Hemoglobinopathies and workup with anemia

Dr. ARWA RAWASHDEH

Hemoglobin Structure

- Hemoglobin consists of 4 subunits (2 α + 2 β in adults).

- Each subunit contains a protein (globin) and a non-protein (heme) group.

- The heme group includes iron (Fe^{2+}) bound to a protoporphyrin ring.

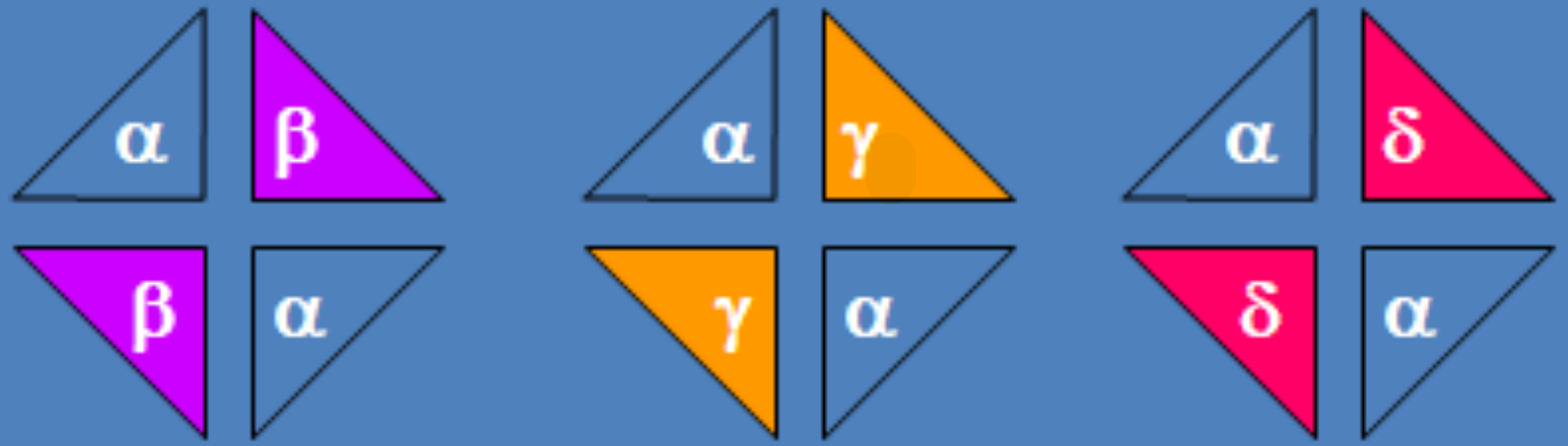
Types of Hemoglobin:

- HbA($\alpha_2\beta_2$) makes up 95% of adult hemoglobin.

- Fetal Hb (HbF, $\alpha_2\gamma_2$)is 1% in adults but dominant in fetuses.

- HbA2 ($\alpha_2\delta_2$) accounts for 1.5–3%in adults.

Hemoglobins in normal adults



HbA	HbF	HbA_2
98%	~1%	<3.5%

THE DISTRIBUTION OF DIFFERENT TYPES OF HEMOGLOBIN IN NORMAL ADULTS

Types of Hemoglobin in Adults:

1. HbA (Hemoglobin A) – 98%

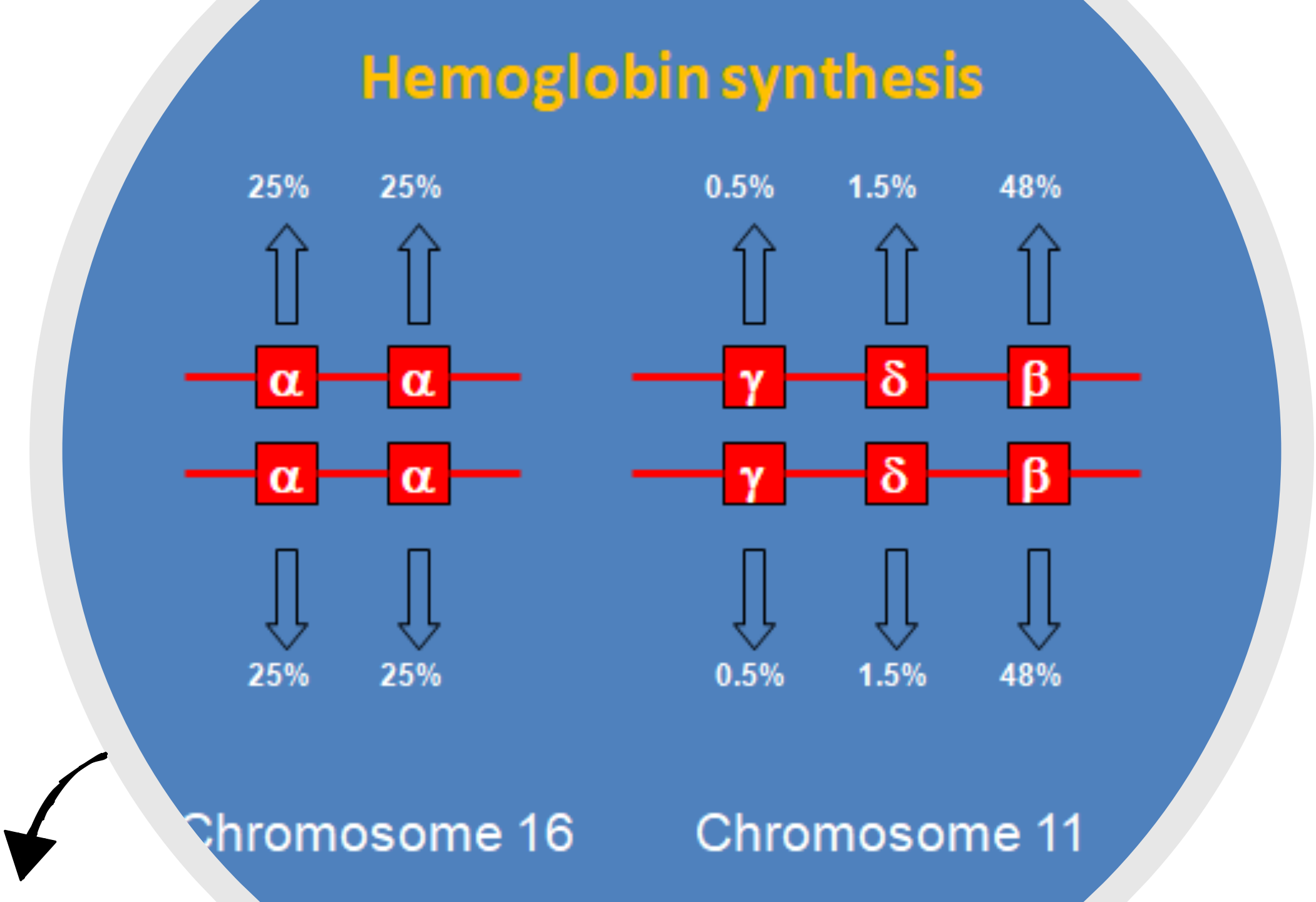
Composition: α2β2 (two alpha and two beta chains).
This is the most abundant hemoglobin in adults.
Responsible for oxygen transport in red blood cells.

2. HbF (Fetal Hemoglobin) – ~1%

Composition: α2γ2 (two alpha and two gamma chains).
This is the dominant hemoglobin in fetal life, allowing higher oxygen affinity.
Normally, its level decreases after birth, but it remains ~1% in adults.
Increased levels may be seen in conditions like beta-thalassemia or hereditary persistence of fetal hemoglobin (HPFH).

3. HbA_2 (Hemoglobin A2) – <3.5%

Composition: α2δ2 (two alpha and two delta chains).
It is a minor component in adults.
Elevated HbA_2 levels are associated with beta-thalassemia trait.


Hemoglobin synthesis
25%
25%
0.5%
1.5%
48%
α
α
α
α
γ
δ
β
γ
δ
β
25%
25%
0.5%
1.5%
48%
Chromosome 16
Chromosome 11

Hemoglobin Formation:

Hemoglobin is composed of two α-globin chains (from chromosome 16) and two non-α chains (from chromosome 11).

HbA (Adult Hemoglobin): α2β2 (major form)
HbA2: α2δ2 (minor form)
HbF (Fetal Hemoglobin): α2γ2 (dominant in fetal life, declines after birth)

* Alpha mutation is more dangerous than Beta

1. Chromosome 16:

Contains α-globin genes (α).
Each α-globin gene contributes 25% to overall hemoglobin synthesis.

Since there are two α-globin genes on each chromosome (two from each parent), they make up 50% of the hemoglobin structure.

2. Chromosome 11:

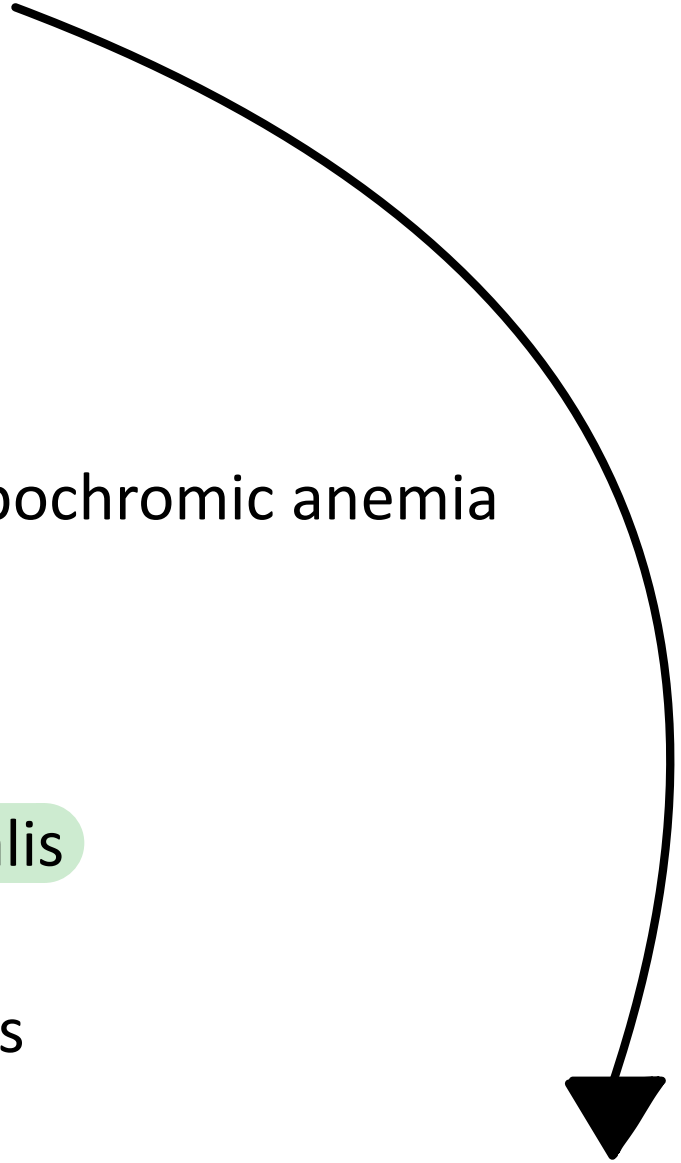
Contains γ (gamma), δ (delta), and β (beta) globin genes.
The contribution to hemoglobin synthesis varies:
γ (gamma): 0.5% (fetal hemoglobin)
δ (delta): 1.5% (minor component in adults, forms HbA2)
β (beta): 48% (major component in adults, forms HbA)

Thalassemia alpha and beta

- Autosomal recessive
- Globin
- alpha chromosome 16 2 copies at 2 loci
- 1 locus asymptomatic
- 2 locus asymptomatic minor microcytic hypochromic anemia misdiagnosed with iron deficiency
- 3 loci B4 tetramers HbH hemolytic anemia
- or Hb Barts gamma tetramers in fetals
- 4 loci not effective oxygenation hydrops fetalis

B minor is asymptomatic microcytic anemia

B major blood transfusion live max to 15 -25yrs



Thalassemia (Alpha and Beta)

Thalassemia is an autosomal recessive blood disorder caused by mutations affecting the production of globin chains in hemoglobin. It is classified into alpha-thalassemia and beta-thalassemia, depending on which globin chain is affected.

Alpha-Thalassemia

- The alpha-globin gene is located on chromosome 16, with two copies at two loci per chromosome (total of four copies).
- The severity of alpha-thalassemia depends on how many gene copies are affected:

- **One deleted locus** (Carrier State): **Asymptomatic**, no clinical impact.

- **Two deleted loci** (Alpha-Thalassemia Minor): Usually **asymptomatic** or presents with mild microcytic hypochromic anemia, often misdiagnosed as iron deficiency anemia.

- **Three deleted loci** (Hemoglobin H Disease): Formation of beta-globin **tetramers** (HbH) leads to hemolytic anemia with varying severity. In fetuses, this can also result in Hb Barts (gamma-globin tetramers), which has a high oxygen affinity but is ineffective in oxygen delivery.

- **Four deleted loci** (Hydrops Fetalis): Complete absence of alpha-globin chains, leading to severe oxygenation failure and intrauterine death.

Beta-Thalassemia

- The beta-globin gene is located on chromosome 11, with two copies (one per chromosome).
- Severity depends on whether one or both beta-globin genes are affected:

- **Beta-Thalassemia Minor** (Heterozygous Beta-Thalassemia): Usually **asymptomatic** but **may present with mild microcytic anemia**.

- **Beta-Thalassemia Major** (Cooley's Anemia, Homozygous Beta-Thalassemia): **Severe anemia requiring lifelong blood transfusions.** Without treatment, affected individuals typically **survive only 15 to 25 years due to iron overload and complications**.

Feature	Alpha thalassemia	Beta thalassemia
Gene location	Chromosome 16	Chromosome 11
Severe form	Hydrops fetalis	β-thalassemia major
Mild form	Microcytic anemia (2-gene deletion)	Microcytic anemia (β-thalassemia minor)

Key terms :-

- Deoxygenation = Low O_2 → Hgb clumps → cells sickle
- polymerization = Sticky hgb fibers form.
- Loss of deformability = cells become rigid (can't squeeze through small vessels)

Sickle cell anemia

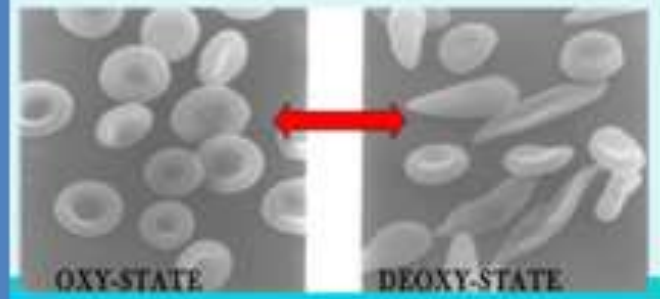
Sickle Cell Anemia

Sickle-cell anaemia
Is caused by a point mutation in the β-globin chain of haemoglobin, causing the hydrophilic amino acid glutamic acid to be replaced with the hydrophobic amino acid valine at the sixth position.

Red blood cells typically live 90–120 days, but sickle cells only survive 10–20 days.

Red Blood Cells from Sickle Cell Anemia

Deoxygenation of SS erythrocytes leads to intracellular hemoglobin polymerization, loss of deformability and changes in cell morphology



- The Hb molecules in their deoxygenated state begin to aggregate with one anther to form long sickle shaped fiber

Sickle cell anemia is a genetic blood disorder caused by a tiny change (point mutation) in the β-globin gene, which is part of hemoglobin (the protein in red blood cells that carries oxygen).

What Goes Wrong?

- **Normally, hemoglobin has glutamic acid (a water-soluble amino acid) at a key position.**
- **In sickle cell anemia, this is replaced by valine (a water-insoluble amino acid).**
- **This small change makes hemoglobin stick together when oxygen levels are low.**

What Happens to Red Blood Cells?

- **Healthy RBCs: Round, flexible, and last 90–120 days.**
- **Sickle RBCs:**
  - **When they lose oxygen, the abnormal hemoglobin forms long, stiff fibers.**
  - **This turns the cells into a sickle (C) shape.**
  - **These sickle cells are fragile and die after just 10–20 days, causing anemia.**
  - **They also block blood flow, leading to pain and organ damage.** (due to the aggregation)
- Extravascular hemolysis for the sickle RBCs

↳ hypoxia

Sickle cell anemia

- Malaria
- Autosomal recessive both parents' carrier
- B globulin , HBB gene , chromosome 11
- GLU VAL number 6
- Deoxygenated polymerization (long fibers)
- Right shift dissociation curve
- Ca influx , K and H2O outflux dehydration
- HbA sickle HbF not sickle up to 6 months
- Hydroxyurea increase HbF and not sickle
- HbS > 60%
- deoxy HbS in vein and oxy in artery
- Extravascular anemia
- Vaso – occlusive crisis

Sickle Cell and Malaria:

Sickle cell trait (HbAS) provides resistance to malaria caused by Plasmodium falciparum.

Sickle cell anemia is an autosomal recessive disorder. Both parents must be carriers for a child to inherit the disease.

Genetic Cause:

- Caused by a mutation in the HBB gene on chromosome 11, affecting beta-globin.
- Glutamic acid (Glu) is replaced by valine (Val) at position 6 of the beta-globin chain.

Pathophysiology:

- Deoxygenation causes polymerization of HbS, forming long fibers inside red blood cells.
- This leads to sickling of erythrocytes, making them rigid and fragile.
- Right shift in the oxygen dissociation curve occurs, reducing oxygen affinity.
- Calcium influx and potassium and water efflux lead to dehydration of red blood cells.
- HbS polymerizes in veins (deoxygenated state) but remains soluble in arteries (oxygenated state).

الظروف اللي لازم تتوفر لل Hb sickle حتى ترتبط مع Hb
ثاني انه لازم يكون وضع ال
RBCs in the deoxygenated state
لإنه ال Valine في حالة oxygenated Hgb بكون حاشرها
ومش مبينة داخل المركب
بينما بحالة ال deoxygenated بتكون مبينه وبسهل
ارتباطها مع valine ثاني
لهيك the sickle cell anemia ما بتصير بال Arteries
بينما بتصير بال Veins!

Clinical Features:

- Vaso-occlusive crises occur due to blocked blood vessels.
- Extravascular hemolysis leads to anemia and jaundice.
- Fetal Hemoglobin (HbF) and Treatment:
- HbF does not sickle, providing protection for up to 6 months after birth.
- Hydroxyurea increases HbF levels, reducing sickling.
- HbS is usually >60% in affected individuals.

Thalassaemia

In health, equal quantities of α- and β-globin chains are produced. Abnormalities in the transcription of either α- or β-globin genes lead to the excessive production of the other chain, and these chains may precipitate, causing haemolysis and anaemia.

The gene for the α-globin chain is duplicated on each chromosome 16, so in health, four α-globin genes exist. α-Thalassaemia results from the deletion of between one and all four genes, with an associated variation in clinical severity. The deletion of all four genes is incompatible with life.

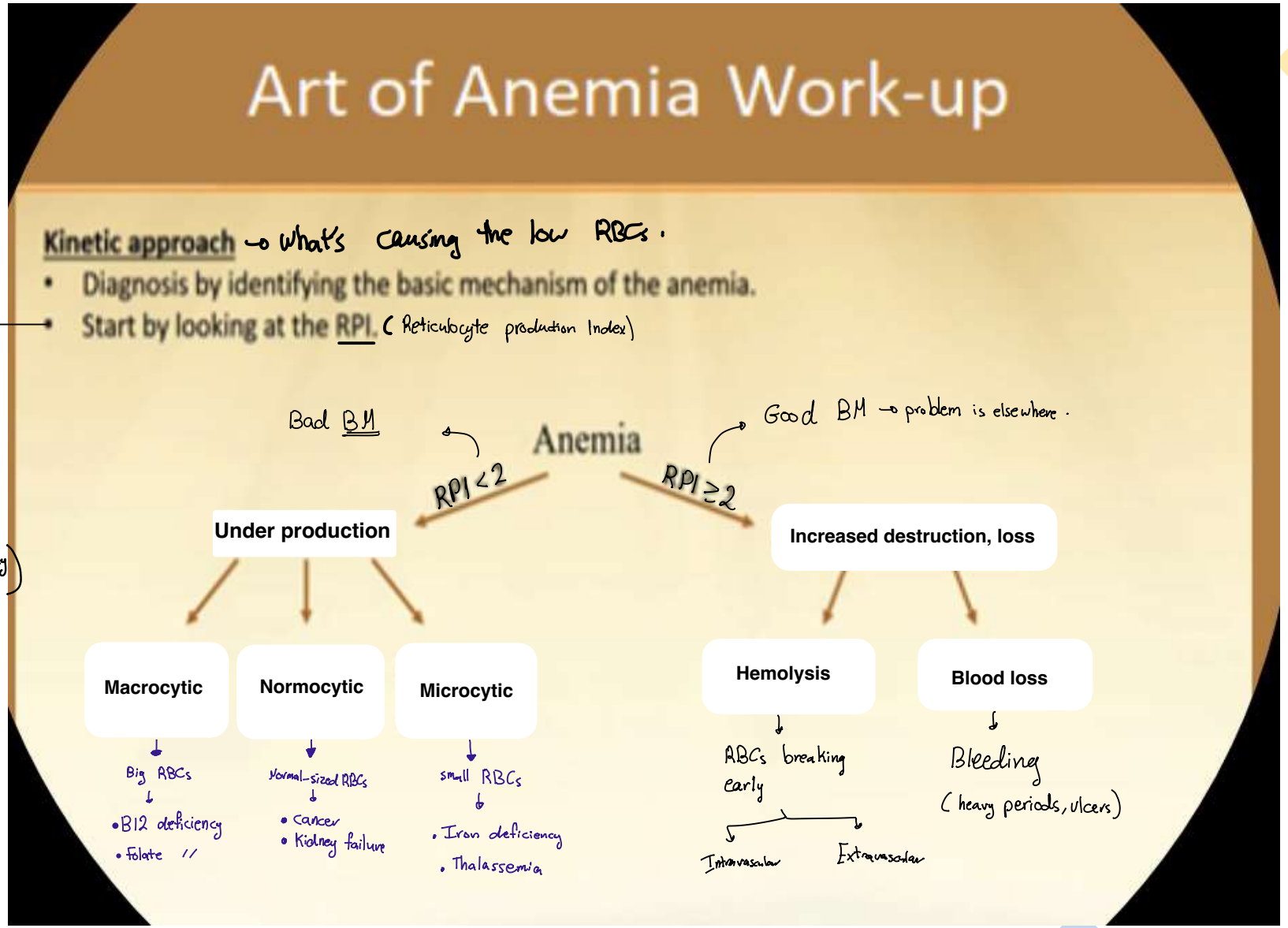
β-Thalassaemia is usually due to a single-gene mutation and results in the reduced production of β-globin chains. It normally becomes clinically apparent at between 3 and 6 months of age, when fetal haemoglobin begins to be replaced by HbA. The excess α-globin chains combine with the available β, δ, or γ chains, forming abnormal amounts of HbA_2 (δ-chains) and HbF (γ-chains

Art of Anemia Work-up

Kinetic approach → what's causing the low RBCs.

- Diagnosis by identifying the basic mechanism of the anemia.
- Start by looking at the RPI. (Reticulocyte production Index)

RPI
- High → Body is making more RBCs to fix to fix the problem (: bleeding or hemolysis)
- Low → Body is failing to produce enough RBCs (nutrient deficiency or BM disease)



Morphologic approach

- Diagnosis by observation of cell changes.
- Start by looking at the MCV.