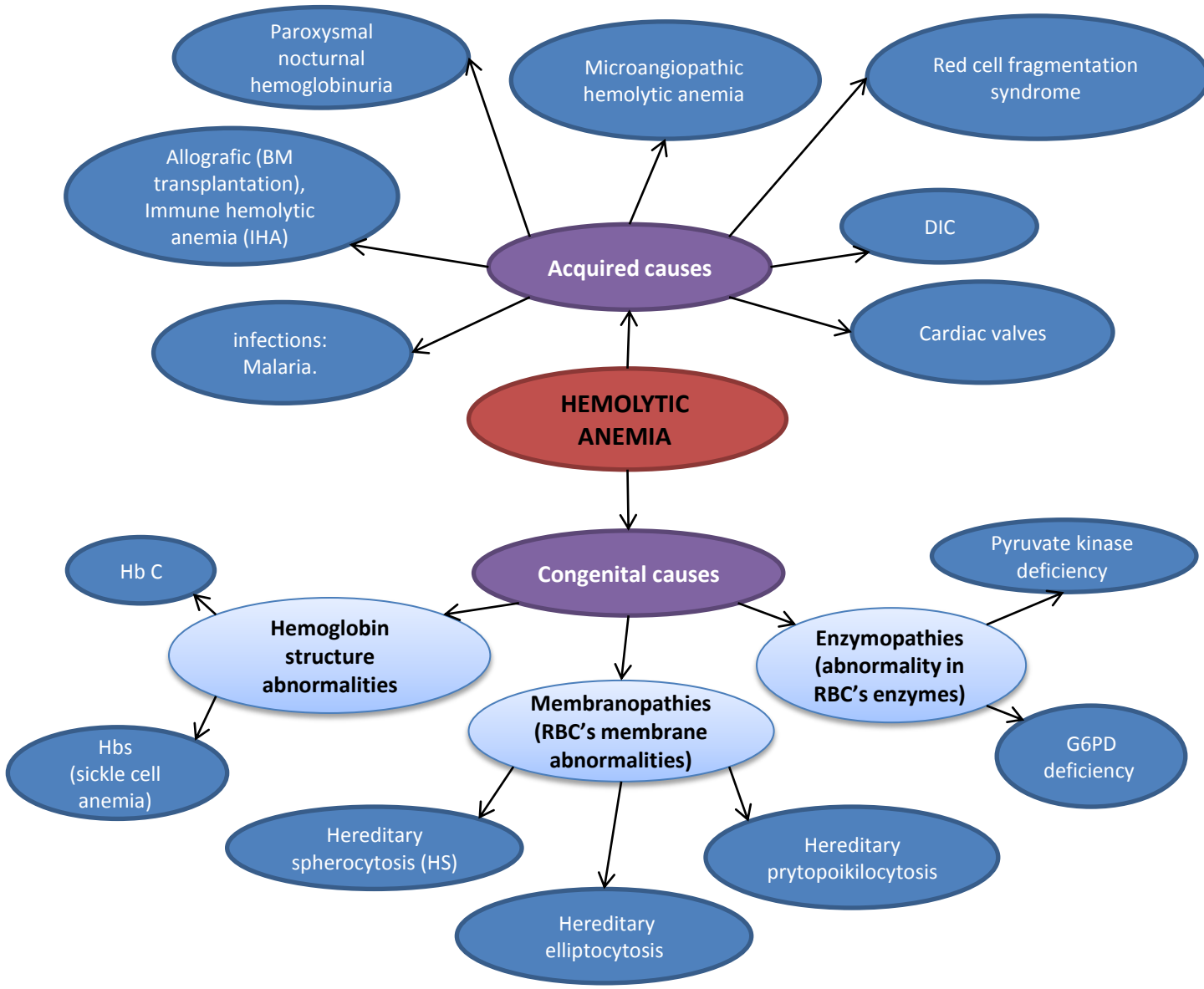
- Girls&Boys Doctors Slides and Notes.
- Lecture notes pathology (chapter 12)
- Pathoma (chapter 5)
- Team 434 & 433.

Revised by
خولة العماري & هشام الغفيلي

➢ Correction File: (HERE)

❖ Lecture outline:





❖ Hemolysis:

Definition: It is the premature destruction of RBCs. (normal life span is 120, in hemolysis the RBCs die in less than 30 days) (it should be less than 30 days to say it's hemolysis > in more than 30 days then it's not hemolysis)

What causes Hemolysis?

a) Defect in the RBCs themselves **(intra-corpuscular)** as in **congenital** (not always) hemolytic Anemia.
b) Defect in the surrounding environment **(extra-corpuscular)** as in **acquired** (not always) Anemia. الخلايا طبيعية بس الخلل برا الخلايا

❖ Hemolytic Anaemias: تكثر ال spherocytosis بالهيموليتك انيميا (مهم)

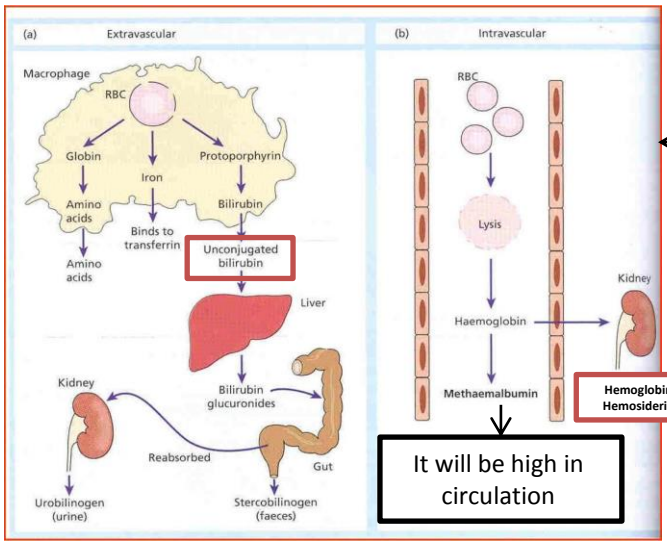
1. Haemolysis is the shortening of the lifespan of a mature red blood cell.
2. Since there is an increase in red cell destruction, there will be an **increase in red cell output from the marrow** (which is stimulated by **erythropoietin***) (compensatory mechanism)
3. This mechanism compensates the loss of RBCs, and this requires an adequately function bone marrow and effective erythropoiesis*
4. If there is more marked reduction in RBC life span (5-10 days) instead of 120 days
5. This is what you call **haemolytic anaemia**
6. Also, a suboptimal* marrow response is seen.

**Definitions:*
Erythropoietin: an important hormone secreted by the kidneys to stimulate RBC production
Erythropoiesis: The name of the process of producing RBCs
Suboptimal: less than the highest standard or quality.

❖ Clinical Features of Hemolysis: لازم كل الي عندهم هيموليتك انيميا يصير عندهم أول ٦أعراض

1. Pallor
2. Lethargy
3. Juandice
4. Splenomegaly
5. Gallstones (pigment – bilirubin)
6. Dark urine (urobilinogen)
7. Bone deformity (in some types of haemolytic anaemia) (because of increase erythrocyte production)
8. Leg ulcers (in some types of haemolytic Anaemia) in HbS & spherocytosis.

Intravascular & Extravascular Hemolysis	
Intravascular Haemolysis	Extravascular Haemolysis
It is the process of the breakdown of red cells **directly** in the circulation (within the vascular compartment)	The more common mode of RBCs destruction. It is the excessive removal of red cells by cells **of RE (reticuloendothelial system) system** in the **spleen and liver** in macrophages.
Main Lab findings of intravascular hemolysis: a. Haemoglobinaemia and haemoglobinuria b. Hemosiderinuria (iron storage protein in urine)	

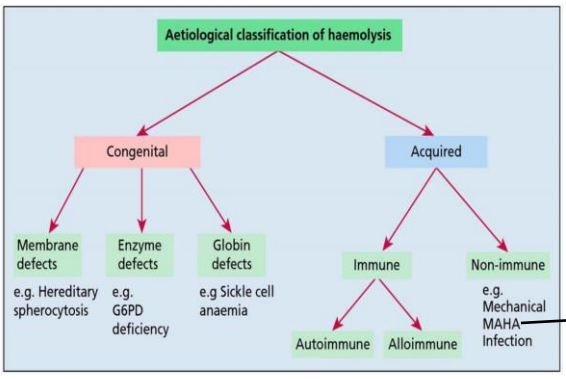


and it's more than enough. اعرفوا هذي الصورة

Laboratory Features of Haemolysis

1-Feature of Increased Red Cell Breakdown	There will be an **increase** in: 1-Serum Bilirubin (**unconjugated** and bound to albumin). 2-urine urobilinogen. (dark urine) 3-faecal stercobilinogen. 4-lactate dehydrogenase (LDH).	**Absent serum Haptoglobins** (which is a protein that attaches to free Hb) it's a strong evidence for haemolysis if it's normal then there's no haemolysis.
2-Features of Increased Red Cell Production	1-Reticulocytosis (when bone marrow is under stress)	2-Bone Marrow Erythroid Hyperplasia
3-Damaged Red Cells	**Morphology**: 1-microspherocytes. 2-elliptocytes. 3-red cells fragmentation. **Increased Osmotic fragility**, auto haemolysis, etc.	**Shortened red cell survival** (This can be shown by 51 CR labelling with study of the sites of destruction)*

*51Cr Cell Labelling is used to distinguish between release of radiolabelled amino acids from primary astrocyte cultures being due to efflux or cell damage.



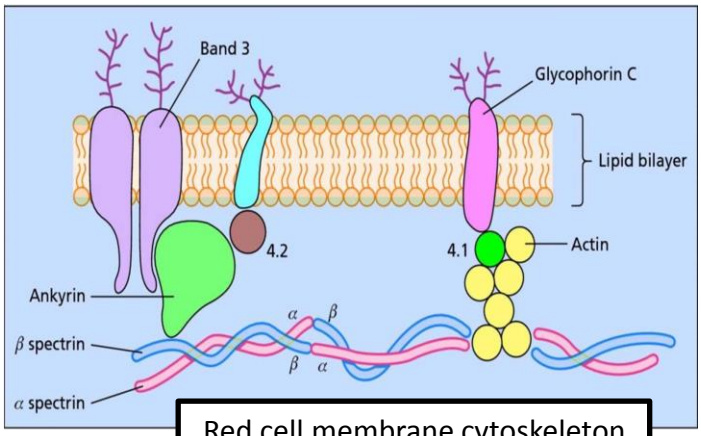
اعرفوا هذي الصورة and it's more than enough.

Microangiopathic hemolytic anemia.

Haemolytic Anaemia

1- congenital	2- Acquired
-Abnormal Haemoglobin (Hb S, Hb C, unstable)	-Allografts, especially marrow transplantation. -drug associated. -Red cell fragmentation syndrome.
-Thalassemia	-Arterial grafts, cardiac valves. (important) -Microangiopathic.
-Membranopathy Hereditary spherocytosis, Elliptocytosis, Acanthocytosis.	-Thrombotic thrombocytopenic purpura, -Haemolytic uremic syndrome. -Meningococcal sepsis. -Pre-eclampsia.
-Enzymopathy G6PD deficiency.	-Disseminated intravascular coagulation (DIC). -March haemoglobinuria.(solider's shoes after long walking ,it will press on the feet and cause RBCs hemolysis) **-Infections:** Malaria, clostridia **-Chemical and physical agents:** Especially drugs, industrial/domestic substances, burns **-Secondary to:** Liver and renal disease -Paroxysmal nocturnal haemoglobinuria

This picture shows the membrane structure of red blood cells. It has an important characteristic which gives the RBC its flexibility, allowing it to enter through blood vessels easily. Any defect of its proteins (ankyrin, alpha and beta spectrin, actin, protien 4.1) will lead to less flexibility of RBCs and shortens its life span.



Red cell membrane cytoskeleton

❖ Hereditary hemolytic anemias:

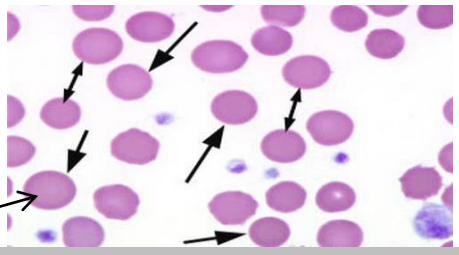
First: Hemolytic Anemia due to Membrane defects.

1-Hereditary spherocytosis (HS):

- **most common** hemolytic anaemia due to a membrane defect.
- 60% of patients have mutations affecting the **Ankyrin** gene. (most commonly involve ankyrin,spectrin or band 3)
- This leads to a loss of membrane surface area with cells adopting a **spheroid** rather than biconcave shape. (more than 30%)
- Destroyed by splenic macrophages, leading to a reduction in red cell survival. (resulting in anemia)

Complications:

development of pigment gallstones من كثر الهيموليسس
Aplastic crises may occur 2ry to parvovirus B19 infection
Megaloblastic anaemia due to folate deficiency



Spherocytes with loss of central pallor.

Diagnosis:

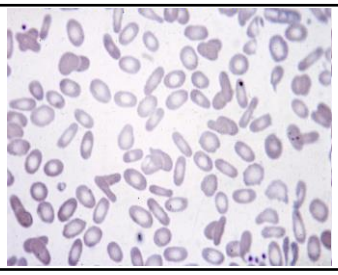
Family history
Mild jaundice
Pallor and splenomegaly
Presence of spherocytes on the peripheral blood film. (60%-70% of RBCs, too high)
The red cell membrane proteins study. (the best test) دايما اول ما نشوف السفيروساتوسس عالية مرة نطلب هذا
Electrophoresis
The eosin-5-maleamide (EMA) binding test (may be used if more definitive evidence for the diagnosis is needed)

Treatment:

Splenectomy (best treatment)
Folic acid supplementation.
Vaccination.

2-Hereditary elliptocytosis (cigar-like)

- It's also a relatively common condition
- Defects in **α spectrin**
- Most patients are clinically asymptomatic, some will have a chronic symptomatic hemolytic anaemia.



3-Hereditary pyropoikiolocytosis: (not important)

-severe disturbance of the multimerization of spectrin
-Severe hemolytic anemia from infancy
-Bizzare peripheral; blood morphology including microspherocytes and poikilocytes.

❖ Second: Hemolysis due to abnormalities of red cells enzymes:

1-Glucose-6-phosphate dehydrogenase (G6PD) deficiency:

X-linked recessive disorder resulting in reduced half-life of G6PD that will prevent the normal generation of NADPH and make erythrocytes sensitive to **oxidative stress.**

-(since the gene is fount in chromosome X, the affected individuals are males**)**

(NADPH, a by product of G6PD is needed to regenerate reduced glutathione (an antioxidant)

So, ⬇G6PD> ⬇NADPH> ⬇Glutathione> oxidative injury by H2O2> intravascular hemolysis.

G6PD variants:

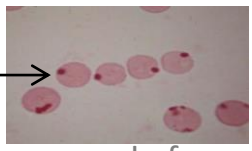
1-"African variant" (type A) mildly reduced half-life of G6PD leading to mild intravascular hemolysis with oxidative stress.

2-"mediterranean variant" markedly reduced half-life of G6PD leading to marked intravascular hemolysis with oxidative stress.

-both have a protective role against falciparum **malaria**.

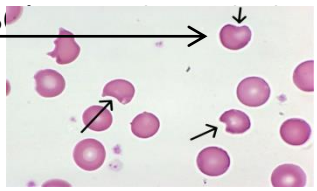
Thus antimalarial drugs should be avoided in pts with G6PD deficiency.

-Oxidative stress precipitates Hb as **Heinz bodies** and is detected by several staining.



What are Heinz bodies? are inclusions within red blood cells composed of denatured hemoglobin.

Heinz bodies and the portion of red cell membrane to which they become attached, are removed by splenic macrophages as the RB[illegible] spleen resulting in **"bite cells"**



(The bile cell, is an abnormally shaped red blood cell with one or more semicircular portions removed from the cell margin. These "bites" result from the removal of denatured hemoglobin by macrophages in the spleen.)

Clinical features of G6PD deficiency:

1-Patients may present with **dark urine** due to **hemoglobinuria.**

2-**Favism**- a syndrome in which an acute haemolytic anaemia occurs after the ingestion of the **broad bean** (Vicia fava) in individuals with a **deficiency of G6PD** (commonly of the **Mediterranean type**) and it usually affects children.

❖ Second: Hemolysis due to abnormalities of red cells enzymes:

2-PYRUVATE KINASE DEFICIENCY:

there's usually chronic hemolytic anemia and some patients may benefit from splenoctomy.

- Under Blood film: "Acanthocytosis cells" (a form of red blood cell that has a spiked cell membrane)

❖ Third: Hemolysis due to hemoglobin defects:

Hemoglobinopathy is a kind of genetic defect that results in abnormal structure of one of the globin chains of the hemoglobin molecule.

Hemoglobinopathies are inherited single-gene disorders. Common hemoglobinopathies include sickle-cell disease (SCD)

The term sickle cell disease (SCD) describes a group of inherited red blood cell disorders. People with SCD have **abnormal hemoglobin**, called hemoglobin S or sickle hemoglobin, in their red blood cells.

People who have SCD inherit **two** abnormal hemoglobin genes, one from each parent. In all forms of SCD, at least one of the two abnormal genes causes a person's body to make hemoglobin S. When a person has two hemoglobin S genes, Hemoglobin SS, the disease is called sickle cell anemia. This is **the most common and often most severe kind of SCD**.

Hemoglobin C (HbC): is a variant hemoglobin with a mutation in the β globin gene causing substitution of glutamic acid for lysine at position 6 of the globin chain

We have 3 types of sickle cell disease:

1-Homozygous sickle cell disease (ss) >> the disease. (2 abnormal Hb)

2-sickle cell trait. (when only one gene is mutated and the other gene is normal)

3-doubly heterozygous sickle cell disease, ex: sickle cell with HbC or sickle cell with thalassemia. (when HbS is present with other Hemoglobins e.g: HbA, HbC, HbF)

The disease	Hb S	Hb C
type	Autosomal recessive mutation in beta chain of hemoglobin in position **number 6**.	
Change in	Glutamic acid (hydrophilic) is replaced by **Valine** (hydrophobic).	Glutamic acid is replaced by **lysine.**

❖ SICKLE CELL DISEASE: الدكتور ما قرأهم ودكتورة فاطمة قالت الي بالاحمر مهم ما ندري نسمع كلام مين

Sickle cell anemia is the most common familial hemolytic anemia in the world. Sickle cell disease arises when **two** abnormal beta genes (HBB gene) are present.

What are the prosperities of HbS?

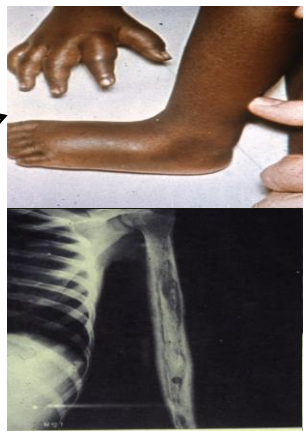
1-decreased solubility. (not fit to go to all tissues)
2-tactoid formation>sickle cells> irreversibly sickled cells.
3-increase mechanical fragility> hemolysis.
4-increase viscosity> organ infarction.

Factors affecting sickling:

1-oxygen tension, 50-60mmHg for SS and 20-30mmHg for AS (normal O2 level is 75 to 100mm Hg). (increase sickling with hypoxemia)
الASما يبان عليهم السيكلنق الا لمن يصير عندهم الاكسجين قليل مره لانهم حاملين للمرض بس, اما الSSاول ما ينزل الاكسجين شوي على طول تطلع الsickle cells.
2-PH, sickling is inhibited by alkaline PH and exacerbated by acidification. How? The abnormal Hb (HbS) gets polymerized when deoxygenated, polymers aggregate into needle-like structure resulting in sickle cells. Acidosis decreases the Hb affinity for O2 thus increasing the sickling.
3-concentartion of HbS (the polymerization of deoxygenated HbS is strongly concentration dependent)
4-presense of other Hb (HbS>HbC>HbF>HbA) (HbC has greater tendency to aggregate with HbS. HbA and HbF interact weakly with HbS)
5-slow blood flow (which has high tendency for Hb to lose their affinity)

Clinical manifestation in sickle cell anemia:

1-pallor.
2-jaundice and dark urine.
3-apathy and anorexia.
4-**hand-foot syndrome** (young children).
5-**leg ulceration and skeletal deformity.**
6-splenic sequestration (young children).
7-hepatic sequestration.
8-bone and joints pain.
9-abdominal pain. (pain crisis)



Diagnosis:
Sickle Solubility test.

10-autosplenoctomy-shrunken, fibrotic spleen leads to increase risk of infection.
11-acute chest syndrome (small vessels occlusion in pulmonary microcirculation)
12-extramedullary hematopoiesis with hepatosplenomegaly. (early childhood and in association with thalassemia)
13-CNS presentation.

❖ Acquired haemolytic anaemias

In the acquired haemolytic anaemias, red cells may be destroyed either by immunological or by non-immunological mechanisms.

1. Immune haemolytic anaemias: (immunological mechanism)

- In these conditions, antigens on the surface of red cells react with antibodies sometimes with complement activation.
- IgG-coated red cells interact with the Fc receptors on macrophages in the spleen, and are then either completely or partially phagocytosed.
- When the phagocytosis is **partial**, the damaged cell will return to the circulation as a **spherocyte**.
- Red cells that are also coated with the activated complement component C3 may interact with C3 receptors on macrophages and are usually **completely** phagocytosed.
- In most instances where **complement is activated**, the cascade sequence only proceeds further and permits deposition of the membrane attack complex (C5-C9) with resultant intravascular haemolysis.

The immune haemolytic anaemias may be due to **autoantibodies**; that is, antibodies formed against one or more antigenic constituents of the individual's own tissues. These include:

- Autoimmune haemolytic anaemia (AIHA) (someone develops antibodies against his own RBCs)
- Some drug-related haemolytic anaemias.
- Alloimmune haemolytic anaemia is also possible to develop, consequent on the production of antibodies against red cells from another individual, as in haemolytic **transfusion reactions** and **haemolytic disease of the newborn**.

Classification of autoimmune haemolytic anaemias (AIHA)

Caused by warm-reactive antibodies

Idiopathic

Secondary (chronic lymphocytic leukaemia, Lymphoma, systemic lupus erythematosus (SLE), some drugs)

Caused by cold-reactive antibodies

Cold haemagglutinin disease

Idiopathic

Secondary (*Mycoplasma pneumoniae* infection, infectious mononucleosis, lymphomas)

Paroxysmal cold haemoglobinuria

Idiopathic

Secondary (some viral infections, congenital and tertiary syphilis)

Very important:
If they ask you in the exam **what types of antibodies in CLL (chronic lymphocytic leukemia)?** It's **WARM** antibodies.

SLE is the most common cause

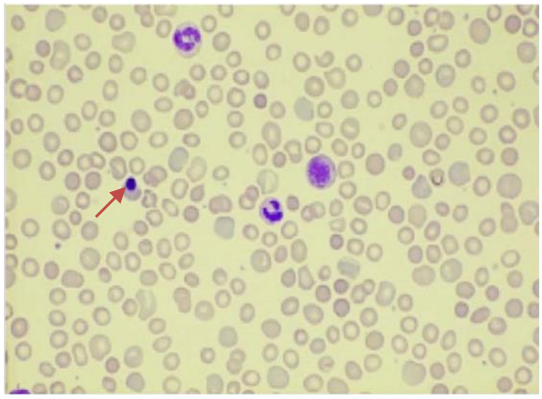
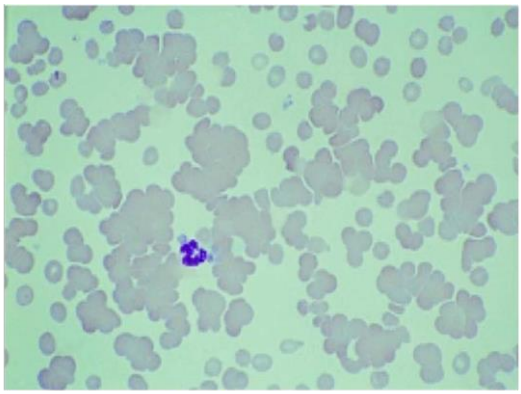
IgM mediated disease (cold reactive antibodies) are associated with infections

❖ Autoimmune haemolytic anaemias (AIHA)

Immunohemolytic anemias are classified on the basis of, 1-the nature of the antibody 2-the presence of predisposing conditions.

	Warm AIHA (MORE COMMON)	Cold haemagglutinin disease (CHAD)
What is it? أهم شي تعرفوا هذا والباقي اقرأووه.	'Warm' autoantibodies react best with the red cell antigen at 37ºC and are usually of IgG subtype.	Cold' antibodies react best at temperatures below 32ºC (usually below 15ºC) and, since they are usually of IgM subtype their pentameric structure permits direct **agglutination** of red cells coated with antibody; they are therefore sometimes termed **cold agglutinins.**
Clinical picture	1) In idiopathic warm AIHA, **haemolysis** dominates the clinical picture and **no** evidence can be found of any other disease. 2) In secondary AIHA, the **haemolysis** is associated with a primary disease such as chronic lymphocytic leukaemia (CLL) or systemic lupus erythematosus (SLE). ➢ The antibody-coated red cells undergo partial or complete phagocytosis in the spleen and by the Kupffer cells of the liver (Extravascular) . There may be partial activation of the complement cascade.	Since cold antibodies react with red cells only at temperatures below about 32ºC, they typically bind to the red cell surface in the cooler superficial blood vessels of the peripheries. • Symptoms due to cold AIHA are worse during cold weather. Exposure to cold provokes acrocyanosis "blue or cyanotic discoloration of the extremities", due to the formation of agglutinates of red cells in the vessels of the skin (Intravascular). • The direct activation of the **complement** system leads to red cells lysis and, consequently, to **haemoglobinaemia and haemoglobinuria**.

❖ Autoimmune haemolytic anaemias (AIHA) cont.

	Warm AIHA	Cold haemagglutinin disease (CHAD)
Diagnosis	Haematological findings include anaemia, spherocytosis, reticulocytosis and occasional nucleated red cells in the peripheral blood. The critical **diagnostic investigation is the direct anti-globulin test**. (direct coombs test)	-
Treatment غير مطالبين فيهم	➢ Haemolysis can be limited by treatment with prednisolone. ➢ If reduction in haemolysis is not maintained when the dose of steroids is lowered, **splenectomy or alternative immunosuppressive therapy** should be considered. ➢ The anti-CD20 monoclonal antibody ***rituximab***, as well as immunosuppressants such as *azathioprine* or *cyclophosphamide*, may be beneficial in reducing autoantibody production.	• Chronic idiopathic CHAD is managed initially simply by keeping the patient **warm**. • Treatment with *rituximab* may be effective.
Blood film	 - Blood film from a patient with AIHA (warm-reactive antibody) showing prominent spherocytosis and polychromasia. - The arrow points to a nucleated RBC	 - Numerous red cell agglutinates on blood film from a patient with idiopathic CHAD.

- Other causes of haemolytic anaemia with an immune element include:
  1) paroxysmal nocturnal haemoglobinuria;
  2) paroxysmal cold haemoglobinuria;
  3) drug-related haemolytic anaemias.

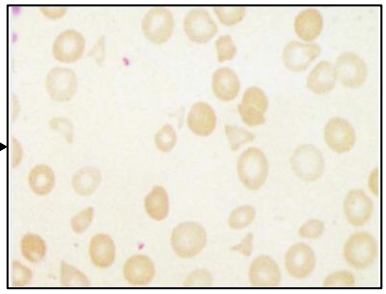
2. Non-immune haemolytic anaemias (read it)

a. Mechanical damage to red cells.

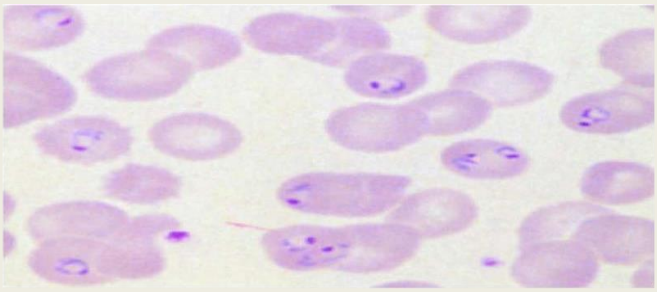
Red cells are mechanically damaged when they impact upon abnormal surfaces.
For example, In disseminated intravascular coagulation (DIC) inappropriate activation of the coagulation cascade produces fibrin strands which are thought to cause mechanical destruction of red cells.
Such damage usually results in the presence of red cell fragments in the blood film.

Several of the mechanical causes of acquired non-immune haemolytic anaemia. **(cardiac valve is the most important one)**

Fragmented RBCs (schistocytes) (is a fragmented part of a red blood cell. Schistocytes are typically irregularly shaped, jagged, and have two pointed ends in the blood film of a patient with a malfunctioning aortic valve prosthesis.



b. infections

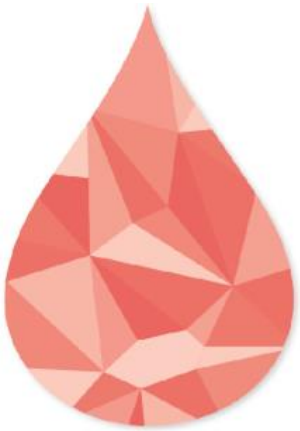


Blood film from a patient with *plasmodium falciparum* **malaria** ((اكثر انفكشن يسوي هيموليسس showing several parasitized red cells, which can be subjected to intravascular lysis. .

c. Hypersplenism

Hypersplenism describes **the reduction in the lifespan** of red cells, granulocytes and platelets that may be found in patients with splenomegaly due to any cause.
The cytopenias found in patients with enlarged spleens are also partly caused by **increased pooling of blood** cells within the spleen.

➢ Check your Understanding! (HERE)



= Haematology 435 =

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