EDUCATIONAL COMMENTARY – BLOOD CELL ID: ETIOLOGIC FEATURES AND DIFFERENTIATION OF RBC INCLUSIONS

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LEARNING OBJECTIVES

On completion of this exercise, the participant should be able to:

- identify morphologic features of normal peripheral blood leukocytes.
- discuss the etiologic features of Howell-Jolly bodies, basophilic stippling, and Pappenheimer bodies.
- describe morphologic characteristics of Howell-Jolly bodies, basophilic stippling, and Pappenheimer bodies.
- contrast the morphologic features of Howell Jolly bodies, basophilic stippling, and Pappenheimer bodies.

Case study

A 78 year old female was seen in the emergency room after falling down the stairs. Her CBC data was as follows: WBC=8.4 x 10^9/L, RBC=2.48 x 10^{12}/L, Hgb=8.8 g/dL, Hct=26.1%, MCV=105 fL, MCH=35.5 pg, MCHC=33.7 g/dL, RDW-SD=83.8 fL, Platelet=549 x 10^9/L.

Educational Commentary

The images provided in this testing event represent normal white blood cells and various abnormal inclusions that may be seen in red blood cells (RBCs).

Image BCI-15 shows a segmented neutrophil. The dense and clumped nuclear chromatin indicates that this cell is mature. The nucleus has at least two lobes, connected by narrow strips of chromatin. The cytoplasm is a characteristically light pink, reflecting numerous specific granules, which are associated with mature neutrophils.
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Image **BCI-16** illustrates a normal lymphocyte. Lymphocytes vary in size. The cell pictured here is an example of a small lymphocyte. Note the thin rim of blue cytoplasm and the relatively large, round nucleus. The nuclei may also appear oval or slightly indented. The nuclear chromatin is clumped, dense, and dark purple.

Image **BCI-17** depicts an RBC with a specific inclusion called a Howell-Jolly body. Red blood cells should have no inclusions. When inclusions are present, they often indicate anemia. The data provided for the patient in this testing event indicate that she has anemia, with a hemoglobin level of 8.8 g/dL (to convert to g/L, multiply by 10.0) and hematocrit level of 26.1% (to convert to proportion of 1.0, multiply by 0.01).

Howell-Jolly bodies are usually small, but can vary in size. They are round and generally stain purple or purple-blue. These inclusions typically appear singly or, occasionally, in multiples if the anemia is severe. Howell-Jolly bodies tend to be located toward the periphery of the cell and represent nuclear remnants.

Howell-Jolly bodies form when a chromosome is separated from the mitotic spindle during cell division or as a result of abnormal nuclear fragmentation as the maturing RBC expels its nucleus. The spleen is usually efficient at removing RBC inclusions. However, if the spleen is absent, dysfunctional, or overwhelmed, Howell-Jolly bodies may be seen in the peripheral blood. They are also associated with megaloblastic anemias, severe hemolytic anemia, and congenital dyserythropoietic anemia.
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The arrow in BCI-18 identifies an RBC with basophilic stippling. These inclusions are fine or coarse deep blue or blue-gray granules dispersed throughout the cell. Fine stippling represents an artifact that forms during the slow drying of the blood smear. Coarse stippling is evident as larger, more distinct granules that are evenly distributed in the cytoplasm. The presence of coarse basophilic stippling is clinically significant: it suggests impaired hemoglobin synthesis. The inclusions are abnormally aggregated ribosomes and polyribosomes that result from dysfunctional RNA degradation. Several diseases and conditions are associated with the appearance of basophilic stippling on the peripheral blood smear and include lead poisoning, thalassemia, megaloblastic anemia, sickle cell anemia, and sideroblastic anemia.

Editor’s note: Some participants reported "Platelet(s), giant" for Image BCI-18. The granules in a giant platelet are generally larger and more packed together than the diffuse, smaller granules of basophilic stippling. In a stippled cell, it is possible to count individual granules as they are so evenly distributed. Platelet granules are more dense and clustered and cannot easily be seen as individual granules. In the bottom left section of image BCI-21 is another example of a polychromatic RBC with basophilic stippling, and this cell is adjacent to an enlarged platelet. Note the packed granularity and the purple color of the platelet versus the diffuse distribution of blue to blue-gray granules within the red cell.

Image BCI-19 shows an eosinophil. Eosinophils characteristically have numerous bright, red-orange granules in the cytoplasm. These specific granules are generally large and uniform in size. The nucleus in eosinophils is often bilobed with dense, clumped, purple chromatin. Because eosinophils are normally present in the peripheral blood in low numbers, the maturation stage does not need to be reported.

Image BCI-20 shows an RBC with inclusions called Pappenheimer bodies. In contrast to the Howell-Jolly body and basophilic stippling seen in other pictures for this testing event, Pappenheimer bodies
appears as small, irregularly shaped clusters near the periphery of the cell. They generally stain light purple or purple-blue. Pappenheimer bodies contain iron that cannot be used in hemoglobin production because of an abnormal condition or disease state. In such situations, damaged ribosomes and mitochondria cause vacuoles to form around them, releasing digestive enzymes. The vesicles then contain excess iron released from mitochondria. The Pappenheimer bodies that subsequently form may be removed by the spleen. But, as mentioned previously, if the spleen is absent, dysfunctional, or overwhelmed, the inclusions remain and are visible on a peripheral blood smear. Disorders associated with Pappenheimer bodies include sideroblastic anemia, thalassemias, megaloblastic anemia, and hemochromatosis.

**Editor’s note:** Some participants reported a result of siderocyte or siderotic granules for Image BCI-20. Granules staining positive for iron using Prussian blue or Perl’s stain are known as siderotic granules. However, the images included in this case study are from a Wright’s stained peripheral blood smear, so these inclusions are recognized as Pappenheimer bodies.

Although Howell-Jolly bodies, basophilic stippling, and Pappenheimer bodies may be seen in many of the same processes, it is important to morphologically distinguish them. The Table defines physical characteristics of these three inclusions and summarizes their composition and basic cause. Note also that Pappenheimer bodies can be confirmed with a positive iron stain, which will be negative if Howell-Jolly bodies and basophilic stippling are present.

**Table. Features Distinguishing RBC Inclusions**

<table>
<thead>
<tr>
<th></th>
<th>Howell-Jolly Bodies</th>
<th>Basophilic Stippling</th>
<th>Pappenheimer Bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composition</strong></td>
<td>DNA</td>
<td>Ribosomes</td>
<td>Iron</td>
</tr>
<tr>
<td><strong>Basic Cause</strong></td>
<td>Chromosome separates; or nucleus fragments</td>
<td>Abnormal aggregation</td>
<td>Vacuoles with digestive enzymes release iron from mitochondria</td>
</tr>
<tr>
<td><strong>Morphologic Features</strong></td>
<td>Round, single or rarely multiples; purple</td>
<td>Evenly distributed, small, round granules; blue-gray</td>
<td>Irregular clusters; purple or purple-blue</td>
</tr>
</tbody>
</table>
Image BCI-21, the last image in this testing event, depicts a nucleated RBC. Nucleated RBCs are not usually seen in the peripheral blood, and, when present, indicate abnormal or accelerated erythropoiesis. These cells are immature and have not yet expelled their nucleus. Note the dense, clumped nucleus - there is no evidence of chromatin or parachromatin. The cytoplasm is pink. There appears to be basophilic stippling in this cell.

Nucleated RBCs do not need to be classified by maturation stage when seen in the peripheral blood.

Summary

The patient in this case study had anemia and an abnormal peripheral blood smear that included several RBC inclusions. These inclusions reflect a severe anemia, and relate to the patient’s splenectomy. The spleen is an efficient organ for pitting abnormal inclusions. Therefore, when the spleen is absent, dysfunctional, or overwhelmed, inclusions will increase.

The laboratorian plays a key role in reporting abnormalities in the peripheral blood, such as the Howell-Jolly body, basophilic stippling, and Pappenheimer bodies seen in this testing event. Properly identified inclusions provide information critical to diagnosis.

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