Hematology

438 teamwork

Approach to Hemolysis

Color index:
Red: Important
Gray: notes
Blue: extra

Editing file

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.

Hemolysis: is a state with a short of the lifespan of a mature red blood cell.

IF hemolysis is not marked and can be compensated

- Increased red cell output from the marrow.
- Stimulated by erythropoietin.
- Will be sufficient.

IF hemolysis is marked and can not be compensated

- 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 a shorten in life span of RBC that can not be overcome by ability of bone marrow production.

Extravascular Haemolysis

Usually doesn't happen as an acute insult.

(The majority of hemolytic anemias)

In Which the macrophages in the spleen, liver and bone marrow remove red cells from the circulation by phagocytosis.

Intravascular Haemolysis

In Which 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

involves RBC destruction by reticuloendothelial system.

Extravascular Haemolysis	Intravascular Haemolysis involves destruction of RBCs within vessels.
Hyperbilirubinemia (unconjugated): A rise in the **unconjugated bilirubin** concentration in the plasma.	
Increase Serum Lactate dehydrogenase (**LDH**,marked in intra), an enzyme present in red cells	
Reticulocytosis: Increased **reticulocyte count**. 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 20-100x10^9/L. Increase in the absolute reticulocyte count is an indication of increased **erythropoietic activity.**	
Macrocytosis: As haemolysis will also increase the marrow's demand for folic acid, **High MCV,** may also develop secondary to folate deficiency. Due to the presence of reticulocytes as the reticulocytes is larger than RBCs	
Spherocytosis on the peripheral blood film. (Spherocyte) Spherocytes are caused by the action of the spleen (sculpturing) on the reticulocytes in an attempt to make them look like normal cells	**Reduction** of serum **Haptoglobin,** molecule binds to free Hb.
	Methemalbumin-aemia: when free heme can bind to albumin. (Now rarely used in investigating a patient.)
The most common findings in extravascular hemolysis is Splenomegaly	Free Hb in the urine: **Hemoglobinuria,** (note the difference from hematuria, which describes the presence of intact red cells in the urine). **Haemoglobinaemia** and **Hemosiderinuria** .
	Polychromasia: a bluish discoloration of RBC, due to reticulocytosis
	Schistocytes: red cell fragmentation

The most common findings in intravascular hemolysis is A-reduced haptoglobin B-schistocytes

Laboratory Evidence of Hemolysis

If examination of the bone marrow is undertaken, there will be evidence of

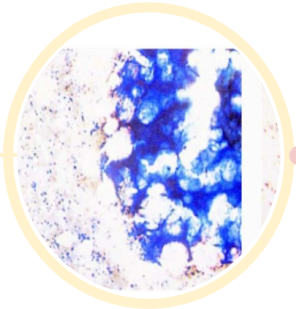
Blood film is the golder investigation in Hemolytic anemia

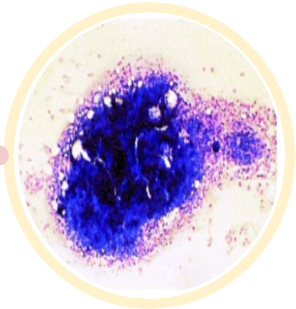
increased **Erythropoiesis**

Erythroid hyperplasia are also hypercellular, due to the replacement of fat cells by **erythroid precursors**

-Signs of ineffective erythropoiesis
-increased erythropoietic activity means that bone marrow responds, if bone marrow diseased or damage → No reticulocytosis

(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.

Laboratory Evidence of Hemolysis

Extravascular haemolysis	Intravascular haemolysis
Hyperbilirubinemia (unconjugated)	Hyperbilirubinemia (unconjugated)
Increased serum lactate dehydrogenase (LDH)	Reduced or absent serum haptoglobin
	Haemoglobinaemia, haemoglobinuria, haemosiderinuria
	Methaemalbuminaemia *
	Markedly increased serum LDH
Reticulocytosis	Reticulocytosis
Spherocytes	Red cell fragments (schistocytes)
* Now rarely used in investigation a patient	

Clinical features of hemolysis

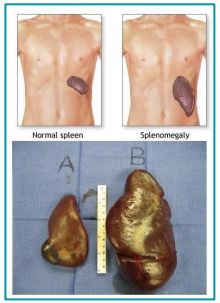
1

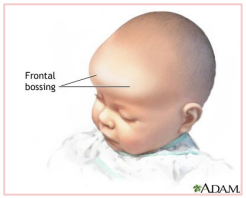
Pallor and **Jaundice**

secondary to the elevated bilirubin levels.

Splenomegaly
may be seen.

2





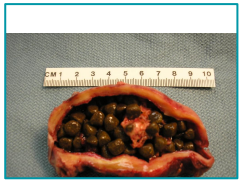
3

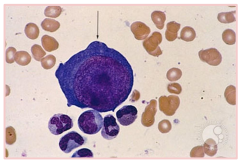
Bone Deformities

Long-term complications of chronic hemolysis; expansion of erythropoiesis in the marrow cavities, thinning of cortical bone,(e.g. frontal and parietal bossing) and, very occasionally, pathological fractures.

Pigment gallstones
are seen commonly.

4





5

Risk of episodes of Pure Red Cell Aplasia especially with parvovirus B19.

Not important

Classification of Hemolytic Anemias

Aetiology

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

congenital

With the **congenital causes**, the underlying defect is typically **intrinsic** to the red cell itself, affecting the red cell:

- **Membrane** — hereditary spherocytosis
- **its enzymes** — G6PD deficiency
- **or, its hemoglobin** — sickle cell anaemia

Acquired

Acquired causes, are typically due to **extrinsic** defects, outside the red cell (except *PNH), and can be divided into those with an **immune basis** and those **without**.

- ****Immune**
  - Autoimmune
  - Alloimmune
- **Non-immune**
  - 1.Mechanical MAHA (microangiopathic hemolytic anemia)
  - 2.Infection: malaria

*Paroxysmal nocturnal hemoglobinuria; it's Acquired **intrinsic**, not extrinsic as the most acquired hemolytic anemias are.

**Meaning Igs coat the RBCs, then as a result of coating it get engulfed and destroyed by the spleen may be because an increase Release of Igs like in SLE (Autoimmune), Or across reaction by an infection (Alloimmune)

Congenital Hemolytic Anemias

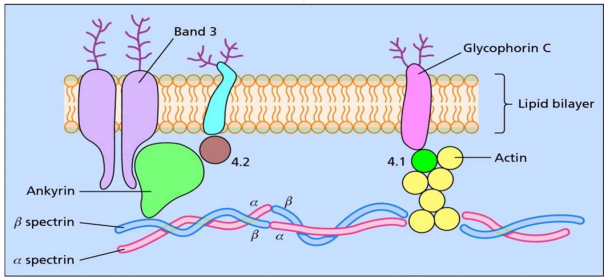
A Red Cell Membrane "Membranopathy"

➤ The red cells undergo significant deformations while traversing the circulation. Thus, flexible red cell cytoskeleton is essential.

Key components of the cytoskeleton include

- α spectrin
- β spectrin
- actin
- protein 4.1

connections linking the cytoskeleton to the overlying red cell phospholipid bilayer include band 3, Rh-associated glycoprotein and glycophorin C (see figure)



➤ Defects in any of these proteins can jeopardize the **integrity of the red cell and shorten its lifespan.**

Congenital Hemolytic Anemias: Red Cell Membrane "Membranopathy"

1 Hereditary spherocytosis (HS)

HS

The **most common** membranopathy is hereditary spherocytosis (HS) with ~ 60% related to **Ankyrin gene.**

Causes

Loss of **Ankyrin** then leads to secondary reductions in **spectrin,** Band 3,and **protein 4.1** leading to **a spheroid shape, vertical.**

Affecting the vertical structure of RBCs membrane

Pathogenesis

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

→ Pathoma

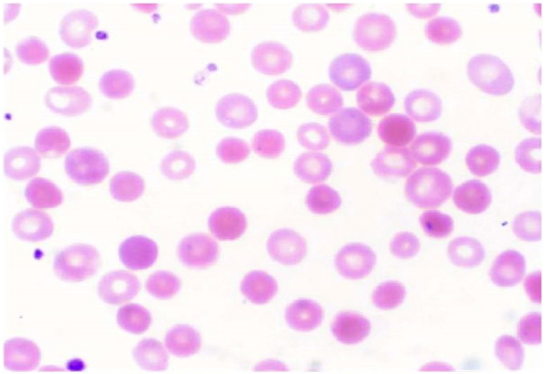
Spherocytes are less able to maneuver through splenic sinusoids and are consumed by splenic macrophages, resulting in anemia.

Complications

Complications of the chronic hemolysis in HS include the development of **pigment gallstones.** Aplastic crisis may occur secondary to parvovirus B19. **Megaloblastic anemia** is occasionally found.

Congenital Hemolytic Anemias:

Red Cell Membrane "Membranopathy"



A blood film from a patient with HS showing many spherocytes.

1 Hereditary spherocytosis (HS)

According to male doctor this slide is not important :)

The Diagnosis of HS:

- **Family historye** , 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.

Some Treatment of Significant HS:

- **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).

Congenital Hemolytic Anemias:

Red Cell Membrane "Membranopathy"

2 Hereditary Elliptocytosis (HE)

3 Hereditary Pyropoikilocytosis (HPP)

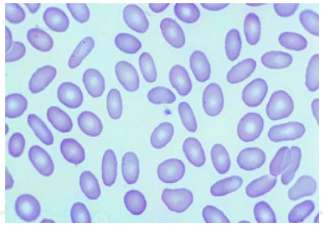
The male doctor skipped this slide

Hereditary Elliptocytosis (HE)

Hereditary elliptocytosis (HE) is also a relatively common condition, with many cases showing defects in **α spectrin**, **horizontal interaction**. it Affects the horizontal structure of RBCs membrane

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 (Figure).



A blood film from a patient with hereditary elliptocytosis showing a high proportion of elliptical red cells.

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**.

Congenital Hemolytic Anemias:

B Enzymopathy (RBC enzymes)

- 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.

In G6PD deficiency, hemolysis can be either
A-intravascular during acute insult (using tetracyclines)
B- extravascular (chronic) (usually mild)

1- Glucose-6-Phosphate Dehydrogenase Deficiency

Definition:

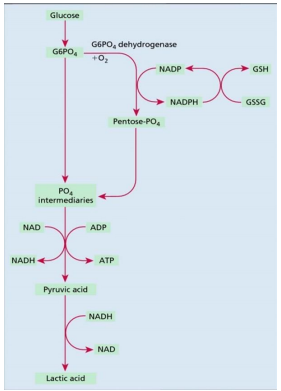
mutations in the G6PD gene on the **X** chromosome. Screening tests for detecting G6PD deficiency are available.

Pathogenesis:

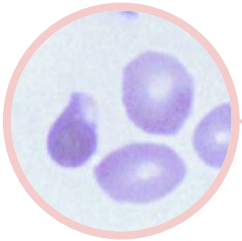
G-6-P	Deficiency	Denaturation	Bite cells	Hemolysis
1	2	3	4	5
glucose-6-p is the first enzyme of the hexose monophosphate/pentose-phosphate shunt	A deficiency will prevent the normal generation of NADPH, with subsequent erythrocyte sensitivity to oxidative stress.	When the red cell is exposed to oxidants, Hb is converted to methemoglobin and denatured. Denatured Hb then precipitates forming inclusions in the red cell (termed Heinz bodies)	Splenic macrophages remove Heinz bodies(extravascularly) resulting in an inclusion-free cells with unstained areas at their periphery ('bite' cells)	Hemolysis begins 1-3 days after exposure to the oxidative stressor, with anemia being maximal about 7-10 days after exposure. Patient may report dark urine due to hemoglobinuria, intravascular.

1- Glucose-6-Phosphate Dehydrogenase Deficiency

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.

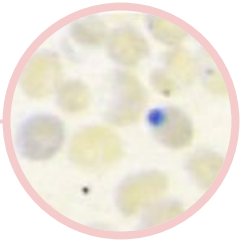


a pathway for glucose metabolism in red cells, to show the role of G6PD. A decreased activity of the enzyme leads to deficiency of the reducing compounds NADPH and GSH.
to reduce free radicals eg, H2O2



'bite' cells

Are in the blood of a patient with G6PD deficiency who had received primaquine. These RBCs are irregular in shape, abnormally dense and show a poorly staining area just beneath part of the cell membrane (MGG stain



Heinz bodies

membrane-bound, consisting of denatured haemoglobin (detected by supravital staining with methyl violet)

Treatment generally focuses on:

the avoidance of oxidative precipitants to hemolysis

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.

Congenital Hemolytic Anemias:

B Enzymopathy (RBC enzymes)

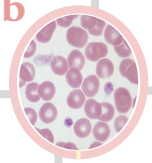
2 Pyruvate kinase deficiency is another relatively common example.
There is usually a chronic hemolytic anemia and some patients may benefit from splenectomy.

C Hemoglobinopathy (RBC Hemoglobin)

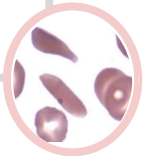
Defects in the structure of Hb

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.

Structural variants of the globin chains may affect the lifespan of the red cell, with sickle cell anemia being the best-described example.



HbS



Sickle cell

Normal RBC

Acquired Hemolytic Anemias

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

a Immunological Causes

01 IgG-coated red cells interact with the Fc receptors on macrophages in the spleen,

02 then either completely or partially phagocytosed (extra).

03 When the phagocytosis is partial, the damaged cell will return to the circulation as a spherocyte.

04 Sometimes, membrane attack complex (C5-C9), complement, leading to intravascular hemolysis.

01 **due to Autoantibodies**

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.

02 **Caused by 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.

Acquired Hemolytic Anemias

a Immunological Causes

Both warm and cold autoantibodies activate the complement system

	Warm-reactive antibodies	Cold-reactive antibodies	Paroxysmal cold haemoglobinuria
	autoantibodies react best with the red cell antigen at 37ºC and are usually of IgG subtype.	antibodies react best at temperatures below 32ºC (usually below 15ºC) and, since they are usually of IgM subtype, are capable of agglutinating red cells.	
etiology	Idiopathic	Idiopathic Cold haemagglutinin disease	Idiopathic
Secondary to	chronic lymphocytic leukaemia	Mycoplasma pneumoniae infection	some viral infections
	Lymphoma	Lymphoma	congenital syphilis
	SLE	infectious mononucleosis	tertiary syphilis
	some drugs		

| Acquired Hemolytic Anemias

| warm Autoimmune hemolytic anemia (AIHA)

idiopathic warm AIHA

hemolysis dominates the clinical picture and no evidence of any other disease.

VS

secondary AIHA

the hemolysis linked with a primary disease like; chronic lymphocytic leukemia (CLL) or (SLE).

The antibody-coated red cells **undergo** partial or complete phagocytosis in :

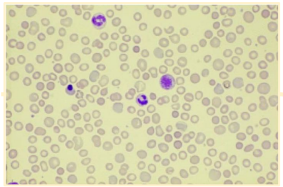
- the spleen
- by the Kupffer cells of the liver

There may be partial activation of the complement cascade.

Findings in the peripheral blood:

- anemia
- reticulocytosis
- spherocytosis
- rare nucleated red cells

The critical diagnostic investigation is the direct antiglobulin test (DAT)

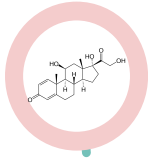


pic: blood film from a patient with idiopathic AIHA (warm reactive antibody) showing prominent spherocytosis and polychromasia.

Treatment

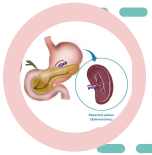
prednisolone

can limit hemolysis.



splenectomy

If reduction in hemolysis is not maintained when the dose of steroids is lowered



immunosuppressive therapy

An alternative option: **anti-CD20** monoclonal antibody **rituximab**, as well as immunosuppressants such as **azathioprine** or **cyclophosphamide**

| Acquired Hemolytic Anemias

| Cold Hemagglutinin Disease (CHAD)

Cold antibodies

bind to the red cell surface in the cooler superficial blood vessels of the peripheries.

Symptoms

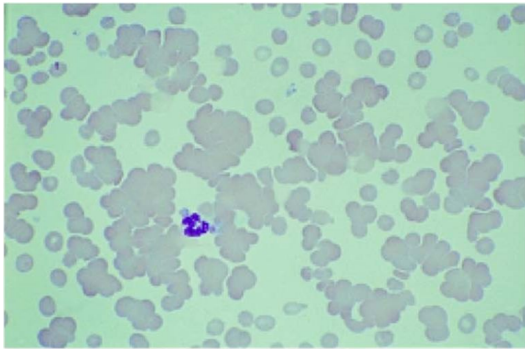
worse during cold weather. Exposure to cold provokes acrocyanosis.

IgM subtype

pentameric structure, permits direct agglutination of red cells coated with antibody therefore sometimes termed cold agglutinins

RBCs lysis

due to direct activation of the complement system and, consequently, to hemoglobinemia and hemoglobinuria (intra).



pic: Numerous red cell agglutinates on a blood film from idiopathic CHAD patient

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

| Acquired Hemolytic Anemias

| B) Non-immune hemolytic anemias

1

Mechanical Damage to Red Cells: Red cells are mechanically damaged when they impact upon abnormal surfaces.

- **Disseminated intravascular coagulation (DIC):**

Such damage usually results in the presence of red cell fragments (schistocytes) in the blood film.

inappropriate activation of the coagulation cascade produces fibrin strands which are thought to cause mechanical destruction of red cells.

Mechanical trauma to red cells		other causes		
Abnormalities in the heart and large blood vessels	Thrombotic thrombocytopenic purpura	**Burns**	**Drugs, chemicals and venoms:**	**Hypersplenism**
Aortic valve prostheses	Metastatic malignancy	**Infections :**	-Oxidant drugs and chemicals	note: Some drugs cause haemolysis by immune mechanisms
Severe aortic valve disease	Malignant hypertension	-Clostridium perfringes (welchii)	-Arsine	
Microangiopathic haemolytic anaemia	Disseminated intravascular coagulation	-Malaria " Dr notes : plays an important role in intravascular hemolytic anemia "	-Acute lead poisoning	
Haemolytic uraemic syndrome	March haemoglobinuria	-Bartonellosis	-Copper toxicity	get rekt LuL

| Acquired Hemolytic Anemias

| B) Non-immune hemolytic anemias

2 Some Drugs

non-immune mechanisms

the red cell lifespan may be shortened by Chemicals, such as

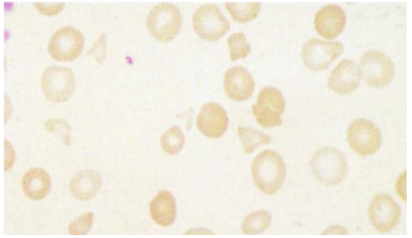
- benzene
- toluene
- saponin,

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

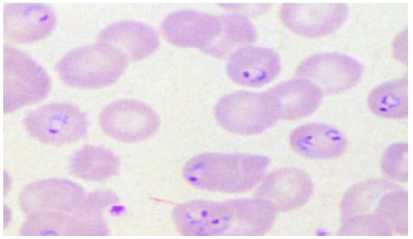
Hypersplenism:
results in the reduction in the lifespan of **red cells**, **granulocytes** and **platelets** that may be found in patients with splenomegaly due to any cause.

Increased pooling of RBCs

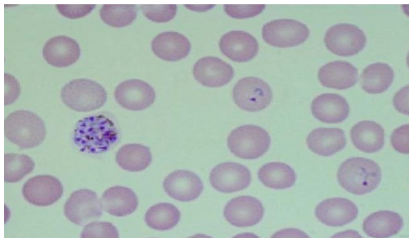
The cytopenias found in patients with enlarged spleens are also partly caused by increased pooling of blood cells within the spleen and might be treated with a **splenectomy**.



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



Blood film[4] from a patient with *Plasmodium Falaciparum* malaria showing several parasitized red cells. RBCs heavily parasitized with malaria may be subject to intravascular lysis



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 cell contains a schizont. some of the parasitized cells are slightly enlarged

Quiz

Key answers:
1-D 2-C 3-B 4-B 5-C6-B

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

A. It is NOT a cause of neonatal jaundice.
B. Carrier females have approximately 10% G6PD levels.
C. It commonly presents as a chronic hemolytic anemia.
D. It protects against malaria.

2-Spherocytosis in the blood film is a feature of which ONE of the following?

A. Thalassemia major.
B. Glucose-6-phosphate dehydrogenase (G6PD) deficiency.
C. Autoimmune hemolytic anemia.
D. Reticulocytosis.

3-Which ONE of the following is an only cause of intravascular hemolysis?

A. Glucose-6-phosphate dehydrogenase (G6PD) deficiency.
B. Red cell fragmentation syndrome.
C. Rhesus incompatibility.
D. Hereditary spherocytosis.

4-Which ONE of these statements is TRUE regarding hereditary spherocytosis?

A. It is more frequent in southern Europe.
B. It can be treated by splenectomy.
C. It is caused by an inherited defect in hemoglobin.
D. It is more common in males.

5-Which ONE of the following is TRUE about autoimmune hemolytic anemia?

A. Hemolytic anemia is minimal.
B. It is associated with pernicious anemia.
C. It may complicate B-cell chronic lymphocytic leukemia.
D. It is associated with a positive indirect antiglobulin test.

6- which type of bilirubin accumulated in hemolytic anemia ?

A. conjugated
B. Unconjugated
C. conjugated and Unconjugated

THANKS

| TEAM LEADERS

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