EDUCATIONAL COMMENTARY – IDENTIFYING PERIPHERAL BLOOD CELLS IN A CASE OF ACUTE MYELOCYTIC (MYELOBLASTIC) LEUKEMIA

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To view the blood cell images in more detail, participants enrolled in program #224 or 225 for Blood Cell Identification can click on the sample identification numbers underlined in the paragraphs below. After logging on with a Paperless Proficiency Testing user name and password, you will see 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. To avoid the need to log in for each image, use the online tool to choose the cell you want to view. Click on this link for the API ImageViewer Instructions.

Learning Objectives

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

- describe features of normal peripheral blood leukocytes;
- discuss morphologic characteristics of immature leukocytes and erythrocytes; and
- identify morphologic features associated with normal platelets.

Case History

A 52-year-old female visited her primary care physician with chief complaint of excessive bruising. Her CBC results are as follows: WBC=31.6 x 10^9/L, RBC=2.69 x 10^{12}/L, Hgb=8.1 g/dL, Hct=24%, MCV=89.2 fl, MCH=30.1 pg, MCHC=33.8 g/dL, RDW-CV=13.1% and Platelet=26 x 10^9/L.

Introduction

The patient presented in this testing event initially presented to her physician with the chief complaint of excessive bruising. Laboratory evaluation resulted in a diagnosis of acute myelocytic (myeloblastic) leukemia (AML). The images for discussion represent both normal and abnormal cells that may be seen in the peripheral blood in this condition.
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Image BCI-08 shows a monocyte. Monocytes are large cells, the largest that can normally be seen in the peripheral blood. The cytoplasm is blue or blue-gray, abundant, and often vacuolated, as in this particular cell. It appears rough, uneven, and resembling ground glass or grains of sand. Fine, small, pink or lilac (azurophilic) granules are also sometimes present. Monocytic nuclei may assume many different shapes, such as oval, round, lobulated, indented, or kidney shaped. The nucleus in this cell appears to have several folds. The nuclear chromatin is fine, shows minimal clumping, and stains a lighter purple. The nuclear vacuoles seen in this monocyte are not typical.

The cell identified for Image BCI-09 is a nucleated red blood cell (RBC). It is not normal to see a nucleated erythrocyte in the peripheral blood of an adult. However, it is not unexpected to see this cell in a patient with acute leukemia and subsequent hematopoietic bone marrow stress. When nucleated RBCs are seen in the peripheral blood, they must be counted and reported, but it is not necessary to categorize them by maturation stage. Circulating nucleated erythrocytes often resemble the cell in this image. The size of these cells varies depending on the stage of maturation. In general, overall size decreases as the cells mature. The nucleus also becomes smaller as the nucleated RBC matures, resulting in more abundant cytoplasm. Cytoplasmic color in more immature stages is typically dark blue and gradually transitions to pink as the cell matures. The intensity of pink seen in any nucleated erythrocyte reflects the amount of hemoglobin synthesized by the cell. Nuclei in these cells are typically round or oval. Chromatin structure varies with maturation stage; dense and clumped chromatin are associated with more mature stages. It is unusual that this particular nucleated RBC has such basophilic cytoplasm whereas the nuclear chromatin is so condensed. It is also atypical to see cytoplasmic vacuoles; these may be artifacts associated with smear preparation or age of the original blood sample.

Author's notes on participant performance: Note that BCI-09 is not a plasma cell. Though the nucleated RBC may initially appear similar to a plasma cell, there are important morphologic differences. The nucleus is eccentrically located as in a plasma cell, but the chromatin is completely “featureless” with no areas of parachromatin visible. In a plasma cell, the chromatin pattern is often referred to as
appearing like a cartwheel or spoke wheel as radial strips of parachromatin and chromatin may be seen. Likewise, a perinuclear halo or hof (representing the Golgi body) is pronounced in a plasma cell. In this nucleated red blood cell, several discrete vacuoles are present, but there is no defined hof. Finally, plasma cells have deeply basophilic cytoplasm. This nucleated RBC has a blue-gray cytoplasm. It is important to always evaluate all morphologic features before identifying and classifying a cell.

Image BCI-10 is a neutrophil. Neutrophils are medium-sized and characteristically have two to five nuclear segmentations. The cytoplasm in segmented neutrophils is usually pink, tan, or a light violet, resulting from numerous small granules. The nuclear lobes are typically separated by thin threads of chromatin. It is difficult to appreciate the chromatin strands in this cell, but there are clearly several lobes. The chromatin is dense and clumped as is expected in this mature cell.

Author's notes on participant performance: Image BCI-10 shows a normal segmented neutrophil. This cell is not a hypersegmented neutrophil. The majority of segmented neutrophils have 3-4 nuclear lobes, though as few as two and as many as five may sometimes be seen. Segmented neutrophils also often have nuclear lobes that are folded and overlap. Hypersegmented neutrophils should be considered when more than five lobes are present in a single cell or when three or more 5-lobed neutrophils are observed on a peripheral blood smear. In these situations, hypersegmentation associated with megaloblastic anemia may be suspected. The patient had leukemia, therefore hypersegmented neutrophils would be unlikely. Furthermore, the folding of the nuclear lobes in this cell is such that it is difficult to clearly define all the lobes. It appears that the horizontal lobe towards the bottom of the nucleus is one single lobe as no thin filaments, which usually separate nuclear lobes, are present to suggest two lobes. At the most, this cell appears to have only three lobes, as no thin strands connect the top and right lobes either. Therefore, a combination of hematologic data and morphologic features of normal segmented neutrophils can be used to identify this cell.

The arrow in Image BCI-11 identifies a normal platelet. Although platelets are not actually cells because they lack a nucleus, they originate from nucleated cells in the bone marrow called megakaryocytes and represent cytoplasmic fragments of these cells. Normal platelets vary in size, but are typically small, ranging from 1 to 4 µm in diameter. They also vary in shape, although they are usually round or oval.
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Platelets have light purple or blue-gray cytoplasm and may appear grainy. Note that very few platelets are seen in any of the images associated with the present case study: the patient's platelet count was reported as $26 \times 10^9/L$ (reference range, 150-400 x $10^9/L$), a critically low value. A decreased platelet count is often associated with acute leukemia. The malignant leukemic cells overcrowd the bone marrow and restrict maturation and development of normal cells, such as megakaryocytes, leading to a peripheral blood thrombocytopenia.

**Image BCI-12** shows a lymphocyte. Lymphocytes vary in size; this example is a medium-sized lymphocyte. The amount of cytoplasm visible in lymphocytes depends on the size of the nucleus. The amount of cytoplasm in this cell is intermediate, neither abundant nor scanty. The agranular cytoplasm with a perinuclear clear area is typical. Nuclei in normal lymphocytes are generally round or oval with coarse and clumped chromatin.

Lymphocytes and nucleated RBCs are sometimes confused. These cells may be similar in size, depending on the maturation stage of the nucleated erythrocyte. Therefore, it is important to note subtle differences in cytoplasm and nuclear features. Nucleated RBCs may have more cytoplasm that is often a blue-gray rather than the “true” blue associated with lymphocytes. In comparing Images BCI-09 and BCI-12, the color of the cytoplasm is not as useful in distinguishing the two cells as is the amount of cytoplasm. Nucleated RBCs in more mature stages often have increased amounts of cytoplasm when compared with normal lymphocytes. However, nuclear characteristics in the two cells are distinctive. The nucleated RBC in Image BCI-09 is so dense and clumped, it is nearly “featureless,” in that areas of parachromatin and chromatin are not apparent. Likewise, the nucleus is eccentrically located in the cell. In contrast, the nucleus in the lymphocyte shown in Image BCI-12, although dense and clumped, still has visible areas of lighter and darker staining.

The cell depicted in **Image BCI-13** is a blast. It is not unexpected to see a blast in the peripheral blood of a patient diagnosed with AML. However, blasts should normally not be seen in the peripheral blood. Note the large size of this cell, typical for blasts, as well as the high nuclear to cytoplasmic ratio, also typical. The cytoplasm is characteristically scanty, agranular, and dark blue. Blasts, as well as their
nuclei, are usually round or oval. The cytoplasmic and nuclear indentations in this particular cell most likely resulted from preparation of the blood smear. The nuclear chromatin is loose and fine. Sometimes, multiple and prominent nucleoli are visible. This blast has at least one apparent nucleolus, located toward the bottom of the cell at about 6 o’clock. Note that the small, round, clear area situated just slightly below the center of the nucleus is a vacuole, not a nucleolus. It is challenging to classify blasts according to cell lineage using only morphology because blasts of various cell lines are similar in appearance. Therefore, additional techniques, such as cytochemistry and immunophenotyping, are used to determine the cell type. It is always important, however, to count and report blasts when they are present in a peripheral blood smear.

The arrow in Image BCI-14 highlights an inclusion called an Auer rod (sometimes called an Auer body). Auer rods form through an abnormal fusion of primary (azurophilic) granules in the cytoplasm of malignant myeloblasts and promyelocytes, although they may occasionally be visible in neoplastic myelocytes and metamyelocytes. When present in a blast, Auer rods confirm that the cell is a myeloblast. The presence of Auer rods in the cytoplasm of a blast is one of the few situations in which a morphologic feature identifies an immature cell in all cases. Auer rods are only seen in cells of granulocytic origin. Although Auer rods indicate a blast is a myeloblast, cytochemistry and immunophenotyping are still used to verify any cell as a myeloblast and to confirm a diagnosis of acute leukemia. These additional tests are also necessary to subclassify the category of acute leukemia. Auer bodies are typically pink or purple-red, rod-shaped, and variable in length. They contain myeloperoxidase and lysosomal enzymes. Although usually rod-shaped, multiple Auer rods may be seen as bundles or clusters. Cells with these formations are sometimes called faggot cells, derived from the British word for “bundle of wood.” The Auer rod in this particular cell is adjacent to the nucleus, but they may overlap the nucleus. Note that although this cell has more abundant cytoplasm than the blast in Image BCI-13, the presence of the Auer rod confirms that the cell in Image BCI-14 is a myeloblast and that the cell in Image BCI-13 is also probably a myeloblast. Notice that the nuclear chromatin is still loose and open and at least one nucleolus is visible.
Acute Myeloid (Myeloblastic) Leukemia

Acute myeloid (myeloblastic) leukemia, or AML, is a hematologic cancer that results from an uncontrolled and clonal overproduction of immature hematopoietic cells. In AML, the differentiation and maturation of a common myeloid progenitor cell in the bone marrow is abnormal. Subsequent neoplastic precursor cells may actually be myelocytic, monocytic, erythrocytic, or megakaryocytic in origin. The World Health Organization classifies AML into several different groups based on morphologic features, genetic and molecular studies, and immunophenotypes. The presence of myeloblasts with Auer rods in the present patient suggests that this AML is myeloid in origin.

Hematologic abnormalities are common in AML and are evident in this patient sample. The white blood cell count is elevated. Anemia and, as previously discussed, thrombocytopenia, are usually present. Not only do malignant, immature cells (in this case, myeloblasts) overcrowd the bone marrow and reduce production of platelets, they impede normal red blood cell development as well. The anemia that occurs in AML is usually normocytic and normochromic. The erythrocyte indices in the patient in this case study are normal.

Summary

The patient presented in this testing event was diagnosed with AML. The images provided include cells and an object for recognition and discussion that may be seen in this condition. Although some normal cells are present, the blast cells and Auer rod are significant for this disorder. The identification of immature cells with inclusions was the basis for additional testing that further defined this abnormality. This case study emphasizes the important role the laboratory professional plays in identifying abnormal cells, thereby contributing to quality patient care.

References