Hematology

HISTOLOGY

☑ Sheet ☐ Slide ☐ Handout

Number: **3**

Subject: **Hematopoiesis**

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Price:

Topics to be discussed in this sheet:

1. Hemopoiesis (hematopoiesis):
   - Erythropoiesis
   - Granulopoiesis
2. Functions and components of bone marrow

❖ Hemopoiesis
The production and maturation of blood cells.

- The stem cell that produces all types of blood cells is called **pluripotent.**
- Stem cell divides and differentiates into **progenitor** cell
- **Progenitor cell** also divides and differentiates into **precursor** cell that gives rise to **mature** cell.

Stem cell works through two different production lineages (cell lineages);

- **lymphoid lineage** produces lymphocytes (lymphoid cell)
- **Myeloid lineage** produces the rest; granulocytes, monocytes, erythrocytes and megakaryocytes. Megakaryocytes in turn produce **platelets**.

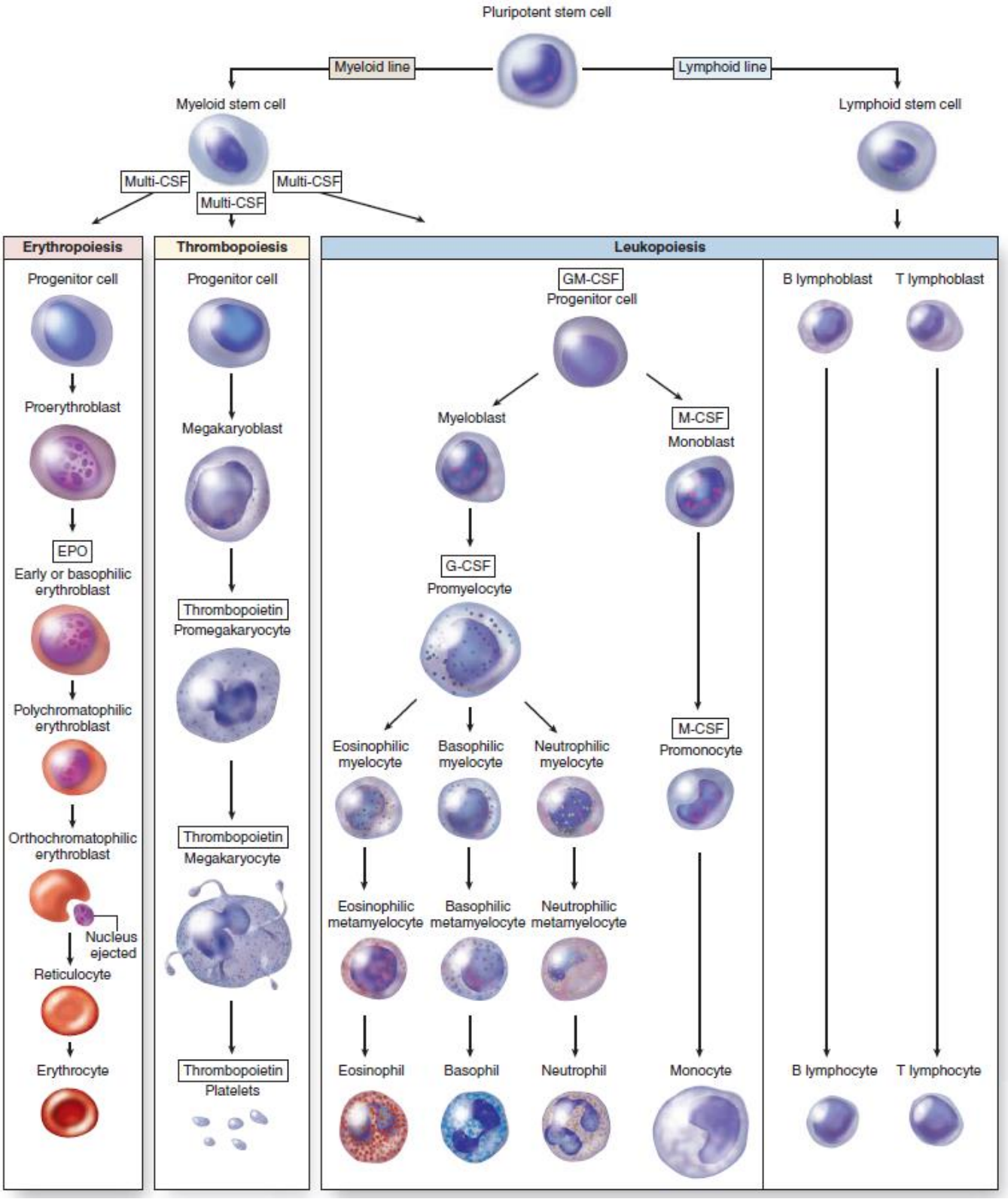
Stem→ progenitor → precursor.

- The stem cell is characterized by its **multipotential** dividing ability (it can divide and give us <u>four types of progenitor cells</u>).
- These progenitor cells are also known as "colony forming units"
  (أي أنَّه إذا تمَّت زراعتها فإنَّها تُنتج مستعمرة من نوع محدَّد من الخلايا)

➢ These **four** types are:

1. Erythroid lineage produces RBCs- the progenitor cell is: CFU-E (colony forming unit- erythrocytes)
2. Thrombocytic lineage for the production of platelets: CFU-Meg
   Meg: refers to megakaryocytes.
3. Granulocyte-monocyte lineage (CFU-GM). This lineage produces granulocytes as well as the monocytes.
4. Lymphoid lineage (CFU-L).

- To make a clear image in your head of what we talked about above, here's this nice figure from Junqueira. The rest of the details in this figure will be mentioned soon.

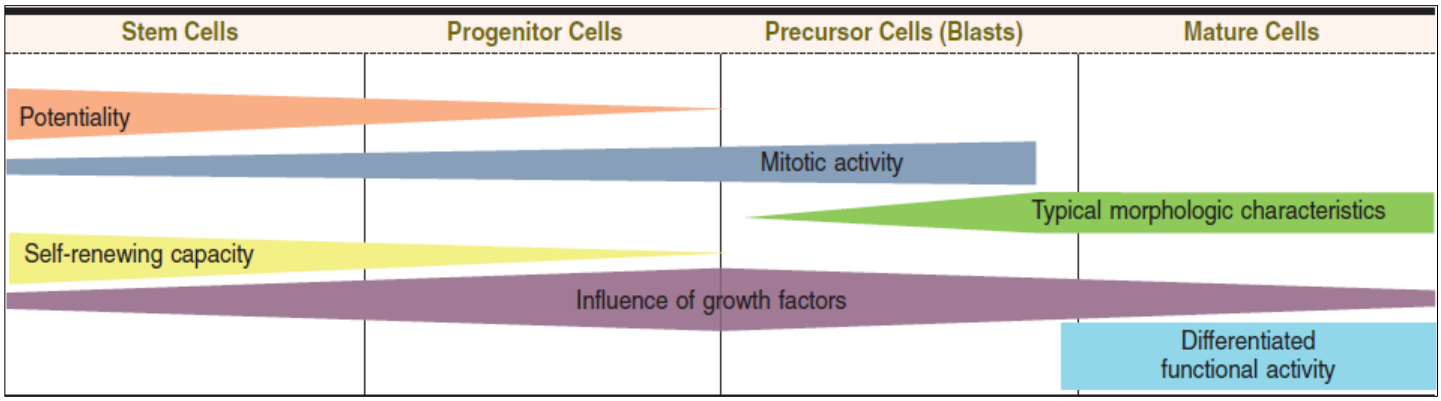


The **progenitor** cell divides to give us:

1. Progenitor cell (self-renewal)
2. Precursor cell (also known as Blasts).

The **precursor** cell <u>only</u> gives a **mature** cell (no self-renewal capacity).

- Stem and progenitor cells are **hard** to differentiate and identify in the bone marrow (they look a lot like a large lymphocyte).
- Stem cell divides **slowly**, the other two divide **quicker**.



<u>About the figure</u>:

- ***Potentiality***; Being able to give more than one type of cells. Stem cells give 4 types of cells, progenitor cells give progenitor or precursor (2 types) while the precursor only gives mature.
- ***Mitotic activity***; division is slow for stem cells, and quick for progenitor and precursor cells.
- ***Self-renewal***; for stem cells, and progenitor cell happens less readily.
- ***Growth factors*** affect progenitor and precursor cells mostly.
- ***Differential* functional activity:** for mature cells.

- Hematopoiesis needs certain growth factors, which we call "**hematopoietic growth factors**" or "**colony stimulating factors**".
  - These factors are proteins that act: either in an **endocrine fashion** or locally **(paracrine)**.

❖ **Clinical Correlate:**

- Let us consider a patient who has a cancer, and he's on chemotherapy (or radiotherapy). After some sessions, the doctor ordered a CBC test (Complete Blood Count), then the results came back and told us the patient has a WBC count of 1,000 **(normal WBC count is between 10,000 and 4,000)**. That is very low, and the patient could die from repeated infections. Therefore, the doctor should not give him any

more sessions; instead, he should give him growth factors to raise up his WBC count.

- Giving growth factor to a bone marrow transplant patient would increase the transplantation efficiency by stimulating cell proliferation.

❖ Some of the most important growth factors are:

1. **Erythropoietin**: produced by interstitial cells from the healthy kidney
   - ❖ Important for the formation of erythrocytes.
   - ❖ Renal failure patients are at risk of developing **anemia** if the failing kidney was not able to produce erythropoietin.
   - ❖ Erythropoietin works both, in vivo and in vitro.

2. **Thrombopoietien**: stimulates the formation of platelets from megakaryocytes (platelets are **anucleated** cells, they don't contain nucleus, they are rather a part of the cytoplasm of megakaryocytes).

3. **Steel factor (stem cell factor)**: produced from stromal cells in the bone marrow. Important in the first stages to stimulate the stem cells.

- The doctor read all the information mentioned in the table below, which are basically more growth factors.

Table 13–2. Main characteristics of five hemopoietic growth factors (colony-stimulating factors, CSF).

Name	Human Gene Location and Producing Cells	Main Biologic Activity
Granulocyte (G-CSF)	Chromosome 17 macrophages Endothelium fibroblasts	Stimulates formation (*in vitro* and *in vivo*) of granulocytes. Enhances metabolism of granulocytes. Stimulates malignant (leukemic) cells.
Granulocyte + macrophage (GM-CSF)	Chromosome 5 T lymphocytes Endothelium fibroblasts	Stimulates *in vitro* and *in vivo* production of granulocytes and macrophages.
Macrophage (M-CSF)	Chromosome 5 macrophages Endothelium fibroblasts	Stimulates formation of macrophages *in vitro*. Increases antitumor activity of macrophages.
Interleukin 3 (IL-3)	Chromosome 5 T lymphocytes	Stimulates *in vivo* and *in vitro* production of all myeloid cells.
Erythropoietin (EPO)	Chromosome 7 renal interstitial cells (outer cortex)	Stimulates red blood cell formation *in vivo* and *in vitro*.

- **Notes on the table:**
  - **CSF** = Colony stimulating factor.
  - **G-CSF** can **stimulate malignant leukemic cells.**
  - **IL-3** (plays the role of a growth factor): stimulates **ALL myeloid cells production**.

Erythropoiesis

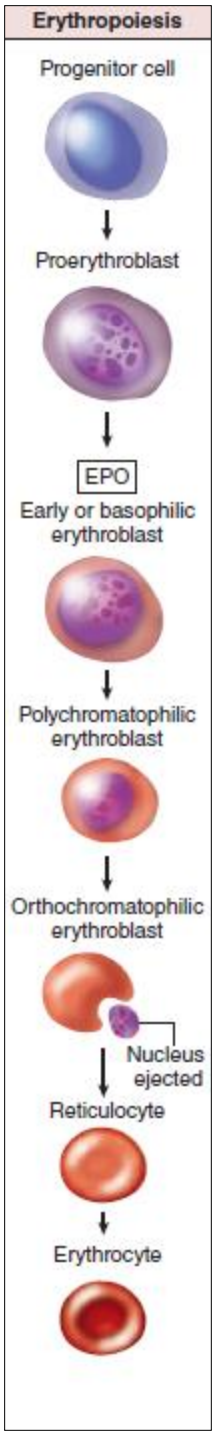
- The production and maturation of erythrocytes (red blood cells).

- General information:
  - Takes nearly **one week** (from stem cell to a mature RBC).
  - Essential factors: erythropoietin, Iron, folic acid, vitamin B12, protein precursors (heme+ globin. Globin is a protein).
  - Each factor absence contributes to a certain type of anemia, for example:
    - Iron deficiency anemia: microcytic hypochromatic (small cell with a pale cytoplasm).
    - Vitamin B12 or folic acid deficiency anemia: Megaloblastic anemia (very large cells).

- Now let's talk about the stages of erythrocytes production. Look at the huge figure in page 2 and follow the steps;

1. Pluripotent stem cell
2. Myeloid stem cell (multipotent stem cell)
3. Myeloid progenitor cell (erythropoid CFU-E)
4. Proerythro*blast* (precursor cell, the first one to be identified)
5. Basophilic erythroblast (early normoblast)
6. Polychromatophilic erythroblast (intermediate normoblast)
7. Orthochromatophilic erythroblast (late normoblast)
8. Reticulocyte
9. Erythrocyte

❖ <u>Now let's bring some of the figure and discuss it with more detail</u>:



1. ***Proerythroblast*:**
   - The fist recognizable erythrocyte precursor.
   - It is a large cell (15-20 µm in diameter) with a large nucleus with fine granular chromatin and prominent nucleoli.
   - Cytoplasm is basophilic (contains ribosomes).
   - This cell contains no hemoglobin.

   During the coming stages there will be progressive **decrease in cell size**, and **progressive loss of organelles**, especially the **ribosomes** (they synthesize hemoglobin; so the **more hemoglobin** you have synthesized the less ribosomes you need). In addition, the nucleus will undergo **pyknosis** (shrinkage) during these stages.

2. ***Basophilic erythroblast*** *(aka early normoblast):*
   - The cell got smaller (12-16 µm in diameter).
   - Coarse chromatin and no nucleoli.
   - Deeply basophilic cytoplasm (still lots of ribosomes, the cell has just started synthesizing hemoglobin).
   - The cell ceases division.
   - Marks the beginning of progressive loss of cytoplasmic organelles.

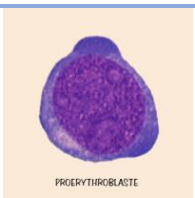
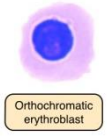
   Note: increasing the amount of hemoglobin accounts for increasing the eosinophilicity of the cytoplasm because hemoglobin stains **red**.

3. ***Polychromatophilic erythroblast*** (عديدة الألوان) *(Intermediate normoblast):*
   - 10-12 µm in diameter.
   - Small nucleus with denser, more compact chromatin.
   - **Why is it called (polychromatic: multicolored)?**
     Some of the hemoglobin is formed so part of the cytoplasm appears red and part appears blue. The blue part contains ribosomes, and the red part contains hemoglobin.

4. ***Orthochromatophilic erythroblast*** *(late normoblast or acidophilic erythroblast):*
   - Acidophilic = Red = most of the hemoglobin has been already synthesized.
   - Here the nucleus is extruded; either through **karyolysis** (dissolution in the cell) or **karyorrhexis** (extrusion out of the cell).

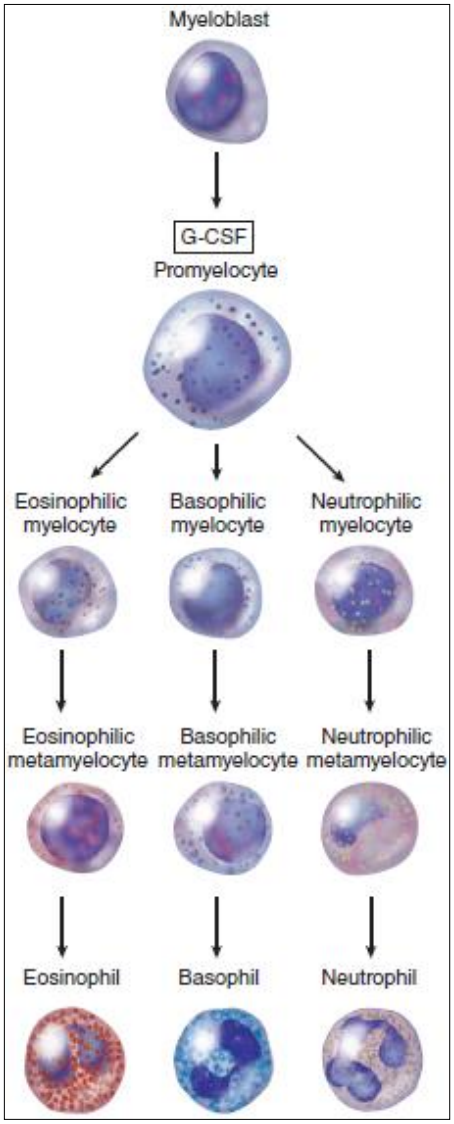
5. ***Reticulocytes:***
   - The RBCs that are released to the circulation are still somehow immature; they contain only 80% of hemoglobin. Where does the remaining come from?
   - The cell synthesizes the remaining 20% of the hemoglobin, so we do still have minimal amounts of organelles. It also contains minimal enzymes for the maintenance of the cell membrane.
   - Could not be easily distinguished from the mature RBCs unless stained with **brilliant cresyl blue stain**.

6. ***Mature Red blood cell***

Proerythroblast -1st precursor	**Basophilic erythroblast *(Early normoblast)***	**Polychromatophilic erythroblast *(Intermediate normoblast)***	**Acidophilic erythroblast (*late normoblast)* *(Orthochromatophilic erythroblast)***	**Reticulocyte**
				
Numerous organelles	Loss of organelles starts	→	→	Minimal organelles
No hemoglobin	→	→	Almost all is formed	Hemoglobin synthesis completed by its end
Basophilic cytoplasm	Deeply basophilic	Varies from blue-gray to slate-gray	Eosinophilic	Eosinophilic
Large nucleus with prominent nuclei	No nucleoli in the nucleus	Small nucleus	Pyknotic nucleus → extruded	Anucleated
Granular chromatin	Coarse chromatin	Dense chromatin	Compact chromatin	--
High mitotic activity (precursor cell)	Cell division ceases	No cell division	No cell division	No cell division
				<1% of circulating the blood

Granulopoiesis

- The production and formation of granulocytes.
- Again, go back to the figure in page 2 and follow the stages and the phases of granulopoiesis:
    1. Pluripotent stem cell
    2. Myeloid stem cell
    3. Granulocyte-monocytes lineage (CFU-GM). (Progenitor).
    4. Myeloblast (precursor, first cell to be identified)
    5. Promyelocyte
    6. Myelocyte
    7. Metamyelocyte
    8. Band cell (If neutrophils are the ones produced) *(not in the figure).*
    9. Mature Granulocyte



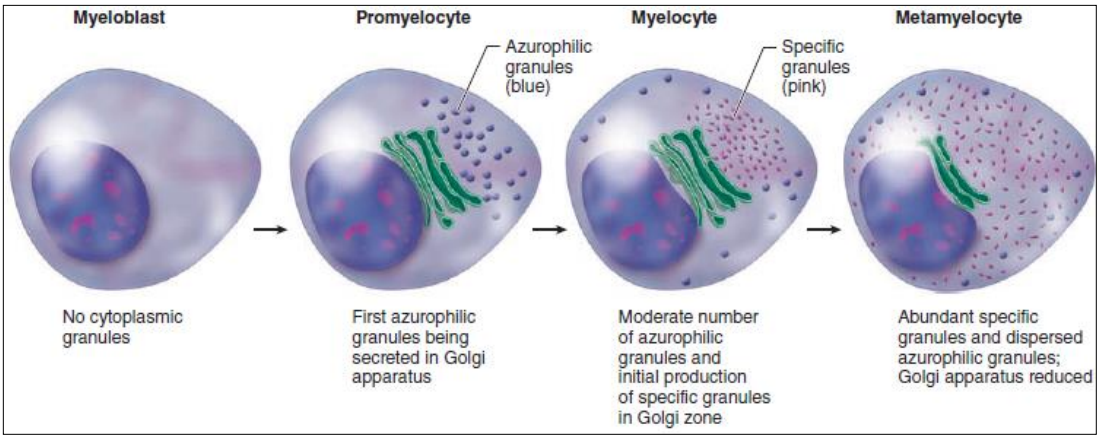
- <u>Now with more details</u>:

1. ***Myeloblast*** *(precursor cell):*
    - The first cell to be identified.
    - The cell is large, 15-20 µm in diameter.
    - Large nucleus, minimal cytoplasm.
    - Prominent nuclei.
    - The most important characteristic is the cytoplasm (pale blue, with no granules)
    - Clinical correlate:
      The second you see a blood film full of **myeloblast** in **excess**, this **could** indicate **acute leukemia** (not 100%; you should take history).

2. ***Promyelocyte:***
    - Myeloblast divides and gives **Promyelocyte**.
    - The difference is that the latter contains azorophilic granules (lysosomes).

3. ***Myelocyte:***
   - The promyelocyte further differentiates into myelocyte.
   - This one has the characteristics of the cell it will give, and therefore, named after the type of the cell given; neutrophilic myelocyte, basophilic myelocyte, eosinophilic myelocyte.
   - It has specific granules (that is what makes it characteristic for the type of the cell it will give).
   - Note: when we say early myelocyte or late myelocyte there is no big difference.
   - *In the following stages, we will assume that the cell will become a **neutrophil**.*

4. ***Metamyelocyte:***
   - The nucleus becomes **kidney shaped** and **eccentric (on the rim of the cytoplasm)**
   - Contains both azoruphilic granules, and specific granules.



5. ***Band cell*** *(stab cell):*
   - The nucleus becomes **horseshoe shaped.**
   - This further matures into mature granulocyte.

6. ***Neutrophil:***
   - The nucleus contains many lobes, ranging from 3 to five lobes (the 3 lobed one is less mature than the one with 5 lobes)
   - Once the bone marrow releases the neutrophils into the blood they will be either **marginating** (on the walls of the vessels) or **circulating.**
   - Clinical correlate 1:
     If the **marginating** become circulating (this means that we have apparent (false) neutrophilia) that lasts only for **few hours.**

- Clinical correlate 2:
  A patient with an **acute bacterial infection** will have neutrophilia; meaning that his body will recruit huge amounts of white blood cells to fight the infection. And that actually will also lead immature WBCs to be filtered into the blood. Therefore, if you ordered a blood film of the patient's blood, you will find:
  - A blood film full of **band cells**, **mature cells**, and **metamyelocytes.**
  - The increase in band cells is called **shift to the left**

Leukemia

- **Malignant** proliferation of **precursor cells** in bone marrow.
- Increase in number of WBCs while all other types decrease in numbers (RBCs, platelet).
- The cells that are released to the blood are mostly immature cells that are not efficient to kill and phagocytose bacteria, so even though the number of cells increases, the patient will have low immunity, and recurrent infections.
- Blood film of a patient with AML (Acute Myelogenous Leukemia) contains large cells (mostly myeloblast).
- Nevertheless, even if we see such blood film we never give diagnosis without history, we cannot say we're 100% sure this is acute leukemia.
- We relate our lab findings to clinical case history:
  Possible history: onset is stormy, with sudden fever (due to infection) and weakness (due to anemia, remember RBCs are low), and bleeding (low platelet count).

Note: we said that both, **acute bacterial infection** and **leukemia** have an increase in immature WBCs, so how can we differentiate between them?

- The immature cells found in **acute bacterial infections** are band cells and metamyelocytes.
- The immature cells found in **leukemia** are the cells of earlier stages of development; myeloblasts, promyelocytes, and myelocytes.

- **Chronic leukemia :**
  - Increase in number of mostly **mature cells**, **band cells**, metamyelocytes, and maybe myelocytes.
  - No recurrent infections (The cells are mostly mature and able to kill pathogens)
  - Incidental finding during lab tests for other causes.

Bone Marrow

- **Morphology of bone marrow:**
  - Stroma: formed from a meshwork of reticular cells and reticulin fibers (contain collagen type 1 and 3)
  - In the spaces between fibers, we find hemopoietic cells, and sinusoidal capillaries.
  - The lining endothelium of the capillaries is of the fenestrated type (contains tight junctions and pores within cells)

- Our body has **releasing factors**; they are substances that stimulate the release of cells from the bone marrow to the blood, and they are:
  - Complement component 3 (C3).
  - Hormones: glucocorticoids, androgens.
  - Bacterial toxins.

- **Functions of bone marrow:**
  1. Production of blood cells and lymphocytes.

  2. **Destruction of old RBCs**:
     Old RBCs become rigid, with low capacity for O_2, so macrophages in the bone marrow destroy them to save space and use their content to make new ones. A function shared with both, the liver and spleen.

  3. Central role in the **immune system**:
     a) Release of neutrophils when needed.
     b) Production of both B Lymphocytes and T lymphocytes occurs in the bone marrow.
     c) Mature B lymphocytes become immunocompetent (able to recognize its specific antigen) in the **bone marrow**, while mature T lymphocytes become immunocompetent in the **thymus.**
     d) Both cells migrate to secondary lymph organs like: lymph nodes, mucosa associated lymphatic tissue (MALT) in the GI tract.

- Recall that we have two types of bone marrow: **red**, and **yellow**.

  - All the bone marrow at birth is red and active in hemopoiesis, and with age, it is replaced by yellow bone marrow (that is full of adipose tissue).
  - Yellow bone marrow can reverse and become red bone marrow when there's a severe need for blood production, as in severe bleeding and hypoxia (like lung fibrosis).
  - We must be careful with radiotherapy, as not to reach the toxic dose and burn the patient's bone marrow
  - Places that remain full of red bone marrow are the sternum, the hip bone, the cap of the skull, the spine, the epiphysis of long bones (especially the femur).
  - The best site to take red bone marrow from is the sternum.

- We have two types of immune reactions:
  - Cell mediated immune reaction.
  - Antibody mediated immune reaction (B lymphocytes become plasma cells that produce immunoglobulins (antibodies)).

"Life is 10% what happens to you and 90% how you react to it." be the ruler of your reactions.

Forgive us for any mistakes <3