EDUCATIONAL COMMENTARY – BLOOD CELL ID: PERIPHERAL BLOOD FEATURES IN MEGALOBLASTIC ANEMIA

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To view the blood cell images in more detail, click on the sample identification numbers underlined in the paragraphs below. This will open a virtual image of the selected cell and the surrounding fields. If the image opens in the same window as the commentary, saving the commentary PDF and opening it outside your browser will allow you to switch between the commentary and the images more easily. Click on this link for the API ImageViewer™ Instructions.

Learning Outcomes

On completion of this activity, the participant should be able to:

- describe morphologic characteristics of normal peripheral blood leukocytes;
- identify morphologic changes in red blood cells associated with megaloblastic anemias; and
- discuss morphologic features in leukocytes associated with megaloblastic anemias.

Case Study

A 36 year old male was seen by his physician for fatigue and weight loss. His CBC results are as follows: WBC=4.2 x 10⁹/L, RBC=1.76 x 10¹²/L, Hgb=7.6 g/dL, Hct=23.4%, MCV=133.0 fl, MCH=43.2, MCHC=32.5 g/dL, RDW-SD=73.3 %, RDW-CV=15.1%, Platelet=230 x 10⁹/L.

Educational Commentary

The patient presented in this testing event has been diagnosed as having a megaloblastic anemia. This condition affects not only red blood cells, resulting in anemia, but also white blood cells and platelets. The images provided represent both normal and abnormal cells seen in the peripheral blood in megaloblastic anemia.

The cell in image BCI-01 is a polychromatophilic red blood cell. The term polychromasia refers to the appearance of these cells on a peripheral blood smear. These cells represent reticulocytes, the stage of red blood cell maturation immediately before the mature erythrocyte. Reticulocytes have no nuclei, but they retain residual RNA (ribonucleic acid). Therefore, the cell appears blue-gray or bluish when Wright’s stain is used. Sometimes reticulocytes look larger than normal erythrocytes, although that is not the case in this example.
Reticulocytes typically mature for approximately 48 hours in the bone marrow and another 24 hours in the peripheral blood. The presence of polychromasia indicates accelerated bone marrow activity in response to an anemia. The appearance of polychromatophilic cells in the smear from this patient is not surprising given the low red blood cell count and decreased hemoglobin and hematocrit values.

Editor's note: Some participants reported "spherocyte" for this cell. This cell is a polychromatophilic erythrocyte and not a spherocyte. Note the slightly darker, blue-gray cytoplasm that is characteristic of polychromatophilia. Likewise, the cell in question is not smaller than normal RBCs, as is characteristic of spherocytes. Polychromatophilic cells may even appear larger than normal RBCs. While both polychromatophilic erythrocytes and spherocytes lack central pallor, it would be unusual to see a spherocyte that is also polychromatophilic. Polychromatophilic cells represent reticulocytes, or immature cells that are released early from the bone marrow in response to an increased need for more peripheral oxygen carrying capacity. Spherocytes may be artifacts, especially in the thin area of a smear as in this image, or as a result of inherited deficiencies in RBC proteins, as in Hereditary Spherocytosis. Finally, spherocytes would not be an expected finding in the peripheral blood from a patient with a megaloblastic anemia, as in this case study. This test event emphasizes the importance of evaluating RBCs for size, shape, coloration, inclusions, and distribution.

Image BCI-02 shows a monocyte. Monocytes are large cells and are normally present in small numbers in the peripheral blood. This cell is a classic example of a monocyte. It has abundant, blue-gray cytoplasm that appears uneven or rough. The vacuoles seen in this monocyte are typical. Sometimes, fine lilac or purple granules may be apparent in the cytoplasm. A few of these granules are scattered in the cytoplasm of this cell. The nuclei in monocytes vary in shape and can be round, oval, lobulated, or kidney-shaped. The chromatin stains a light purple and is generally fine, with minimal clumping, and no nucleoli are visible.
Image **BCI-03** shows an eosinophil. Eosinophils are medium-sized cells, generally about the same size as segmented neutrophils. They characteristically have numerous, red-orange cytoplasmic granules. These distinctive granules are typically large and uniform in size and shape. Eosinophils usually are bilobed, although this cell has three lobes. The nuclear chromatin appears clumped and dense, reflective of a mature cell.

The cell in image **BCI-04** is a normal lymphocyte. Lymphocytes vary in size. This cell is an example of a small lymphocyte. Note the scanty amount of blue cytoplasm rimming the nucleus, a characteristic seen in this size lymphocyte. Nuclei in normal lymphocytes are usually round, although sometimes they may appear oval or slightly indented. The nuclear chromatin stains a dark purple and is condensed and clumped. At times, nucleoli may be present, but they are often not visible.

Image **BCI-05** shows a segmented neutrophil. Segmented neutrophils are medium-sized cells. They represent the most common leukocyte normally seen in the peripheral blood of an adult. Their cytoplasm has numerous, small, light violet or pink granules. Contrast the size and color of these granules with those seen in the eosinophil in image BCI-03. The distinguishing feature of segmented neutrophils is the shape of the nucleus. Neutrophils characteristically have 2 to 5 nuclear lobes connected by thin strands of chromatin. The nucleus stains a darker purple and the chromatin is dense and clumped.
The red blood cell identified in image BCI-06 is classified as macrocytic. Macrocytic red blood cells may be seen in the peripheral blood of a patient, such as this one, who has a megaloblastic anemia. Many times, the cells are also not only large, but slightly oval as well. The smear should be reviewed for macrocytes when the mean corpuscular volume (MCV) is greater than 100 femtoliters (fL). Note that in this case the MCV was reported as 133.0 fL. A helpful internal reference for determining erythrocyte size is to view the nucleus of a small, normal lymphocyte, like the one in image BCI-04. Red blood cells should be about the same size as the nucleus in a small lymphocyte. The red blood cell in this image is much larger. Macrocytes also sometimes appear hyperchromic because they are thicker cells and stain without any area of central pallor. Other cells in this image do not have defined areas of central pallor as the red blood cells are distributed too thinly in this section of the smear.

The last image in this test event, BCI-07, is a hypersegmented neutrophil. It is expected that such a cell would be seen in the peripheral blood of a patient with megaloblastic anemia. Hypersegmented neutrophils represent the most unmistakable morphologic feature associated with this condition. These cells are generally larger than normal segmented neutrophils. The cytoplasm has numerous small, light violet or pink granules as does a normal segmented neutrophil, but hypersegmentation is the distinguishing characteristic. A true hypersegmented neutrophil has at least 6 nuclear lobes, in contrast to the 2 to 5 lobes seen in a normal segmented neutrophil. However, if more than 10% of the neutrophils have at least 5 lobes, hypersegmentation should be suspected. This particular cell may have at least 6 to 7 lobes. Also note that the nuclear chromatin may appear less clumped and condensed in the hypersegmented cell.

Megaloblastic Anemia
The patient presented in the case study for this testing event was diagnosed as having a megaloblastic anemia. The two most common causes of this anemia are deficiencies in vitamin B\textsubscript{12} or folate. Both these nutrients are necessary for normal cellular DNA metabolism. Therefore, when vitamin B\textsubscript{12} or folate levels are decreased, DNA synthesis in all cell lines is impaired. The most noteworthy morphologic findings associated with megaloblastic anemia are seen in erythrocytes and leukocytes. Because DNA
synthesis occurs in the nuclei of cells, normal nuclear maturation is delayed while cytoplasmic development proceeds as usual. The result is a nuclear-cytoplasmic asynchrony. Peripheral blood manifestations of this unbalanced maturation are seen as macrocytic red blood cells and hypersegmented neutrophils. However, these findings are seen in any megaloblastic anemia, regardless of whether the cause is related to a vitamin B₁₂ deficiency or a folate deficiency. Additional tests to determine vitamin B₁₂ level and serum and red blood cell folate values are necessary to confirm a diagnosis.

Summary
The images presented in this exercise represent both normal and abnormal peripheral blood findings associated with megaloblastic anemia. Several of the morphologic abnormalities, including polychromasia, macrocytosis, and hypersegmented neutrophils, are not unexpected for this condition. A careful review of the blood smear is important so that the characteristic features are recognized and reported.

References

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