بسم الله الرحمن الرحيم

Physiology

﴿ وَقُل رَّبِّ أَدْخِلْنِي مُدْخَلَ صِدْقٍ وَأَخْرِجْنِي مُخْرَجَ صِدْقٍ وَاجْعَل لِّي مِن لَّدُنكَ سُلْطَانًا نَّصِيرًا ﴾

ربنا آتنا من لدنك رحمة وهيئ لنا من أمرنا رشدًا

MID | Lecture 2

Introduction: Red Blood Cells, Anemia and polycythemia (Pt.2)

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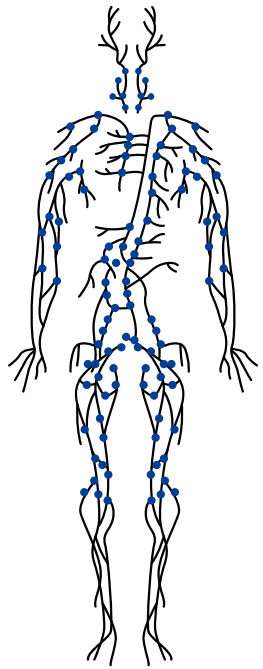


Hemoglobin and Hematocrit

- 280 million /RBC
- Normal hemoglobin concentration is 34 g per 100 ml of packed cells
- Hemoglobin constitutes 33% of RBC weight
- Normal hematocrit ("packed cell volume") is 40-45% (slightly lower in women)
- Thus normal hemoglobin is 14-15 g per 100 ml of blood
- When hemoglobin is measured in 100 ml packed cells only (RBCs) it's condensed, but if it's measured per 100 ml of whole blood then the number will be almost the half (because the hematocrit is 40-45%)
- O_2 carrying capacity is 1.34 ml / g Hgb, or 19-20 ml O_2 / 100 ml blood
- transports 25% CO2

The doctor said that we need to identify different units
You don't have to memorize numbers but you should be able to answer questions when you're given the normal values

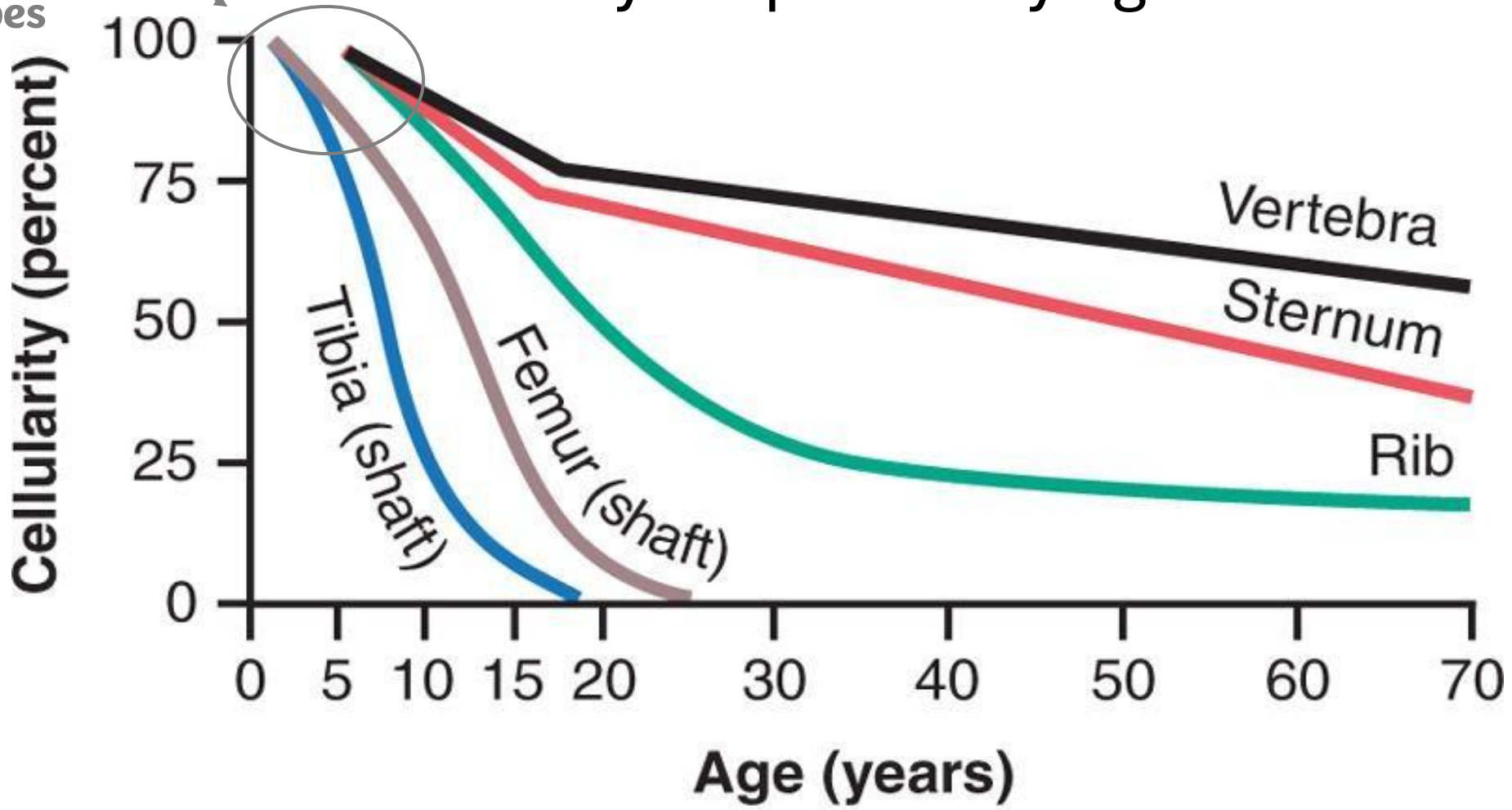
Sites of Erythropoiesis

- Hematopoiesis: production of all blood cell types in the bone marrow.
- Erythropoiesis: production of erythrocytes, it's a continuous process that starts before we are born until death, with continuous destruction because they have limited lifespan (so they have to be continuously replaced).

- First few weeks of gestation – RBCs production takes place in the yolk sac
- Mid-trimester – mainly in the Liver (+ spleen, lymph nodes)
- Last month of gestation through adulthood – mainly in the Bone marrow

From 0-5 years almost 100% cellularity in all bone types

Sites of Erythropoiesis by Age



See the next slide for further explanation

Further explanation of the previous slide

In the graph, we have the age in years on the x-axis & the cellularity on the y-axis.

Cellularity of the bone marrow: indicates the proportion of RBC-producing cells in the BM so it's a reflection of the activity of erythropoiesis, the higher the cellularity the higher the activity will be.

- Before the age of 5

The marrow of all bones (long & short) produces RBCs .

- After the age of 5

There is a decline in the production (& cellularity) of RBCs in the marrow of long bones (tibia & femur shafts with preserved cellularity in their heads) until the age of 20 .

- After the age of 20

RBCs production takes place only in the marrow of short membranous bones (vertebra, sternum, ribs, ilia…) which become the main sites for erythropoiesis.
While in the long bones, RBCs-producing cells are replaced by fat cells so they don't have active production of RBCs.

Generally, cellularity decreases with age in all bone types but that doesn't mean that we won't be able to produce RBCs (this is just a measure of cellularity).

Hematopoiesis

- **Pluripotent hematopoietic stem cells (they're present in the bone marrow after birth) give rise sequentially to committed stem cells and mature cells.**

- Pluripotent hematopoietic stem cells are undifferentiated & have no identity so they can differentiate to all blood cell types, but when they further proliferate & differentiate they become committed .
- Committed stem cells: they are still stem cells (have no identity) but they are committed to produce certain type of blood cells (if they are cultured they give their specific line).

- **Driven by**
  - **Growth inducers (factors; e.g. *interleukin-3*):** they induce increase in size or number.
  - **Differentiation inducers:** they induce cell differentiation to become more specific to its final mature cell (through the expression of certain genes & producing certain proteins & organelles required to perform the cellular functions).

- Rate of **Hematopoiesis responds to changing conditions** including;
  - **Hypoxia** (low tissue oxygen level): increases the rate of **erythropoiesis** (in order to compensate this low oxygen level).
  - **Infection / inflammation: WBC production** (some infections may increase the production of a certain line of WBCs).

Blood Cell Lineages

This figure explains the different cell lines that are derived from the original hematopoietic stem cell (MHSC)

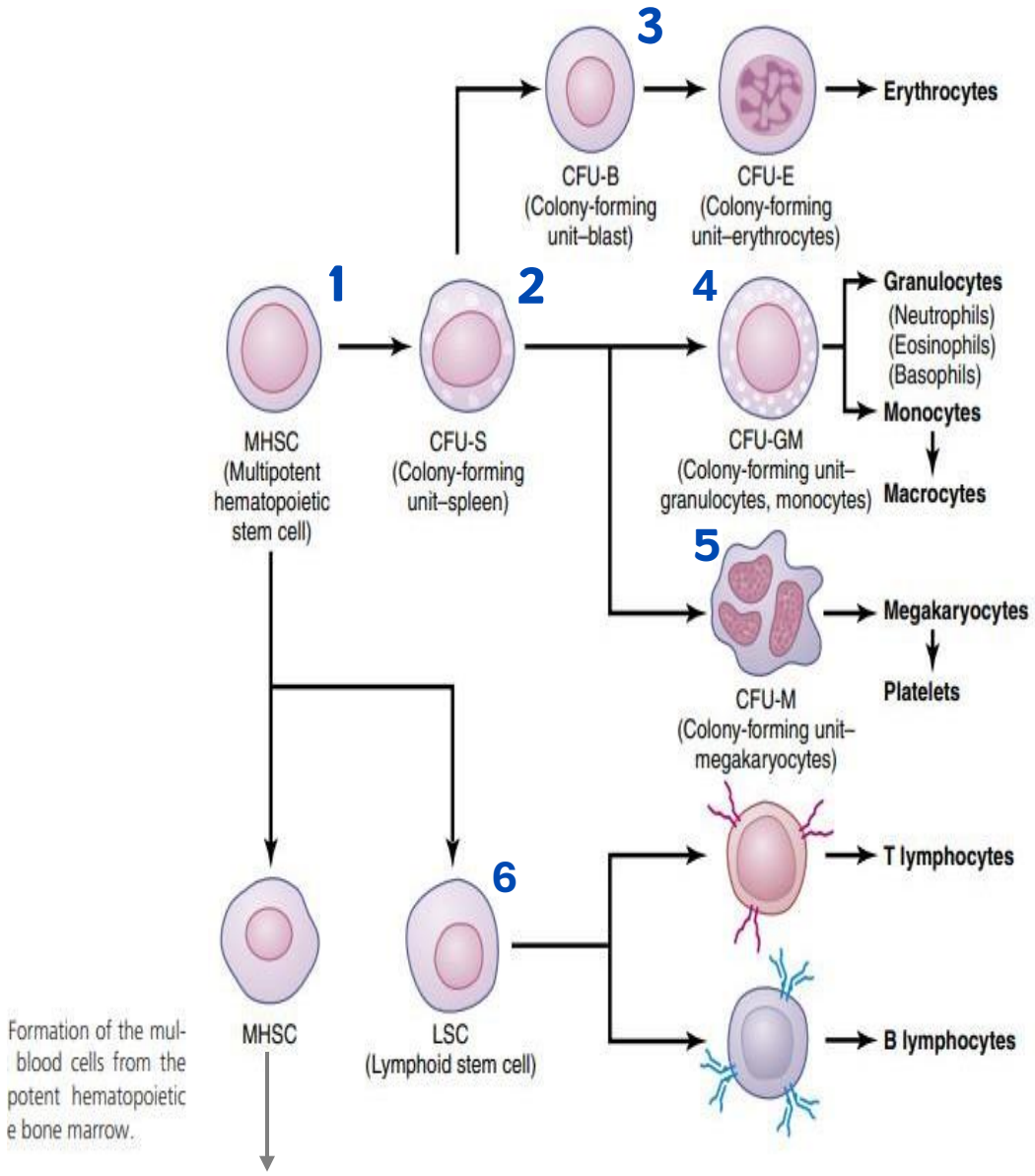
1. MHSC: will further differentiate into two main stem cells (more committed stem cells; CFU-S & LSC).

2. CFU-S: called colony-forming unit because it proliferates to high number of same cells, also called myeloid stem cell, it will only give rise to one of three cell types (CFU-B, CFU-GM & CFU-M).

3. CFU-B: will further differentiate into CFU-E that will finally give rise to a mature erythrocyte.

4. CFU-GM: (another committed stem cell) this will either give granulocytes (neutrophils, eosinophils or basophils) or monocyte that will be a mature macrophage later on.

Please refer to the figure while going through the text



As these cells reproduce, a small portion of them remains exactly like the original MHSCs & is retained in the bone marrow to maintain their supply

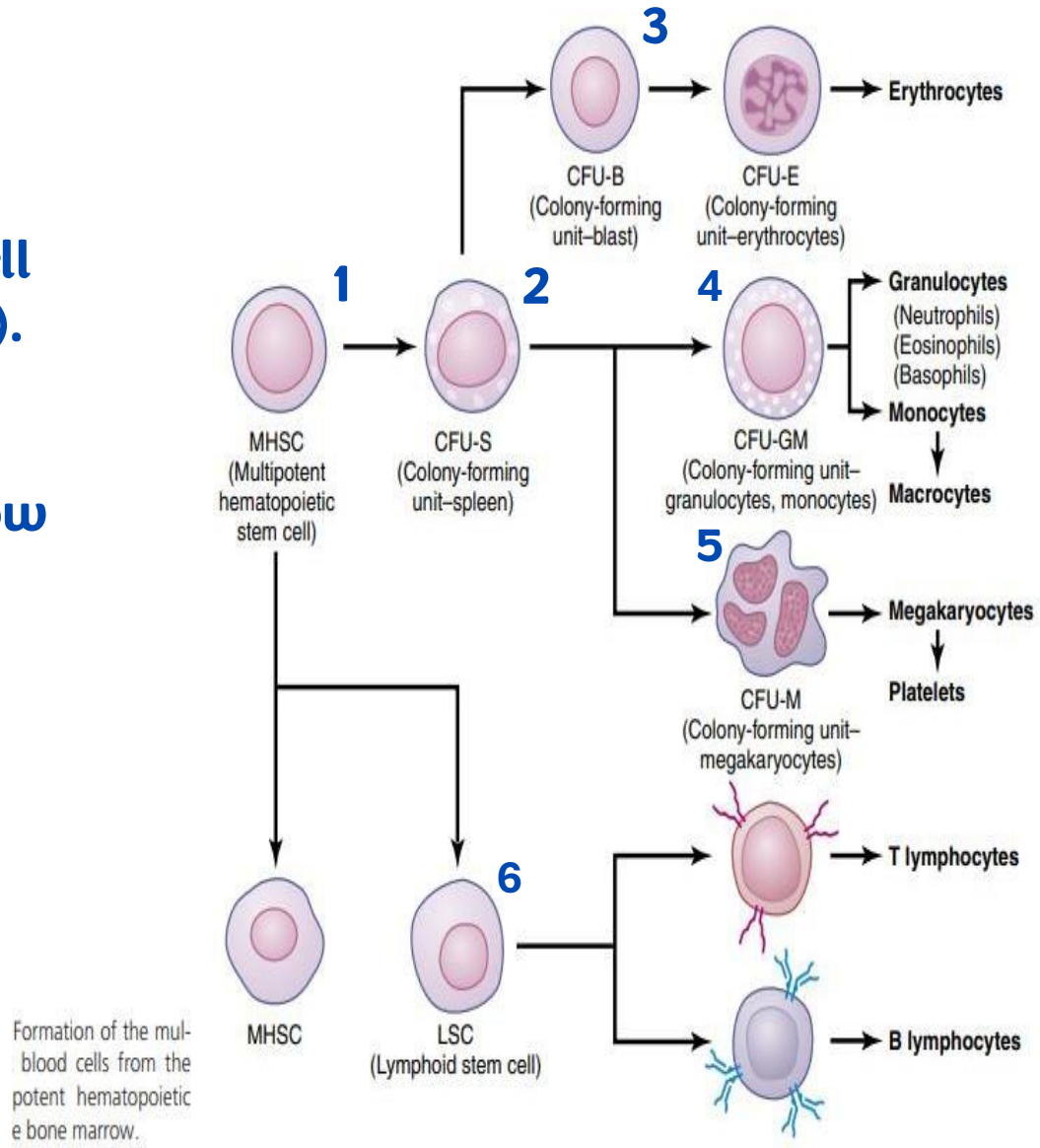
Blood Cell Lineages

5. CFU-M: this gives rise to megakaryocyte (mature cell that undergoes fragmentation & gives rise to platelets).

*** The previous cells (3-5) originate from the myeloid stem cell (CFU-S), once they get out of the bone marrow they don't divide (they completed their division in the BM) & they are semi-mature** (will be discussed later).

6. LSC: it gives rise to B & T lymphocytes & <u>unlike myeloid-derived cells, lymphocytes can proliferate outside the bone marrow</u> (in the lymphoid tissues).

Please refer to the figure while going through the text



Formation of the mul- blood cells from the potent hematopoietic e bone marrow.

Erythropoiesis and Anemia

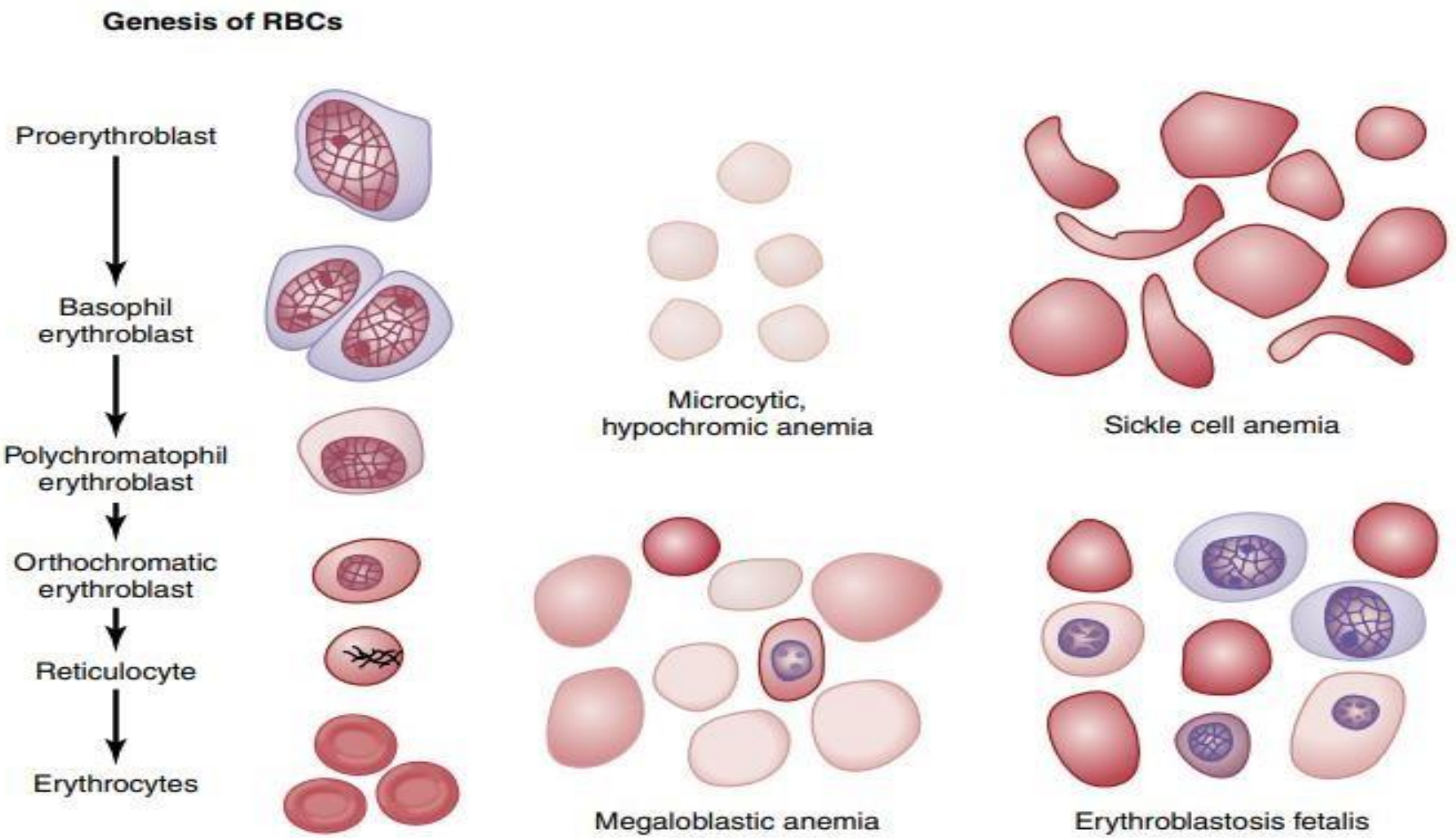


Figure 33-3. Genesis of normal red blood cells (RBCs) and characteristics of RBCs in different types of anemias.

See the next slide for further explanation

Further explanation of the previous slide

There are several stages from CFU-E until we reach the final mature erythrocyte.

- Proerythroblast is still nucleated with a basophilic cytoplasm, since it's blast it can divide & increase in number.
- When it starts to have red color it's called polychromatophil erythroblast (polychromatic: more than one stain), this is the first cell where hemoglobin starts to appear.
- Nucleus decrease in size (atrophy) & the cytoplasm becomes more eosinophilic (more hemoglobin) & some of the organelles will start to be reabsorbed & the nucleus will be extruded, in the reticulocyte stage we only have remnants Golgi & ER, now reticulocytes are ready to leave BM to the circulation by diapedesis through the sinusoids in the BM.
- These reticulocytes aren't considered fully mature so they require about 1-2 days until they reach the final normal shape of erythrocytes.
- The percentage of reticulocytes represents 1-2% of all RBCs in the blood & they reflect the newly produced RBCs (within two days they mature), this percentage is clinically important because it reflects the activity of the BM especially in cases of BM stimulation like anemia or haemorrhage, if this percentage is increased this indicates that the BM is active & is responding to the stimulus .

  But if the percentage is low despite having anemia or haemorrhage this indicates a problem in the BM, erythropoietin or any step of erythropoiesis .

Erythropoiesis and Anemia

These different forms of RBCs can indicate certain diseases:

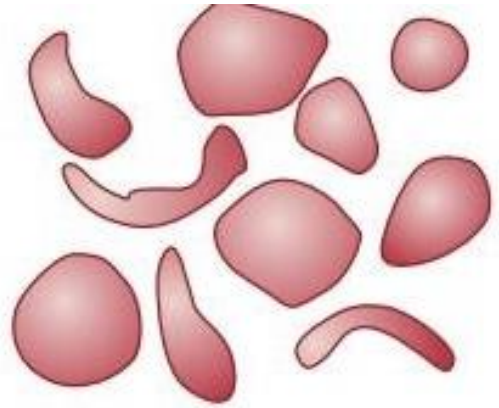
Microcytic hypochromic anemia: small & pale red RBCs can indicate low content of Hgb & this reflects low iron level.

Megaloblastic anemia: RBCs are large & they have abnormal nucleus also they're more fragile than the normal RBCs, this anemia results from vitamin B12 & folate deficiency which are important for RBCs maturation of the DNA & the nucleus (which can lead to abnormal division).

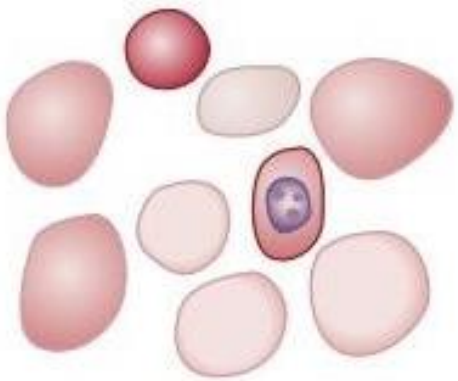
Sickle cell anemia: Malformation of hemoglobin due to a genetic disease.

Erythroblastosis fetalis: Will be discussed later.

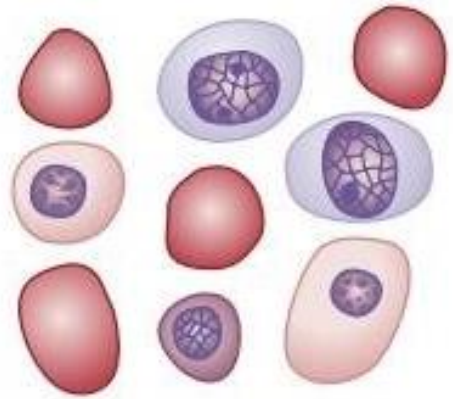
Microcytic, hypochromic anemia



Sickle cell anemia



Megaloblastic anemia



Erythroblastosis fetalis

Regulation of Red Cell Production

We can't produce higher than what we need of RBCs
Why? There is only enough number of RBCs that can cover our need, not higher, not lower

- So Red blood cell mass (hematocrit) is regulated within a relatively narrow range to:
- Maintain adequate oxygen carrying capacity
- Avoid excessive blood viscosity, if the production increases (polycythemia) , that may lead to increased resistance and impede blood flow (more sluggish blood flow)
- If the bone marrow is damaged (due to chemotherapy or radiotherapy) or if demand for erythropoiesis is extreme (due to hypoxia) , other parts of the bone marrow may become hyperplastic (increase in number in a contained space) , or *extramedullary hematopoiesis* may occur.
- Extramedullary hematopoiesis: RBCs formation in sites outsides the BM, like the liver or lyphmatic tissues (sites of early stages of hematopoiesis) .

Tissue O2 and Erythropoietin

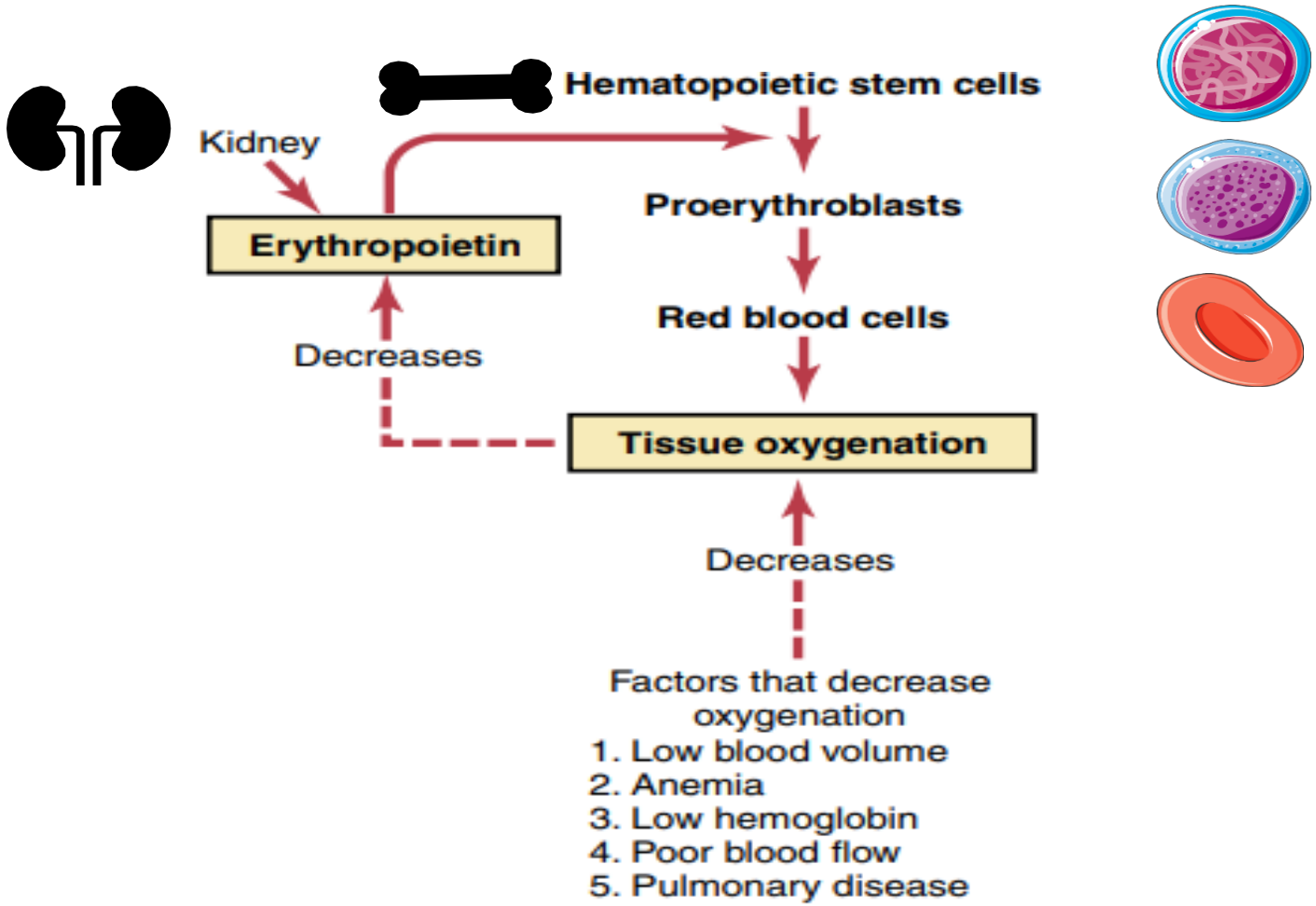


Figure 33-4. Function of the erythropoietin mechanism to increase production of red blood cells when tissue oxygenation decreases.

See the next slide for further explanation

Further explanation of the previous slide

Feedback mechanism of regulating RBCs production:

✓ **The key factors are hypoxia & erythropoietin.**

Erythropoietin: a hormone is mainly produced by the kidney (in fibroblasts or epithelial tubular cells), that is responsible for regulating the rate of RBCs production, kidney cells respond to hypoxia by increasing the production of erythropoietin into the circulation where it can affect the sites of erythropoiesis the BM, stimulating the production of proerythroblast from the hematopoietic stem cells & increasing their rate of proliferation & differentiation and thus increasing the number of RBCs.

Any condition that decreases tissue oxygenation will be sensed by kidney cells to increase erythropoietin production , these conditions can be:

1. **Low blood volume : due to hemorrhage (blood loss)**
2. **Anemia: insufficient RBCs or Hgb**
3. **Low hemoglobin: insufficient transportation of O2**
4. **Poor blood flow : due to stenosis , thrombosis, heart failure…**
5. **Pulmonary Disease : decreases normal tissue oxygenation**

- **For example: patients with asthma or any respiratory disease will have higher RBCs level** (don't forget the relationship between these conditions & erythropoiesis).

Clinical Perspective

Compensatory Polycythemia

=Secondary polycythemia

- **Sustained hypoxia can result in red cell mass above the usual normal range…**

- **Causes of polycythemia :**
- **Prolonged stay at high altitude** (physiological) , lower atmospheric oxygen levels will lead to hypoxia and thus more RBCs production.
- **Lung disease** like chronic asthma.
- **Heart failure:** low O2 thus compensation occurs to increase the number of RBCs.

Erythropoietin (EPO)

- **Circulating hormone, mw ~34,000 kDalton**
- **Necessary for erythropoiesis in response to hypoxia, when there is a hypoxia there will be increase in production of erythropoietin.**
- **~90% made in the kidney mainly**
- **10% of erythropoietin can arise from the liver**
- **Cells of origin not established.**
- **The signaling that is responsible for stimulating erythropoietin transcription is hypoxia.**

Hypoxia → *HIF-1* → binds hypoxia response element → *EPO* transcription

***HIF-1: hypoxia inducable factor , it's a gene expression promoter.**

- **Without erythropoietin, only few RBCs are produced, so a basal level of erythropoietin is required even if no hypoxia (because of the continuous destruction).**

Erythropoietin (cont'd)

- If the kidney is removed by nephrectomy or other reasons like cancer or kidney failure due to damage, so the body will mainly have only the liver erythropoietin, this isn't enough to support the normal RBCs production.

- **Extra-renal hypoxia** (hypoxia outside the kidney, like certain organ) **can stimulate EPO production** from the kidney.
  - Some factors (hormones) are activated and stimulated in that organ including, **epinephrine, norepinephrine, and some prostaglandins can promote EPO production** in the kidney.

- **In anephric or in kidney failure; severe anemia ????**

- **In anephric individuals, 10% residual Epo (mainly from liver), supports 30-50% needed RBC production…**
- **Hematocrit (packed cell volume) ~23-25% rather than 40- 45%,** this isn't enough, we need to replace it with erythropoietin for patient with kidney failure.

Response to Hypoxia

- Within **Minutes to hours** of hypoxia, it will lead to ↑**Erythropoietin**
- **New circulating reticulocyte** (new RBCs) will appear from **(3-5) days.** 
  (RBCs number won't increase for at least 5 days until the final increase in number of RBCs counts appears, but erythropoietin increases from first day).
- **Erythropoietin** is important because it :
  - drives production of proerythroblasts from HSCs (Hematopoietic Stem Cells).
  - accelerates their maturation into RBCs, both growth & differentiation .
- **EPO** can increase RBC production up to 10-fold, so it is considered very powerful .
- **Erythropoietin remains high until normal tissue oxygenation is restored,** due to negative feedback. When oxygenation is normal, the EPO level decreases because we don't need more than necessary.

Vitamin B_{12} and Folic Acid

Requirements of RBCs production

They are also important during early pregnancy.

- **Rapid, large-scale cellular proliferation requires optimal nutrition**
- **Cell proliferation requires DNA replication,** and requires normal level of vitamin B12 and folic acid,if there is a lack of them, this will lead to abnormal DNA replication. Any cell needs to replication, it needs to replicate its DNA to get normal proliferation and division.
- **Vitamin B_{12} and folate both are needed to make thymidine triphosphate (thus, DNA)**
- **Abnormal DNA replication causes failure of nuclear maturation and cell division ➡ *maturation failure* ➡ *large, irregular, fragile,* *abnormal, bizarre shaped* *"macrocytes"* , but the macrocyte still has hemoglobin and it can carry oxygen but its membrane is fragile, has a short life span, thus the person will have anemia "megaloblastic anemia ".**

Clinical Perspective

Pernicious Anemia

- **Failure to absorb vitamin B_{12}**
- **Atrophic gastric mucosa…**
  - **Failure to produce *intrinsic factor,* which is secreted from gastric secretion.**
- **Intrinsic factor binds to vitamin B_{12}, and facilitate its absorption.**
  - **Protects it from digestion**
  - **Binds to receptors in the ileum where the absorption occurs.**
  - **Mediates transport by pinocytosis**
- **Vitamin B_{12} is largely stored in liver, released as needed**
- **Usual stores: 1 – 3 mg Daily needs: 1 – 3 µg**
- **Thus normal stores are adequate for 3 – 4 years until anemia appears.**

Clinical
Perspective

Folic Acid Deficiency

- **Folic acid is present in green vegetables, some fruits, and meats like liver.**
- **Destroyed during cooking**
- **Subject to dietary deficiencies**
- **May also be deficient in cases of intestinal malabsorption**
- **Maturation failure may reflect combined B_{12} and folate deficiency**

Formation of Hemoglobin

- **Occurs from proerythroblast through reticulocyte stage**
- **Reticulocytes retain a small amount of endoplasmic reticulum and mRNA, supporting continued hemoglobin synthesis**

Shapes of RBC and Hemoglobin

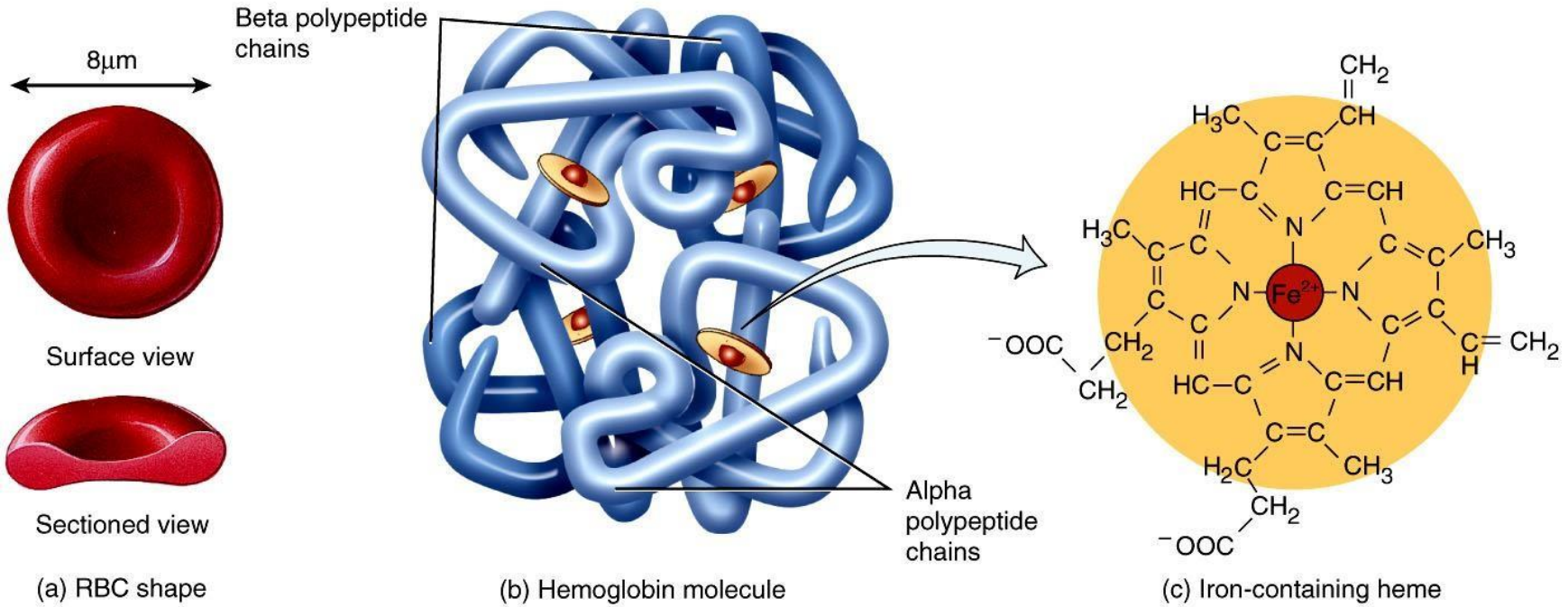


Figure 19.04 Tortora - PAP 12/e

Physiology Quiz 2

QUIZ

External Resources

1. Guyton & Hall textbook of medical physiology chapter 33 (p 439-443)
2. Blood cell lineages
3. Erythropoiesis

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			