Charting New Horizons in Education

Hemoglobinopathies and anemia work up

03

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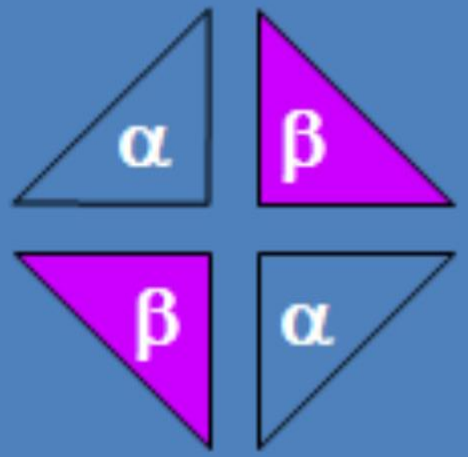
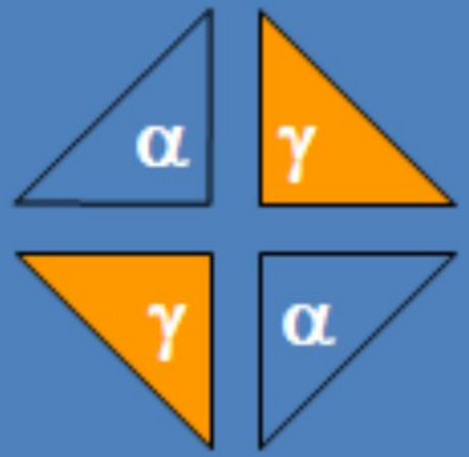
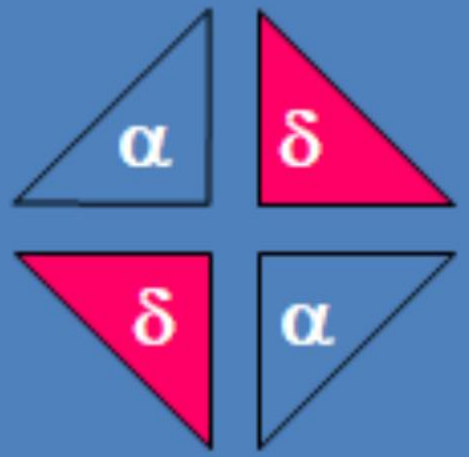
Hemoglobin structure

•**Hemoglobin (Hb) Structure**:
•**Protein Subunits**: Hemoglobin consists of four subunits, typically two alpha (α) and two beta (β) chains in adult hemoglobin (HbA).
•**Non-Protein Component (Heme)**: Each subunit contains a heme group, which is a non-protein component that binds to oxygen. The heme is an iron-containing protoporphyrin molecule.
•**Adult Hemoglobin (HbA)(α2β2):**
•**Composition**: The majority of hemoglobin in adults is HbA, which is about 95% of the total hemoglobin.
•**Subunits**: HbA is made up of two alpha (α) chains and two beta (β) chains.
•**Fetal Hemoglobin (HbF)(α2γ2):**
•**Composition**: In fetuses, hemoglobin consists of two alpha (α) chains and two gamma (γ) chains. HbF is present in small amounts after birth, but is the predominant form of hemoglobin during fetal development.
•**Percentage**: In adults, HbF is typically around 1% of total hemoglobin.
•**HbA2(α2δ2):**
•**Composition**: HbA2 consists of two alpha (α) chains and two delta (δ) chains.
•**Percentage**: The normal adult percentage of HbA2 is between 1.5% and 3%.

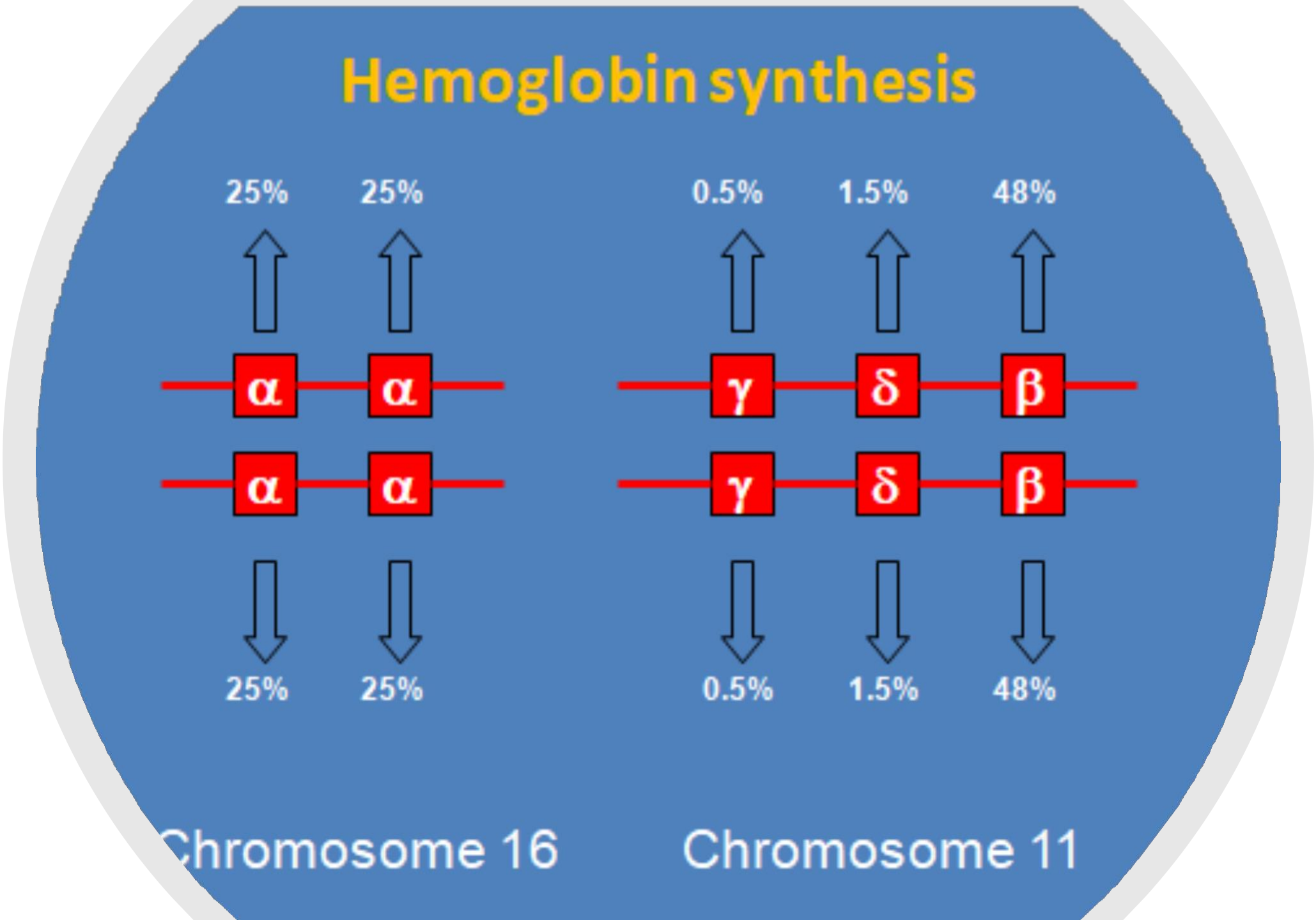
Hemoglobins in normal adults







HbA	HbF	HbA_2
98%	~1%	<3.5%


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

Alpha-Thalassemia and Its Genetic Mechanism

•The **alpha-globin genes** are located on chromosome **16**, and each individual has **two loci** (locations) for these genes—one on each chromosome 16. This means a total of **four alpha-globin gene copies** (two from each parent). The severity of alpha-thalassemia depends on how many of these copies are deleted or mutated.

Clinical Manifestations Based on the Number of Loci Affected

1. Locus Affected (Silent Carrier or Asymptomatic):

1. **Gene Deletion**: A deletion in one of the alpha-globin loci.
2. **Clinical Outcome**: This typically results in a **silent carrier state**, meaning the person is asymptomatic and does not exhibit any symptoms of anemia. It is often undiagnosed without genetic testing because it does not cause significant health issues.
3. **Severity**: No clinical symptoms.

2. Loci Affected (Alpha-Thalassemia Minor):

1. **Gene Deletion**: A deletion of two alpha-globin loci (can either be on the same chromosome or on different chromosomes).
2. **Clinical Outcome**: Often causes **mild microcytic hypochromic anemia** (small, pale red blood cells), which can be misdiagnosed as **iron deficiency anemia** because the blood test results might appear similar.
3. **Severity**: This form is usually **asymptomatic or mildly symptomatic**, but individuals may have slightly reduced hemoglobin levels and might need to be monitored. This type of thalassemia is often undiagnosed unless genetic testing is done.

3. Loci Affected (HbH Disease):

3. **Gene Deletion**: A deletion in three of the four alpha-globin genes.
4. **Clinical Outcome**: This results in the production of **HbH** (hemoglobin H), which consists of **beta-globin tetramers (B4)**. These tetramers are unstable and cannot function properly in oxygen transport. **HbH** leads to **hemolytic anemia**, where red blood cells are prematurely destroyed.
5. **Severity**: **HbH disease** can cause **moderate to severe anemia**, spleen enlargement, and jaundice due to hemolysis. Treatment may include blood transfusions to manage symptoms.

4. Loci Affected (Hydrops Fetalis):

1. **Gene Deletion**: A deletion of all four alpha-globin genes.
2. **Clinical Outcome**: This results in the absence of alpha-globin chains, and the production of **gamma-globin tetramers (Hb Barts)**. **Hb Barts** is composed of four **gamma-globin chains (γ4)** and is incapable of effectively carrying oxygen.
3. **Severity**: This leads to **hydrops fetalis**, a severe condition where there is widespread fluid accumulation in the fetus, causing heart failure and other organ dysfunctions. **Hydrops fetalis** is typically fatal, and affected babies rarely survive to birth, or they die shortly after birth.

Alpha thalassemia summary

Number of Loci Affected	Condition	Genetic Mechanism	Symptoms	Severity
1 locus	Silent Carrier	1 alpha-globin gene deleted	Asymptomatic, no anemia	No symptoms
2 loci	Alpha-Thalassemia Minor	2 alpha-globin genes deleted (heterozygous)	Mild microcytic hypochromic anemia	Mild anemia, often misdiagnosed as iron deficiency
3 loci	Hemoglobin H Disease (HbH)	3 alpha-globin genes deleted	Moderate hemolytic anemia, spleen enlargement, jaundice	Moderate to severe anemia, blood transfusions needed
4 loci	Hydrops Fetalis	4 alpha-globin genes deleted	Severe anemia, **Hb Barts** (γ4 tetramers), heart failure, organ dysfunction	Fatal, usually occurs in utero or shortly after birth

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

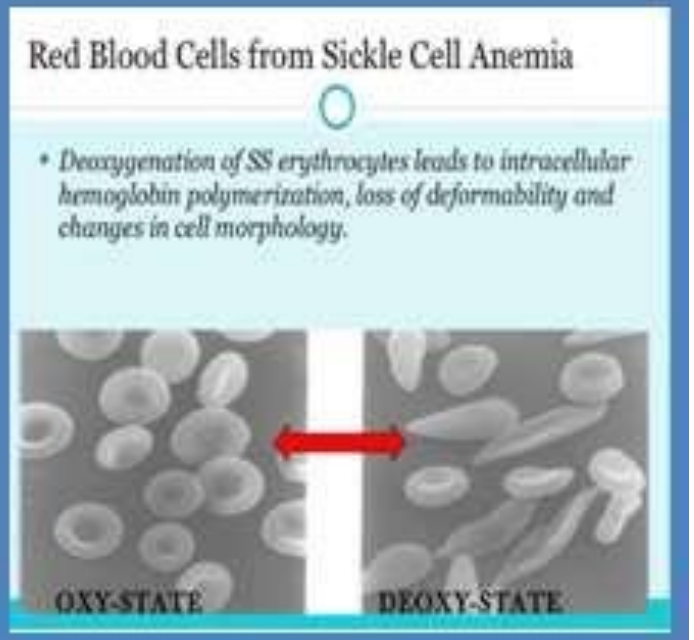
B major blood transfusion live max to 15 -25yrs

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.



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

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 anemia

Sickle Cell Anemia and Genetics

•**Inheritance**:

- **Autosomal recessive** inheritance means that both parents must be carriers (heterozygous for sickle cell trait, **HbS**) for a child to inherit the disease. If both parents carry the sickle cell trait, there is a **25% chance** for each pregnancy that the child will inherit **two copies of the mutated gene** (homozygous **HbSS**), causing sickle cell anemia.

•**HBB Gene (Beta-globin gene)**:

- The mutation causing sickle cell anemia occurs in the **HBB gene** on chromosome **11**, which encodes the **beta-globin** chain of hemoglobin.
- **Mutation**: The specific mutation involves the substitution of **glutamic acid (GLU)** with **valine (VAL)** at position **6** of the beta-globin chain (β6). This single amino acid change significantly alters the structure of hemoglobin.

Sickle cell anemia

Mechanism of “sickling”:

- **Deoxygenated Hemoglobin Polymerization**:
  - Under low oxygen conditions, the mutated hemoglobin (**HbS**) undergoes **polymerization**—it forms long, rigid fibers. This polymerization causes the red blood cells to become **rigid** and **sickle-shaped** (instead of the normal biconcave disc shape).
  - The sickle-shaped cells are less flexible, causing them to block small blood vessels and impede normal blood flow.
- **Right Shift of Oxygen Dissociation Curve**:
  - Sickle cell hemoglobin (**HbS**) causes a **rightward shift** of the oxygen dissociation curve. This means hemoglobin has a decreased affinity for oxygen, releasing oxygen more readily but also making it harder for oxygen to be carried effectively in the blood.
  - The **right shift** is typical of **hypoxia** (low oxygen levels), which exacerbates sickling under conditions of low oxygen.
- **Cellular Changes**:
  - **Calcium (Ca^{2+}) influx** and **potassium (K^{+})** and **water (H_2O) outflux** occur in sickle red blood cells, leading to dehydration and further making the cells more prone to sickling.
  - This process also contributes to **extravascular hemolysis**—the premature destruction of sickled red blood cells, primarily in the **spleen** and **liver**.
- **Hydration Issues**:
  - Dehydration exacerbates the sickling process, making the cells more likely to polymerize and "stick" together, blocking blood flow.

Sickle cell anemia

Fetal Hemoglobin (HbF) and Sickle Cell Disease

•**HbF (Fetal Hemoglobin)**:

- In newborns, the predominant hemoglobin is **HbF** (composed of alpha and gamma globin chains). **HbF does not sickle** because it has a higher affinity for oxygen and does not polymerize under low oxygen conditions.
- **Up to 6 months**, the high levels of HbF may protect against some sickling, which is why infants typically have milder symptoms until their HbF levels decrease.

•**Hydroxyurea**:

- **Hydroxyurea** is a drug that increases the production of **HbF**, effectively reducing the percentage of **HbS** in red blood cells and decreasing the frequency and severity of sickle cell crises.
- It does not directly "reverse" sickle cell disease, but it helps prevent the **sickling** of cells by increasing **HbF**, which **does not sickle**.

Deoxygenated vs. Oxygenated Hemoglobin

•**Deoxygenated HbS**: In veins, where oxygen levels are lower, **HbS** polymerizes and forms **long, rigid fibers**, leading to sickled red blood cells that obstruct blood flow and cause pain and organ damage.

•**Oxygenated HbS**: In arteries, where oxygen is abundant, the hemoglobin is in its **oxygenated form**, and the red blood cells are usually not sickled. However, when they reach tissues that are low in oxygen, the sickling occurs again.

Sickle cell anemia

•**Sickle Cell Disease (HbSS)**:
•Individuals with **HbSS** (homozygous sickle cell) produce predominantly **HbS** (sickle hemoglobin), and their red blood cells sickle when oxygen levels are low.
•**HbS > 60%**: In a person with sickle cell anemia, more than **60%** of their hemoglobin is **HbS**.
•**Vaso-Occlusive Crisis**:
•A **vaso-occlusive crisis** (or **pain crisis**) is one of the hallmark features of sickle cell disease. It occurs when sickled red blood cells block small blood vessels, leading to pain and ischemia (lack of blood flow) in various parts of the body.
•This can affect bones, joints, the spleen, lungs, and kidneys, and can result in severe **pain crises**.
•**Extravascular Hemolytic Anemia**:
•**Extravascular hemolysis** occurs when sickled red blood cells are prematurely destroyed in the spleen and liver. This leads to **anemia** (low red blood cell count) and other complications such as **jaundice** (yellowing of the skin due to high bilirubin levels) and **splenomegaly** (enlarged spleen).
•**Malaria and Sickle Cell Disease**:
•Individuals with **sickle cell trait (HbAS)** or **sickle cell disease (HbSS)** have some protection against **malaria**. The **Plasmodium falciparum** parasite, which causes malaria, has a harder time infecting sickle-shaped red blood cells, giving individuals with sickle cell a selective advantage in malaria-endemic regions.
•This is why **sickle cell trait** has remained prevalent in malaria-prone areas like sub-Saharan Africa.

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.

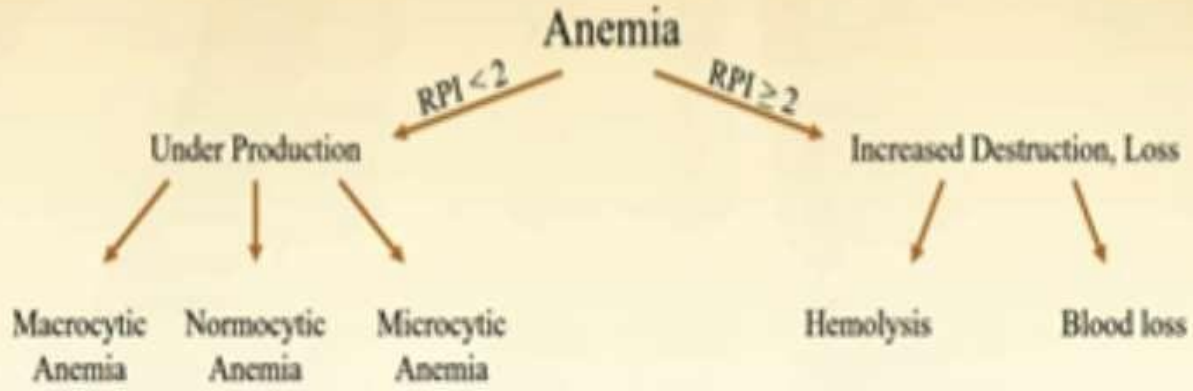
The gene for the α-globin chain is duplicated on each chromosome 16, so in health, four α-globin genes exist. α-Thalassaemia results from the deletion of between one and all four genes, with an associated variation in clinical severity. The deletion of all four genes is incompatible with life.

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

- Diagnosis by identifying the basic mechanism of the anemia.
- Start by looking at the RPI.



Morphologic approach

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