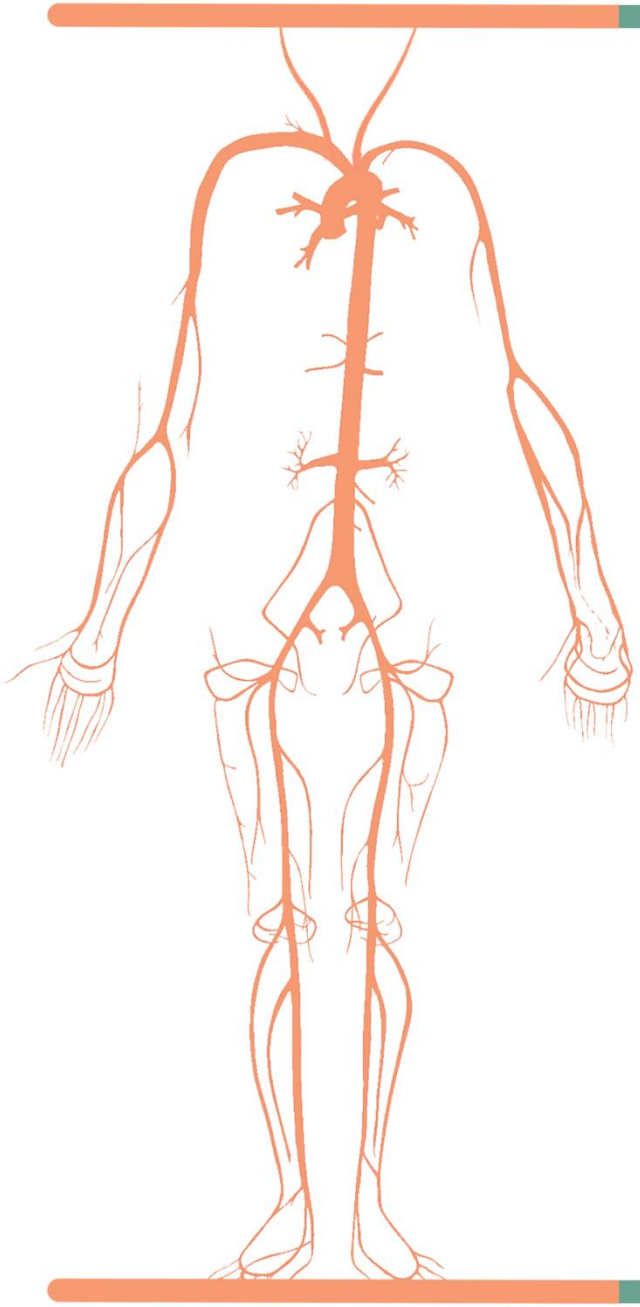
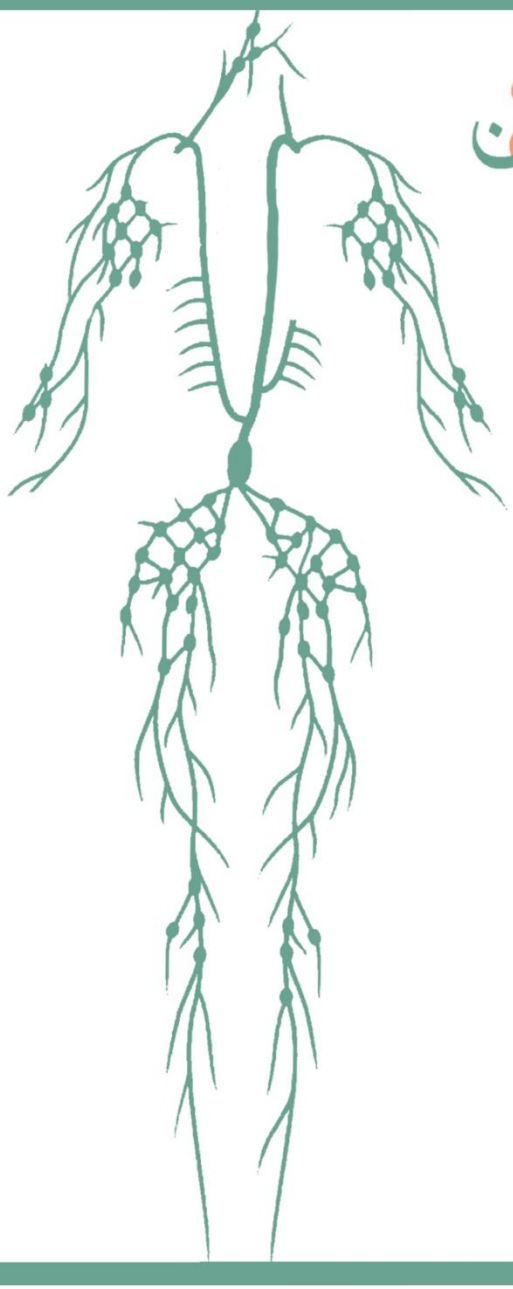
Pathology

HematoLymphatic





Title: Sheet 3 – Anemia of decreased production II + Hemolytic anemia

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**Flashback to previous lectures:

- Anemia is the reduction of oxygen carrying capacity of blood secondary to a decrease in red cell mass.
- We classified anemia according to cause into:
  1. Anemia of blood loss (chronic and acute)
  2. Anemia of decreased production
  3. Hemolytic anemia
- Then, we mentioned general causes for the anemia of decreased production, which are: nutritional deficiency, chronic inflammation, and bone marrow failure. We've already discussed the first two and now we will proceed to the last one.

Anemias resulting from bone marrow failure:

1. Aplastic anemia
2. Pure red cell aplasia
3. Myelophthisic anemia
4. Myelodysplastic syndrome

Other anemias of decreased production:

1. Anemia of renal failure
2. Anemia of liver disease
3. Anemia of hypothyroidism

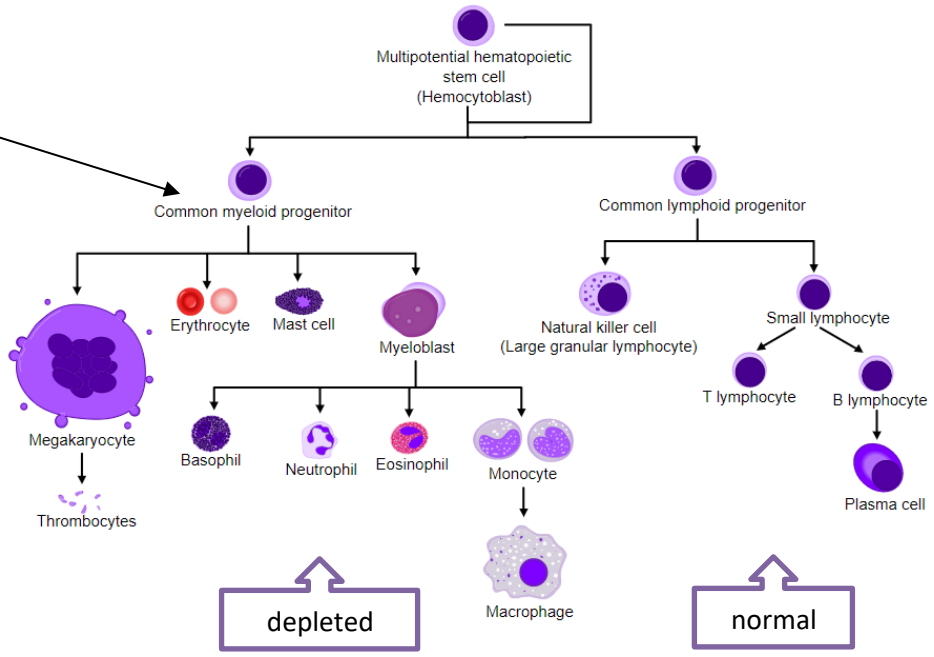
00:00

1. Aplastic anemia

Is a condition where the multipotent myeloid stem cells produced by the bone marrow are damaged.

Remember: the bone marrow has stem cells called **myeloid stem cells** which eventually differentiate into erythrocytes, megakaryocytes (platelets), and myeloblasts (white blood cells).

Notice that lymphocytes are not produced from the myeloid progenitor, therefore, they are not affected.



As a result, the bone marrow becomes depleted of hematopoietic cells. And this is reflected as **peripheral blood pancytopenia** (all blood cells -including reticulocytes- are decreased, with the exception of lymphocytes).

Pathogenesis

There are two forms of aplastic anemia according to pathogenesis:

a. Acquired aplastic anemia

It happens because of an **extrinsic factor** e.g. (drug, virus, environmental factor)

Mechanism: antibodies raised against the extrinsic factor can also react with stem cells in the bone marrow in a process called antigen cross-reactivity. And this in turn activates T-lymphocytes to destroy stem cells. What proves this theory is that immunosuppressive drugs restore bone marrow in 70% of cases.

However, it's important to mention that most cases (60-70%) are idiopathic. Whereas associated factors like chloramphenicol, gold injections (used in rheumatoid arthritis), NSAIDs (as an idiosyncratic reaction* not allergic), pregnancy, and some hepatitis viruses, consist only 30% of the cases.

*Idiosyncratic reactions are adverse effects of drugs that are rare and unpredictable.

b. Inherited aplastic anemia

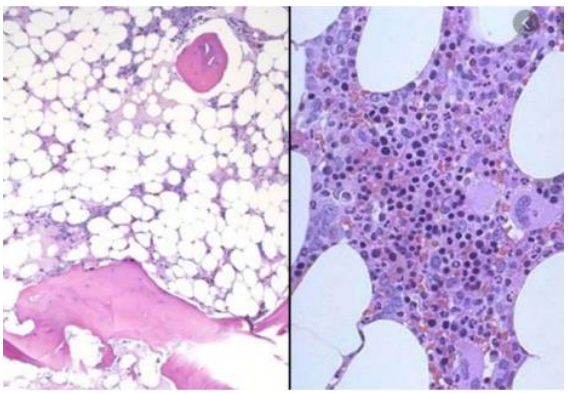
10% of aplastic anemia patients have inherited defects in telomerase enzymes, which give the stability to chromosomes. Thus, stem cells die early due to mutations.

Also, there's a suggestion that these genetically altered (defective) stem cells might express additional abnormal antigen attracting T-cells.

Lab findings

a. **In peripheral blood**: pancytopenia (anemia, leukopenia, thrombocytopenia). Anemia is normochromic or macrocytic. [Remember: problems with stem cells usually give macrocytic anemia]
b. **In bone marrow**: decreased number of hematopoietic cells.

Bone marrow depleted of hematopoietic cells, with only residual fat and some lymphocytes



Normal bone marrow cells

Patients of aplastic anemia might develop severe infections or major bleeding (due to leukopenia and thrombocytopenia, respectively.). That's why aplastic anemia is considered an emergency that needs immediate intervention.

Fanconi anemia: a rare, inherited, special form of aplastic anemia that manifests early in life during infancy and childhood as a result of a defect in DNA repair proteins. Patients develop aplastic anemia and acute leukemia in early life because of accumulated mutations.

07:13

2. Pure red cell aplasia

Less common than aplastic anemia and it affects only erythroid cells. Thus, only nucleated RBCs are absent in the bone marrow. (remember: aplasia = no production, so, only red blood cells are absent, while platelets and WBCs are normal)

This can be:

a. congenital (Diamond-Blackfan anemia)
b. Acquired *(more common)* with:
   - Autoimmune diseases e.g. systemic lupus erythematosus and rheumatoid arthritis.
   - Parvovirus B19 infection.

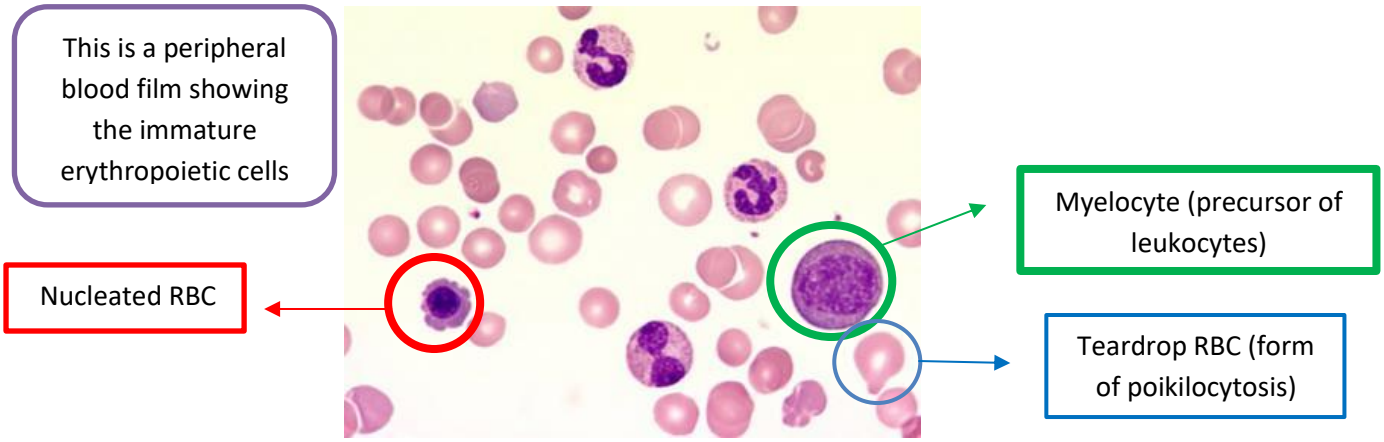
Please read the next part carefully as it's not mentioned in the video

3. Myelophthisic anemia

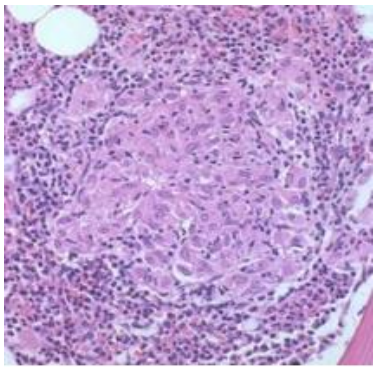
An infiltration of the bone marrow causing **physical** damage to the hematopoietic cells. These infiltrations can be:

a. Cancer: most commonly in acute leukemia, advanced lymphoma, and metastatic cancer.
b. Granulomatous disease: Tuberculosis.
c. Storage diseases: Gaucher disease (buildup of fat).

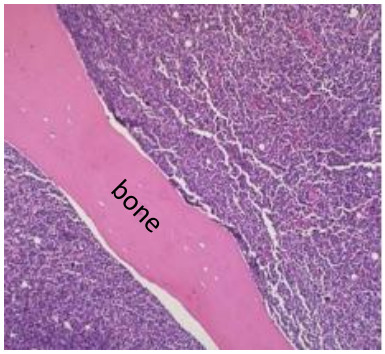
Mechanism: The bone marrow will be crowded with these infiltrations forcing the hematopoietic cells to be pushed out of the bone marrow. As a result, we commonly find immature granulocytic and erythroid precursors in peripheral blood.



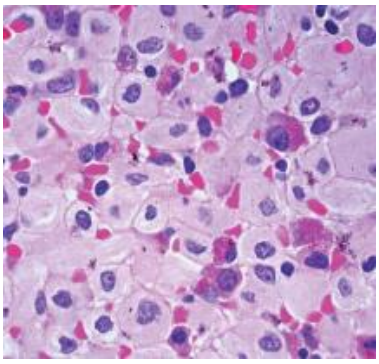
- Peripheral blood: **leucoerythroblastic anemia** (nucleated RBCs + 'left shift' which means there are high levels of immature cells).



Granuloma infiltration of the bone



Hypercellular bone marrow (but NOT erythropoietic cells)



Bone marrow full of infiltrative cells

Symptoms

- Symptoms are usually insidious with accelerated symptoms of anemia
- Thrombocytopenia manifests as skin bleeding
- Neutropenia may result in serious infections and death

So, we can say it's pancytopenia, but anemia symptoms are more evident.

09:08

4. Anemia of renal disease

Mainly results from <u>decreased erythropoietin production from kidneys</u>, due to a disease in kidneys that interferes with this function.

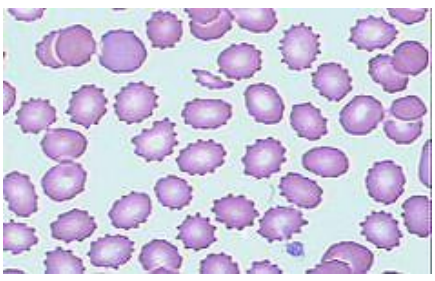
We should know that there is no correlation between kidney function and the severity of anemia. That is, if we measured serum creatinine it does not give an indication about the degree of anemia.

Extra clarification: Creatinine levels reflect kidney functions because creatinine clearance happens in the kidneys. So high levels of creatinine mean low kidney functions, but does NOT mean severe anemia and vice versa.

symptoms

- Decreased RBC production (low reticulocyte count).
- Patients with uremia develop abnormal platelets <u>function</u> (bleeding) despite <u>normal count</u>. And echinocytes (Burr cells) appear.

RBCs appear with many projections on the cell membrane



In chronic renal failure (advanced stages), kidneys are not able to excrete urea, so it accumulates in blood; a condition called uremia

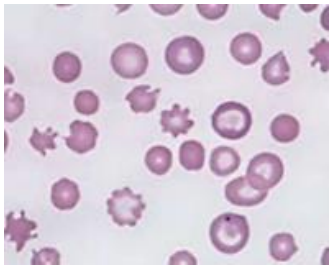
12:16

5. Anemia of liver disease

It's not a specific form of anemia but liver diseases usually result in anemia. and we need a multifactorial process to cause the anemia:

a. Decreased liver synthesis of clotting factors and this leads to bleeding.
b. Bleeding from varices (dilated veins in the GIT).
c. Decreased synthesis of transferrin.

Acanthocytes (spur cells) can be seen. They are longer and larger than burr cells. (although we can't always distinguish between the two).



Liver diseases cause abnormal metabolism of lipids, so it is thought that this causes abnormalities in the plasma membranes of the RBCs resulting in abnormal morphology.

14:23

6. Anemia of hypothyroidism

Hypothyroidism is a relatively common disease with many complications, one of which being anemia.

Mechanism: Thyroid hormones control the metabolism of many cells in the body including bone marrow cells, they also stimulate erythropoiesis and erythropoietin production. So, a deficiency in thyroid hormones can result in decreased erythropoiesis and eventually anemia.

Anemia is most commonly normocytic but can be **macrocytic**.

15:18

7. Myelodysplastic syndrome

An acquired, relatively common disease of old age.

Mechanism: It's a neoplastic disease that results in mutations in the bone marrow stem cells. However, these cells don't die early, rather they have prolonged survival but defective maturation. So, the bone marrow will be full of cells but they are unfunctional.

In most patients the anemia is refractory to treatment (doesn't respond to treatment) because the stem cells are mutant.

RBCs are **macrocytic.**

17:05

Hemolytic anemia

In hemolytic anemia, the problem is in the survival of RBCs, the normal life span of an RBC is around 120 days but in hemolytic anemia it is much less than that.

Hemolytic anemia means that there is an acceleration in red cell destruction (hemolysis).

Characteristics:

1. Bone marrow erythroid hyperplasia and peripheral blood reticulocytosis.

Anemia results in hypoxia which triggers release of erythropoietin from kidneys, which in turn causes growth of erythroid stem cells in bone marrow and increases the release of reticulocytes.

Reticulocytosis is an important difference between hemolytic anemia and anemia of decreased production

2. In severe cases, extramedullary hematopoiesis and hepatosplenomegaly occur.

The elevated erythropoietin can stimulate extramedullary hematopoiesis in the spleen and liver

3. Decreased serum haptoglobin.

Destructed RBCs release hemoglobin which is toxic for tissues, so another molecule called haptoglobin - from the liver- binds this free hemoglobin. Therefore, free haptoglobin decreases in serum.(useful for diagnosis of hemolytic anemia)

4. Development of jaundice.

Due to hemoglobin release from RBC's

General classification of hemolytic anemia:

1. According to main site of hemolysis
   a. **Extravascular**: hemolysis outside the blood stream, primarily in spleen.

RBCs that have abnormal shape or coated with antibodies are removed by macrophages. Patients have jaundice, pigmented gall bladder stones, and splenomegaly.

   b. **Intravascular:** hemolysis inside the blood stream.

A sudden release of Hemoglobin occurs, consuming all haptoglobin molecules and resulting in hemoglobinemia, hemoglobinuria, hemosiderinuria, and iron deficiency.

This type is more critical and acute compared to the first one, however it's less common. Also, serum haptoglobin levels are almost absent.

Hemoglobinemia: increased Hb in plasma

Hemoglobinuria: presence of Hb in urine, which gives it a red color.

Hemosiderinuria: presence of hemosiderin -iron storage complex- in

2. According to cause of hemolysis

a. **Extracorpuscular**: the cause is from outside the RBC e.g. antibodies or microorganisms like malaria.
b. **Intracorpuscular:** the cause is from within the RBC e.g. enzymatic deficiencies, thalassemia.

22:37

Thalassemia

A group of inherited disorders that result in decreased production of either α or β chains or rarely, both. Thalassemia is inherited in an autosomal recessive manner. This disease is common in Middle East, Africa, and South East Asia.

The normal adult hemoglobin is made up of two α chains and two β chains. Therefore, the amount of synthesized hemoglobin is below normal.

The deficiency in one of the globin chains results in relative increase in the other one, and the excessive unpaired chains will cause instability and hemolysis.

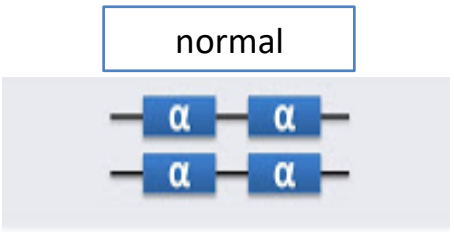
Patients of thalassemia are resistant to infection by **malaria falciparum.**

Hemoglobin types in adults:

1. HgA, accounting for 95% of the total red blood cell hemoglobin. Made of 2 α and 2 β chains. (mostly affected).
2. HgA2, made of 2 α and 2 δ. Important for diagnosis.
3. HgF, made of 2 α and 2 ɣ. least common one in adults.

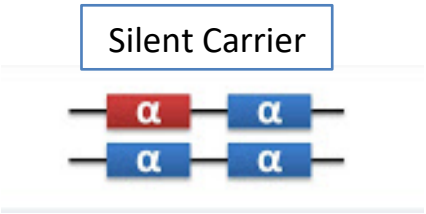
26:02

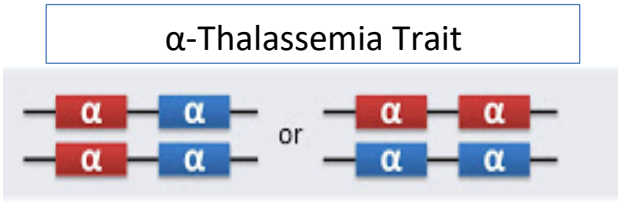
Alpha thalassemia



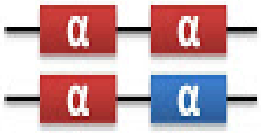
Most mutations in α-thalassemia are <u>deletion</u> of α-chain that's encoded by 2 genes on chromosome 16.

- Deletion in <u>1,2 gene(s)</u> results in a silent carrier (in both cases the patient is asymptomatic).

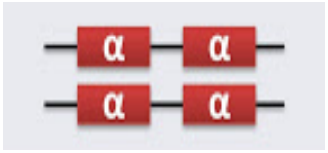




- Deletion of <u>3 genes</u> results in Hemoglobin H disease. Extra β- chains bind each other to a tetramer called HgH, and extra ɣ- chains form a tetramer called Hg-Barts. They both have high affinity to oxygen but they're still functioning. Patients will have lifelong symptomatic anemia.



- Deletion of <u>4 genes</u> results in **hydrops fetalis** that's incompatible with life, because they will not have HbA, HbA2, or HbF. Usually individuals die before or shortly after birth.



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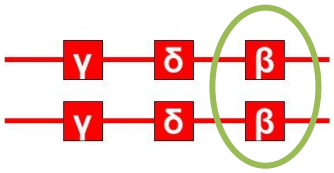
Beta thalassemia

Most mutations in β-thal are <u>point mutations</u> in the β-chain that is encoded by a single gene on chromosome 11.



Common genotypes:

Where: β0 = no production of β-chain

β+ = decreased production of β-chain

β = normal production of β-chain

- β/β+: silent carrier or mild anemia (thal-minor/ trait)
- β+/β+: thalassemia intermedia
- β0/β0 or β0/β+: thalassemia major (Cooley anemia)

There is variation in the degree of thalassemia depending on the amount of β-chains produced since there are more than 100 different mutations and every mutation gives a certain degree of deficiency.

Extra α-chains remain uncoupled, making a solid particle in the cytoplasm and causing hemolysis of RBCs and precursors (ineffective erythropoiesis).

This early hemolysis is even more serious than megaloblastic anemia because each and every RBC has this mutation.

Morphology of thalassemia

- Decreased amount of hemoglobin
- Hypochromic, microcytic anemia
- Target cells, because of abnormal hemoglobinization
- Basophilic stippling (small blue dots -remnants of ribosomes)
- Reticulocytosis

In thalassemia major:

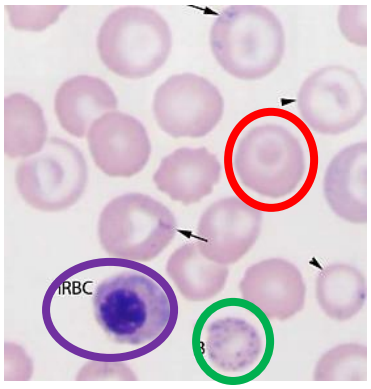
Peripheral blood: Increased poikilocytosis and nucleated RBCs.

Bone marrow: high amount of normoblasts (nRBC) filling bone marrow spaces and expanding into the bone, leading to abnormal bone growth. Bones appear enlarged, thick, and full of blood.

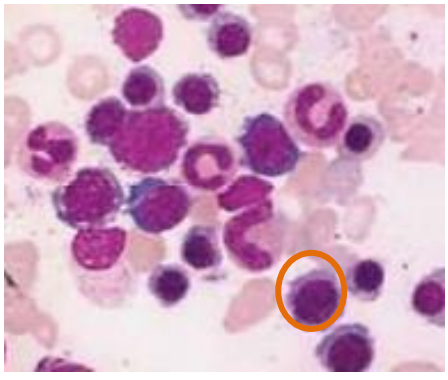
Hemosiderosis (iron overload) may occur in bone marrow and spread to other organs. It is a major and fatal complication of hemolysis.

Causes of hemosiderosis:

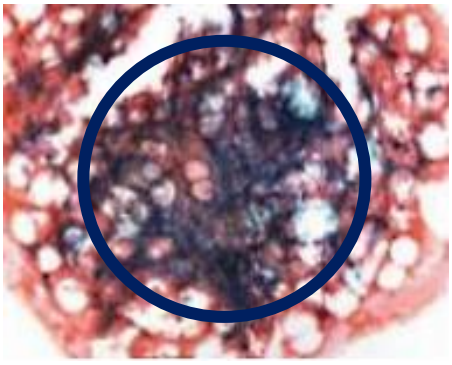
1. As RBCs release iron when they are hemolyzed, the iron does not get excreted. Instead, it accumulates in tissues.
2. High erythropoietin levels inhibit hepcidin synthesis (a molecule that inhibits absorption of iron from the GIT) which means more absorption of iron from the gut.
3. The usual treatment for thalassemia major is blood transfusion, and this also supplies the body with even more iron.



Peripheral blood film: Target cells, basophilic stippling (blue dots), and nRBC.



Bone marrow: most cells are **normoblasts** (secondary to erythropoietin). The normal bone marrow contains myeloid cells 3-4 times the number of normoblasts.



(Perls stain) The **iron** fills the entire bone marrow. Patient has hemosiderosis in bone marrow.

38:50

Clinical symptoms

- **Thalassemia traits** (carriers) are asymptomatic, they have normal lifespan, but the premarital test is important.

> In the premarital test, we can detect the abnormality by doing the complete blood count (CBC) test, the RBCs are **hypochromic and microcytic** but in a mild degree.

- **Thalassemia major**:
  Symptoms begin after age of 6 months (because the dominant hemoglobin before this age is HbF -no need for β-chains).
  Persistent symptoms of anemia, growth retardation, skeletal abnormalities, but they can be ameliorated by regular blood transfusion. However, systemic hemochromatosis and related organ damage occurs in 2nd or 3rd decade of life because of iron overload.

- **Thalassemia intermedia and HbH disease** have moderate lifelong anemia but they do not require regular blood transfusion. The degree of anemia is much less than in thalassemia major.

Bone marrow transplant is suggested as a treatment for thalassemia. (it is still not used in clinical practice).

42:24

Diagnosis

Although we have blood film and the clinical picture, the most important test is **hemoglobin electrophoresis :** [the principle is that we use a device and put the sample of the patient that contains the hemoglobin in it, then we give an electric current so it separates the different chains and can give us the percentage of each type of hemoglobin]

As we mentioned before HbA2 is used for the diagnosis:

In all types of β-thal, there is an increase in HbA2 (2α,2δ), because obviously the HbA2 doesn't need β-chains. HbF levels are also increased, however, it's not reliable as there are normal variation between individuals (people might have a higher HbF percentage normally).

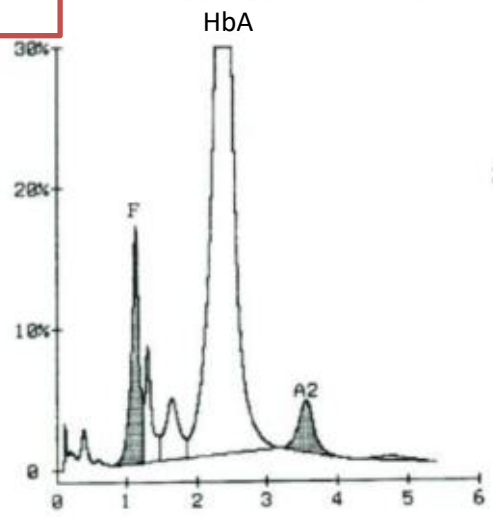
The normal range of HbA2 is up to 3.5%, if it's more than that then the patients has β-thalassemia.

In β-thal major: HbA is absent or markedly decreased.

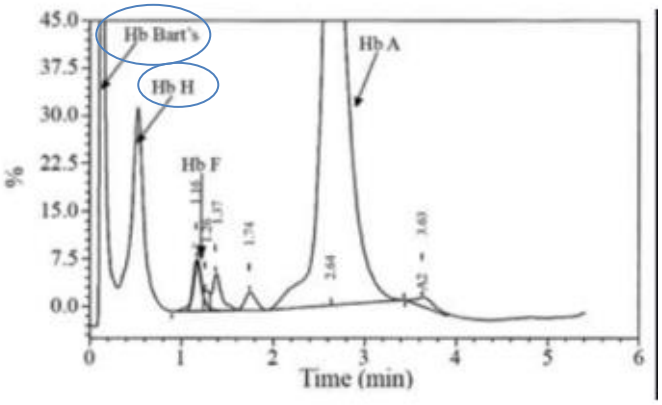
In HbH disease: HbH and Hb Barts bands appear.

ANALYTE ID	%	TIME	AREA
F	8.4	1.12	146914
P2	3.9	1.29	65767
P3	4.2	1.64	70198
Ao	77.9	2.37	1315634
A2	4.9	3.56	67100
Unknown 1	0.8	4.74	13136
	TOTAL AREA		1678749
	8.4% A2		4.9%

HbA2 is above normal = β-thalassemia



Bands of HbH and Hb-barts



In α-thal carrier and minor: no abnormality is found (Because there is a decrease in α-chains → there is an equal decrease in HbA, HbA2, and HbF → percentages stay the same)

But then, how do we diagnose it:

- mild hypochromia and mild microcytosis
- normal electrophoresis
- normal iron test

➔ we suspect α-thalassemia by exclusion. But nowadays we do a DNA genetic test

The end