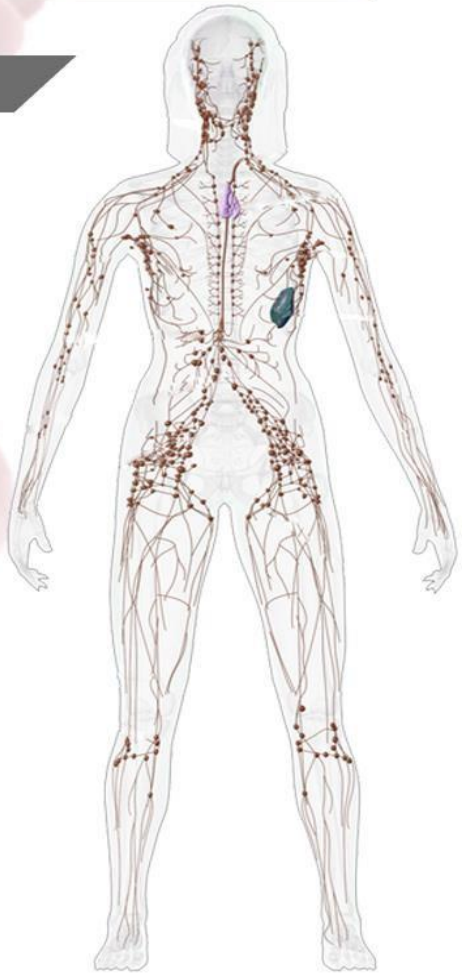
Sheet 5

Hematology and Lymphatic system



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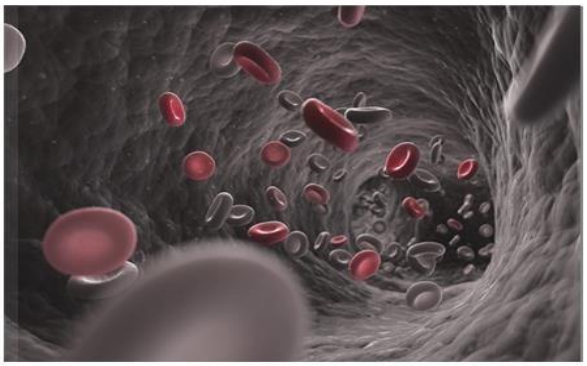


Anemia of Blood Loss cont.:

This sheet will continue with the anemias and we will talk about hemolysis caused by intrinsic factors to the RBC.

The anemias caused by defects in the RBC can be divided into the following categories:

- Membranopathies (spherocytosis)
- Hemoglobinopathies (thalassemias and HbS)
- Enzymopathies (G6PD deficiency)

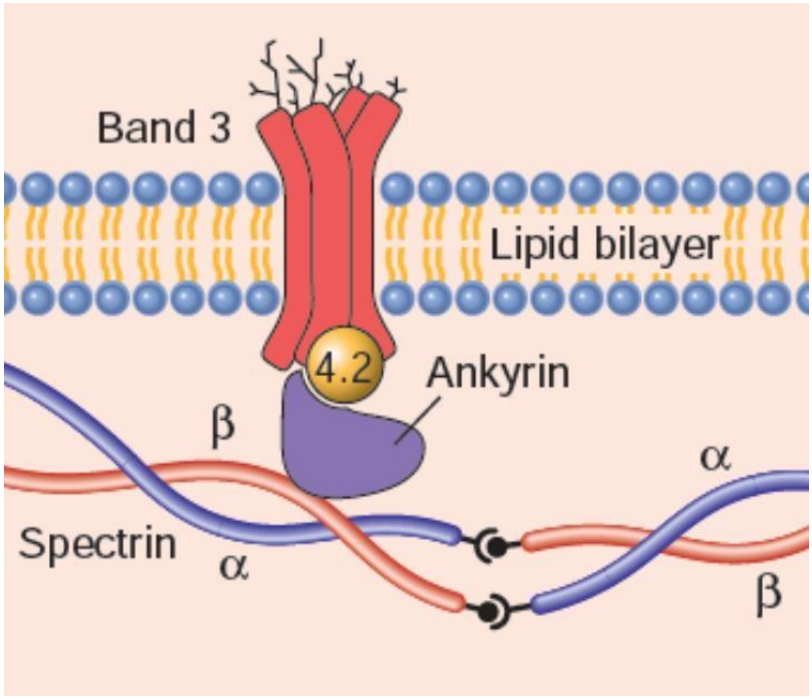
Hereditary

- Autoimmune (paroxysmal nocturnal hemoglobinuria)

Acquired

Hereditary Spherocytosis:

In order for RBCs to maintain their biconcave shape, they require proteins to be bound to the plasma membrane, in order to hold the shape. The three main proteins that end up having mutations are: **spectrin**, **ankyrin**, and **Band 3**.



A mutation in any of these three proteins will cause the RBC to be unable to maintain its biconcave shape. In physics, the shape that can fit the most volume per unit surface area is a sphere, so the RBCs become spherical. The problem these RBCs face, is their **lack of flexibility** and **slow transit time in the spleen**, which decreases glucose and pH, as well as increases the likelihood of phagocytosis by macrophages.

This disease is inherited in an **autosomal dominant** pattern, and is prevalent in northern Europe. The anemia is **mild** and **chronic**. Because it is **chronic** hemolytic anemia, symptoms include **jaundice**, **gallstones**, and **splenomegaly**. The jaundice and gallstones are a result of the intravascular hemolysis, spilling large amounts of free hemoglobin into the blood, which then is metabolized to bilirubin. The gallstones are a result of the large amounts of bilirubin being stored in the gallbladder. The splenomegaly is a result of **macrophage hyperplasia** in the spleen. This is the only hemolytic anemia with a **high MCHC**, because of the high volume to surface area ratio. Diagnosis is done via osmotic fragility test, and treatment of symptoms involving a splenectomy can be done. Aplastic crisis can complicate symptoms, and is caused by Parvo Virus: B19.

Osmotic Fragility Test:

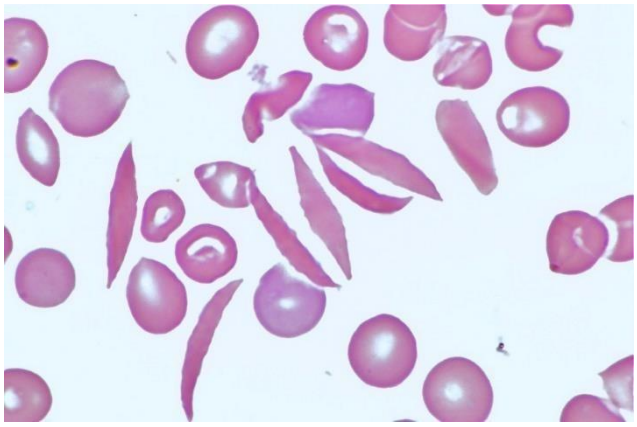
This test is based on the usage of hypotonic solutions of NaCl in order to see at what point cells will lyse. A normal RBC has room to take in water before lysing, and will do so at a lower concentration of salt compared to spherocytes. Normally, RBCs will begin to lyse at a concentration of 0.5, while spherocytes will lyse at a higher concentration.

Sickle Cell Anemia:

The most common hemoglobinopathy in humans caused by a point mutation in the 6th amino acid of the β-chain, replacing glutamate with valine. VAL is hydrophobic, while GLU is hydrophilic. This causes changes in the Hb structure. In homozygotes for the HbS gene, all of their Hb is HbS, while heterozygotes have ½ of their Hb replaced with HbS (2 β-chain genes on chromosome 11). This disease is very common in Africans, which is endemic for malaria. Having 1 copy of the HbS gene actually protects you from malaria, which explains why it is so common in Africans. These hemoglobins form polymers after multiple deoxygenation cycles and cause the RBCs to become sickled in shape. The problem with this is that they are inflexible, and can become stuck in capillaries and cause diffuse ischemia.

Note: when the RBC is still young it is **normal** but as it ages it becomes sickled. Three major factors affect the sickling in RBCs:

- Presence of Hb other than HbS
- Intracellular concentration of Hb
- RBC transit time in vessels



Presence of other Hemoglobins influence on sickling:

HbA: weak HbF: weak HbC: strong

*HbSC individuals have as much sickling as homozygote HbS individuals Intracellular concentration influence on sickling:

↑ concentration = ↑ sickling

Dehydration, hypoxia, infection all increase Hb concentration α-thalassemia

decreases Hb concentration

Transit time influence on sickling:

Slow transit time increases sickling, fast transit time decreases sickling.

Homozygous individuals will have fatty changes in the heart, liver, renal tubules; reticulocytosis, erythroid hyperplasia, prominent cheek and skull bones, as well as extramedullary hematopoiesis. Because these people will be in a constant state of hemolytic anemia, their spleen will atrophy and die over time. Another side effect is their increased risk of infections (mostly salmonella). One of the most important presentations in these patients is pain crises, which are episodes of chest and bone pains caused by the ischemia. They are also at risk of aplastic crisis (parvo virus B:19). Diagnosis via electrophoresis or fetal DNA. As for treatment, bone marrow transplant for severe cases, and milder cases can be treated with a drug called hydroxyurea which:

- Increases expression of HbF
- Decreases leukopoiesis (anti-inflammatory)
- Increases MCV of RBCs
- Produces nitric oxide which acts as a vasodilator

Thalassemias:

A group of inherited hemoglobinopathies that result in a **decrease** in the total hemoglobin. **Normal HbA is still produced in these patients**, but abnormal globin tetramers can form which damage the RBC. Remember, α-chain: 4 genes, chromosome 16 and β-chain: 2 genes, chromosome 11. A sensitive screening test for thalassemia is by using MCV (microcytic might mean thalassemia).

β Thalassemias:

Point mutations that cause a decrease in β-chains which has 2 phenotypes: β^0 = no synthesis of β-chains, and β^+ = decreased synthesis of β-chains.

Three types of mutations occur **outside the protein coding zone.** They are either in the promoter region, splicing region, or cause chain termination.

Why does a decrease in production cause anemia?

By underhemoglobinization of RBCs or chain imbalance. The ratio of α to β chains should be 1:1, and when it is abnormal it causes aggregation of unbound chains to precipitate and damage the membrane, resulting in hemolysis.

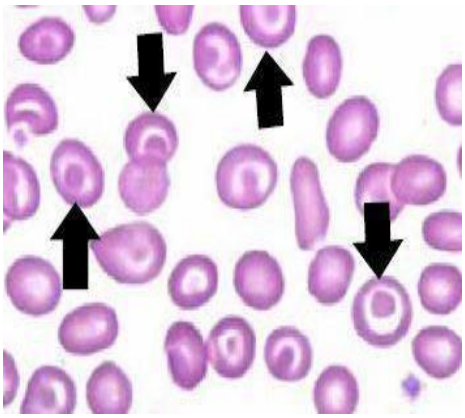
The constant state of anemia causes the body to try and compensate by increasing iron absorption, causing hemochromatosis, and by increasing hematopoiesis in the bones causing skeletal deformities.

β thalassemia has 3 major phenotypes, each with different phenotypes:

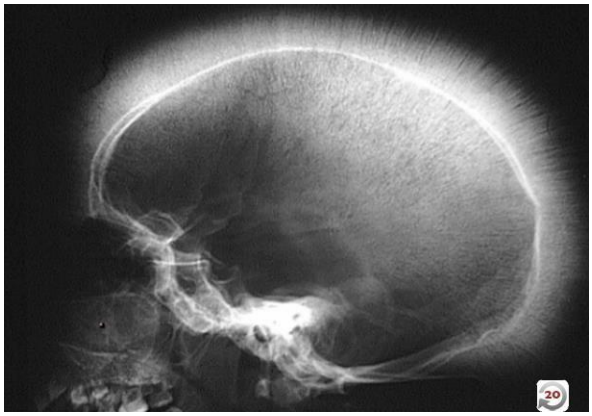
- β thalassemia minor (β^0/β or β^+/β)
- β thalassemia major (β^0/β^0, β^+/β^+, β^0/β^+)
- β thalassemia intermedia (β^0/β^+, β^+/β^+, β^0/β, β^+/β) – is variable

β Thalassemia Major:

- common in Mediterranean and Middle East
- manifests at 6-9 months of life (because of β-chain expression)
- low Hb concentration (3-6 Hb/dL)
- increased HbF and HbA_2



Causes target cells and crew cut skull (because of increased hematopiesis)



These patients have splenomegaly, cardiac disease (caused by iron overload), and require constant transfusions which increases iron overload, so chelation therapy must be used. The only way to cure this is through stem cell transplantation before the patient passes away.

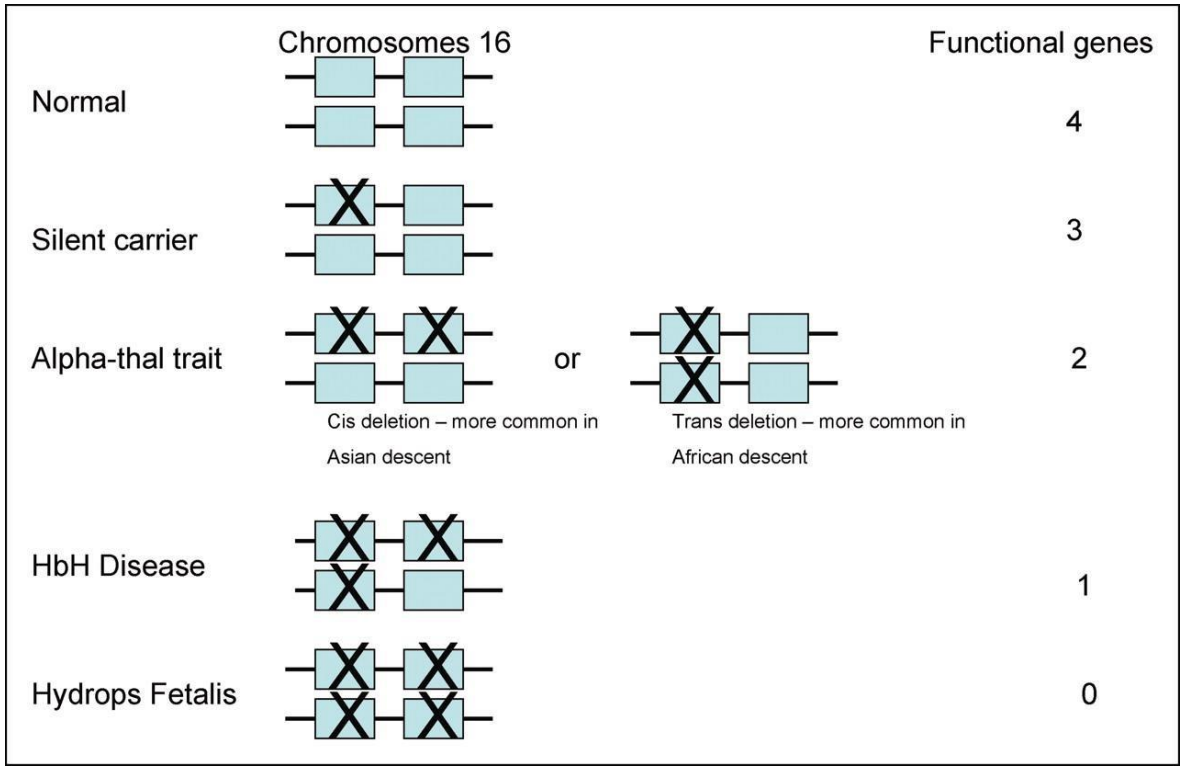
β Thalassemia Minor:

Is a mild disease with microcytic RBCs, and effects the same demographic as β thalassemia major. These patients have increase HbA_2 and slight bone marrow dysplasia, but they are asymptomatic. Despite this it is important to diagnose because you may mistake it for iron deficiency, and it is important to recommend genetic counseling because a patient's children are at a risk of getting β thalassemia major. Now, how do we differentiate it from IDA? By looking at RDW. IDA have a high RDW while β thalassemia has a **low RDW**. Iron studies show that IDA patients have low iron, while β thalassemia patients have normal iron levels. Patients with β thalassemia will have very small MCV,

while IDA has slightly low MCV. One interesting distinction is in the RBC count, IDA have low RBCs, while β thalassemia has **normal or high** RBCs.

α Thalassemias:

The α-chain in hemoglobin has 4 genes on chromosome 16, and can have multiple mutations. Mutations that cause α thalassemia are large deletions.



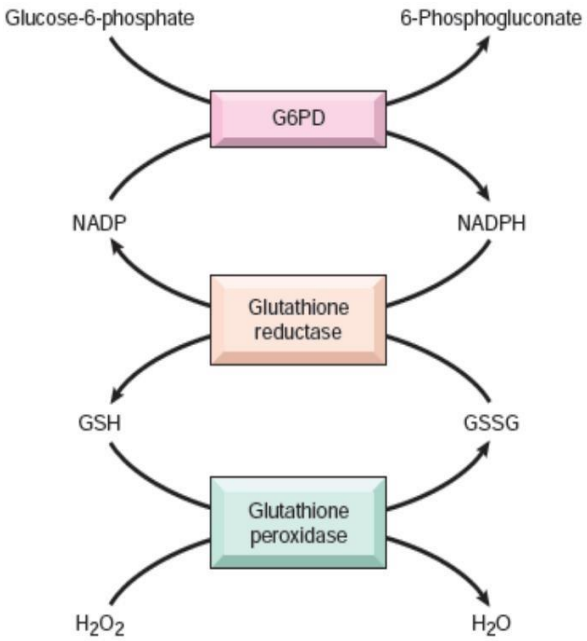
Silent carrier: asymptomatic, microcytosis α

thalassemia minor: mild or no anemia, microcytosis

HbH Disease: moderate to severe anemia

Hydrops Fetalis: lethal without in-utero transfusion

G6PD Deficiency:

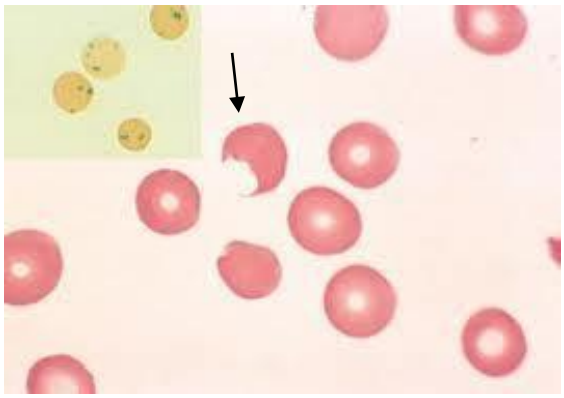


An enzymopathy that causes a decrease in the NADPH production in the RBC, which leads to increased oxidative stress and hemolysis. The main cause of oxidative stress is infection, so they will have anemic attacks when they are sick.

The disease is X-linked, so it is more common in males and it has many mutations that result in the deficiency. The 2 main variants are A- (African) and Mediterranean (Middle East).

In contrast to the other diseases we discussed, this disease is not chronic, rather acute and episodic. The main causes for hemolytic episodes are as follows:

- Infection (most common)
- Drugs: antimalarials, nitrofurantoin
- Foods: fava beans



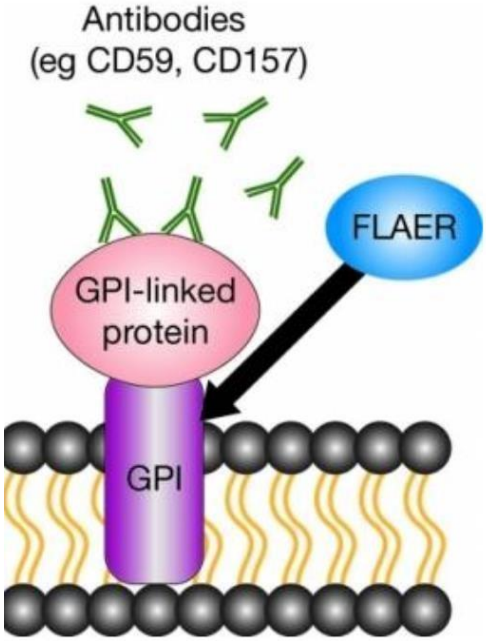
During these episodes the patient will have something called **bite cells.** These cells are the result of the spleen recognizing damaged cells and removing the damaged portion of them.

Between attacks the patient is normal. During an attack the hemolysis stops after old RBCs lysis, even if the cause of the oxidative stress is still present. The hemolysis caused by G6PD can be either intravascular or extravascular, depending on the severity of the mutation (mild G6PD = extra, severe = intra). G6PD patients typically do not have splenomegaly and gallstones because the hemolysis is episodic.

Paroxysmal Nocturnal Hemoglobinuria:

An **acquired** mutation in the PIGA gene (X chromosome) that causes a deficiency in enzymes responsible for synthesizing membrane proteins that regulate the complement system, preventing them from attacking RBCs. Mutations result in the complement system attacking RBCs and punching holes in the membrane, causing hemolysis. It is called nocturnal because during sleep blood pH drops slightly, making hemolysis easier, but

hemolysis is occurring at all times in affected individuals. These cells are monoclonal, but not neoplastic in any other way.



These patients have chronic, mild hemolysis, but they do not present with hemolytic symptoms. The classical presentation is a young (teens – 20s) patient with thromboses (DVT, stroke, etc.). They also have an increased risk of aplastic anemia. The treatment for this disease is by countering the complements responsible for the hemolysis. The only risk of this treatment is an increased risk of Neisseria infections, because the complement system is the main defense against them.