MED439
KING SAUD UNIVERSITY

Approach to Hemolysis

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.

Dr's notes
Important
Extra notes
** Only in girls slide
** Only in boys slide

Revised & Approved
Bassam Alasmari
Rania Almutiri

Hematology Team

Editing file

Introduction to Hemolysis

Pathoma
(Skip to 6:25)

Hemolysis

Explained by a **student** here

- Hemolysis is a state with a short lifespan of a mature red blood cell, in other words it's the rupture or destruction of red blood cells.

Not marked (mild) and can be compensated	Marked and CAN NOT be compensated
1. Reduction in RBC lifespan 2. Increased red cell output from the marrow 3. Stimulated by erythropoietin 4. Will be **sufficient** (no hemolytic anemia produced)	1. More marked reductions in red cell lifespan 2. 5-10 days from usual 120 days. 3. Will result in **hemolytic anemia.** (topic of the lecture)

- Site of hemolysis? Which one is a site of **normal and pathological** hemolysis?

Extravascular hemolysis	Intravascular hemolysis
Excessive removal of RBCs by macrophages of the Reticuloendothelial system (liver, spleen and bone marrow) from the circulation by phagocytosis. It means that hemolysis occurs in the reticuloendothelial system, which occurs in normal and pathological hemolysis. ****Majority of hemolytic anemia**	Red blood cells are caused to rupture and release their hemoglobin (Hb) directly into circulation. It means that hemolysis is taking place within vessels. Never in normal physiology, always pathological.

Why is it important to know the site of hemolysis when studying hemolytic anemias? The extra/intravascular site of red cell destruction may give clues to the **underlying etiology** of the hemolysis.

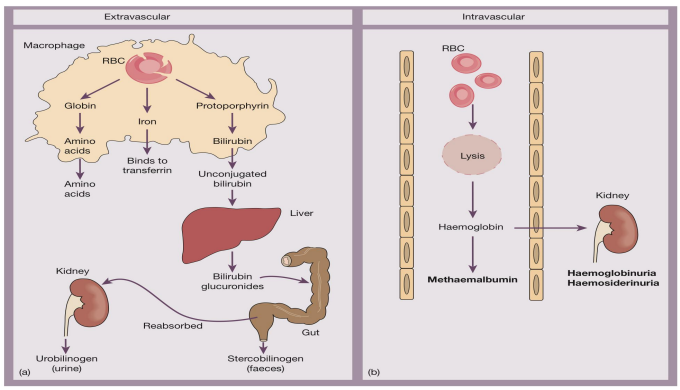


Figure 6.1 **(a)** Normal red blood cell (RBC) breakdown. This takes place extravascularly in the macrophages of the reticuloendothelial system. **(b)** Intravascular haemolysis occurs in some pathological disorders.

Hemolytic Anemias

- **Hemolytic anemia:** It is a shorten in the lifespan of an RBC that cannot be overcome by ability of bone marrow production (marked hemolysis that **cannot be compensated**).
- Many diseases can be classified as hemolytic anemias which will be discussed in detail in this lecture.

★ Laboratory evidence of hemolysis

Explained by a **student** here

General findings seen in both extravascular and intravascular hemolysis

Hyperbilirubinemia: A rise in **unconjugated bilirubin** concentration in plasma.

↑ **Serum lactate dehydrogenase (LDH),** an enzyme present in red cells, marked in intravascular hemolysis.

Macrocytosis (high MCV) may also develop secondary to folate deficiency. How? hemolysis will increase the bone marrow demand for folic acid (to create rbc's for compensation) leading to deficiency.

Increased reticulocyte count.
An increase in the absolute reticulocyte count is an indication of increased erythropoietic activity.
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%** and the absolute count is
20 - 100 X 10^9/l

Polychromasia, a bluish discoloration of RBC due to reticulocytosis

If examination of the bone marrow is undertaken, there will be evidence of **increased erythropoiesis**. Marrow shows **erythroid hyperplasia** are also hypercellular, due to the replacement of fat cells by erythroid precursors.

Figure A.

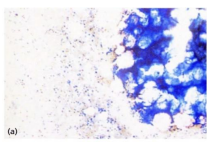


Figure A. A normocellular marrow fragment: about half its volume consists of hematopoietic cells (stained in blue) and the remainder of instained rounded fat cells.

Figure B.

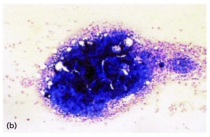


Figure B. A markedly hypercellular marrow fragment as might be seen in the response to hemolysis: virtually all the fat cells are replaced by hematopoietic cells.

Hemolytic Anemias

★ Laboratory evidence of hemolysis

Female Dr: no need to identify each disease as Extra or Intra, just focus on this table and the characteristic features

Male dr: Most important markers to differentiate are **absence of haptoglobin and hemoglobinuria**

Seen in intravascular hemolysis:

↓ **Serum haptoglobin**, molecule binds free Hb, intravascular hemolysis.

★ **Schistocytes: Red cell fragments**

Methaemalbuminaemia: when free heme can bind to albumin to form methemalbumin. (Now rarely used in investigating a patient).

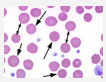
Free Hb in the urine: **hemoglobinuria** (note the difference from hematuria, which describes the presence of intact red cells in the urine).

Hemosiderinuria (note the difference from hematuria, which describes the presence of intact red cells in the urine).

Hemoglobinemia (excess Hb in the circulation)

Seen in extravascular hemolysis:

★ **Spherocytosis** on the peripheral blood film (spherocyte).



Splenomegaly: The most common finding in extravascular hemolysis

★ Comparison between findings:

Extravascular hemolysis	Intravascular hemolysis
1. Hyperbilirubinemia (unconjugated) 2. Increased serum lactate dehydrogenase (LDH) 3. Reticulocytosis **4. Spherocytosis**	1. Hyperbilirubinemia (unconjugated) 2. **Markedly** increased serum LDH 3. Reticulocytosis 4. Red cell fragmentation (**schistocytosis**) 5. Reduced or absent serum haptoglobin **6. Haemoglobinaemia, haemoglobinuria and hemosiderinuria** 7. Methaemalbuminaemia

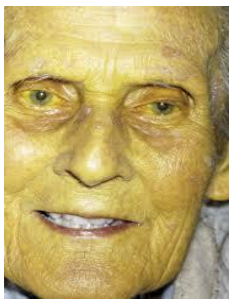
Hemolytic Anemias

Clinical features of hemolysis

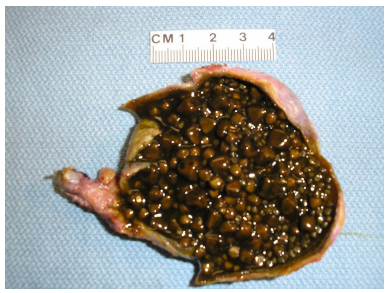
Explained by a **student** here

1. **Pallor** (sign of anemia) and **jaundice** (sign of hemolysis) secondary to the elevated bilirubin levels
2. **Splenomegaly** might be seen (sign of hemolysis)
3. 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 (Signs of increased hematopoiesis)
4. **Pigment gallstones** are seen commonly **(sign of chronic hemolysis)**
5. Risk of episodes of **pure red cell aplasia (PRCA)** especially with **parvovirus B19**

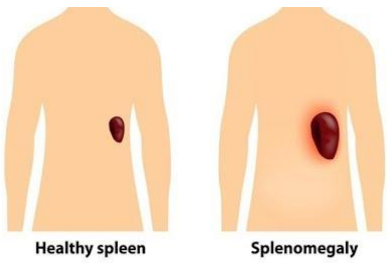
Pallor and Jaundice



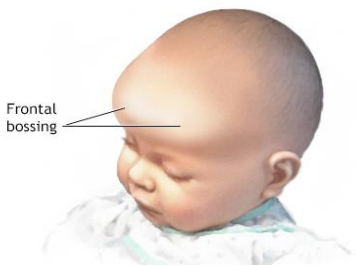
Pigment Gallstones



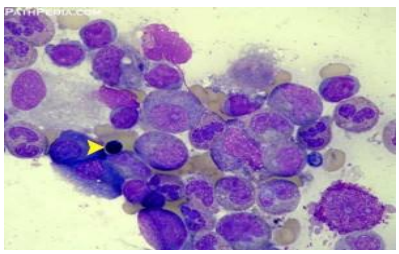
Splenomegaly



Frontal Bossing (Bone deformity)



Pure Red Cell Aplasia



Hemolytic Anemias

Hemolytic anemias are defined as anemias characterized by an excessive breakdown of red blood cells (RBCs). They can be classified according to the cause of hemolysis (intrinsic or extrinsic) and by the location of hemolysis (intravascular or extravascular).

By RBC Pathology

Type	Definition
Intrinsic hemolytic anemia	Increased destruction of RBCs **due to a defect within the RBC**
Extrinsic hemolytic anemia	**Abnormal breakdown** of normal RBCs

By location of RBC Breakdown

Type	Definition
Intravascular hemolytic anemia	Increased destruction of RBCs **within the blood vessels**
Extravascular hemolytic anemia	Increased destruction of RBCs **by the reticuloendothelial system** (primarily the spleen)

Based on presence at birth

Type	Definition
Congenital hemolytic anemia	Hereditary conditions including defects of erythrocyte membrane proteins, red cell enzymes and globin chains
Acquired hemolytic anemia	Normal RBC's. However, some other disease or factor causes the body to destroy red blood cells and remove them from the bloodstream.

Hemolytic Anaemias

Definition

- It is a shorten in the lifespan of an RBC that **cannot be overcome** by ability of bone marrow production

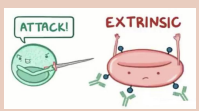
Classification

- Hemolytic anemias can be classified simply either **congenital or acquired**
- The underlying defect of the **congenital** causes is typically **intrinsic** to the red cell itself, **affecting the red cell's membrane, its enzymes, or its hemoglobin.**
- The underlying defect of **acquired** causes are due to defects **extrinsic** or outside the RBCs (except PNH= Paroxysmal nocturnal hemoglobinuria)and can be divided into those with an immune basis and those without.

Note: The details of each condition will be explained in this lecture

Male dr: the two tables here are very important you need to memorize it
الدكتور عاد ذا الجملة مرتين*

	Congenital Hemolytic Anemia (**Intrinsic** cause) 		
Etiology	Membrane defects **(Membranopathies)**	Enzyme defects **(Enzymopathies)**	Globin defects
Conditions	• Hereditary spherocytosis **(HS)** • Hereditary Elliptocytosis **(HE)** • Hereditary Pyropoikilocytosis **(HPP)**	• G6PD def. • Pyruvate kinase def.	• Sickle cell anemia

	Acquired Hemolytic Anemia (Extrinsic cause mostly)  		
Etiology	Immune		Non-immune
	Autoimmune	Alloimmune	
Conditions	• Warm autoimmune hemolytic anemia **(WAHA)** • Cold Hemagglutinin Disease **(CHAD)**	• Hemolytic transfusions reactions • Hemolytic disease of the newborn	• Microangiopathic haemolytic anemia **(MAHA)**

Membranopathies
(Congenital Hemolytic Anemias)

Overview of Membranopathies

Explained by a **student** here

- The red cells undergo significant deformations while traversing the circulation. Thus, flexible red cell cytoskeleton is essential.
- RBCs membranes are made of **Cytoskeleton** attached to **Bilayer phospholipid**
- Key components of The RBCs cytoskeleton is formed by structural proteins that include:
  - **α Specretin**
  - **β Spectrin**
  - **Protein 4.1**
  - **Actin**
  - **Ankyrin**
- While connections linking the cytoskeleton to the overlying red cell phospholipid bilayer include:
  - **Band 3**
  - Glycophorin C
  - Rh-associated glycoprotein
- The cytoskeleton proteins form **(horizontal connections)** on the internal side of the red cell membrane and are important in maintaining the **biconcave shape**.
- At the head end, of the cytoskeleton, the β spectrin chains attach to ankyrin which connects to band 3, the transmembrane protein that acts as an anion channel forming **(vertical connections)**.
- Defects in any of these proteins can jeopardize the **integrity of the red cells and shorten its lifespan**
  - Defects in proteins of the **vertical interactions**→ Hereditary spherocytosis **(HS)**
  - Defects in proteins of the **horizontal interactions**→ Hereditary Pyropoikilocytosis **(HPP)**
  (both discussed in the next slides)

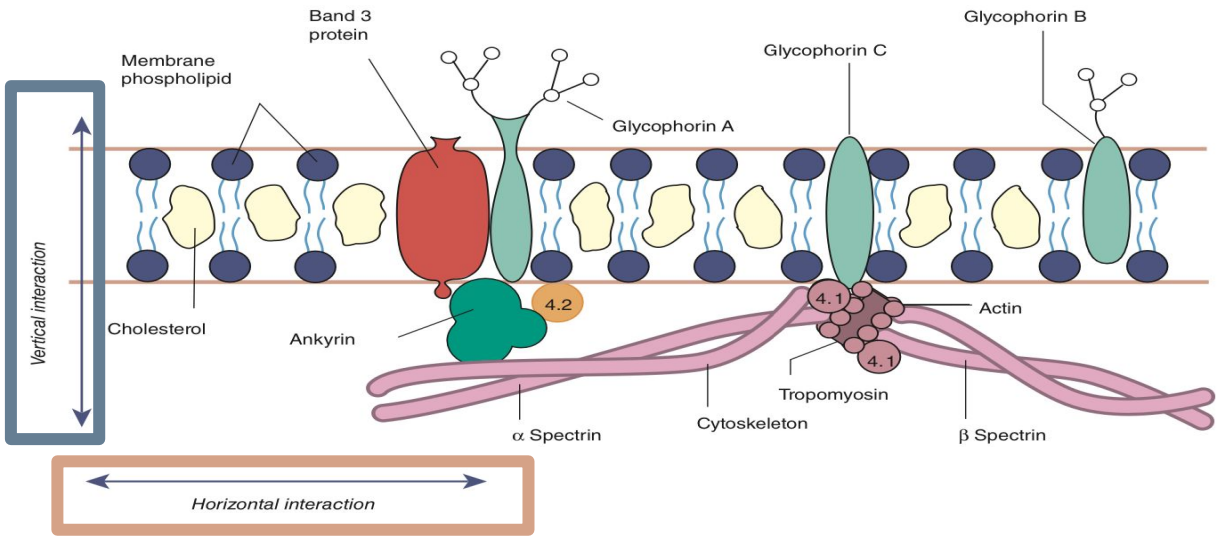


Figure 2.12 The structure of the red cell membrane. Some of the penetrating and integral proteins carry carbohydrate antigens; other antigens are attached directly to the lipid layer.

Membranopathies
(Congenital Hemolytic Anemias)

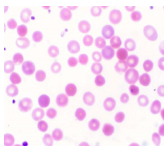
Pathoma

Hereditary Spherocytosis

Explained by a **student** here

- it is an autosomal dominant disease caused by mutations of proteins that attach RBC cytoskeleton to RBC membrane leading to loss of the membrane, RBC becomes more spherical **(spherocyte)**
- HS is the most common membranopathy **with 60%** related to Ankyrin protein gene. Loss of Ankyrin gene leads to secondary reductions in spectrin and protein 4.1 leading to a **spheroid shape**, (vertical interaction, explained in the previous slide)
- RBCs are destroyed by splenic macrophages **(extravascular hemolysis)**

Presentation	• 20% of all HS patients have **mild disease** • The majority of patients have **moderate disease** characterized by a Hb concentration of 8-11 g/dl • A small percentage have **severe disease** requiring intermittent or even regular transfusions.
Complications	• Pigment gallstones. Increased hemolysis of red blood cells leads to increased bilirubin levels, which may cause the formation of a pigmented gallstones and jaundice • Megaloblastic anemia occasionally found Folate and vitamin B12 deficiency may develop due to chronic hemolysis leading to megaloblastic anemia • Aplastic crisis may occur **secondary to** parvovirus B19. **How?** The main target cells of parvovirus B19 are erythroid precursor cells in bone marrow leading to further destruction of RBCs and Aplastic crisis which is when the body fails to produce blood cells in sufficient numbers

Diagnosis
 	
Female dr: Family history and morphology are enough
	• Laboratory findings (anemia, reticulocytosis and elevated plasma bilirubin).
	• **Family history,** mild jaundice, pallor and **splenomegaly, How?** The spleen is a filtering organ in the body and the main site of destruction for the abnormal red cells in HS, spherocytes have a hard time passing through due to their irregular shape causing back up in the spleen and eventually splenomegaly
	• Special tests:
	  ○ Eosin-5-maleamide (EMA) binding test **(definitive evidence, test of choice)** using flow cytometry, shows decreased binding between dye (eosin-5-maleimide) and RBC membrane proteins, which shows decreased RBCs fluorescence. Binding is quantified
	  ○ Molecular testing of red cell membrane proteins gene
	  ○ Protein electrophoresis on a denaturing polyacrylamide gel to check for RBC abnormal proteins.
	• Presence of **spherocytes** on peripheral blood film. 

Membranopathies
(Congenital Hemolytic Anemias)

Hereditary Spherocytosis contd..

Treatment

- Folic acid supplementation to maintain erythropoiesis
- Splenectomy (children with severe disease).

  The absence of the spleen causes increased risk of significant infection by encapsulated organisms (especially in children under the age of 5), So patients with asplenia require:
  - Splenectomy **preoperative** preparation: administration of pneumococcal, meningococcal and haemophilus influenzae type b vaccine
  - **Post** splenectomy: Prophylactic penicillin V is advised lifelong

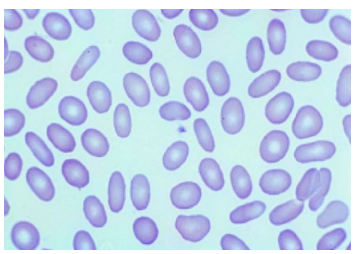
Why splenectomy? Having spherocytes isn't the problem, spleen eating them is, so a Splenectomy removes the primary "graveyard" for spherocytes eliminating anemia and hyperbilirubinemia and lowers the high reticulocyte number to nearly normal levels.

Hereditary Elliptocytosis

Explained by a **student** here

- Relatively common condition, with many cases showing defects in α spectrin protein, (**horizontal interaction)**
- Most patients are clinically asymptomatic, some will have a chronic symptomatic hemolytic anemia.
- Has similar clinical and laboratory features to HS except for the appearance of the blood film showing very characteristic elongated (elliptical) red cell shape on peripheral blood film

Elliptical RBC's



Hereditary Pyropoikilocytosis

- Described as disturbance of multimerization of spectrin with severe hemolytic anemia from infancy and a bizarre peripheral blood morphology, including **microspherocytes and poikilocytes** (Abnormally shaped blood cells)
- More severe than hereditary elliptocytosis

Male dr: Here the gene defect of **α spectrin** protein is in the form of **homozygous**, in hereditary elliptocytosis its in the **heterozygous** form .

Enzymopathies
(Congenital Hemolytic Anemias)

Overview of Enzymopathies

Explained by a **student** here

- 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
  - Maintenance of membrane flexibility and cell shape
  - Regulation of sodium and potassium pumps
  - **Maintenance of Hb in the reduced ferrous form which protects from an oxidative stress**

G6PD Deficiency

Explained by a **student** here

Pathoma
(Skip to 7:18)

Refer to biochemistry

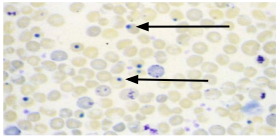
Definition

Glucose-6-phosphate dehydrogenase (G6PD) deficiency results from various mutations in the G6PD gene on the **X chromosome** (recessive) with a **male predominance.** **G6PD deficiency protects against malaria, an infection by plasmodium falciparum.**

Pathogenesis

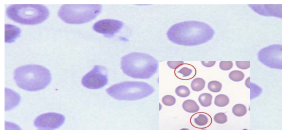
- **Deficiency of G6PD**, the first enzyme of the hexose monophosphate/pentose-phosphate shunt, **will prevent the normal generation of NADPH, with subsequent erythrocyte sensitivity to oxidative stress.**
- When the red cell is exposed to oxidants (for example some medications), Hb is converted to methemoglobin and denatured.
- Denatured Hb then **precipitates** forming inclusions in the red cell called **Heinz bodies** (detected by supravital staining)
- Splenic macrophages (extravascular) **remove Heinz bodies** (this is not hemolysis!); the resulting inclusion-free cells display unstained areas at their periphery **(Bite cells,** caused by removal of Heinz bodies from RBC**)**
- Bite cells lead to **predominantly intravascular hemolysis** (during acute insult) which 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, a finding of intravascular hemolysis. Extravascular hemolysis is seen in chronic insult.

A. Heinz bodies



A. Membrane-bound Heinz bodies consisting of denatured hemoglobin (supravital staining with methyl violet)

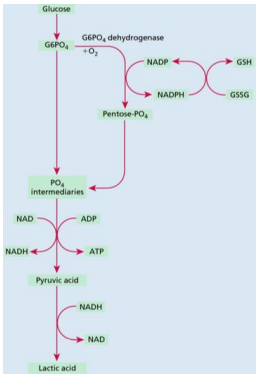
B. Bite cells



B. 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)

Enzymopathies
(Congenital Hemolytic Anemias)

G6PD Deficiency contd..

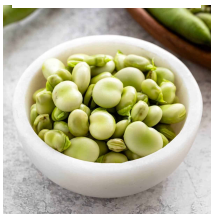


- A schematic diagram of the pathway of glucose metabolism in the red cell, to show the important role of G6PD.
- A decreased activity of the enzyme leads to a deficiency of the reducing compounds NADPH and GSH (reduced glutathione), which makes RBCs more susceptible to oxidative stress and can result in episodic hemolytic anemia

Favism

- 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, **type II**), the degree in deficiency varies, often being mild in african people and more severe in mediterranean people
- **Usually affects children:**
  - Severe anemia develops rapidly
  - Often accompanied by hemoglobinuria.

Vicia fava



Treatment

- Avoidance of oxidative precipitants to hemolysis. E.g. Intake of oxidant drugs (antibiotics), exposure to infections
- In children, rehydration is needed to avoid acute kidney injury.
- In many cases hemolysis is self limiting.
- Packed red cell transfusion may be required in cases of severe hemolysis.

Diagnosis

Screening tests and assays for detecting G6PD deficiency are available.

Pyruvate Kinase Deficiency

- Pyruvate kinase deficiency is another relatively common example of enzymopathies
- There is usually a chronic hemolytic anemia and some patients may benefit from splenectomy.

Hemoglobinopathies
(Congenital Hemolytic Anemias)

Refer to Hemoglobinopathies lecture for details

- Hemoglobinopathies are described as 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.**

Immunological Causes

Explained by a **student** here

- **Antigens** on the surface of red cells react with **antibodies** and might cause complement activation.

Pathogenesis

- **Extravascular hemolysis**
  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**.
- **Intravascular hemolysis:** Sometimes, membrane attack complex (C5-C9), complement, leads to it.

Male Dr: Usually are extravascular but in some cases are intravascular like in cold AIHA because it driven by IgM and cells are stick to each other and lysis inside the vessels

Subtypes

Autoantibodies Autoimmune hemolytic anemias	**Alloimmune hemolytic anemia**
• Antibodies formed by the body against one or more antigenic constituents of the individual's own tissues. • These include autoimmune hemolytic anemia (AIHA) and some drug-related hemolytic anemias.	• Antibodies produced by one individual reacts against red cells of another • As in hemolytic transfusion reactions and hemolytic disease of the newborn (when the IgG molecules produced by the mother pass through the placenta and cause hemolysis)

Autoimmune Hemolytic Anemia (AIHA)
(Acquired Hemolytic Anemia)

Classification of AIHAs

Caused by	Warm-reactive antibodies (Warm agglutinin disease)	Cold-reactive antibodies (Cold haemagglutinin disease CHAD)	Paroxysmal cold haemoglobinuria skipped
Etiology	1) Idiopathic		
	2) Secondary to:		
	• Chronic lymphocytic leukemia (CLL) • Lymphoma • Systemic lupus erythematosus (SLE) • Some drugs	• Infections: Mycoplasma pneumoniae and Infectious mononucleosis • Lymphomas	• Some viral infections • Congenital & tertiary syphilis

Other causes of autoimmune hemolytic anemias: Paroxysmal nocturnal hemoglobinuria (PNH), and Drug-related hemolytic anemias.

Warm AIHA

Explained by a **student** here

Medicosis

Pathoma (Skip to 14:04)

Overview

- **Warm Agglutinin Disease** is characterized by heat sensitive (warm) antibodies binding to RBCs, which triggers inappropriate phagocytosis of these RBCs
- **'Warm'** autoantibodies react best with the red cell antigen at 37°C and are usually of **IgG** subtype.
- Antibody-coated red cells undergo partial or complete phagocytosis in the **spleen** and by the Kupffer cells of the **liver**. (kupffer cells are macrophages of the liver)
- There may be partial activation of the complement cascade.

Etiology

- **Idiopathic**
  Hemolysis dominates the clinical picture and no evidence can be found of any other disease.
- **Secondary to a primary disease like:**
  - Chronic lymphocytic leukemia (CLL)
  - Systemic lupus erythematosus (SLE).

Critical diagnostic investigation: Male dr : very important

Direct antiglobulin test (DAT) also known as **Coomb's test,** helps to distinguish between autoimmune (positive Coombs test) and non-autoimmune anemias (negative Coombs test).

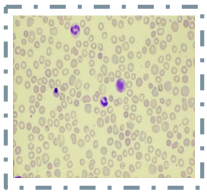
Autoimmune Hemolytic Anemia (AIHA)
(Acquired Hemolytic Anemia)

Warm AIHA contd..

Mnemonic:
- **Warm** weather is Great (IgG)
- **Cold** ice Cream is yuMMy (IgM)

Findings in the peripheral blood.

- Anemia
- Reticulocytosis
- **Spherocytosis**
- Rare nucleated red cells



Blood film from a patient with idiopathic AIHA showing prominent spherocytosis and polychromasia

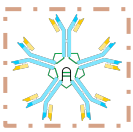
Treatment

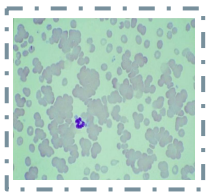
- **Prednisolone** (steroid) can limit hemolysis.
- **Splenectomy:** should be considered if reduction in hemolysis is not maintained when the dose of steroids is lowered
- **Immunosuppressive therapy**. An alternative option, the anti-CD20 monoclonal antibody rituximab, as well as immunosuppressants such as azathioprine or cyclophosphamide.

Cold AIHA

Explained by a **student** here | Medicosis | Pathoma (Skip to 17:17)

Overview	• **Cold Hemagglutinin Disease (CHAD)** is characterized by heat sensitive (cold) antibodies binding to RBCs, which causes agglutination and lysis of these RBCs • 'Cold' antibodies react best at temperatures below 32°C (usually below 15°C) and, since they are usually of **IgM** subtype, are capable of agglutinating red cells. • Cold antibodies bind to the red cell surface in the cooler superficial blood vessels of the peripheries. IgM subtype, **pentameric** structure **(check pic),** permits direct agglutination of red cells coated with antibody; they are therefore sometimes termed cold agglutinins.
Symptoms	**Symptoms of CHAD are worse during cold weather** • Acrocyanosis (painful cyanosis of the extremities): provoked by exposure to cold • The direct activation of the complement system leads to red cells lysis and, consequently, to hemoglobinemia and hemoglobinuria (typical finding of intravascular hemolysis).
Treatment	• Rituximab may be effective, Steroids or Splenectomy is prohibited. • Chronic idiopathic CHAD is managed initially simply by keeping the patient warm





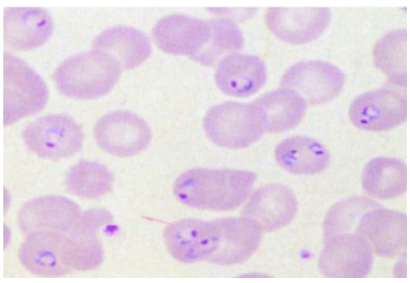
Numerous red cells agglutinated from a patient with idiopathic CHAD

Non-Hemolytic Anemia
(Acquired Hemolytic Anemia)

- Several of the mechanical causes of acquired non-immune hemolytic anemia are **summarized in table. Note that some drugs cause hemolysis by immune mechanisms.**

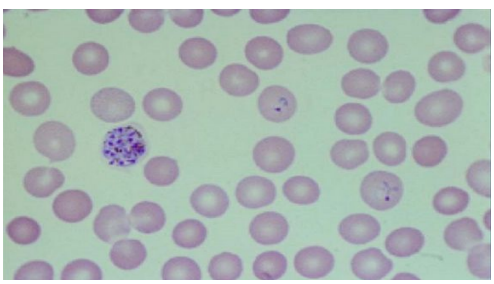
Causes of Acquired Non-immune Hemolytic Anemias	
Mechanical trauma to RBC	• Abnormalities in the heart & large blood vessels • Aortic valve prosthesis, severe aortic valve disease • Microangiopathic haemolytic anaemia • Haemolytic uremic syndrome (HUS) • Thrombotic thrombocytopenic purpura (TTP) • **Disseminated intravascular coagulation (DIC)** • Metastatic malignancy, Malignant hypertension • March haemoglobinuria
★ Infections* **Most common cause**	***DR:** What is the most common acquired cause of hemolytic anemia? Answer would be any of the following infections. Clostridium perfringens (welchii), **Malaria**, bartonellosis
Drugs, *chemicals & venoms	• Oxidant drugs and chemicals, arsine • Acute lead poisoning, copper toxicity • Venoms of certain spiders and snakes
Burns	
Hypersplenism	

A. Plasmodium Falciparum Malaria



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

B. Plasmodium Vivax Malaria



B. Blood film from a patient with **plasmodium vivax malaria** showing two parasitized red cells, each containing a single parasite (ring form or early trophozoite and an ameboid late trophozoite). Another red cells contains a schizont. Some of the parasitized cells are slightly enlarged

Non-Immune Hemolytic Anemia
(Acquired Hemolytic Anemia)

Mechanical Damage to Red cells

Explained by a **student** here

- Red cells are mechanically damaged when they impact upon abnormal surfaces.

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)**, sign of intravascular hemolysis in the blood film.

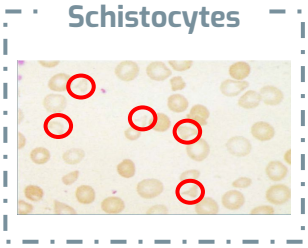


Figure: Fragmented red cells (schistocytes) in the blood film of a patient with a malfunctioning aortic valve prosthesis

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 (methylbenzene) and saponin, which are **fat solvents**, act on the red cell membrane directly and disrupt its lipid components, inducing hemolysis.

Hypersplenism

Explained by a **student** here

- Overactive spleen 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 these patients are also partly caused by **increased pooling** of blood cells within the spleen and might be treated with a **splenectomy**.

Summary

Laboratory Findings of Hemolysis	
Extravascular Haemolysis involves RBC destruction by reticuloendothelial system (The majority of hemolytic anemia)	**Intravascular Haemolysis** involves destruction of RBCs within vessels
Hyperbilirubinemia (unconjugated)	
Increase Serum Lactate dehydrogenase (**LDH**, marked in intra)	
Reticulocytosis: Increased **reticulocyte count**. Which indicates increased **erythropoietic activity.**	
examination of the bone marrow shows: 1-increased Erythropoiesis 2-Erythroid hyperplasia.	
Spherocytosis on the peripheral blood film. (Spherocyte)	**Reduction** of serum **Haptoglobin**
	Hemoglobinuria, Haemoglobinaemia and **Hemosiderinuria** .
	Schistocytes: red cell fragmentation

Hemolytic Anemia: is a shorten in lifespan of RBC that can't be compensated by bone marrow. Hemolytic anemias can be classified into:

A) Congenital Hemolytic Anemia: The defect is **intrinsic** to RBC itself, affecting Red cell:

Membrane	**Hereditary spherocytosis (HS)** Most common	**Causes:** loss of **Ankyrin** then leads to secondary reductions in **spectrin**, and **protein 4.1** leading to a spheroid shape, vertical interaction, Destroyed by splenic macrophages, **extravascular hemolysis.** Peripheral smear shows **spherocytosis**.
	Hereditary Elliptocytosis (HE)	**Causes:** defects in **α spectrin,** horizontal interaction**.** Peripheral smear shows **elliptical/elongated RBC's**.
	Hereditary Pyropoikilocytosis (HPP) sever form from HE (homozygous mutation)	
Its Enzymes	**1- G6PD Deficiency** (G6PD gene mutation on **X** chromosome) **predominantly intravascular, could be extravascular**	**Blood film shows: 1-Heinz bodies 2- Bite cells** (in a patient exposed to **Oxidants**). **Favism:** causes an **acute hemolytic anemia** after the ingestion of the broad bean in G6PD deficiency patients. Affects **children**. **Severe anemia** develops & accompanied by **hemoglobinuria**.
	2 - Pyruvate kinase deficiency Extravascular	
Its hemoglobin	Sickle cell anemia	

B) Acquired Hemolytic Anemia: The cause is typically due to **extrinsic** defects, outside the red cell (**except** ***PNH**)

Immune	**Autoimmune**	**Warm Autoimmune Hemolytic Anemia** Extravascular	Autoantibodies react best with the red cell antigen at 37ºC and are usually of **IgG**. Secondary to: SLE and CLL. **Peripheral smear shows spherocytosis. Critical diagnostic investigation:** Direct antiglobulin test (DAT), known as Coomb's test
		Cold Hemagglutinin Disease (CHAD) Intravascular	Antibodies react best below 32ºC, they are usually of **IgM**.
	Alloimmune		
Non-immune	**Most commonly due to infection of: Malaria,** Clostridium perfringen, and bartonellosis. **Some drugs**		

Quiz

***The questions in red are from the Dr's slides**

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	Glucose-6-phosphate dehydrogenase (G6PD) deficiency	D	Autoimmune hemolytic anemia

Q3) Which ONE of the following is an **only** a cause of **intravascular** hemolysis?							
A	Glucose-6-phosphat e dehydrogenase (G6PD) deficiency	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 hemoglobin	B	It is more common in males	C	It can be treated by splenectomy	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 is minimal	C	It may complicate B-cell chronic lymphocytic leukemia	D	It is associated with a positive indirect antiglobulin test

Q6) What is the type of immunoglobulins commonly associated with **warm** autoimmune hemolytic anemia?							
A	IgG	B	IgM	C	IgE	D	IgA

Q7) Which of these conditions will most likely show **schistocytosis** on blood film?							
A	HS	B	Warm Autoimmune Hemolytic Anemia	C	Sickle Cell anemia	D	Disseminated Intravascular Coagulation (DIC)

Q8) Which of the following is **NOT** associated with non-immune hemolytic anemia?							
A	Clostridium perfringens	B	Malaria	C	Mycoplasma pneumoniae	D	bartonellosis

Q9) Which of the following is the best option for treatment of CHAD?							
A	Prednisolone	B	rituximab	C	Splenectomy	D	Exposure to cold

Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9
B	D	C	C	C	A	D	C	B

Leaders

Sarah Alobaid Sarah Alqahtani Albara Aldawoud

Organizer

Sarah Alaidarous

Members

Ghada Alothman Fahad Alrasheedd

Note Takers

Ghada Alothman Faisal Alshehri

Summary

Sarah Alobaid

Feel free to contact us :
Hematologyteam439@gmail.com