ADVANCED BLOOD CELL ID: PERIPHERAL BLOOD CELLS IN A CASE OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY

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. 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 Outcomes

After completion of this exercise, participants should be able to:

- Identify abnormalities in red blood cell morphology that may be associated with anemia resulting from a deficiency of G6PD.
- Describe morphologic features of normal peripheral blood leukocytes.
- Discuss morphologic characteristics of platelets.

Case History

A CBC with differential was ordered on a 24-year old male post splenectomy. His CBC results are as follows: WBC=4.9 x 10^9/L, RBC=2.7 x 10^{12}/L, Hgb=8.1 g/dL, Hct=24.3%, MCV=95 fl, MCH=30.5 pg, MCHC=33.0 g/dL, RDW=19.5%, Platelet=237 x 10^9/L.

Educational Commentary

The young man presented in the case study for this advanced blood cell identification virtual testing event was diagnosed with a G6PD deficiency. His medical history is also significant because he received a previous splenectomy.

The cell annotated for ABI-08 is a basophil (note there is a second basophil situated to the right and above the selected cell). Basophils are usually slightly smaller than the other two types of granulocytes, eosinophils and segmented neutrophils. Basophils are distinguished by their large, round, purple or blue-black cytoplasmic granules. Typically, these granules obscure the nucleus. Basophilic granules are water soluble and may fade during staining. Nuclear detail is difficult to appreciate because of the numerous granules. However, the nucleus is generally segmented with clumped chromatins.
The cell selected for discussion in ABI-09 is a nucleated red blood cell. Nucleated RBCs are immature erythrocytes that have not yet expelled their nuclei. They are not normally seen in the peripheral blood of adults. Sometimes, they may be present in the peripheral blood of newborns and infants. When seen in the peripheral blood of an adult, as in this case study patient, they indicate abnormally increased erythropoiesis. G6PD deficiency causes a hemolytic anemia and stimulation of the bone marrow to release young RBCs. These immature cells attempt to replace those with shortened survival resulting from the intrinsic enzyme deficiency. Therefore, it is not unexpected to see nucleated RBCs in the peripheral blood in this situation. The nucleated erythrocyte annotated in ABI-09 is a stage often seen when nucleated RBCs are present. The cell is small, with a moderate amount of pink cytoplasm. The color of the cytoplasm is variable, depending on how much hemoglobin has been synthesized in any individual cell. In some more immature nucleated erythrocytes, when less hemoglobin has been produced, the cytoplasm may appear blue-gray or dull blue. Likewise, the nucleus in this particular stage of red blood cell maturation is dense and clumped with no evidence of parachromatin. In younger erythrocytes, the chromatin may not be so condensed. Nucleated RBCs, though not classified according to maturation stage when seen in the peripheral blood, must be enumerated and reported.

The cell identified in ABI-10 has an inclusion called a Howell-Jolly body. Erythrocytes are generally evaluated morphologically to assess their overall size, shape, distribution on the smear, amount of chromicity to include color, and whether or not they have inclusions. RBCs should not have inclusions and when they are seen, they suggest some imbalance in cell maturation or hemoglobin production that results in anemia. Howell-Jolly bodies may also be present when a patient has had a splenectomy, as in this case study scenario.

Howell-Jolly bodies are generally small, but may vary in size. They are round and stain purple or purple-blue. Howell-Jolly bodies are typically seen as single inclusions in RBCs, but may appear as multiples, especially if the anemia is severe. They are generally located toward the periphery of the cell. Howell-Jolly bodies are nuclear remnants that form when a chromosome becomes separated from the mitotic spindle during cell division or abnormal nuclear fragmentation occurs during the process of nuclear extrusion by the maturing erythrocyte. Howell-Jolly bodies are usually efficiently removed by the spleen. However, if the anemia is severe the spleen may be overwhelmed, incapable of removing all inclusions. Likewise, if the spleen is dysfunctional or, as in this patient, absent, Howell-Jolly bodies will be evident in the peripheral blood.

The cell annotated for ABI-11 is a lymphocyte. Lymphocytes vary in size and this is an example of a medium-sized cell. This cell has minimal amounts of blue cytoplasm. The nucleus is characteristically
round, but may sometimes appear oval or slightly indented. The nuclear chromatin is clumped and condensed.

The cell shown in ABI-12 is a giant platelet. The term “giant” usually refers to platelets that are as large as or larger than a normal RBC. However, this platelet is larger than the other platelets visible in this area of the slide. Platelets generally range in size from 1-4 µm. Note that a giant platelet can also be seen in the field of view for ABI-11, adjacent to the RBC which is above the lymphocyte. Platelets may be round, oval, or irregularly shaped. The cytoplasmic edges may be smooth, frayed, or scalloped. Light purple or blue-gray granules are present. Sometimes a slight clear area may be visible around a central core of granules, as is evident in this cell.

The cell chosen for ABI-13 is another nucleated RBC, although this erythrocyte is less mature than the cell selected for discussion in ABI-09. Note that this cell is medium-sized, but much larger than the cell in ABI-09. There is a minimal amount of dark blue cytoplasm. The nucleus has areas of chromatin that appear more open, in contrast to the dense and clumped chromatin apparent in ABI-09.

It is important not to mistake the nucleated erythrocyte in ABI-13, which is at a very early stage of maturation, with a lymphocyte, as seen in ABI-11. The cells are approximately the same size, though the nucleated RBC is slightly larger. However, there are distinguishing cytoplasmic and nuclear features evident in the two cells. The cytoplasm in nucleated erythrocytes may be a deep blue, dull blue, blue-gray, or even pink, depending on the amount of hemoglobin synthesized by the cell. The cytoplasm color in lymphocytes is a more “true” blue. The chromatin in the nucleated RBC may look dense, but with lighter areas that give a patchy appearance to the nucleus. The nuclear chromatin pattern in the lymphocyte is typically uniformly clumped, with only some areas of lighter purple parachromatin visible.

The last cell annotated for discussion, ABI-14, is a polychromatophilic RBC. The polychromatophilic erythrocyte is the stage of maturation just prior to the normal red blood cell, also called a reticulocyte. Polychromatophilic RBCs have retained a small amount of ribonucleic acid (RNA). The RNA present allows the cells to appear bluish when Wright stained. The RBC can also be precipitated within the cell using a supravital stain like new methylene blue. This is the basis for a manual procedure that provides the percentage of reticulocytes present in a blood sample. Automated methods are also now commonly utilized to enumerate reticulocytes. The term “polychromasia” is used to describe the presence of polychromatophilic erythrocytes on a peripheral blood smear. Besides their bluish color, polychromatophilic RBCs lack any area of central pallor and are usually slightly larger than normal red blood cells. The presence of polychromatophilic erythrocytes indicates the bone marrow has increased activity in an effort to replace red blood cells lost through extrinsic or intrinsic mechanisms. This loss results in anemia.
G6PD Deficiency

G6PD deficiency is a common worldwide enzymopathy. G6PD is an important enzyme in RBC metabolism, preventing hemoglobin precipitation by accumulated cellular oxidants. When oxidants are increased, they cause hemoglobin to denature as inclusions in RBCs called Heinz bodies. Heinz bodies can only be visualized using supravital stains, but shorten erythrocyte survival. Again, it is also not surprising to see Howell-Jolly bodies in this case of G6PD deficiency. The disorder can result in an overall imbalance in RBC maturation and hemoglobin production. Likewise, polychromasia is expected whenever a hemolytic anemia occurs.

Most G6PD deficient patients are asymptomatic until their ability to reduce cellular oxidants is challenged through drugs, such as the antimalarial primaquine, or ingestion of food sources, like fava beans, that induce oxidative stress. Avoiding exposure to these medications and food items prevents hemolytic episodes. Although splenectomy is not usually necessary to treat G6PD deficiency, this patient did present in a post-splenectomy state. The peripheral blood findings are consistent with a post-splenectomy condition and include Howell-Jolly bodies, visible since the spleen is no longer present to remove any RBC inclusions. Thrombocytosis may be seen post-splenectomy as well because an overproduction of thrombopoietic growth factors occurs during the traumatic event (surgery) and stimulates megakaryocytes to produce platelets. Although the platelet count was not reported as increased in this case study patient, numerous platelets can be seen in most areas of the virtual slide.

Summary

The patient presented in this virtual testing event was diagnosed with G6PD deficiency and had no spleen. The cells annotated for commentary represent normal leukocytes and abnormalities in platelets as well as immature erythrocytes and other RBC morphologic changes. Even though this patient’s abnormality was diagnosed prior to presentation, the laboratory professional still contributes important information for patient monitoring and management.

References

