ADVANCED BLOOD CELL ID: PERIPHERAL BLOOD CELLS IN A CASE OF MICROANGIOPATHIC HEMOLYTIC 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. 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 Objectives
After completion of this testing event, participants will be able to:

- describe morphologic abnormalities in RBCs to include shape changes, an inclusion, and variation in coloration
- distinguish various erythrocyte shape abnormalities based on morphologic evaluation
- identify morphologic characteristics of basophils.

Case History
A CBC with differential was ordered on a 7 year old girl complaining of abdominal pain, fever, vomiting, and diarrhea. Her CBC results were as follows: WBC=5.7 x 10^9/L, RBC=3.11 x 10^{12}/L, Hgb=9.3 g/dL, Hct=28%, MCV=90.4 fL, MCH=29.9 pg, MCHC=33.2 g/dL, Platelet=122 x 10^9/L, RDW-CV=21.9%.

Educational Commentary
The cells and objects annotated for commentary in this exercise represent several morphologic variations in RBCs associated with a condition called microangiopathic hemolytic anemia (MAHA). One leukocyte has also been selected for discussion. These cells were identified from the blood smear of a 7 year old girl who initially presented with pain, fever, vomiting, and diarrhea in addition to anemia and thrombocytopenia.

The RBC chosen in ABI-01 is a schistocyte. Identifying schistocytes in the peripheral blood is important. They are fragmented erythrocytes, varying in shape and size; most are small. Schistocytes are typically irregularly shaped and characteristically have no area of central pallor. Though most often irregular, common shapes include triangles,
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helmets, and commas. Note the unusual tail in this particular cell. Schistocytes have survived physical or mechanical trauma as they circulated in the peripheral blood. Obstructed vessels, as may occur in MAHA, impede the normal flow of RBCs and the cells may shear or fragment. The erythrocytes retain their membrane, but are nevertheless damaged. The presence of schistocytes on a peripheral blood smear is a significant and reportable finding. Identifying schistocytes can be an important indicator regarding the etiology of a hemolytic anemia.

The second cell selected for discussion, **ABI-02**, is a polychromatophilic RBC. The term polychromasia is used to describe the condition associated with the presence of these cells on a peripheral blood smear. Polychromatophilic erythrocytes represent the stage of RBC maturation just prior to the mature red blood cell, also call the reticulocyte. Reticulocytes have no nuclei, but have retained various amounts of RNA (ribonucleic acid). The cell therefore appears blue-gray when Wright-stained. Reticulocytes are sometimes larger than normal RBCs, as in this cell. Reticulocytes normally mature about 48 hours in the bone marrow and an additional 24 hours after their release into the circulation. The presence of polychromatophilic RBCs in the peripheral blood indicates the bone marrow is attempting to compensate for a decrease in oxygen carrying capacity resulting from a loss of erythrocytes. In this testing event, the patient has been diagnosed with a hemolytic anemia that has shortened the lifespan of the red blood cells. Therefore, it is not unexpected to see polychromasia on the blood smear of this patient.

**ABI-03** shows a spherocyte. Spherocytes are smaller than normal RBCs because they have lost cellular membrane. They also have decreased surface to volume ratio and appear dense, lacking any area of central pallor. Spherocytes may be seen in patients with hereditary spherocytosis, immune-mediated hemolytic anemia or, as in this situation, if the patient has a MAHA. Obstructions in small blood vessels not only result in fragmented erythrocytes, but if the membrane can completely reseal, then spherocytes will form.

The cell identified in **ABI-04** is an echinocyte, also called a burr or crenated cell. These cells are usually the same size as normal RBCs. They are characterized by their evenly spaced surface projections. Echinocytes typically have 10-30 short, rounded, or slightly pointed spicules. In contrast to spherocytes,
they do have an area of central pallor. Echinocytes are most commonly artifacts produced during blood smear preparation or from sample condition. Blood slides that are made too thick, prepared from old specimens, or permitted to air dry too slowly can cause echinocyte formation. Likewise, erythrocytes are sensitive to environmental and physiological conditions that can affect their shape. Variations in pH, for example, can cause echinocytes to be produced. An increase in pH on the glass slide, resulting from the diffusion of basic substances in the medium surrounding the RBCs, may induce echinocyte formation. Although not often seen associated with clinical conditions, echinocytes may sometimes be seen in pathologic states such as severe renal disease (uremia) and pyruvate kinase deficiency as well as in patients with burns. Mechanisms of echinocyte formation in these other conditions may be related to accumulations of surface lipids or loss of intracellular adenosine triphosphate (ATP) that limit the cell’s ability to maintain a normal discocyte shape.

The RBC selected in **ABI-05** has an inclusion called a Howell-Jolly body. Normal erythrocytes have no inclusions. Howell-Jolly bodies are variable in size, but most are small. They are round and usually stain purple or purple-blue. Howell-Jolly bodies typically present as single inclusions on the periphery of the RBC, but sometimes appear as multiple inclusions in severe anemia. They form either as a consequence of abnormal nuclear fragmentation, which occurs as the developing RBC expels its nucleus, or when a chromosome separates from the mitotic spindle during cell division. The spleen is generally efficient at removing red cell inclusions, but if it is absent, dysfunctional, or simply overwhelmed, Howell-Jolly bodies may be seen in the peripheral blood. Several conditions are associated with the presence of Howell-Jolly bodies and include severe hemolytic anemia, megaloblastic anemia, myelodysplastic syndromes, and hemoglobinopathies.

**ABI-06** is a basophil. Basophils are medium-sized cells and are generally oval or round. They are characterized by their large, round, dark purple (almost black) cytoplasmic granules. These granules are also numerous and often obscure the condensed and clumped nucleus; the nucleus may be
segmented or lobed. Basophilic granules sometimes appear faded or washed-out, as they are water-soluble and their color leaches away during the staining process.

The final cell annotated for discussion, ABI-07, is a helmet cell. Helmet cells are actually a specific type of schistocyte and result from the same fragmentation processes that can produce schistocytes. Helmet cells typically have two projections on either end that are tapered and pointed. When a circulating RBC catches and shears on fibrin deposited abnormally in a small vessel, the cell fragments. Smaller pieces of membrane become classic schistocytes while larger portions may still reseal, but as either spherocytes, as previously discussed, or as helmet cells.

Microangiopathic Hemolytic Anemia (MAHA)

MAHA results when erythrocytes fragment within small blood vessels obstructed primarily by fibrin strands. Sometimes the cells with disrupted membranes can survive and continue to circulate as schistocytes, the membranes may reseal as spherocytes or helmet cells, or complete intravascular lysis of the cells will occur if they are severely damaged. The pathologic deposition of fibrin in vessels is usually associated with disseminated intravascular coagulation (DIC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP). However, other situations, such as malignant hypertension or HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) can also result in a MAHA. Though the microthrombi formation in these conditions is triggered by different mechanisms, the notable finding in all is the presence of schistocytes on the peripheral blood smear.

Summary

The annotations in this testing event represent cells that may be seen in the blood smear of a patient with a MAHA. Although the underlying cause of the MAHA in this case study patient is not specified, the appearance of schistocytes would be especially significant in the initial determination that a MAHA was present. MAHA must be quickly treated and the laboratory professional plays an important role in reporting this key RBC morphologic change.

Bibliography

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