ADVANCED BLOOD CELL ID: PERIPHERAL BLOOD FINDINGS IN SICKLE CELL ANEMIA

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:

- describe morphologic features of normal peripheral blood leukocytes.
- identify morphologic characteristics distinctive of sickle cells.
- distinguish selected RBC inclusions based on morphologic features.
- describe significant morphologic characteristics of nucleated red blood cells.

Case Study

The CBC from a 30 year old African American male is as follows: WBC=9.5 x 10^9/L, RBC=1.66 x 10^12/L, Hgb=5.0 g/dL, Hct=13.9%, MCV=83.7 fl, MCH=30.1 pg, MCHC=36.0 g/dL, RDW-CV=24.9%, MPV=9.6 fl, Platelet=326 x 10^9/L.

Educational Commentary

The cells and RBC inclusions chosen for identification in this testing event were seen in the peripheral blood of a man with a severe anemia resulting from sickle cell disease.

The cell shown in ABI-08 contains a Howell-Jolly body. Howell-Jolly bodies represent fragments of deoxyribonucleic acid (DNA) retained in the cytoplasm and form in one of two possible mechanisms. In one process, a chromosome is abnormally separated from the mitotic spindle during cell division and remains in the cell after nuclear extrusion. Likewise, if miscues in nuclear fragmentation occur when the maturing erythrocyte expels its nucleus, a Howell-Jolly body will be left behind. Regardless of how Howell-Jolly bodies form, they are inclusions that indicate some imbalance in RBC production (and that the spleen is dysfunctional or absent and cannot effectively remove them from red blood cells). Howell-Jolly bodies appear as a single object in a cell, but more than one may be seen in severe anemia. They are typically small, though they can vary in size. Howell-Jolly bodies are usually round, appear purple, purple-blue, or blue-black and are generally located near the periphery of the cell. It is not surprising to see Howell-Jolly bodies in this patient diagnosed with sickle cell anemia, which causes both impaired erythropoiesis and splenic abnormalities.
ABI-09 is a classic example of a sickle cell. The appearance of these cells is diagnostic for the inherited condition, sickle cell disease (sickle cell anemia). Sickle cells characteristically have two pointed ends, are elongated, and may have a slight crescent shape; they have no area of central pallor. Sickle cells, or drepanocytes, contain an abnormal hemoglobin, hemoglobin S (HbS), that results when one single amino acid on the beta hemoglobin chain is substituted with a different amino acid. Note that in sickle cell anemia, no normal hemoglobin is produced. The consequence is hemoglobin with markedly reduced solubility when deoxygenated. Decreased oxygen tension causes HbS molecules to polymerize, forming filaments that distort the normal red blood cell into a sickle cell. When a sickled cell circulates and becomes oxygenated, the polymerized strands of hemoglobin dissociate and the cell can assume its normal biconcave shape. However, when the HbS again becomes deoxygenated, tactoids of hemoglobin form rods and once more alter the shape of the cell. This process of continuous sickling and non-sickling of RBCs occurs as the cells traverse the circulation. Eventually, repeated sickling and non-sickling result in a cell with a damaged membrane and which becomes permanently and irreversibly sickled, regardless of oxygen concentration.

Red cell ABI-10 contains another type of inclusion called Pappenheimer bodies. In contrast to Howell-Jolly bodies, Pappenheimer bodies consist of iron. Several characteristics morphologically distinguish Pappenheimer and Howell-Jolly bodies. Pappenheimer bodies are small, like Howell-Jolly bodies, but are irregularly shaped. Their color is similar to Howell-Jolly bodies; however, Pappenheimer bodies appear in light purple or purple-blue clusters within the cell. As with Howell-Jolly bodies, they tend to be visible near the periphery of the cell.

It is not unexpected that Pappenheimer bodies are seen in this case of sickle cell anemia. The abnormal hemoglobin disrupts normal erythropoiesis. Pappenheimer body formation is a complex process, but involves damaged ribosomes and mitochondria that result in aggregates of iron and protein and that subsequently form inclusions in the red cells. Inclusions such as Pappenheimer and Howell-Jolly bodies are usually removed by the pitting action of the spleen. However, patients with sickle cell disease often experience severe damage to the spleen because of recurrent blockages of splenic vessels by the sickle cells. These repeated splenic infarcts result in a dysfunctional spleen no longer capable of removing RBC inclusions.

ABI-11 shows an example of a reversible sickle cell. Sickle cells that have the potential to reverse back to a normal discocyte often appear in shapes that are not characteristically a “sickle,” that is, elongated, slightly curved, with pointed ends. These reversible sickle cells may assume many different shapes, including having more rounded ends like the cell in this image. Many terms have been used to describe these various cells that are morphologically “in-between” true sickle cells and normal RBCs. They may
resemble a holly leaf, boat, envelope, or blister, or appear ovoid. The actual mechanism resulting in irreversibly sickled cells is multifactorial, but the processes ultimately affect the RBC membrane. The membrane becomes permanently altered due to abnormalities in structural proteins related to oxidative damage and increased calcium levels, all associated with a subsequently rigid and leaky cell. A scan of this field of view reveals several other reversibly sickled cells. It is important to identify reversibly sickled cells as they are still abnormal and contain aberrant hemoglobin. Furthermore, the presence of sickle cells on a peripheral blood smear, reversible or otherwise, is diagnostic for sickle cell disease. Even one classic sickle cell confirms that other RBC shapes seen are also sickle cells, just reversible and capable of still becoming normally shaped red blood cells.

The cell selected for **ABI-12** is a target cell, or codocyte (from the Greek word meaning “bell”). These cells actually circulate as bells or Mexican hats. They appear as targets when flattened and dried on a glass slide. Several mechanisms can cause target cells to form, including when RBC membrane surface is increased relative to the hemoglobin content. This is the process resulting in codocytes in sickle cell anemia. Target cells have excess membrane for their surface area because of the decrease in intracellular hemoglobin. The extra red cell membrane condenses into the center of the cell where the area is relatively less compact. Then, the membrane is naturally squeezed up from the middle, forming a bell-shaped cell. But, during the process of smear preparation, this “bell” is flattened. Therefore, on a stained blood smear, target cells appear with a central core of hemoglobin surrounded by a ring of pallor or white area, and then another rim of hemoglobin.

**ABI-13** is the only white blood cell chosen for identification in this testing event; it is a monocyte. Monocytes are normally large cells. They have abundant cytoplasm that may be vacuolated or contain faint lilac or purple granules, neither of which is easily seen in this monocyte. The cytoplasm is typically blue-gray and appears rough, uneven, or what is sometimes described as “sandy.” Often, as in this example, the cellular margins are irregular with cytoplasmic projections. The shapes of nuclei in monocytes vary and may be round, oval, lobulated, or kidney-shaped. The nuclear chromatin generally stains a lighter purple and has little clumping.

The last cell chosen for identification in this exercise, **ABI-14**, is a nucleated RBC. Nucleated erythrocytes are immature cells that have not yet expelled their nuclei. They are not normally seen in the peripheral blood, and when present suggest abnormal or accelerated erythropoiesis. In this case study patient diagnosed with sickle cell disease, circulating nucleated RBCs most likely reflect the overall imbalance in erythropoiesis that occurs in this condition. Generally, when nucleated red blood cells are present in the peripheral blood, it is only later maturation stages that are seen, as with this cell. The nuclear chromatin is condensed and clumped, with no visible areas of parachromatin. The cytoplasm is
pink, though the color can vary in nucleated RBCs depending on how much hemoglobin has been synthesized by the cell. Likewise, the amount of cytoplasm is variable. Less mature nucleated erythrocytes will have less cytoplasm. It is unnecessary to classify nucleated RBCs by maturation stage when they are seen on a peripheral blood smear, though their presence must be reported.

**Sickle Cell Anemia**

Sickle cell anemia is a devastating global disorder that affects thousands of people in many countries. It is the most common worldwide symptomatic hemoglobin abnormality. The symptoms associated with this disease directly relate to the RBC’s inability to maintain a normal shape and to be flexible and deformable. Sickle cells block the microvasculature and numerous clinical manifestations result, including signs of a chronic hemolytic anemia, pain associated with vaso-occlusion, and splenic fibrosis and calcification.

The laboratory features, as seen in this testing event, are characteristic for the disorder. While the appearance of sickle cells is diagnostic, the presence of inclusions such as Howell-Jolly bodies and Pappenheimer bodies, nucleated RBCs, and target cells are also common findings. The sickle cells may be reversibly or irreversibly sickled. Likewise, the markedly decreased hemoglobin and hematocrit levels are significant. Though observing sickle cells on the peripheral blood smear is important, the condition is confirmed by identifying the abnormal HbS through electrophoresis or other methods.

**Summary**

The cells selected for identification and discussion in this exercise underscore the significant peripheral blood findings that can be seen in a patient with sickle cell anemia. It is particularly important for the laboratory professional to understand the mechanisms associated with the formation of sickle cells and that these cells may be not only classic sickle shapes (irreversible sickle cells), but also reversibly sickled and appear as many different shapes. It is nevertheless important to identify these reversibly sickled cells.

**References**