**ADVANCED BLOOD CELL ID: LEUKOCYTES AND ERYTHROCYTES IN AN ACUTE LEUKEMIA**

Educational commentary is provided for participants enrolled in program #259- Advanced Blood Cell Identification. This virtual blood cell identification program includes case studies with more difficult challenges. 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**

After completing this exercise, participants should be able to:

- Discuss morphologic characteristics of normal peripheral blood leukocytes.
- Describe morphologic features of immature granulocytes.
- Identify morphologic abnormalities in erythrocyte shape and chromaticity/coloration.

**Case Study**

An 18 year old male was seen by his physician for bruising and severe nosebleeds. His CBC results are as follows: WBC=7.5 x 10^9/L, RBC=2.79 10^{12}/L, Hgb=8.7 g/dL, Hct=24.6%, MCV=88 fL, MCH=31 pg, MCHC=35 g/dL, RDW=18.4%, Platelet=41 x 10^9/L, MPV=10.4.

**Educational Commentary**

The cells selected for identification and discussion in this exercise are from the peripheral blood smear of an 18 year old man diagnosed with acute promyelocytic leukemia (APL). APL is also referred to as acute myeloid leukemia, M3 (AML-M3). As with other acute leukemias, APL has a rapid onset. Though it represents less than 10% of all AMLs, APL has a high rate of remission if timely treatment is provided.

Image **ABI-01** is a normal lymphocyte. This cell is a small, resting lymphocyte. The cytoplasm is characteristically scanty and blue. The nucleus is generally round or oval and may sometimes be slightly indented. The chromatin is dense and clumped and stains a dark purple.

The cell chosen for **ABI-02** is a segmented neutrophil. These cells are recognized by their multiple nuclear lobes, usually two to five, that are connected by thin strands of chromatin. As a mature cell, the chromatin is coarsely clumped and dense. The abundant, small, pink cytoplasmic granules also help differentiate this cell. The granules in this example are a nice pink color, but sometimes they appear more tan or violet, depending on the quality of the staining process.
ADVANCED BLOOD CELL ID: LEUKOCYTES AND ERYTHROCYTES IN AN ACUTE LEUKEMIA (cont.)

The cell identified in image **ABI-03** is a promyelocyte. Note this cell is larger than the mature segmented neutrophil in ABI-02. This is one clue that this cell is immature, as cells generally decrease in overall size as they mature. It is not normal to see promyelocytes in the peripheral blood, but is not unexpected in this case. Nuclei in promyelocytes are usually round, but may be oval as in this cell. The deep purple chromatin is open and loose; sometimes nucleoli are visible. The distinguishing morphologic feature of promyelocytes is the presence of nonspecific, azurophilic, or primary granules. These violet or purple granules are more coarse and larger than the secondary or specific granules associated with mature granulocytes, such as the segmented neutrophil. Often, the granules also overlie the nucleus. The cytoplasm is generally blue. This particular promyelocyte is unusual in that the primary granules are more clustered in pockets within the cytoplasm. However, it is not surprising to see such an abnormality since these cells are malignant.

**ABI-04** is a nucleated red blood cell (NRBC). NRBCs are immature erythrocytes that have not yet fully matured and retain their nucleus. It is abnormal to see them in the peripheral blood of an adult. In this patient with leukemia, however, it is not unusual to see NRBCs. Leukemia causes hematopoietic bone marrow stress and nucleated erythrocytes can be released prematurely into the peripheral blood. The cell identified here is typical of those often seen. The nuclear chromatin is dense and clumped. Usually the nucleus is round, but in this example it is slightly indented (possibly because of smear preparation). The cytoplasm is blue-gray. Sometimes the cytoplasm may appear more pinkish, depending on how much hemoglobin the cell has synthesized. The amount of cytoplasm also may be more scanty as related to the stage of maturation.

The cell selected for **ABI-05** is a dacryocyte or teardrop erythrocyte. Note the pear shape with only one elongated end. This tail may be long or short, tapered or blunt, and an area of central pallor is usually visible. Dacryocytes form as an artifact during smear preparation, when RBCs have inclusions (such as Howell Jolly bodies) and attempt to navigate narrow spaces of the spleen, or if a hematologic or non-hematologic malignancy in the bone marrow obstructs the normal passage or exodus of erythrocytes. Artifactual teardrop cells appear with short, sharp and pointed tips. Sometimes the tails of artifactual dacryocytes will line up, all facing in the same direction. When RBCs with inclusions try to negotiate the spleen, the inclusions become trapped and the erythrocytes stretch and elongate. The RBC membrane is permanently damaged and results in the dacryocyte. Likewise, if malignant or non-malignant cells are overproduced in the bone marrow, they impede the normal passage of RBCs as they exit the marrow vessels. Again, the erythrocytes stretch and deform into a teardrop cell. It is likely the dacryocytes seen
in this patient’s smear resulted from the latter mechanism. Leukemias are often associated with a massive increase of abnormal cells that crowd the bone marrow.

Image **ABI-06** is a polychromatophilic RBC. The term *polychromasia* may also be used to refer to the presence of these cells on Wright-stained peripheral blood smears. Polychromatophilic red cells represent the final stage of erythrocyte maturation that actually occurs in the circulation. The nucleus has been extruded and some ribonucleic acid (RNA) is retained by the cell. The small amount of RNA remaining causes the cell to appear a blue-gray when stained. These cells may also be called *reticulocytes* and can be specifically enumerated by using a special stain to precipitate the ribosomes from RNA into a filamentous network (or reticulum). Notice that even though these cells are characteristically larger than normal RBCs, they should not be described as macrocytic. The term *macrocyte* is used to identify cells without polychromasia. The cell in this example also has a small area of central pallor. However, central pallor may or may not be visible in polychromatophilic erythrocytes. This RBC abnormality is not unexpected in this patient, who has developed anemia secondary to his leukemia. The polychromasia indicates a bone marrow trying to compensate for a peripheral decrease in oxygen carrying capacity.

The last cell identified in this testing event, **ABI-07**, is a blast with an Auer rod. A small percentage of blasts were reported on the automated differential cell count. Blasts should not be seen in the peripheral blood, but it is not surprising that some were noted in this patient diagnosed with an acute leukemia. Blasts are generally large, round or oval cells with a high nuclear to cytoplasmic ratio. Nuclei are also usually round or oval. The chromatin is loose and open with areas of parachromatin more evident. Nucleoli are often present and prominent. The cytoplasm is scanty and blue, sometimes appearing very basophilic. Assigning lineage to blast cells based only on Wright-stained morphology is often challenging because blasts from different cell lines are so similar in appearance. However, the Auer rod in this particular cell distinguishes it as a myeloblast; no other blast cell type is associated with the presence of Auer rods. These pink-red inclusions are small, sometimes round, but usually rod-shaped. They consist of azurophilic or primary cytoplasmic granules that have accumulated within the cytoplasm.

**Acute Promyelocytic Leukemia**

APL is often seen in young adults, as in this case study patient. Two morphologic subtypes may be seen. The typical or hypergranular variant is characterized by the presence of promyelocytes with abundant azurophilic granulation. The WBC in this type of APL is usually not increased. The second variant is the hypogranular or microgranular subtype. In hypogranular APL, the cytoplasmic granules are fine, barely
visible, or sometimes not visible at all. The nuclei are often folded, bilobed, multilobed or may even resemble the nuclei in monocytes. The WBC may be markedly elevated in hypogranular APL.

A diagnostic cytogenetic translocation involving chromosomes 15 and 17 is associated with both subtypes of APL. Therefore, cytogenetic studies, immunophenotyping by flow cytometry, and sometimes cytochemical staining are procedures that confirm APL even when the hypergranular variant is present. It is critical to identify APL quickly as both types are associated with a high risk of disseminated intravascular coagulation (DIC) that can be life-threatening. Fortunately, the abnormal promyelocytes are sensitive to differentiation-based therapy with a high cure rate possible.

Summary
The patient presented in this testing event was diagnosed with APL, most likely the hypergranular variant based on morphology of the abnormal cells and the total WBC. All the cells seen in the peripheral blood are not unexpected given this condition. Because of the potential severity of DIC and the need to initiate curative treatment, rapid diagnosis of APL is essential. The laboratory professional plays a key role in identifying this disorder.

References