ADVANCED BLOOD CELL ID: VARIATIONS IN RBC MORPHOLOGY

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:

- identify abnormalities in RBC morphology that may be associated with anemia.
- describe morphologic characteristics of nucleated red blood cells.

Case Study
A 70 year old male was seen by his physician for weakness and fatigue. His CBC results are as follows: WBC=29.6 x 10^9/L, RBC=2.98 x 10^{12}/L, Hgb=7.5 g/dL, Hct=23.9%, MCV=80.3 fL, MCH=25.1 pg, MCHC=31.2 g/dL, RDW=31.9%, Platelet=362 x 10^9/L.

Educational Commentary
The patient presented in the case study was diagnosed with an anemia of unspecified origin. The cells selected for this virtual educational activity represent variations in red blood cell morphology that may be seen in any anemia.

Image **ABI-08** is a polychromatophilic RBC. The condition represented when these cells are seen in the peripheral blood smear is termed polychromasia. Polychromatophilic red blood cells are actually reticulocytes, the stage of erythrocyte maturation just before the mature RBC. Residual RNA is retained in these cells even though the nucleus has been extruded. Wright staining causes the cells to appear blue gray. Reticulocytes are often larger than normal RBCs, as in this example. They mature for approximately 48 hours in the bone marrow and an additional 24 hours once released into circulation. The presence of polychromasia suggests that the bone marrow is actively producing and releasing RBCs in response to a decrease in oxygen reaching cells and tissues of the body. This defines anemia and is consistent with the patient’s diagnosis.

Image **ABI-09** is a classic target cell or codocyte with a central, dense area of hemoglobin surrounded by a white ring and then more hemoglobin. Target cells form when there is excess surface membrane relative to cell volume. One mechanism that can cause target cells to form is decreased or abnormal hemoglobin
content associated with various anemias. Therefore, as with polychromasia in image ABI-08, it is not unexpected to see target cells in this case study patient.

The RBC in image ABI-10 has inclusions called Pappenheimer bodies. Red blood cells should not have inclusions, so when they are present, some type of abnormality in cellular maturation or hemoglobin synthesis is suggested. Pappenheimer bodies contain iron and some protein. They form in pathologic conditions when cytoplasmic organelles, such as mitochondria, are damaged. The small Pappenheimer bodies are irregularly shaped and may be seen as a single inclusion or as multiples in clusters. They stain purple or purple blue. Pappenheimer bodies are usually removed by the pitting capabilities of the spleen. However, if the spleen is absent, dysfunctional, or overwhelmed, the inclusions remain and are apparent on a peripheral blood smear. The inclusions are also stained by Prussian blue and may appear more greenish or blue. When these inclusions are seen with an iron stain, the red blood cells are referred to as siderocytes. The term Pappenheimer body is used to describe the inclusions when visualized with a Wright Stain. Several disorders can be associated with Pappenheimer body formation and include thalassemias, sickle cell anemia, megaloblastic anemia, and sideroblastic anemia.

ABI-11 is a red blood cell with another type of inclusion, called a Howell-Jolly body. Howell-Jolly bodies represent nuclear remnants retained in the cytoplasm after the nucleus has been expelled during RBC maturation. As with Pappenheimer bodies, the spleen normally removes these inclusions. Howell-Jolly bodies are generally small, but vary in size. Likewise, they typically appear as a single inclusion, but several may be seen in any one RBC, especially in severe anemias. Howell-Jolly bodies are round and are dark purple or purple-blue. They are seen in megaloblastic anemia, severe hemolytic anemia, and congenital dyserythropoietic anemia. It is important to morphologically differentiate Howell-Jolly bodies from Pappenheimer bodies. Note that Howell-Jolly bodies generally appear as a single, round inclusion whereas Pappenheimer bodies are usually irregular and cluster within the RBC. Also, Howell-Jolly bodies will not stain with Prussian blue.

The cell identified in image ABI-12 is a nucleated red blood cell. Nucleated erythrocytes are immature cells that have not yet expelled their nuclei. These cells are not normally seen in the peripheral blood of an adult. Therefore, their appearance in this case study patient indicates abnormal or accelerated erythropoiesis associated with the unspecified anemia. The nucleated RBC seen in this image is one that is frequently seen when these cells are present in the peripheral blood. The nucleus is dark purple, dense, and clumped; areas of parachromatin are not visible. The cytoplasm is pinkish with faint bluish granules. Granules are not typically seen and may represent inclusions that are forming in this particular
cell. Nucleated erythrocytes are not identified according to stage of maturation, but the total number present on a differential cell count should be reported.

Image **ABI-13** is a schistocyte or fragmented cell. The designation “schistocyte” is a broad name that includes many shapes that can be specifically defined, as well as several unclassifiable shapes. For instance, some fragmented cells appear as helmets, bite cells, and horn cells. The cell shown in this picture is a classic unclassifiable fragment. The size of these fragments can vary as well. Some are tiny bits of cells, whereas others may approach the size of a normal RBC. This particular schistocyte has an area of central pallor, but often this is not present in fragmented cells. Numerous conditions can result in schistocyte formation. Some situations include the obstruction of small blood vessels by fibrin strands as occurs in disseminated intravascular coagulation (DIC), hemolytic uremic syndrome (HUS), or thrombotic thrombocytopenic purpura (TTP) (microangiopathic hemolytic anemias). Extreme blood pressure or mechanical force can also shear RBCs into fragments. Heat denatures spectrin, an important structural protein in the erythrocyte membrane, and fragmentation may result. Other factors can likewise cause RBC fragmentation.

The final picture for this test event is **ABI-14**, a spherocyte. It is not unexpected to see such a cell when so many schistocytes are present. If the disrupted or fragmented cell is able to reseal any portions of its membrane, spherocytes can result. Because cellular membrane has been lost, spherocytes have a decreased surface to volume ratio and therefore have no area of central pallor. Note how densely stained this example appears. They are also generally smaller than normal red blood cells and are round. One important functional issue related to the shape of the spherocyte is that the cell is far less flexible than a normal RBC. This rigidity results in a cell that cannot deform effectively as it circulates and as it attempts to negotiate narrow splenic vessels. Therefore, the spherocyte is easily filtered by a functional spleen and removed from circulation. Spherocytes may be seen in hereditary spherocytosis, immune-mediated hemolytic anemias (when antibody associated with the RBC is removed by the spleen), in burn patients, and in the microangiopathic hemolytic anemias.

**Summary**
The peripheral blood smear provided for this testing event is from a patient diagnosed with an unspecified anemia. Note the low RBC count, hemoglobin, and hematocrit values. Although the anemia is not identified, it is a severe condition given the spectrum of abnormal RBCs present. Inclusions are visible as well as many abnormal shapes including target cells, schistocytes, and spherocytes. The polychromasia and nucleated RBCs demonstrate that the bone marrow is responding to loss of peripheral erythrocytes.
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It is important for the laboratory professional to identify and report the variety of RBC morphologic changes apparent on this peripheral blood smear. This information can help in defining a diagnosis.

References