ADVANCED BLOOD CELL ID: IDENTIFYING NORMAL LEUKOCYTES AND ABNORMAL ERYTHROCYTES IN THE PERIPHERAL BLOOD

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. You will need Adobe Flash to use this feature. 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 features of abnormalities in RBC size, shape, and chromicity/color.

Case Study
A 62 year old male was seen by his physician for weakness and fatigue. His CBC results are as follows: WBC=9.5 x 10^9/L, RBC=3.03 x 10^12/L, Hgb=10.0 g/dL, Hct=30.2%, MCV=100 fL, MCH=33 pg, MCHC=33 g/dL, Platelet=373 x 10^9/L.

Educational Commentary
The patient presented for this Advanced Blood Cell Identification exercise was diagnosed with a vitamin B₁₂ deficiency. The images selected for this educational activity represent normal leukocytes but also several changes in RBC morphology.

Image ABI-15 is a basophil. Note the large, round, deep purple, almost black cytoplasmic granules that are characteristic of this cell. Frequently, these granules obscure the nucleus. However, the granules are also water-soluble and may be washed out during the staining process. The nucleus stains purple and, in this example, is visible. Because basophils are normally present in such low numbers in the peripheral blood, it is not necessary to report the maturation stage when these cells are seen.

Image ABI-16 is a monocyte. When identifying blood cells, it is important to determine the cell size, view cytoplasmic characteristics, and evaluate features of the nucleus. The monocyte is the largest cell that can normally be seen in the peripheral blood. The cytoplasm of monocytes is usually abundant and stains a blue-gray color. The cytoplasm is also uneven, rough, or
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“sandy” in appearance; vacuoles are frequently seen. Sometimes small, red-purple granules may be visible. Cytoplasmic projections may also be seen, though this cell is round and uniform. The nuclear shape is variable and may be oval, round, lobulated, or kidney-shaped. The chromatin shows minimal clumping and is a lighter purple color.

The cell identified in Image ABI-17 is a normal lymphocyte. Lymphocytes are variable in size, and this is an example of a small cell. Note a larger lymphocyte is in the same field of view. In small lymphocytes, the cytoplasm is scanty and generally a darker blue. The nucleus may be round, oval, or slightly indented. The nuclear chromatin is characteristically condensed, clumped, and stains a dark purple.

The cell annotated in Image ABI-18 is the first in a series chosen to show morphologic variations in RBCs; it is a macrocytic red blood cell. Macrocytes are larger erythrocytes and may be expected in the peripheral blood when the MCV is greater than 100 fL. Although the MCV in this case study patient is not greater than 100 fL, it is at the upper limit of normal. Also keep in mind that the MCV represents the average size of the RBCs. Therefore, it is still possible to see macrocytes even when the MCV is normal.

A useful internal gauge to help determine RBC size is to compare the red blood cells to the nucleus of a small normal lymphocyte. The nucleus of a normal lymphocyte is about the same size as a normal erythrocyte. A small, normal lymphocyte is not in this image, but a scan of the virtual slide shows several red blood cells much smaller than this cell, as well as cells of intermediate size which can be considered normocytic RBCs.

Frequently, macrocytes have no area, or only a small area, of central pallor. This reflects the fact that these larger cells may be thicker than normal cells, so the area of central pallor is not visible. Macrocytes may be associated with a vitamin B₁₂ deficiency, though in this condition the cells often appear oval in addition to macrocytic.

Image ABI-19 is a target cell, or codocyte. Target cells are red blood cells that have a dense, center area of hemoglobin, then a rim of white, and a final section of hemoglobin. Their true circulating shape is a bell or a Mexican hat. “Targeting” forms when the cells become flattened and dried during smear preparation. There are three basic mechanisms that result in target cell development. If the red blood cell membrane surface area is increased relative to the hemoglobin content, as can occur when excessive amounts of lipids accumulate on the RBC membrane, codocytes can form. Liver disease is one condition that results in an increase in lipids depositing on the red cell surface. Likewise, a decrease
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in hemoglobin content as can be seen in hemoglobinopathies, thalassemias, and iron deficiency anemia causes excess membrane surface in the red blood cell and the formation of target cells. Lastly, artifactual target cells can appear as a result of improper smear preparation, when a wet slide is blown dry rather than air-dried.

The cell selected for Image ABI–20 is a spherocyte. In contrast to target cells with excess membrane surface area compared to the amount of hemoglobin in the cell, spherocytes have a decreased surface-to-volume ratio. Because of a loss of surface area, the cell is smaller than normal, usually round, with no area of central pallor. Since the cell is thicker than normal, spherocytes may be considered hyperchromic, with the MCHC at the upper end of normal or above normal. Though reference values for the indices for this patient are not specified, the MCHC of 33 g/dL may be near the upper range of a normal number. Spherocytes are more commonly associated with hereditary spherocytosis and immune hemolytic anemias. However, they may be seen in any hemolytic condition that results in disruption of the RBC membrane. If the membrane can reseal and repair itself, a spherocyte will be evident.

The last image identified in this testing event, Image ABI-21, is a polychromatophilic erythrocyte. These cells are actually reticulocytes. A reticulocyte is an immature red blood cell that has no nucleus, but retains small amounts of RNA. The RNA allows the cell to stain bluish or a blue-gray. Polychromatophilic RBCs usually lack any area of central pallor. Sometimes polychromatophilic RBCs are slightly larger than mature red cells. Normally, reticulocytes mature one day in the bone marrow and another day in the peripheral blood. When increased RBC destruction results in lack of oxygen to tissues, reticulocytes can be released early from the bone marrow. Therefore, it is not unexpected to see polychromatophilic RBCs in this patient diagnosed with an anemia.

Summary
The images selected for this testing event are from a peripheral blood smear of a patient diagnosed with a vitamin B_{12} deficiency. The WBCs chosen are not unique to this condition. However, some of the selected RBCs are important abnormalities that may be associated with this disorder.

Vitamin B_{12} deficiency is a type of anemia classified as megaloblastic and results in defective DNA synthesis. Since DNA metabolism occurs in the nucleus, nuclear division of RBC precursors is also disrupted in megaloblastic anemias, resulting in macrocytic cells. Overall maturation and development of erythrocytes is inefficient, and an anemia can develop. Polychromatophilic cells indicate a bone marrow response to a deficit of peripheral RBCs. Codocytes and spherocytes are not commonly associated with megaloblastic anemias, but can represent artifacts of smear preparation or a general manifestation of
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ineffective red cell production. This testing event underscores the importance of carefully reviewing the entire peripheral blood smear to note any abnormalities in red blood cell morphology.

References