

EDUCATIONAL COMMENTARY - GRANULOCYTE FORMATION AND CHRONIC MYELOCYTIC LEUKEMIA

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Learning Outcomes

On completion of this exercise, the participant should be able to

- describe the key characteristics that identify each stage in granulocyte maturation;
- describe the various granules that appear in granulocytes; and
- identify additional peripheral blood cell changes in chronic myelocytic leukemia.

Granulocytes found in the normal peripheral blood smear develop from myeloid stem cells in the bone marrow. Cells of the first four maturation stages are not normally found in the peripheral blood; however, in chronic myelocytic leukemia immature cells of almost all stages are seen in increased numbers depending on the phase (onset, aggression, or progression of the leukemia). The six maturation stages and their approximate cell diameters are as follows:

Stage	Name	Diameter
1	Myeloblast	10-20 μm
2	Promyelocyte	10-20 μm
3	Myelocyte	10-18 μm
4	Metamyelocyte	10-18 μm
5	Band	10-18 μm
6	Segmented	12-14 μm

The prefix *myelo-* refers to the bone marrow, where these cells originate. The cells are also referred to as *granulocytes* because specific granules identify the maturing cells. These terms are often interchangeable; leukemia of this cell lineage can be referred to as *myeloid*, *myelogenous*, *myelocytic*, or *granulocytic* leukemia. The cells can be identified as granulocytes by these characteristics:

- Cell size and color
- Nuclear size, shape, and chromatin pattern
- Nucleoli
- Granule formation

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Cell Size

Typically, as a cell matures it becomes smaller. However, when it is about to undergo mitosis, it becomes slightly larger before dividing into two daughter cells, which are, of course, smaller. Thus, size cannot always be a major criterion in cell identification. Often, the promyelocyte is larger than the myeloblast.

Cell Color

In the first two stages (myeloblast and promyelocyte), the cytoplasm is basophilic (dark to light blue). This is indicative of the RNA present in the cell. By the third stage (myelocyte), the overall color of the cytoplasm reflects the predominant granules the cell is programmed to produce. Thus, a neutrophil contains tiny pink-lavender granules, often overlaying some residual blue cytoplasm.

Nuclear Size

As the cell matures and becomes smaller, so does the nucleus within the cell; therefore, more of the cytoplasm becomes apparent.

Nuclear Shape

In the first two stages of maturation, the nucleus is round or slightly oval. At the third stage (myelocyte) there may be a slight indentation, which becomes quite obvious by the fourth stage (metamyelocyte). The nucleus ultimately forms into a kidney bean shape, and by the fifth stage (band) into a cigar shape, or C shape, or S shape. Often the nucleus becomes twisted. Opposite sides of the nucleus are typically of the same diameter. As the cell finally matures, the indentation extends to more than half the width of each side. Indentation continues, and forms a fine filament (segmenter) between the nuclear segments, or *lobes*. Normal segmented neutrophils have two to four lobes.

Nuclear Chromatin Pattern and Color

The chromatin made of DNA appears as a fine network of filaments in the first stage and stains red-purple. As the cell matures, the filaments begin to clump together and the color becomes more purple-blue. This clumping process continues until total cell maturation. In the mature segmented form, the chromatin clumps stain purple-blue, and a clear area, referred to as parachromatin, may be visible.

Nucleoli

Nucleoli are present in the first three stages. They are typically seen in the myeloblast and promyelocyte, and rarely in the early myelocyte stage. Nucleoli, mostly RNA, stain light lavender to light blue, showing a delicate membrane. These can vary in number; up to five can be seen in the earliest stage.

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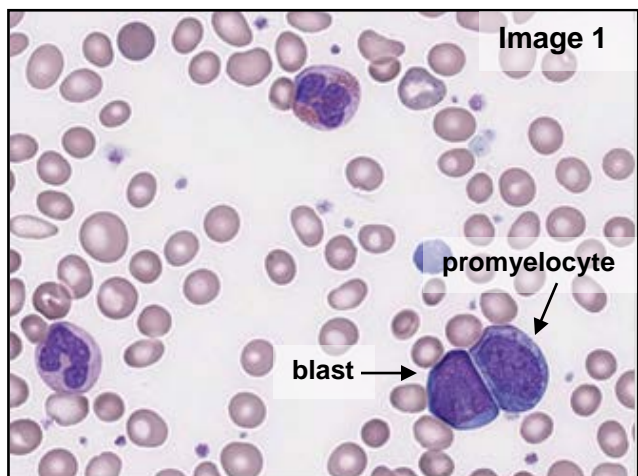
Granule Formation

Granules are not observed in the first stage (myeloblast). In the second stage (promyelocyte), reddish-purple granules, often referred to as *azurophilic granules*, appear in the cytoplasm and may overlie the nucleus. Because these are the first granules to appear, they are referred to as the *primary granules*. These granules are typically not observed at the third stage (myelocyte); however, some residual primary granules can be seen at this stage as the secondary granules appear in the cytoplasm. The secondary granules are identified by their characteristic staining, and the cell is identified in the same way. Cells with granules that reflect the basophilic stain are called *basophils*; those that reflect the eosin stain are referred to as *eosinophils*; and those that reflect characteristics of both the basophilic and eosinophilic stains are referred to as *neutrophils*. Thus, from the myelocyte stage until total maturity, the cell is identified by these secondary granules.

For the purpose of identifying cells on a smear, it should be noted that the first stage, the myeloblast, is referred to only as a “blast.” Regardless of the cell lineage, all cells in the first stage look alike. Once a diagnosis is made by identifying other maturing cells and performing confirmatory tests, this first cell can be identified by the cell lineage. In addition, basophils and eosinophils are typically referred to by those names and not by the developmental stage. Thus an eosinophilic metamyelocyte is called an eosinophil. For the purpose of this exercise, the cells in the Images will be identified by stage, if it is obvious.

The images below are from a patient with chronic myelocytic leukemia.

Image 1 shows a blast and a promyelocyte that appear to be attached. The nucleus in the blast appears to cover the entire cell, and the chromatin pattern is fine and diffuse without any clumping. A small rim of dark blue cytoplasm is present. The promyelocyte, to its right, is slightly larger and shows the appearance of primary granules around the 12:30 position. The chromatin pattern shows a few small clumps although it is mainly fine and diffuse. Nucleoli are also visible.



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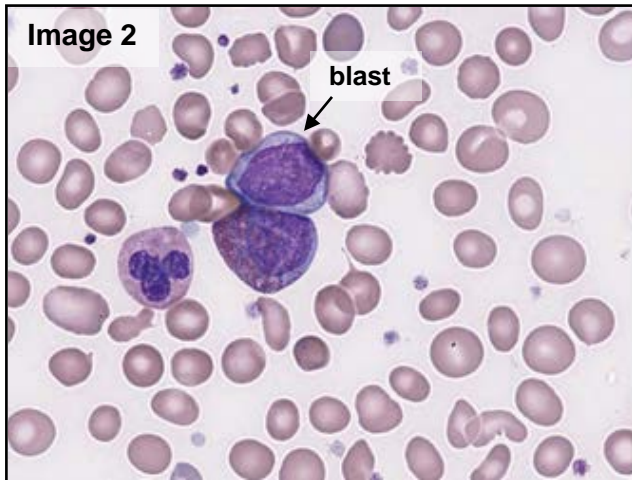
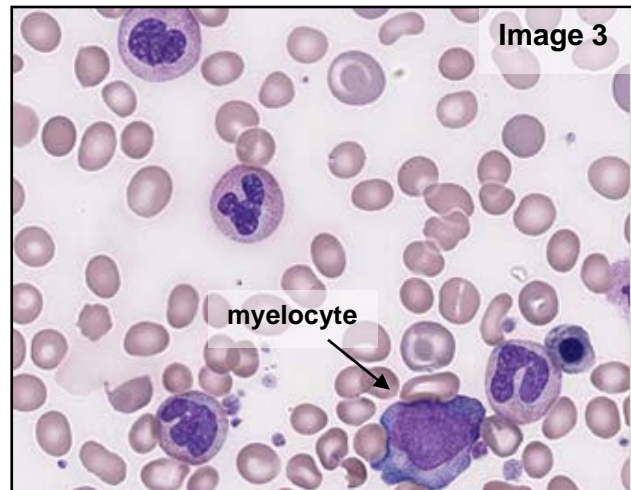


Image 2 again shows a blast with blue cytoplasm, an oval nucleus, and no granules in the cytoplasm. This is in contrast to the cell below it, which has abundant primary granules, some overlying the nucleus. It should be mentioned that when adjacent cells, such as red blood cells or white blood cells, crowd into any cell being studied, it makes it difficult to identify specific cell stages.

In the lower right corner of **Image 3** is a promyelocyte transitioning into a myelocyte. Both primary granules and secondary neutrophilic granules are present. This cell is thus identified as a myelocyte. This is a good example of how cells transition from one developmental stage to another.



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Image 4 is an excellent example of a myelocyte showing neutrophilic granules on the top side of the cell, but still showing residual blue cytoplasm in the bottom. This cell also shows a nucleolus. To the left of the myelocyte are two basophils. Notice the dark blue-black color of the granules, which also vary in shape and size, typical for a basophil. Compare the color of these granules with the reddish blue color of the primary granules in the promyelocyte in Image 2. This is a critical distinction. Confusing promyelocytes with basophils is a serious error in the differential reporting that may affect diagnosis and patient treatment.

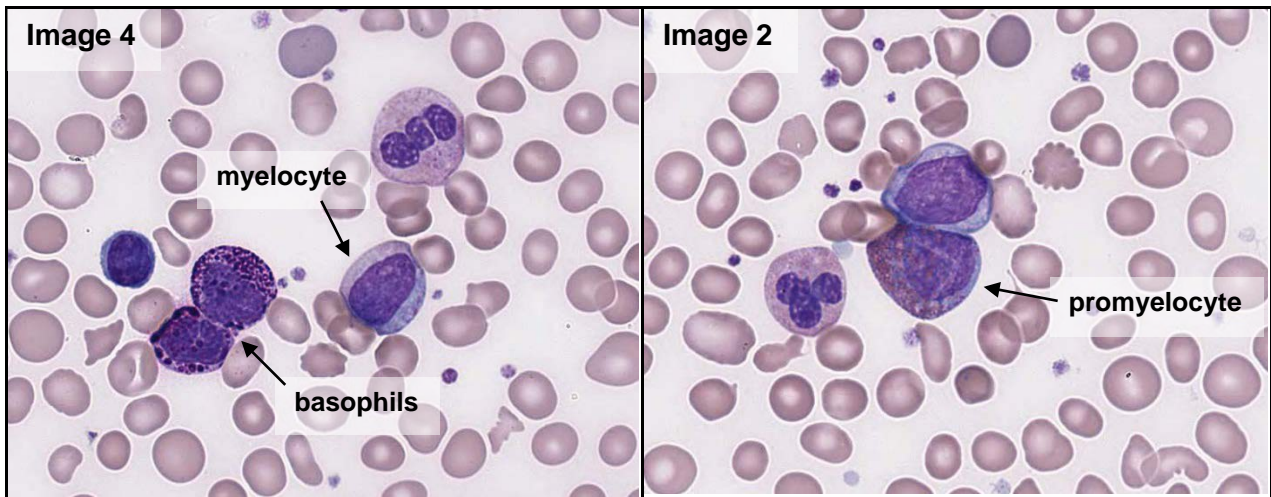
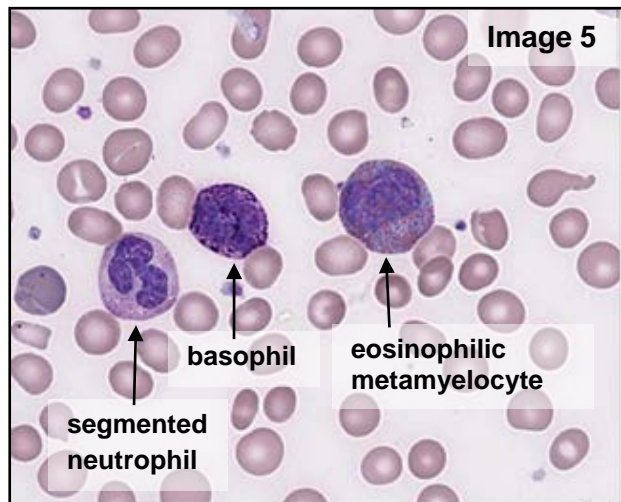


Image 5 demonstrates the granules of the three granulocytic cells. The cell on the left shows tiny pink-lavender neutrophilic granules in a mature segmented cell. The basophil shows the dark blue-black granules overlying an obscure-appearing nucleus (which is typically the case) in contrast to the eosinophilic granules, which appear pink-salmon and have uniformly sized round granules. This particular eosinophil is in the metamyelocyte stage.



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Image 6 shows a neutrophilic metamyelocyte. Notice the chromatin is very densely clumped and the sparse neutrophilic granules are overlying residual blue in the cytoplasm. The nearby mature segmented neutrophil is full of granules.

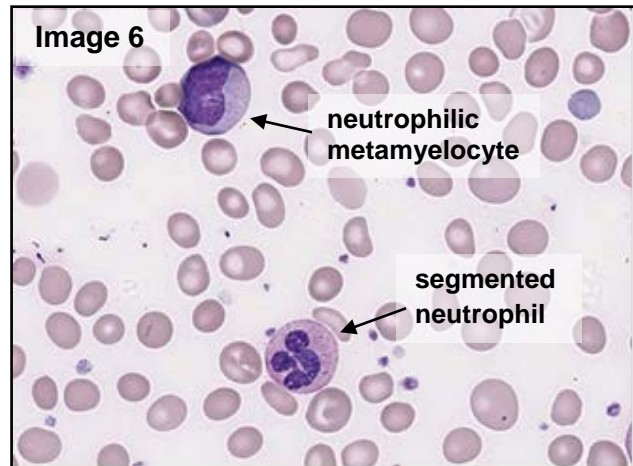
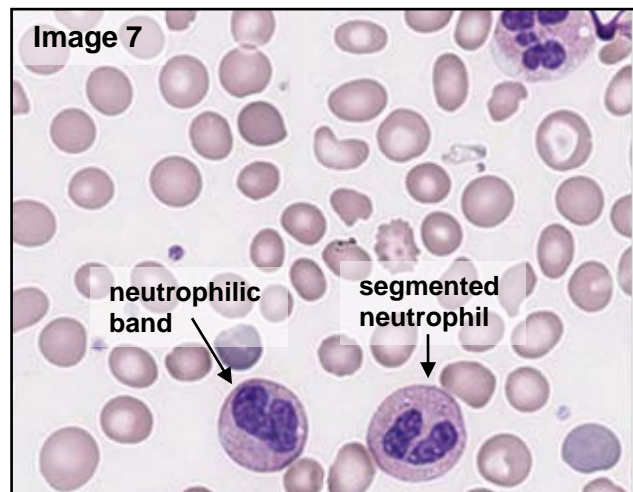


Image 7 shows a neutrophilic band to the left of a bilobed segmented neutrophil.



Chronic myelocytic leukemia (CML) is a type of cancer that begins in the bone marrow when an abnormal gene forms in an immature cell. The Philadelphia chromosome (Ph1) is associated with this leukemia. The leukemic cells do not mature normally: they divide at faster rates, build up in the bone marrow, and finally spill over into the peripheral blood. This results in an increased white blood cell count in the peripheral blood with the presence of immature granulocytes. All of the granulocytes - neutrophils, basophils, and eosinophils - are increased in number. The cells do not function normally and cannot fight infection. Leukemic cells do not undergo normal apoptosis, and crowd out other cells from the bone marrow, such as immature red blood cells and platelets.

The following images are from the same patient with CML as the first seven, and will illustrate the additional details seen in the peripheral blood picture of CML.

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Image 8 shows another nucleated red blood cell at the rubricyte stage. Again, this should not be confused with a blast. This nucleus is very clumped and the royal-blue cytoplasm is more indicative of an immature red blood cell.

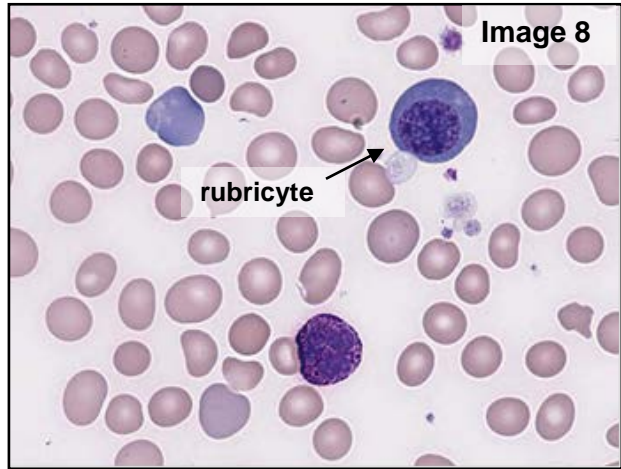


Image 9 shows two nucleated red blood cells (metarubricytes), a segmented neutrophil, a basophil, a small lymphocyte, and a monocyte with a vacuole.

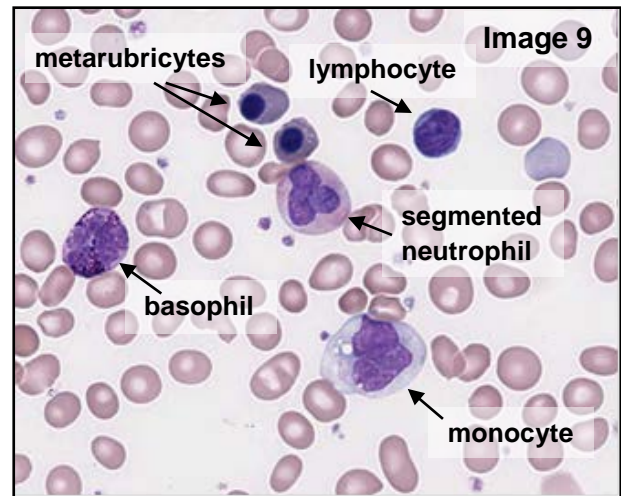
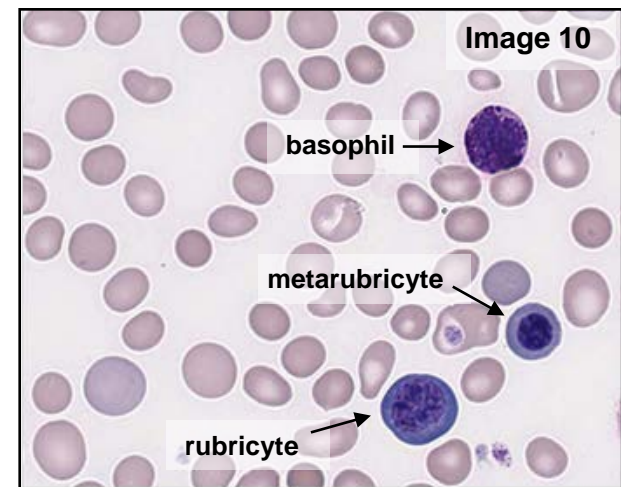
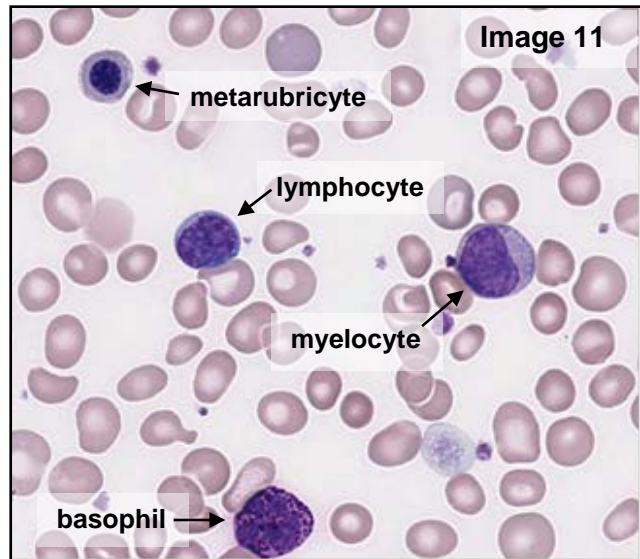


Image 10 demonstrates the results of bone marrow crowding in the presence of the nucleated red blood cells (rubricyte and metarubricyte) along with a basophil. Also, polychromatic red blood cells are increased in number.



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Image 11 also shows a nucleated red blood cell (metarubricyte), a basophil, a myelocyte, and a normal small lymphocyte. It should be noted that as this leukemia progresses, blast-stage cells may continue to divide in larger numbers without maturing and may be confused with small lymphocytes. These blasts are referred to as *microblasts*. That is why critically looking at the chromatin pattern is essential. Also, this slide shows that the platelet count is increased and some platelets are larger than normal and do not have many granules. This indicates a dysfunction of the platelets, which is common in CML.



When CML is suspected by the patient's symptoms or by the results of the complete blood count and peripheral blood smear differential, a bone marrow analysis is performed for confirmation. Genetic studies will also be included. Chronic myelocytic leukemia is a slow-growing leukemia and the blood picture will reflect the stage of its progression. However, CML can also transform into a fast-growing acute leukemia. The World Health Organization (WHO) has described three phases of CML that are identified primarily by the number of blasts in the peripheral blood smear differential along with the number of white cells and platelets.

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