EDUCATIONAL COMMENTARY – BLOOD CELL IDENTIFICATION

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Learning Outcomes
Upon completion of this exercise, the participant will be able to:

- Describe characteristic morphologic and pathophysiologic features of clumped and giant platelets.
- Identify morphologic abnormalities in erythrocytes associated with sideroblastic anemia.
- Compare and contrast the morphology of basophilic stippling, Howell Jolly bodies, and Pappenheimer bodies on a Wright’s-stained peripheral blood smear.

Case History
A CBC was requested as part of a routine physical examination for this 50 year old male, whose history revealed a splenectomy several years earlier. The CBC data was as follows: WBC=8.4 x 10⁹/L, Hgb=8.8 g/dL, Hct=26%, MCV=105 fL, MCH=33.7 pg, RDW=22.5%, Platelets=549 x 10⁹/L. WBC Differential: Neut=50%, Lymphs=38%, Monos=8%, Eos=3%, Baso=1%.

The patient presented in this testing event has been diagnosed with sideroblastic anemia. The photographs for review represent several morphologic changes, especially inclusions in red blood cells, which may be seen in the peripheral blood in this condition.

BCI-08 shows platelets that are clumped. Clumped platelets can indicate a specimen that was clotted at the time the blood smear was prepared. Platelet clumps are also sometimes seen as a nonspecific antibody-induced phenomenon associated with blood collected in EDTA. However, note that in this photograph other platelets are still evenly disbursed throughout the smear. Platelets within the clump morphologically resemble the individual platelets in regard to size, color, and granularity. Whenever platelet clumps are observed, it is important to evaluate the remainder of the smear and ensure that adequate numbers of platelets are present. If individual platelets are not seen and large clumps are noted at the end of the smear, then a new blood smear should be prepared. It is also sometimes necessary to recollect a new blood sample if platelet clumping results because of EDTA. Platelet clumping can falsely decrease the total platelet count as determined by automated cell counters. The platelet count in this patient was reported as 549 x 10⁹/L.
A normal lymphocyte is shown in BCI-09. Lymphocytes vary in size. The one pictured here is a small lymphocyte. In small lymphocytes, the nuclei are relatively large when compared to the scanty rim of blue cytoplasm. Nuclei are usually round, oval, or slightly indented. The nuclear chromatin is clumped, condensed, and appears a deep purple. Nucleoli may sometimes be present, but are often not visible.

BCI-10 illustrates an erythrocyte with basophilic stippling. Basophilic stippling represents the abnormal aggregation of ribosomes that appear in red blood cells during the drying and staining of peripheral blood smears. Fine stippling is generally an artifact associated with slow drying of the smear. Coarse stippling is clinically significant, suggesting impaired hemoglobin synthesis. These inclusions are more distinct and larger. Coarse stippling is also characterized by evenly distributed, blue-gray granules. Disorders associated with the presence of basophilic stippling include lead poisoning, megaloblastic anemias, thalassemias, sickle cell anemia, and, as in this case, sideroblastic anemia.

Photograph BCI-11 identifies a nucleated red blood cell. Nucleated RBCs are young erythrocytes that have not yet matured and still retain their nucleus. Although normally present in the bone marrow, the appearance of nucleated erythrocytes in the peripheral blood is indicative of abnormal or accelerated erythropoiesis. Even in abnormal conditions, usually only the very late stages of these cells will be seen in the peripheral blood. The stage of maturation for nucleated RBCs does not need to be identified, but their presence should be reported. The nucleated RBC seen in this picture is typical of those found in the peripheral blood. The nucleus is dense and clumped, whereas the cytoplasm is scanty and bluish-gray.
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The arrow in **BCI-12** identifies a giant platelet. The term “giant” is used to describe a platelet that is larger than a normal red blood cell. Normal platelets are usually only 1-4 µm in diameter, as shown in picture **BCI-08**. The shape of giant platelets is variable. The edges may be smooth and rounded or uneven and ruffled. The cytoplasm may be agranular or, as in this case, contain numerous purple granules. Giant platelets are more often seen in conditions associated with disturbed megakaryopoiesis, such as myelodysplastic syndromes or myeloproliferative disorders. So, it is unusual to see a giant platelet in this case. Giant platelets should not be confused with smudge cells or residual cytoplasm from other cells. Giant platelets contain light and dark areas in the cytoplasm. Cellular artifacts lack any defined structures.

Howell-Jolly bodies are depicted in **BCI-13**. These RBC inclusions are composed of DNA and appear purple on a Wright’s-stained peripheral blood smear. Howell-Jolly bodies are round or oblong and may be single or multiple. Multiple inclusions are more often associated with severe anemias. Howell-Jolly bodies form in one of two possible processes: 1) during erythrocyte maturation, a chromosome may become separated from the mitotic spindle during cell division and be retained in the cell after nuclear expulsion, or 2) from abnormal fragmentation of the RBC nucleus during nuclear extrusion. Small nuclear fragments are separated from the main nucleus and remain in the cell. The presence of Howell-Jolly bodies is associated with megaloblastic anemias, myeloproliferative disorders, some cases of leukemia and hemolytic anemia, and when a patient has no spleen or a dysfunctional spleen. Although not specifically characteristic of sideroblastic anemia, seeing Howell-Jolly bodies in this patient is not unexpected considering that in general, erythropoiesis is impaired.
Image **BCI-14** shows 2 erythrocytes with Pappenheimer bodies. Pappenheimer bodies contain iron. They are small and shaped irregularly. Pappenheimer bodies are purple-blue in color and are generally located near the periphery of the cell in clusters. These RBC inclusions are produced in pathologic conditions from damaged ribosomes and abnormal iron-containing mitochondria. They are most often associated with not only sideroblastic anemias, but also thalassemia, megaloblastic anemias, and after splenectomy. It is important to morphologically distinguish Pappenheimer bodies from other RBC inclusions, especially basophilic stippling and Howell-Jolly bodies. The following chart compares and contrasts these 3 inclusions according to their appearance on a Wright's-stained peripheral blood smear. However, also note that Pappenheimer bodies are confirmed with a positive iron stain whereas basophilic stippling and Howell-Jolly bodies do not stain with an iron stain.

**MORPHOLOGY OF BASOPHILIC STIPPLING, HOWELL-JOLLY BODIES, AND PAPPENHEIMER BODIES**

<table>
<thead>
<tr>
<th><strong>Basophilic Stippling</strong></th>
<th><strong>Howell-Jolly Bodies</strong></th>
<th><strong>Pappenheimer Bodies</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Evenly distributed,</td>
<td>Round, oblong, single</td>
<td>Irregular, purple-blue</td>
</tr>
<tr>
<td>small, round, blue-gray</td>
<td>or multiple (rare),</td>
<td>clusters near cell</td>
</tr>
<tr>
<td>granules</td>
<td>purple inclusions</td>
<td>periphery</td>
</tr>
</tbody>
</table>

It is also important not to confuse RBC inclusions, such as Pappenheimer bodies, with platelets that may be superimposed on erythrocytes. Platelets, such as those seen in BCI-08 and BCI-12, are granular and irregular in shape. Pappenheimer bodies are uniform in shape and generally clustered within the cell. Furthermore, Pappenheimer bodies do contain iron and will stain positive with a Prussian blue or iron stain, whereas platelets will not stain. Technically, the term Pappenheimer body refers to iron deposits in RBCs when seen with a Wright's stain. These inclusions can be confirmed as containing iron by performing an iron stain, at which point the inclusions can be called siderocytes or siderotric granules. Pappenheimer bodies, with their iron, can be visualized on a peripheral blood smear such as is seen in picture BCI-14 because they also contain protein, which is readily visible with a Wright's stain.
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The appearance of multiple RBC inclusions in this patient not only reflects the underlying sideroblastic anemia, but also is seen after splenectomy. The spleen is very efficient at removing or pitting abnormal particles from erythrocytes. When it has been removed, inclusions will be seen in increased numbers. It is also not unusual to see an elevated platelet count in the splenectomized patient.

Sideroblastic anemia is actually a term used to describe a diverse group of disorders characterized by abnormal iron metabolism. Regardless of classification or underlying cause, sideroblastic anemias result when iron cannot be incorporated to form heme during hemoglobin synthesis. This blockage generally results through some process that impairs enzymes that regulate heme biosynthesis. The disturbance of enzymes may be associated with hereditary disorders or secondary to any one of several exposures or conditions including drugs, lead, or malignancy. All types of sideroblastic anemia are characterized by an increase in body iron stores. Iron is present; it just cannot be incorporated into heme. In addition to RBC inclusions, other peripheral blood findings associated with sideroblastic anemias include anisocytosis, as reflected in an elevated RDW, and slight macrocytosis (note the MCV of 105 fL and RDW of 22.5% in this patient).

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