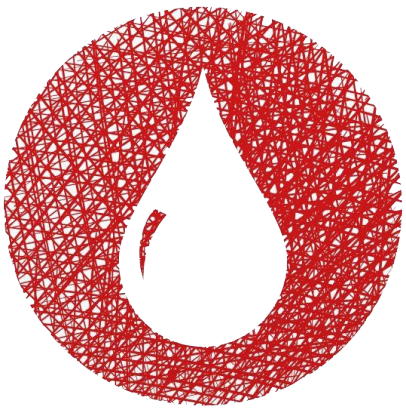


hemolytic anemia.

Objectives :

- To be able to define haemolysis and hemolytic anemia.
- To be able to classify hemolytic anemias into congenital and acquired types,and to know the etiological factors in each division.
- To understand the difference between intravascular and extravascular haemolysis, and to recognize the laboratory features of each.
- To appreciate some major examples of congenital disorders resulting in hemolysis like HS and G6PD deficiency.
- To understand the role of autoantibodies in the production of hemolytic anemias and to know the types of disease with which they are associated
- To understand some causes of non-immune acquired hemolytic anemias.

Team leaders : Abdulrahman Alageel, Ebtesam Almutairi.
Done by : Saif AlMeshari, abdullah alsergani ,
Mansour ALobrah .

Impotent
Notes
Doctor's slides

HEMOLYTIC ANAEMIAS

Hemolysis: is a state with a substantially short lifespan of a mature red blood cell. Hyperbilirubinemia is common, might present as jaundice

If hemolysis is not marked and can be **compensated**	If hemolysis is marked and **can not be compensated**[1]
- increased red cell output from the marrow. - stimulated by erythropoietin. - will be sufficient.	- more marked reductions in red cell lifespan, say to 5-10 days from the usual 120 days. - will result in **hemolytic anemia.**

Hemolytic anemia: is shorten life span of RBC that can not be overcome by ability of bone marrow production.

Extravascular Hemolysis[2]	Intravascular Hemolysis
In the majority of hemolytic anemias, the **macrophages** in the spleen, liver and bone marrow reticuloendothelial system remove red cells from the circulation by phagocytosis.	Here, the red cells are caused to rupture and release their hemoglobin (Hb) directly into the circulation.

The intra/extravascular site of red cell destruction may give **clues** to the underlying etiology of the haemolysis.

Laboratory Evidence of Hemolysis

Features of increased red cell breakdown	Features of increased red cells production.	Damaged red cells.
▪ A rise in the **unconjugated bilirubin** concentration in the plasma. ▪ **Lactate dehydrogenase[LDH]** , an enzyme present in red cells, more with **intra**,. ▪ Reduction of serum haptoglobin, molecule binds to free Hb, intra,. ▪ Free heme can bind to albumin to form **methemalbumin**. ▪ Free Hb in the urine: **hemoglobinuria**, **intra**. (note the difference from hematuria which describes the presence of intact red cells in the urine[3]).	▪ **reticulocytosis** ▪ **Polychromasia**, a bluish discoloration of RBC, due to **reticulocytosis**. ▪ **erythroid hyperplasia**	▪ As haemolysis will also increase the marrow 's demand for folic acid, **macrocytosis**, high MCV, may also develop secondary to folate deficiency. ▪ Generally extravascular haemolysis is associated with spherocytosis on the peripheral blood film. ***Intravascular haemolysis*** is characterized by red cell fragmentation (***schistocytes***).

[1] the body can't response to broken RBCs.
[2] in the tissues , and There is no hemoglobinuria compared to Intravascular hemolysis.
[3] hematuria = normal RBCs in the urine ,, hemoglobinuria = lysis RBCs in urine.

Laboratory Evidence of Hemolysis con..

- Increased **reticulocyte** count [1] . The number of reticulocytes in the blood is expressed either as a **percentage** of the total number of red cells or as an **absolute number** per liter of blood; in normal adults, the percentage is in the range of 0.5-3.0% and the absolute count is 20-100x109/L. Increase in the absolute reticulocyte count is an indication of **increased erythropoietic activity.**
- If examination of the bone marrow is undertaken, there will be evidence of increased erythropoiesis. Marrows showing **erythroid hyperplasia**[2] are also hypercellular, due to the replacement of fat cells by erythroid precursors. (Figure).

This table shows the difference between intra and extra (**important**)

Extravascular	Intravascular
• Spherocyte	• Reduced or absent serum haptoglobin. • **Haemoglobinuria, haemoglobinaemia** , hemosiderinuria . • Methaemalbuminaemia • Red cell fragments (schistocyte).
• Hyperbilirubinemia (unconjugated). • Increase serum LDH (marked in intra). • Reticulocytosis .	

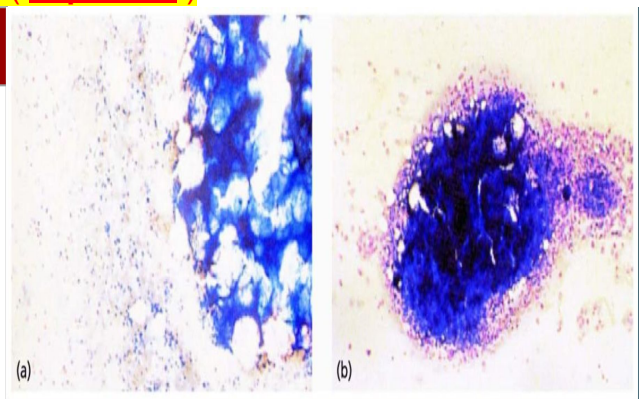


Figure 3.1 (a) A normocellular marrow fragment: about half its volume consists of haemopoietic cells (staining blue) and the remainder of unstained rounded fat cells. (b) A markedly hypercellular marrow fragment, as might be seen in the response to haemolysis: virtually all the fat cells are replaced by haemopoietic cells.

Clinical Features of Hemolysis

- **Pallor**, and **jaundice** secondary to the elevated **bilirubin** levels.
- **Splenomegaly** may be seen.
- Long-term complications of chronic hemolysis; expansion of erythropoiesis in the marrow cavities, thinning of cortical bone, **bone deformities** (e.g. frontal and parietal bossing) and, very occasionally, pathological fractures.
- **Pigment gallstones** are seen commonly.
- Risk of episodes of **pure red cell aplasia**[3] especially with **parvovirus B19.**[4]

Classification of Hemolytic Anemias

Classified simply as either **I] congenital** or **II] acquired**.

- With the congenital causes, the underlying defect is typically **intrinsic** to the red cell itself, affecting the red cell's:
  A) membrane, B) its enzymes or**, C) its hemoglobin.**
- Acquired causes, are typically due to defects **extrinsic**, outside the red cell,(**except PNH**) and can be divided into those with an
  - **immune** basis.
  - **without**.

[1] normally there is very low amount of reticulocyte in circulation , in hemolytic Anemia will increase to compensate .
[2] خلايا الدم تتكسر و يصير فيه طلب لنخاع العظم انها تنتج خلايا اكثر فيكون الناتج اوفر بودكشن اللي هو erythroid hyperplasia
[3] the bone marrow ceases to produce red blood cells.
[4] in immune deficiency.

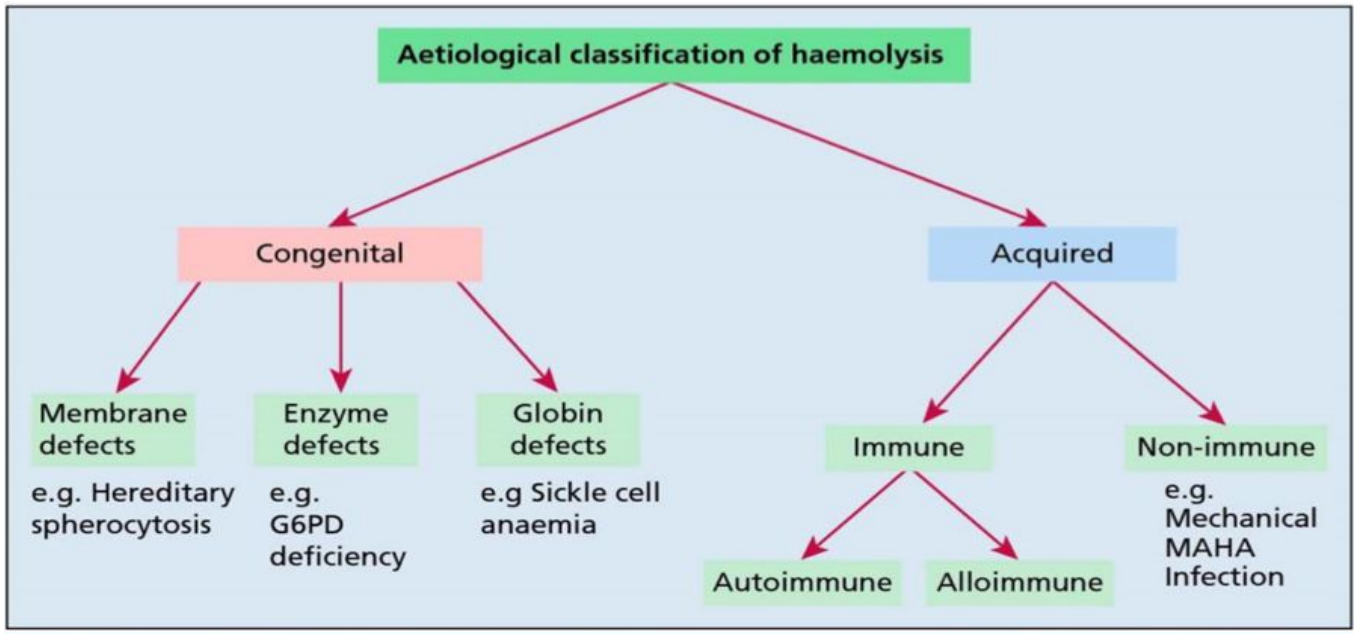


Figure 3.2 A classification of haemolytic anaemia by aetiology. Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; MAHA, microangiopathic haemolytic anaemia.

Congenital Hemolytic Anemias

- The red cells undergo significant deformations while traversing the circulation. Thus, **flexible red cell cytoskeleton** is essential.[1]
- Key components of the cytoskeleton include **α and β spectrins**, **actin** and **protein 4.1**, while connections linking the cytoskeleton to the overlying red cell phospholipid bilayer include **band 3**, Rh-associated glycoprotein and glycophorin C. .
- Defects in any of these proteins can jeopardize the integrity of the red cell and shorten its lifespan.

A) Red Cell Membrane "Membranopathy":

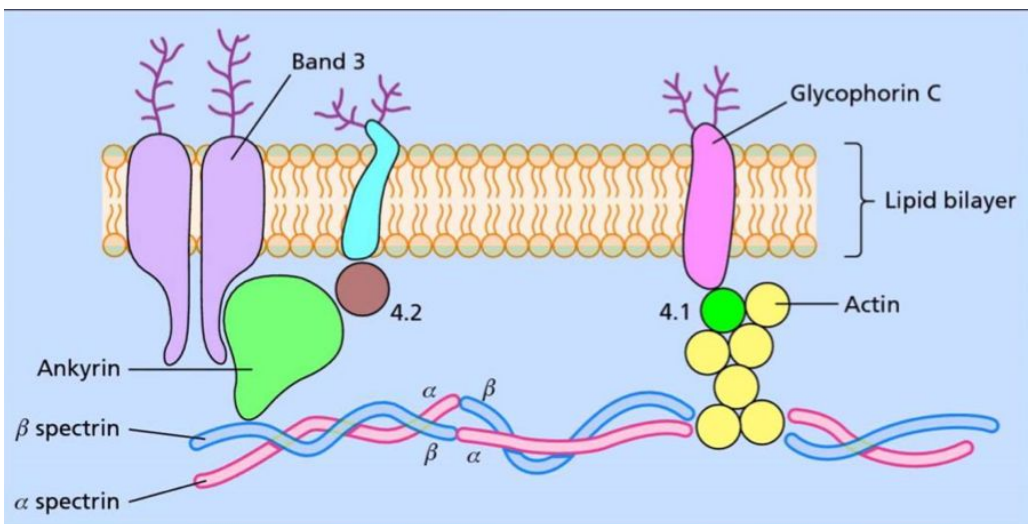
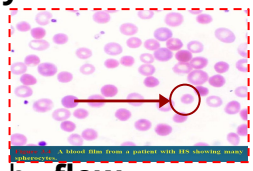
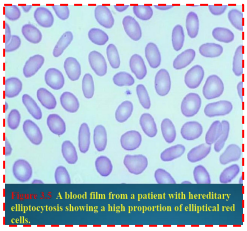


Figure 3.3 Schematic diagram of the red cell membrane cytoskeleton.

[1] if there is a defect in cell membrane , this will lead to decrease the flexibility.

Membranopathy

Hereditary spherocytosis (HS)	**Characters**	• The **most common** membranopathy is hereditary spherocytosis (HS), with ~ 60% related to **Ankyrin gene**. Loss of Ankyrin then leads to secondary reductions in **spectrin and protein 4.1** leading to a **spheroid shape, vertical.** • Destroyed by **splenic** macrophages, **extravascular hemolysis**, with 20% of all HS have mild disease. The majority of patients have **moderate disease** characterized by a **Hb concentration of 8-11g/dl**, while a **small percentage have severe disease** requiring intermittent or even regular transfusions. • Complications of the chronic hemolysis in HS include the development of **pigment gallstones**. **Aplastic crises** may occur secondary to **parvovirus B19. Megaloblastic anemia** is occasionally found**.**
	Diagnosis	• **Family history**[1] , mild jaundice, pallor and splenomegaly. • Laboratory findings (anemia, **reticulocytosis** and elevated plasma bilirubin). • Presence of **spherocytes** on the peripheral blood film • Special Tests: The eosin-5-maleamide (**EMA**) binding test (definitive evidence) by **flow cytometry**. The red cell membrane proteins' genes, by **molecular testing**. **Protein electrophoresis** on a denaturing polyacrylamide gel. 
	Treatment	• **Folic acid** supplementation. • **Splenectomy** (children with severe disease), which increases the risk of significant infection, encapsulated organisms. • This risk is especially marked in children under the age of 5. • Administration of pneumococcal and meningococcal vaccine and Haemophilus influenzae type b vaccine (splenectomy preoperative preparation). • Prophylactic penicillin V is advised lifelong (post splenectomy).
Hereditary Elliptocytosis (HE)		• a relatively common condition, with many cases showing defects in **α spectrin**, **horizontal** interaction. • Most patients are clinically asymptomatic, some will have a chronic symptomatic hemolytic anemia. • All show the very characteristic **elongated red cell shape** on peripheral blood films. 
Hereditary Pyropoikilocytosis (HPP)		Severe disturbance of the multimerization of spectrin with a severe hemolytic anemia from infancy and a bizarre peripheral blood morphology, including microspherocytes and poikilocytes. Such patients are described as having **hereditary pyropoikilocytosis.**

[1] family history in hematological disorders is very important.

Congenital Hemolytic Anemias Cont..

Enzymopathy

- Hemolytic anemias may also result from congenital abnormalities of the **enzymes required for energy** transfer in glucose **metabolism**.
- The red cell needs a continuous supply of energy for the maintenance of membrane flexibility and cell shape, the regulation of sodium and potassium pumps, and the maintenance of Hb in the reduced ferrous form which protects from an **oxidative stress**.
- **Pyruvate kinase deficiency** is another relatively common example.
- There is usually a **chronic hemolytic anemia** and some patients may benefit from **splenectomy**.

G6PD

- Deficiency of **glucose-6-phosphate dehydrogenase (G6PD)**, the first enzyme of the **hexose monophosphate/pentose-phosphate shunt**, will prevent the normal generation of **NADPH**, with subsequent erythrocyte sensitivity to oxidative stress. Various mutations in the G6PD gene on **the X chromosome** results in this disorders with **a male predominance**.
- When the red cell is exposed to **oxidants, e.g. some medications**, Hb is converted to methemoglobin and denatured. Denatured Hb then precipitates forming inclusions in the red cell (termed **Heinz bodies** and detected by **supravital staining**, as in Figure). Splenic macrophages, extra, remove Heinz bodies; the resulting inclusion-free cells display unstained areas at their periphery (‘**bite’ cells** [or basket cells], seen in Figure next slide). **Screening tests and assays** for detecting G6PD deficiency are available.
- Hemolysis begins 1-3 days post exposure to the oxidative stressor, with anemia being maximal about 7-10 days after exposure. Patient may report dark urine due to **hemoglobinuria**, **intravascular**.
- ***Favism:*** a syndrome in which an acute hemolytic anemia occurs after the ingestion of the broad **bean (*Vicia fava*)** in individuals with a deficiency of G6PD (commonly of the **Mediterranean type**), usually affects children; severe anemia develops rapidly and is often accompanied by hemoglobinuria.
- Treatment generally focuses on the **avoidance of oxidative precipitants** to hemolysis. In many cases, hemolysis is **self limiting**. In children, **rehydration** is needed to avoid acute kidney injury. Packed **red cell transfusion** may be required in cases of severe hemolysis.

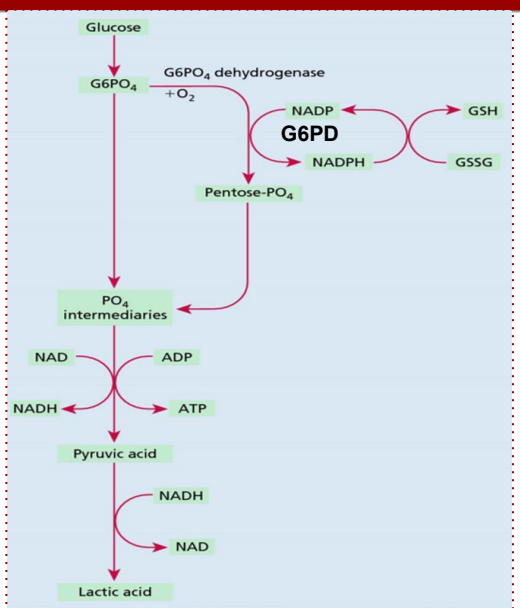


Figure 3.6 A schematic diagram of the pathway of glucose metabolism in the red cell, to show the important role of G6PD. A decreased activity of the enzyme leads to a deficiency of the reducing compounds NADPH and GSH.

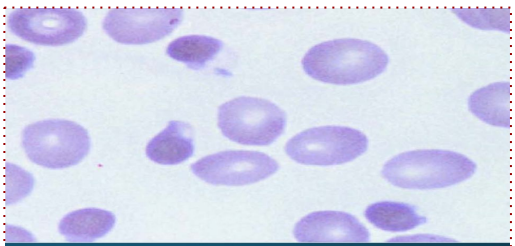


Figure 3.8 ‘Bite’ cells in the blood film of a patient with G6PD deficiency who had received primaquine. These red cells are irregular in shape, are abnormally dense and show a poorly staining area just beneath part of the cell membrane (MGG stain).

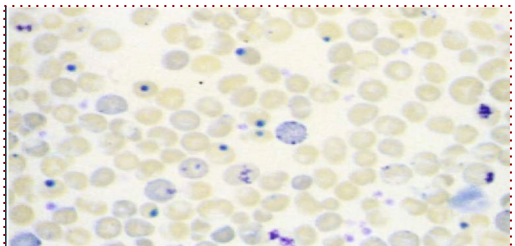


Figure 3.7 Membrane-bound Heinz bodies consisting of denatured haemoglobin (supravital staining with methyl violet).

Congenital Hemolytic Anemias Cont..

Hemoglobinopathy	• Defects in the structure of Hb. Structural variants of the globin chains may affect the lifespan of the red cell, with sickle cell anemia being the best-described example. • A tendency of the HbS variant to polymerize under conditions of low oxygen tension leads to distortion of the erythrocyte in the well-recognized sickle shape.

Acquired Hemolytic Anemias

In the acquired hemolytic anemias, red cells may be destroyed either by **immunological** or by **non-immunological** mechanisms.

A) Immunological Causes

- Antigens on the surface of red cells react with antibodies and might complement activation.
- IgG-coated red cells interact with the Fc receptors on macrophages in the spleen, and are then either completely or partially phagocytosed (extra). When the phagocytosis is partial, the damaged cell will return to the circulation as a **spherocyte**. Sometimes, membrane attack complex (C5-C9), complement, leading to **intravascular hemolysis.**
- The immune hemolytic anemias may be due to **autoantibodies**; that is, antibodies formed against one or more antigenic constituents of the individual's own tissues. These include **autoimmune hemolytic anemia (AIHA)** and some drug-related hemolytic anemias.
- It is also possible to develop **alloimmune hemolytic anemia**, consequent on the production of antibodies against red cells from another individual, as in hemolytic transfusion reactions and hemolytic disease of the newborn.

Autoimmune Hemolytic Anemias

- **'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, are capable of agglutinating red cells.

- autoimmune = body attack itself.
- Alloimmune = antibody from outside the body , like in case of blood transfusion

Warm AIHA	Cold Hemagglutinin Disease (CHAD)
• In **idiopathic warm AIHA**, hemolysis dominates the clinical picture and no evidence can be found of any other disease. In **secondary AIHA**, the hemolysis linked with a primary disease like; chronic lymphocytic leukemia (CLL)[1] 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. There may be partial activation of the complement cascade. • Findings like;anemia, **spherocytosis**, reticulocytosis and rare nucleated red cells in the peripheral blood. The critical diagnostic investigation is the direct antiglobulin test (DAT). • Hemolysis can be limited by treatment with **prednisolone**. If reduction in hemolysis 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**.	• Cold antibodies bind to the red cell surface in the cooler superficial blood vessels of the peripheries. IgM subtype, **pentameric** structure, permits direct agglutination of red cells coated with antibody; they are therefore sometimes termed **cold agglutinins**. • Symptoms due to cold AIHA are worse during cold weather. Exposure to cold provokes acrocyanosis. The direct activation of the complement system leads to red cells lysis and, consequently, to hemoglobinemia and hemoglobinuria (intra). • Chronic idiopathic CHAD is managed initially simply by **keeping the patient warm**. Treatment with **rituximab** may be effective.

Other causes of hemolytic anemia with an immune element include:

1) Paroxysmal nocturnal hemoglobinuria (PNH);

2) Paroxysmal cold hemoglobinuria;

3) Drug-related hemolytic anemias.

Table 3.3 Causes of acquired non-immune haemolytic anaemias.

Mechanical trauma to red cells

Abnormalities in the heart and large blood vessels

Aortic valve prostheses (Figure 3.11), severe aortic valve disease

Microangiopathic haemolytic anaemia

Haemolytic uraemic syndrome, thrombotic thrombocytopenic purpura, metastatic malignancy, malignant hypertension, disseminated intravascular coagulation

March haemoglobinuria

Burns

Infections

Clostridium perfringes (welchii), malaria (Figures 3.12 and 3.13), bartonellosis

Drugs, *chemicals and venoms

Oxidant drugs and chemicals, arsine, acute lead poisoning, copper toxicity, venoms of certain spiders and snakes

Hypersplenism

***Note:* *Some drugs cause haemolysis by immune mechanisms.**

Table 3.2 Classification of AIHAs.

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

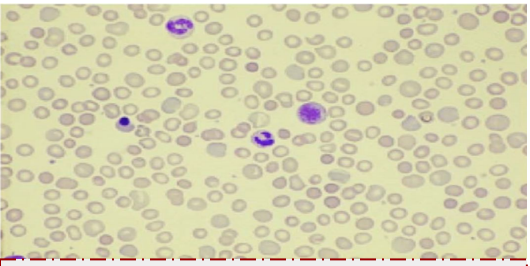
Secondary (*Mycoplasma pneumoniae* infection, infectious mononucleosis, lymphomas)

Paroxysmal cold haemoglobinuria

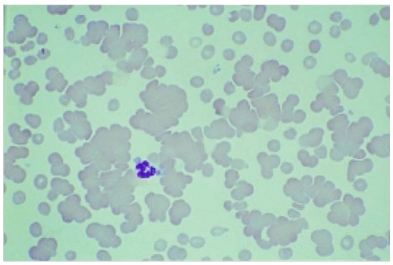
Idiopathic

Secondary (some viral infections, congenital and tertiary syphilis)

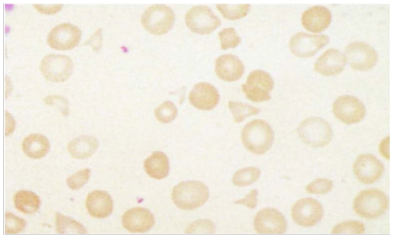
[1] the most common cause (important)



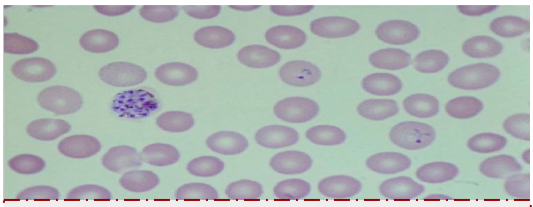
Blood film from a patient with idiopathic AICH (warm-reactive antibody)showing prominent spherocytosis and polychromasia



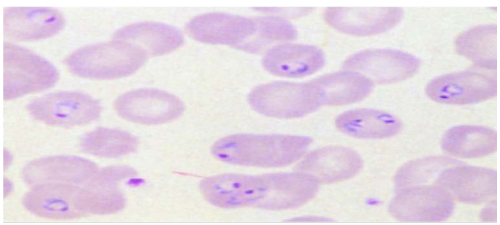
Numerous red cells agglutinate on a blood film from a patient with idiopathic CHADA



Fragmented red cell (spherocytes) in blood film from patient with a malfunctioning aortic valve prosthesis



Blood film from patient with a plasmodium vivax malaria showing two parasitized blood cells, each containing a single parasite (ring or early trophozoite and a amoeboid late trophozoite). Another red cell contain a schizont. Some of the parasite cells are slightly enlarged.



Blood film from patient with a plasmodium falciparum showing several parasitized red cell. Red cells heavily parasitized with malaria may be subject to intravascular lysis.

Mechanical Damage to Red Cells

- Several of the mechanical causes of acquired non-immune hemolytic anemia are summarized in Table. Red cells are mechanically damaged when they impact upon abnormal surfaces.
- 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 (schistocytes) in the blood film.

Some drugs

While immune mechanisms of drug-induced haemolysis are well described, there are also non-immune mechanisms by which the red cell lifespan may be shortened. Chemicals, such as benzene, toluene and saponin, which are fat solvents, act on the red cell membrane directly and disrupt its lipid components, inducing hemolysis.

Hypersplenism

- Hypersplenism results in 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 and might be treated with a splenectomy.

Doctors notes:

- Hyperbilirubinemia is common with hemolysis (might present as jaundice.
- Bleeding: blood comes out as a whole, Hemorrhage: confined to a one place, hemolysis is shortening of RBC life
- Extravascular hemolysis: works on reticuloendothelial system.
- There is no hemoglobinuria compared to Intravascular hemolysis
- Most disease are a mix between Intra and Extravascular hemolysis (the difference is in predominance)
- Lactate dehydrogenase increase in due to mechanical or complement activation (C5-9) which causes morphong in RBC
- Haptoglobin is responsible for cleansing the circulation from HB
- Hemoglobinuria is presence of RBC in urine, while hematuria is presence of blood in urine
- Increased reticulocyte is usually seen in both intra and extravascular
- Hemocellulo-urea is the presence of Iron precipitation in urine and it is seen in intravascular hemolysis
- Bluish discoloration of polychromasia is due to RNA precipitation
- Schistocytes are also seen in severe thrombocytopenia
- Erythroid hyperplasia is seen in both
- Pigment gallstones are seen in extravascular hemolysis most commonly
- PNH (paroxysmal nocturnal hemoglobinuria) : is a case of a bone marrow defect which prevents complement activation on cell due to anchor proteins
- Membranopathy is usually seen due to decrease in flexibility of red cell which damages it, resulting in the spleen recognizing it as an old cell and destroys it (so it is mostly extracellular)
- Hereditary spherocytosis usually causes mild to moderate anemia
- Spherocytosis is characterized by rigidity of vertical proteins
- The EMA binding test is linked to ankyrin
- Gold standard for diagnosis of hereditary spherocytosis is gene analysis. However if gene is not a known pathogenic gene we have to do a protein assay (protein electrophoresis)
- Splenectomy is indicated for hereditary spherocytosis if patient has ongoing hemolysis and jaundice while not responding to classical therapy. We need to give penicillins and vaccines before.
- Hereditary elliptocytosis is caused by a defect in the horizontal genes and is usually milder than hereditary spherocytosis. However hereditary pyropoikilocytosis is the advanced form of the disease which has a very severe presentation and bizzare looking RBC (it is a defect in the same gene as elliptocytosis)
- IDA usually looks cigar shaped (difference between it and elliptocytosis)
- Most common cause of enzymopathy is G6PD deficiency

CONT'D

- G6PD is part of the hexose monophosphate/pentose-phosphate shunt which is responsible for transforming the oxidized form of glutathione to the reduced form
- Heinz bodies are a result of precipitation of Hemoglobin
- G6PD is X linked therefore it is seen is males more than females
- Bite cells are the cardinal signs of G6PD deficiency
- If G6PD deficiency causes slow shortening of RBC (chronic form), it is usually extravascular if it is caused by some sort of stress (acute form) it is intravascular
- If G6PD deficient patients don't have abnormal vital signs then there is no need for transfusion
- Presentation in emergency is usually acute (Intravascular)
- Most common hemoglobinopathy are thalassemia and sickle cell anemia
- All acquired causes of hemolytic anemia are extrinsic except PNH
- Spherocytosis in immunological anemias are seen due to partial phagocytosis
- To differentiate between spherocytosis seen in Hereditary spherocytosis and immunological hemolytic anemia, we check family history and perform DAT (Immunological hemolytic anemias are usually seen with a coated red cell)
- Autoantibodies type of hemolytic anemia is usually seen due to antigenic mimicry.
- Warm type of autoimmune hemolytic anemia are usually extravascular while the cold type is an intracellular type
- PNH is IGg mediated yet it is a cold type of autoimmune hemolytic anemia because it is usually caused by formation of antibodies during the cold whereas activation happens in warm weathers.
- Most common cause of schistocytes are Microangiopathic hemolytic anemias.
- One of the causes of non immune hemolytic anemias is Malaria (intravascular)
- Hypersplenism is seen in extravascular anemia

Doctors Q

Q1) Which ONE of the following is TRUE about glucose-6- phosphate dehydrogenase (G6PD) deficiency?

A- it is not a cause of neonatal jaundice.
B- it protects against malaria.
C- it commonly presents as a chronic hemolytic Anemia.
D- carrier females have approximately 10% G6PD levels.

Q2) Spherocytosis in the blood film is a feature of which ONE of the following?

A- thalassemia major.
B- Reticulocytosis .
C- G6PD.
D- Autoimmune hemolytic Anemia.

Q3) Which ONE of the following is an only cause of intravascular hemolysis?

A- G6PD .
B- Rhesus incompatibility.
C- red cell fragmentation syndrome.
D- hereditary spherocytosis.

Q4) Which ONE of these statements is TRUE regarding hereditary spherocytosis?

A- it is caused by an inherited defect in Hb.
B- it can be treated by splenectomy.
C- it is more common in males .
D- it is more frequent in Southern Europe.

Q5) Which ONE of the following is TRUE about autoimmune hemolytic anemia?

A- it is associated with pernicious Anemia.
B- hemolytic Anemia in minimal.
C- it may complicate B-cell chronic lymphocytic leukemia.
D- it is associated with a positive indirect antiglobulin test.

1) C
2) D
3) A
4) B
5) C