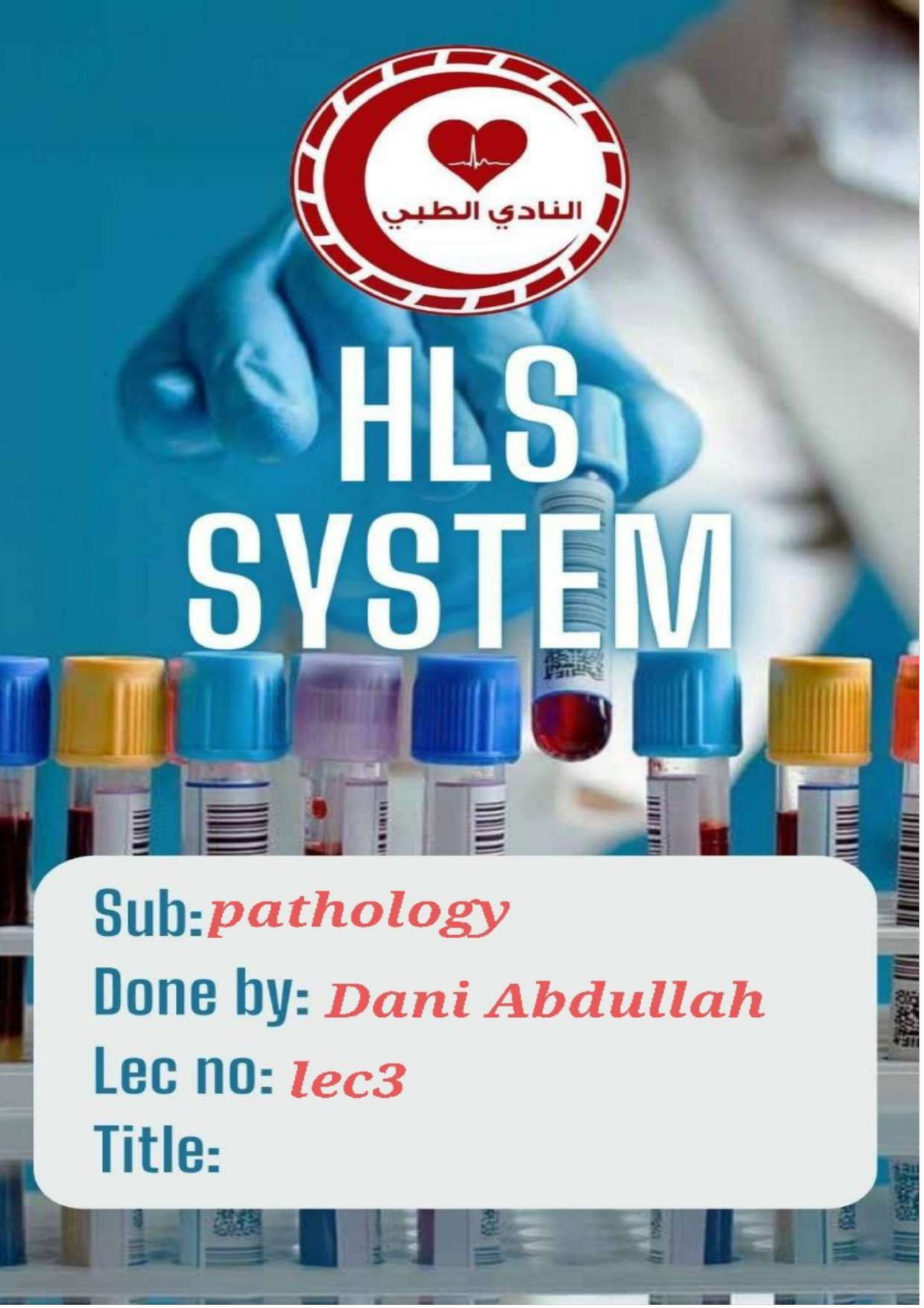


HLS SYSTEM

Sub: *pathology*

Done by: *Dani Abdullah*

Lec no: *lec3*

Title:

LECTURE(3) SUMMARY

Normocytic Anemia

Normocytic anemia: is decreased RBC mass with normal-sized RBC (MCV - 80-100 µm3).
Divides to:
*High retic count
-Peripheral destruction of RBC (will have reticulocyte >3%)
-Extravascular hemolysis (RBC destroyed by liver, spleen and lymph)
-Intravascular hemolysis (RBC destroyed within blood vessels)
*Low retic count
Underproduction of RBC (no increased reticulocytes)

- Aplastic anemia is a bone marrow disorder characterized by pancytopenia dueto ineffective hematopoiesis in the absence of any underlying neoplasia or fibrosis.
- Mostly sporadic but can be constitutional (congenital)
- Bimodal age distribution: first peak at 10 - 25 years; second peak at > 60 years.

Etiology:

Acquired aplastic anemia (most common):

- nfectious agents: parvovirus B19, HIV, EBV, Hepatitis C virus
- Toxins such as benzene
- Drugs, chemicals, or radiations (example of drugs:chloramphenicol)
- Autoimmune disease - most common SLE
- Idiopathi

Constitutional "congenital" aplastic anemia,example "Fanconi anemia"

Morphology:

CBC:

- shows pancytopenia (including normochromic normocytic anemia)
- Low reticulocyte count (< 30 x 109/L)
- Normal vitamin B12, folate and iron (to exclude vitamin deficiency anemias)

Bone marrow biobsy:

- Bone marrow markedly hypocellular (cellularity < 5%)
- Lacunar spaces replaced by fatty cells
- Residual nucleated cells include mostly lymphocytes, plasma cells, macrophages, mast cells
- Marrow lacunar spaces are replaced by fat, and very scant hematopoietic cells

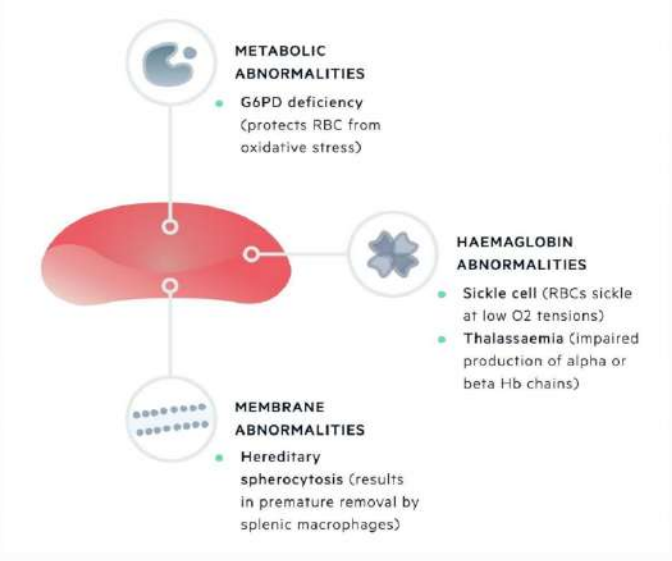
Clinical features:

Signs and symptoms related to severity of pancytopenia

- Anemia: most common are fatigue, shortness of breath.
- Thrombocytopenia: bleeding and bruising.
- Leukopeni : frequent or prolonged infections.

Treatment :

-Bone marrow transplant is the only curative treatment
-Treat underlying cause if present (toxic, drugs, infections)
-Immunosuppression for cases with abnormal T-cell activation
-Transfusion support only to relieve symptom



Features of hemolytic anemia ' intra and extra vascular'

- Shortened RBCs survival
- Elevated erythropoietin level leadingto increased erythropoiesis and earlyrelease of RBCs from marrow
- Reticulocytosis
- Elevation in unconjugated Bilirubin(indirect) and LDH

Extravascular hemolysis:

- Hemolysis done by reticuloendothelial system (macrophage in liver, spleen and lymph nodes)
- Globin is broken to AA; Iron is recycled.
- Unconjugated (indirect) bilirubin is carried by albumin to liver and then conjugated in liver and excreted to bile.

clinically present with:

*Anemia with splenomegaly Jaundice due to unconjugated bilirubin (too much bilirubin to be conjugated by liver).
*High risk for bilirubin gallstones.
*Marrow hyperplasia with corrected reticulocyte >3.

Intravascular hemolysis:

- RBC is destroyed in blood vessels. Unlike macrophage breaking down hemoglobin to bilirubin, hemoglobin simply leaks out to blood.
- Patients will have hemoglobinemia and hemoglobinuria (hemoglobin water soluble)
- Then, hemosiderinuria occurs after few days -Hemoglobin in urine is picked up by renal tubular cells. Iron is recycled back and stored as hemosiderin. Renal tubular cells slough off and hemosiderin will be seen in urine.
- Hemoglobin is carried by haptoglobin. Haptoglobin is not enough to bind all Hgb. So, patients will quickly have hemoglobinemia and hemoglobinuria
- Also, patients will show marked decrease in Haptoglobin "almost absent"

Immediate	After few days
- Decreased serum haptoglobin	- Hemosiderinuria
- Hemoglobinemia	
- Hemoglobinuria	

Marrow Response To Hemolysis:

1. Erythroid hyperplasia with decreased Myeloid :Erythroid ratio.
2. In chronic cases, extramedullary hematopoiesis may take place.
3. Erythropoiesis can increase up to 8 times its normal level. Thus, hemolysis may take place without development of anemia.

Anemia develops if

- Rate of hemolysis increases beyond the compensatory rate (hemolytic crisis).
- The bone marrow stops producing RBCs (aplastic crisis).

Test	Intravascular hemolysis	Extravascular hemolysis
Serum Haptoglobin	↓↓	Normal or ↓
Plasma Hb	Present	Absent
Hemoglobinuria	Present	Absent
Hemosiderinuria	Present	Absent
Serum lactate dehydrogenase LDH	↑	↑
Serum unconjugated bilirubin	Normal or ↑	↑

SICKLE CELL DISEASE:

- Most common familial hemolytic hemoglobinopathy
- Molecular basis:single point mutation (A to T substitution) in the first exon of the β globin gene,converting glutamic acid into valine.
- It is an autosomal recessive inheritance.

Phenotype	Hemoglobin composition
Sickle cell disease (homozygous mutation)	90% HbS, 8% HbF, 2%HbA_2, **no HbA**
Trait (one mutated and one normal B chain)	**55% HbA**, 43% HbS, 2% HbA_2

- *HbS – sickle cell hemoglobin (in $\alpha 2\beta 2$ protein, both copies of β are mutated)*

Incidence:

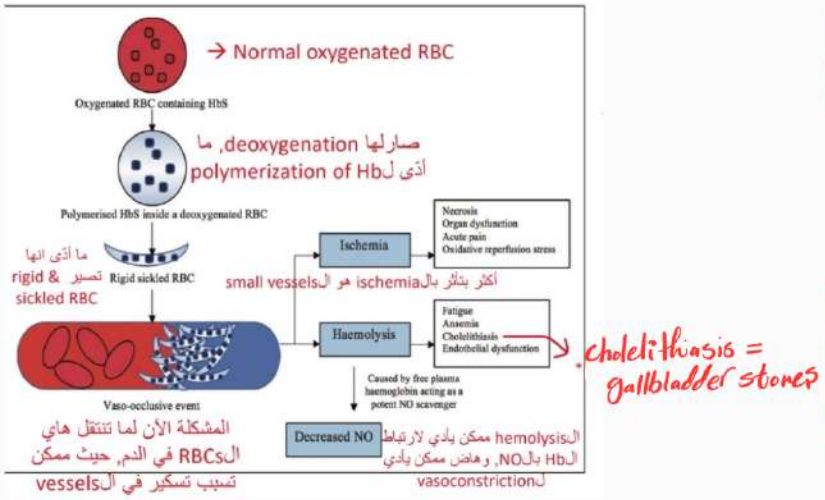
*It is more common among African & Asian population

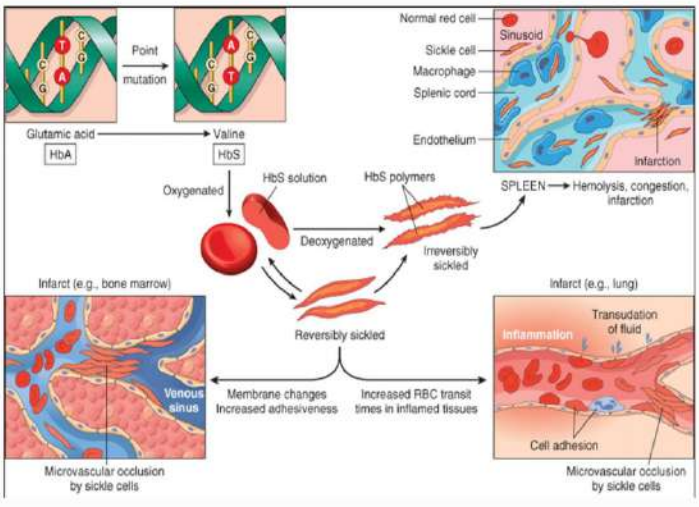
*It was found that HbS has a protective effect against Plasmodium Falciparum malaria infection.

Pathogenesis:

- HbS polymerizes when deoxygenated(reversible).
- The polymers accumulate into needle shaped structures and make RBCsickle cell.
- Sickling increases with hypoxemia,dehydration and acidosis.
- Note HbF protects against sickling. Kids protected for first few months of life.
- Sickling and de-sickling damages membrane leading to both intravascular and extravascular hemolysis (spleen eats damaged RBC); sickled RBC cause vasoocclusion.
- Massive erythroid hyperplasia occur in BM to replace RBC.Ca influx, efflux of K and water, ↑ MCHC.

Note:repeated episodes of sickling cause membrane damage; the cell accumulate Ca, loose K & water & then become irreversibly sickled retaining their abnormal when fully oxygenated.





FACTORS AFFECTING THE DEFGREE OF SICKLING

Type of Hb

*Hemoglobin SC Disease (α 2 β 2 6 Val, 2α 2 β 6 Lys) shows a milder disease.

*homozygous vs. heterozygous.

Hb. Concentration

**red cell dehydration increases HbS concentration which will greatly facilitate sickling during deoxygenation and can trigger occlusion of small blood vessels .

**coexistence of α thalassemia reduces the HbS concentration; due to low MCHC.

Blood circulation

***sickling is confined to microvascular beds where blood flow is sluggish - bone marrow , spleen & possibly kidneys.

***inflammation slows the flow by increasing the adhesion of leukocytes and RBC's.

clinical consequences:

chronic hemolysis:

- marked reticulocytosis and hyperbilirubinemia and gall stones formation.
- Expansion of the bone marrow due to increase erythropoiesis causes prominent cheek bones & changes in the skull. (“Hair on end” appearance on skull X-ray)

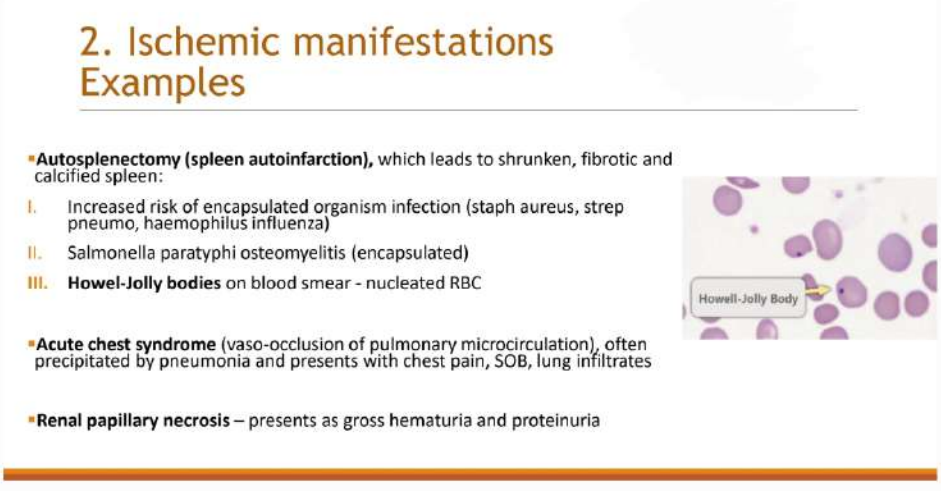
Ischemic manifesations:

(microvasculature obstruction): bones, liver, kidneys, skin, retina,...etc

Examples

■ Dactylitis is due to vasoocclusive infarcts in the bones of fingers in hands and feet, causing painfulswelling.

■ Early common presentation in infants.



Diagnosis:

- Asymptomatic till 6 months of age.
- Moderate to severe anemia (6-8 g/dl).
- Unremitting course punctuated by sudden crises.
- CBC and Hb electrophoresis (HcT is about 18% -30% - normal value 35 %-45%).

Treatment:

* Prophylactic treatment with penicillin to

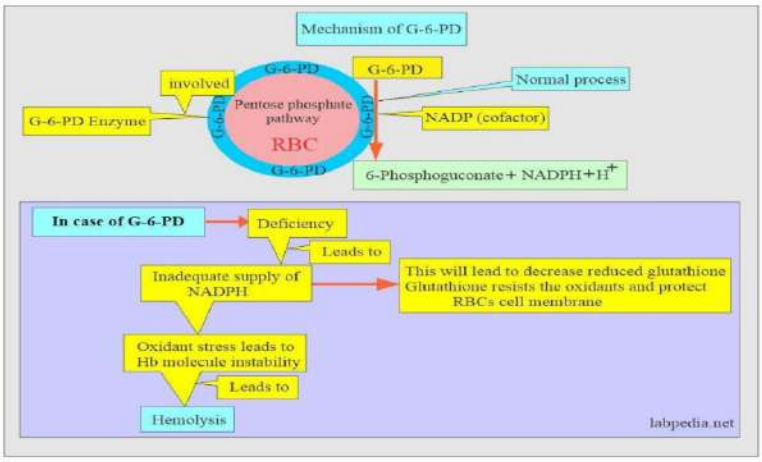
** prevent pneumococcal infection .

*** Adequate hydration and pain relief.

****Use the hydroxyurea therapy“increase HbF” In severe cases, exchange transfusion to reduce the HgbS.

GDPD Deficiency aka "favism"

- X-linked recessive disorder
- G6PD is first enzyme in pentose phosphate pathway and is required to make NADPH. NADPH is important to reduce oxidative stress.
- G6PD deficiency presents as increased oxidative stress including hemolytic anemia.
- The majority of patients are asymptomatic most of the time and go through life without ever being aware of their genetic trait.
- Hemolysis occurs after a lag of 2-3 days Males more vulnerable than female (heterozygous)



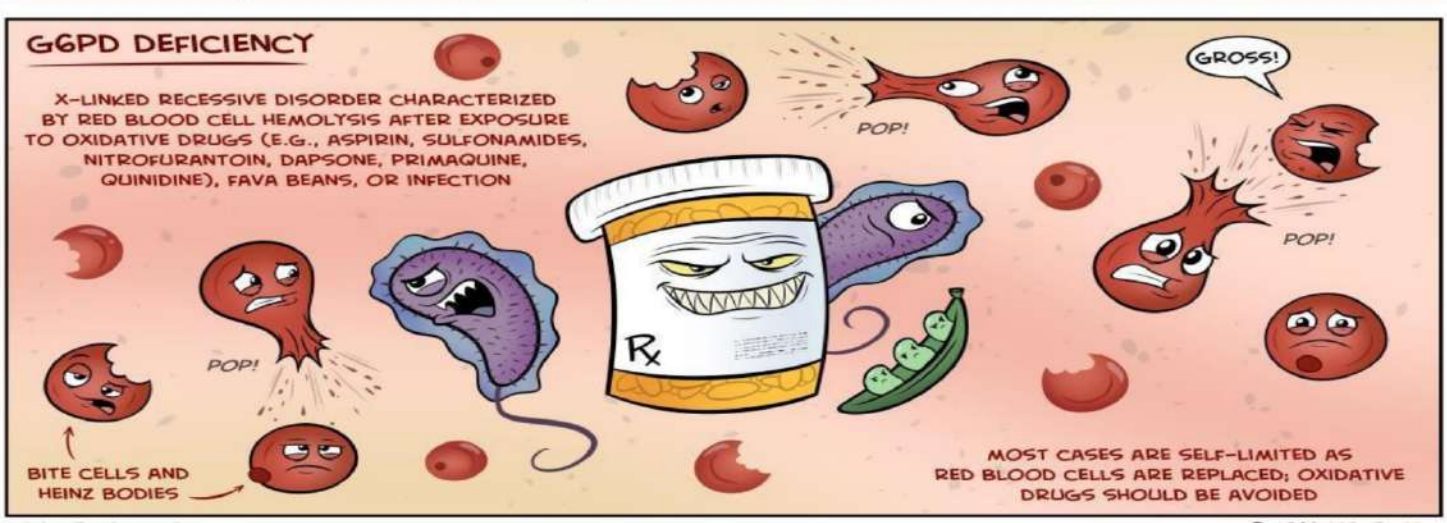
Hemolysis due to oxidant stress:

*Drugs eg: Antimalarials, sulfonamides, furantoins,...etc.
*Favism; chickpeas , green peas , all types of beans should be avoided
*Infections; produces free radicals
*Oxidation leads to denaturation of globin chains, and precipitation at membranes forming Heinz bodies.
*RBCs: Bite cells and Heinz bodies.

*Features of Extra/Intravascular hemolysis.

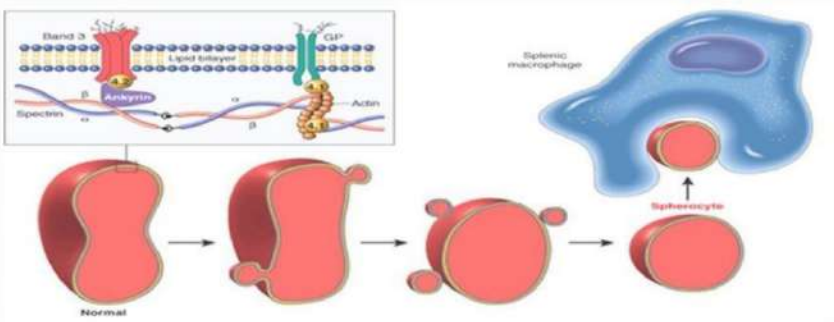
Diagnosis of G6PD deficiency:

- Screening - Heinz preparation – Blood smear will show Heinz bodies.
- Confirm - enzymatic studies (however; in the acute phase, RBCs lacking G6PD are hemolyzed and dead, so they cannot be detected.



HEREDITARY SPHEROCYTOSIS

- Congenital hemolytic anemia (AD) due to genetically determined abnormal spectrin and ankyrin molecules, leading to defects in red blood cell membrane.
- RBC membrane blebs and are lost overtime. RBC becomes more spherical.
- Red blood cells become trapped within spleen and have less than usual 120-day lifespan
- Spherocytes are phagocytosed by splenic macrophages , leading to extravascular hemolysis characterized by anemia, jaundice, increased reticulocytes & splenomegaly.



CBC and blood smear findings:

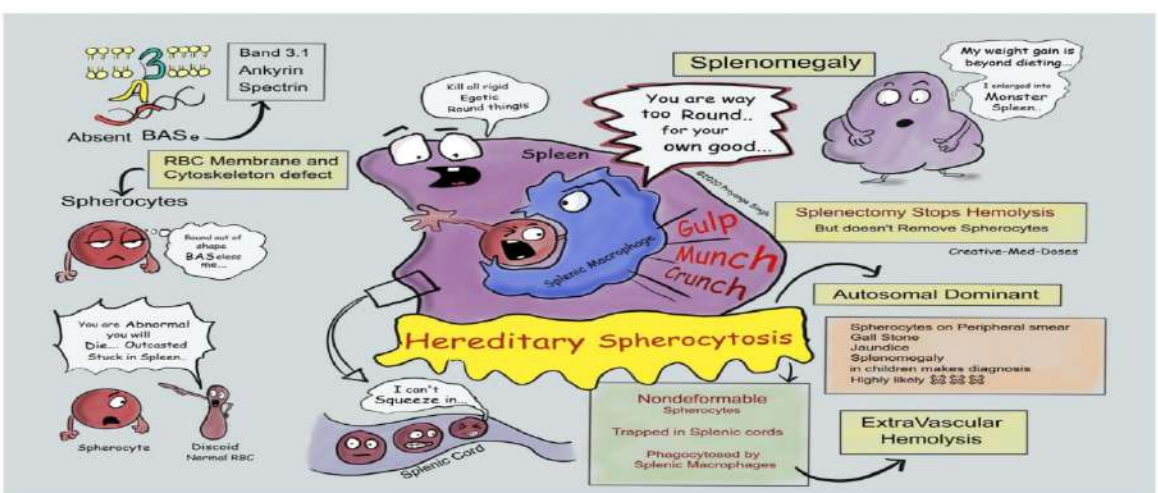
- RBC becomes round instead of disc shaped (loss of central pallor)
- High MCHC - high concentration of hemoglobin as cells are getting small.
- Howell-Jolley bodies in peripheral blood RBCs . The Howell-Jolley body is anuclear DNA remnant.

Treatment:

Splenectomy (prolongs survival of red blood cells, although they still have membrane defects).

Osmotic fragility:

- increased; basis for diagnostic testing
- The osmotic fragility of red cells isincreased i.e . the RBCs are easily hemolysed when kept in a hypotonic saline solution.
- The test consists of exposing RBC tovarying strengths of hypotonic saline solutions and measuring the degree of hemolysis colorimetrically at room temperature .



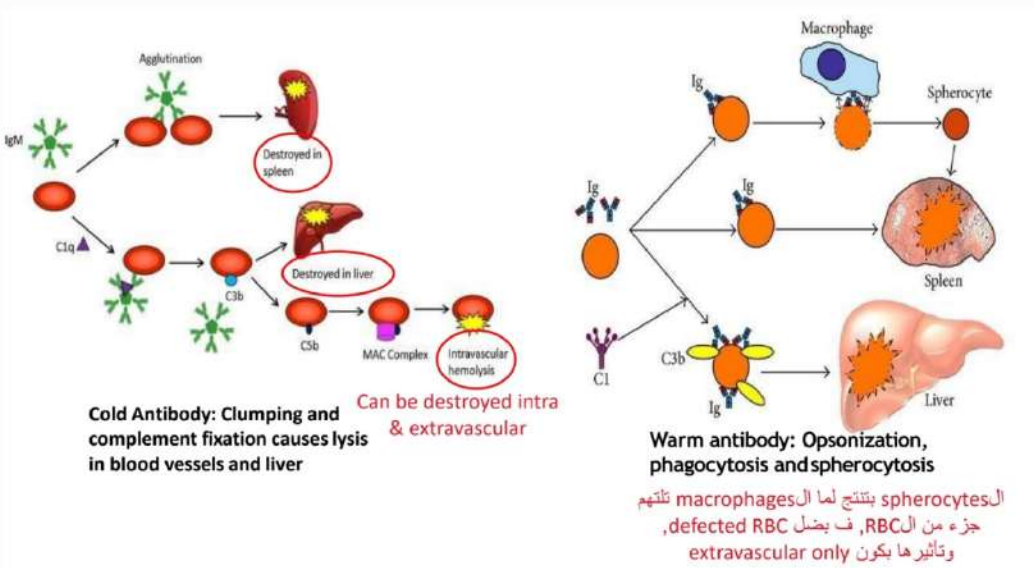
Immune Hemolytic Anemia AIHA

Three broad categories:

Alloimmune: The patient produces alloantibodies to foreign red cell antigens (transfusion, pregnancy, or organ transplant)
Autoimmune: Autoreactive antibodies (loss of self recognition of individual's own red cell antigen)
Drug-induced: Antibodies against red cells coated with drug or it is metabolites.

Causes of autoimmune-IHA,IgG or IgM mediated destruction of RBC

Warm Antibody: IgG/IgA type	Cold Antibody: IgM type
Activated at body temp. (37 c)	Active at 0-4°C IgM binds to RBC in cold temp (extremities) → (Hands & feet)
IgG-coated RBC lysis in spleen (predominantly extravascular)	Clumping and complement fixation causes lysis in blood vessels and liver (intra- and extravascular)
Morphology: spherocytes (splenic macrophage phagocytose tagged RBC leading to formation of spherocytes)	IgM agglutination (hemolysis occurs in the hands & feet in cold weather) (بتربط الRBCs مع بعض)
80% of immune hemolytic anemias: Primary (50-70%) Secondary: - Lymphoproliferative disorders - Autoimmune diseases (SLE) - Drugs (penicillin and cephalosporins)	• Infectious mononucleosis (EBV) (20% of IHA) • Mycoplasma infection • Lymphoproliferative disorders **Very Important**

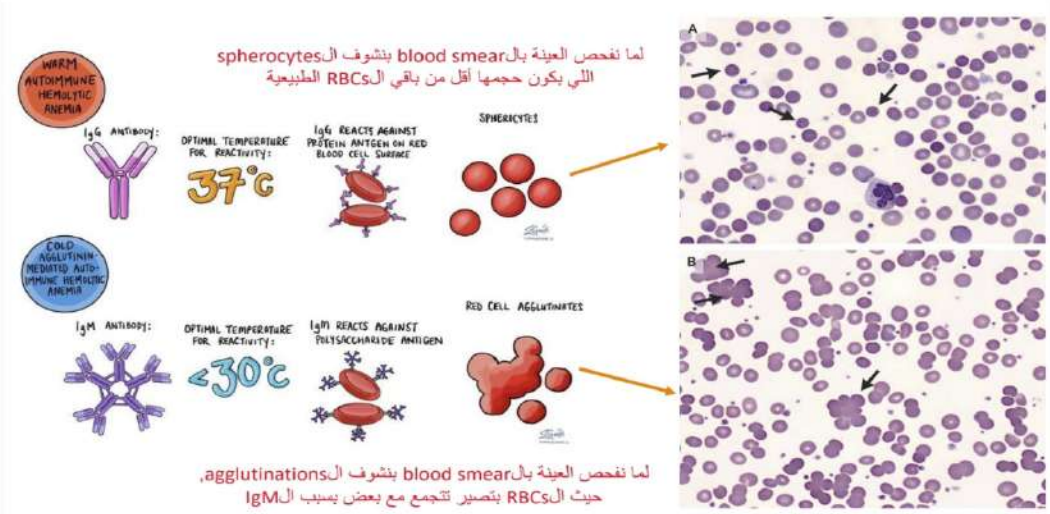


Cold Antibody: Clumping and complement fixation causes lysis in blood vessels and liver

Can be destroyed intra & extravascular

Warm antibody: Opsonization, phagocytosis and spherocytosis

الspherocytes بتنتج لما الmacrophages تلتهم جزء من الRBC, ف بضل defected RBC, وتأثيرها بكون extravascular only



لما نفحص العينة بالblood smear بنشوف الspherocytes اللي بكون حجمها أقل من باقي الRBCs الطبيعية

لما نفحص العينة بالblood smear بنشوف الagglutinations, حيث الRBCs بتصير تتجمع مع بعض بسبب الIgM

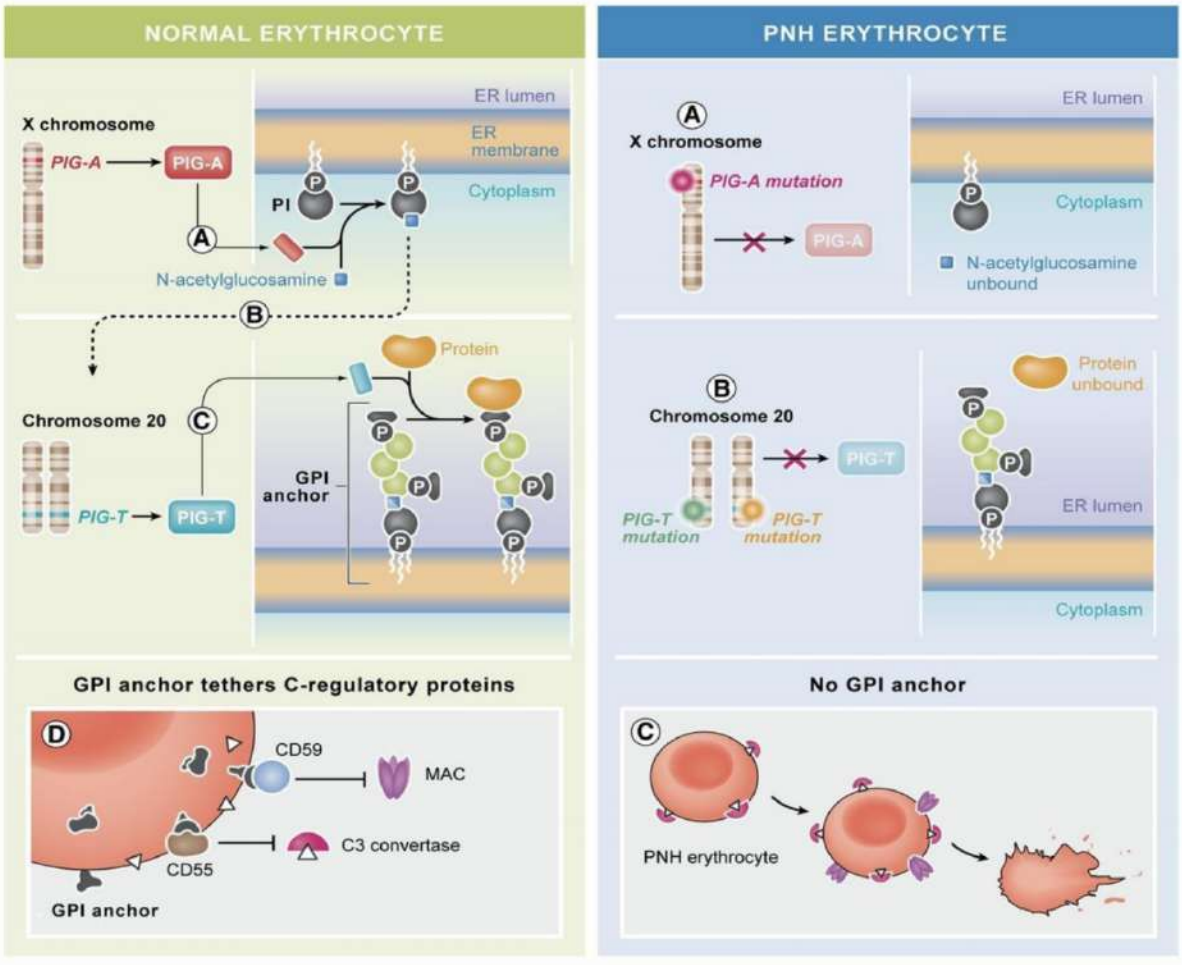
Paroxysmal nocturnal Hemoglobinuria(PNH)

➢ PNH is caused by an acquired somatic (non-germline) mutation in the X-linked phosphatidylinositol glycan class A (PIGA) gene;

➢(PIGA) gene produces the glycosylphosphatidylinositol (GPI) anchor proteins (GPI-APs), that links cell surface proteins to cell membranes.

➢Hematopoietic cells containing PIGA mutations lack GPI anchored cell surface markers, including complement inhibitors (such as CD59 and CD55).

➢ So, mature erythrocytes lacking GPI-APs are unprotected from the membrane attack complex (MAC or C5b9), leading to paroxysmal hemolysis.



Hemolysis occurs mostly at night when there isfixation of complement which is enhanced by decrease of blood PH during sleep.

✓ Chronic intravascularhemolysis with hemoglobinemia, hemosiderinuria -/+ hemoglobinuria

✓ Increased Reticulocytosis.

✓ Venous thrombosis (hypercoagulability due to free Hb in blood).

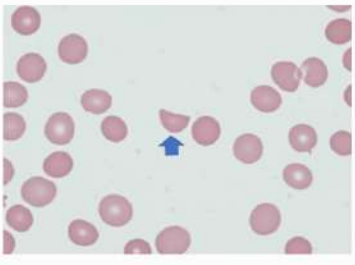
Hemolytic anemias due to mechanical trauma to RBCs

Red cells are disrupted by physical trauma:

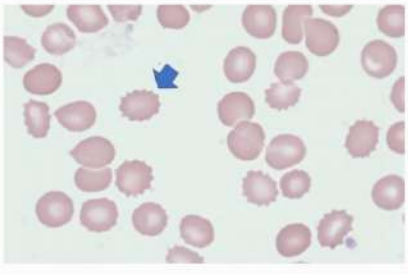
I. Cardiac valve prostheses .

II. Microangiopathic hemolytic anemia as in DIC , malignant hypertension, thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS).

In all these conditions the circulating RBCs are mechanically traumatized , get the appearance of Schistocytes , burr cells or helmet cells B



Helmet cells



Burr cell