EDUCATIONAL COMMENTARY – PERIPHERAL BLOOD CELL MORPHOLOGY IN SEPTICEMIA

Learning Objectives
Upon completion of this exercise participants will be able to:

- identify morphologic features of mature and immature leukocytes.
- distinguish extracellular and intracellular bacteria from contaminants and other cells.
- discuss differences between immature and mature leukocytes.

The images presented in this testing event are both normal and abnormal peripheral blood smear findings that might be seen in septicemia, as diagnosed in this patient.

Photograph BCI-15 illustrates a normal segmented neutrophil. These cells characteristically have a nucleus that is segmented into 2 to 5 lobes. The nuclear lobes are connected by thin strands of chromatin. Note that sometimes the chromatin joining the segments is not visible, as in this example. Pink, tan, or violet granules can be seen in the cytoplasm.

Normal RBCs or erythrocytes are pictured in photograph BCI-16. Red blood cells are evaluated morphologically for their size, shape, size of the area of central pallor, distribution on the smear, and the presence or absence of inclusions. In normal erythrocytes, the central pallor is about one-third of the diameter of the cell. A useful internal gauge for measuring RBC size is to compare the RBC with the nucleus of a small, normal lymphocyte. Erythrocytes should appear close to the same size as the nucleus of a small lymphocyte. Although the RBCs in this image vary in size, most are about the same size as the lymphocyte that is also present in the image. They are also evenly shaped, have an appropriate area of central pallor, are uniformly distributed, and contain no inclusions.
EDUCATIONAL COMMENTARY – PERIPHERAL BLOOD CELL MORPHOLOGY IN SEPTICEMIA (cont.)

A metamyelocyte is shown in photograph BCI-17. This cell is not normally seen in the peripheral blood. The metamyelocyte is an immature neutrophil and the first cell in the neutrophil maturation sequence that can no longer undergo mitosis. The presence of a metamyelocyte in the patient’s peripheral blood is not surprising and reflects the severity of the patient’s infection. The characteristic morphologic feature that distinguishes the metamyelocyte from other cells in the neutrophil maturation continuum is a nucleus that is slightly indented, sometimes referred to as kidney-shaped. The indentation is less than one-half of the diameter of a hypothetical round nucleus. The nuclear chromatin is generally dense and clumped, indicating the postmitotic stage of the cell. Some pink, tan, or violet specific granules are abundant in the cytoplasm. Some darker-staining primary granules may be seen, as in this example. Even though the cytoplasm in this cell is blue, the few primary granules, violet secondary granules, and nuclear shape are features that classify the cell as a metamyelocyte. A general rule of thumb states that when in doubt as to the maturation stage of a cell, identify the cell as the most mature form.

It is important to differentiate metamyelocytes from myelocytes, and occasionally, monocytes. In myelocytes, the nucleus is generally eccentrically located and is often round or oval. Although the nucleus is usually clumped, some lighter areas of parachromatin may be visible. A hof, or clear area, is seen adjacent to the nucleus. As with other cells in the neutrophil maturation sequence, the specific granules are pink, tan, or violet. Darker nonspecific or primary granules may still be retained in the cytoplasm as well as a light blue color.

Monocytes are large cells and are usually slightly larger than myelocytes and metamyelocytes. The cytoplasm is characteristically blue-gray and may appear uneven. The cytoplasm in myelocytes and metamyelocytes is typically filled with pink or lilac granules. Sometimes the cytoplasm in these immature neutrophils may appear a smooth or homogeneous blue with just a few specific granules. Monocytes may also display a few azurophilic or primary granules. Primary granules may be seen in myelocytes and metamyelocytes, but are usually more numerous than those seen in monocytes. Monocytes often have cytoplasmic vacuoles. The chromatin is dense and clumped, but less than what is seen in the neutrophils. The nucleus may be round, oval, indented, or lobulated. Cytoplasmic protrusions are often present.
EDUCATIONAL COMMENTARY – PERIPHERAL BLOOD CELL MORPHOLOGY IN SEPTICEMIA (cont.)

Blood cells are sometimes difficult to classify when characteristics overlap or appear very similar. A review of cell size, as well as nuclear and cytoplasmic characteristics, should be part of cell identification. The Table below compares the morphologic characteristics of metamyelocytes, myelocytes, and monocytes.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Metamyelocyte</th>
<th>Myelocyte</th>
<th>Monocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size</td>
<td>Large</td>
<td>Large</td>
<td>Larger</td>
</tr>
<tr>
<td>Nuclear shape</td>
<td>Kidneylike; slight indentation</td>
<td>Round, oval</td>
<td>Round, oval, indented, lobulated</td>
</tr>
<tr>
<td>Nuclear placement</td>
<td>Center of cell</td>
<td>Eccentric</td>
<td>Center of cell</td>
</tr>
<tr>
<td>Nuclear chromatin</td>
<td>Clumped</td>
<td>Clumped</td>
<td>Minimal clumping</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Moderately abundant; rare primary granules; often specific pink/lilac granules present</td>
<td>Abundant; primary granules may be present; specific pink/lilac granules may be present; clear area near nucleus</td>
<td>Abundant; primary granules and vacuoles may be visible.</td>
</tr>
</tbody>
</table>

Image BCI-18 shows a normal lymphocyte. Lymphocytes vary in size. The one shown here is a good example of a small lymphocyte. Small lymphocytes generally have minimal, blue cytoplasm and a relatively large nucleus. Sometimes, light purple granules may be seen in the cytoplasm, although none are visible in this example. The nuclear shape is generally round, though a slight indentation may occasionally be evident. The nuclear chromatin is clumped, dense, and appears a dark purple. Nucleoli are sometimes seen, but are often not visible.
EDUCATIONAL COMMENTARY – PERIPHERAL BLOOD CELL MORPHOLOGY IN SEPTICEMIA (cont.)

Image **BCI-19** shows a smudge cell or basket cell. These cells result from the process of making the peripheral blood slide and are most often associated with fragile cells. This example can be more characterized as a smudge cell with its smear of chromatin. A basket cell features wisps or strands of chromatin that extend out from a condensed nuclear mass, resembling a basket. Note that no cytoplasm is present in smudge/basket cells; so cell identification is not possible. Smudge/basket cells are most often lymphocytes, but they can be monocytes and other types of leukocytes. Although the presence of significant numbers of these cells is highly associated with chronic lymphocytic leukemia, they may be seen in other malignant as well as benign hematologic disorders and even in normal blood smears. Seeing a smudge/basket cell in this patient is not surprising given the severity of this patient's illness. Sometimes the smudge/basket cells are so prevalent that an accurate evaluation of a peripheral blood smear cannot be completed. In these cases, 1 drop of 22% bovine serum albumin added to 4 to 5 drops of patient's blood will prevent the distortions. A new smear can be made and reviewed. The original smear (without added albumin) should be used to evaluate erythrocyte morphology and to determine a platelet estimate.¹

The cell in photograph **BCI-20** has phagocytized bacteria. Intracellular bacteria are not normally present in peripheral blood cells. The appearance of a cell with microorganisms reflects the patient’s overwhelming infection. Because the cell in the image has engulfed bacteria, its morphology has been distorted. Based on size, nuclear lobulation, light-blue-gray cytoplasm with vacuoles, and minimal nuclear clumping, it is most likely a monocyte. However, in this situation, identifying the cytoplasmic bacteria is more critical than classifying the cell.
EDUCATIONAL COMMENTARY – PERIPHERAL BLOOD CELL MORPHOLOGY IN SEPTICEMIA (cont.)

In photograph **BCI-21**, the bacteria are extracellular. A careful review of the intracellular bacteria in photograph BCI-20 with the extracellular microorganisms in photograph BCI-21 indicates that they are the same. The bacteria seen in either image must be clearly distinguished from stain precipitate or platelets. Although bacteria, artifact, and platelets all stain purple, a close evaluation shows differences. Bacteria are randomly distributed in the smear, are more uniform in size and shape, are few in number, and do not appear as darkly stained as precipitate. Stain precipitate generally forms clusters in the smear, individual granules are irregular and variable in size, and the precipitate is often a deep purple color. Although the extracellular bacteria in photograph BCI-21 appear as small groups, note how all the individual organisms are the same size and shape. Also, intracellular inclusions will focus with the cell whereas any artifact will focus in a different plane from the cell.

Likewise, platelets should not be confused with bacteria. Normal platelets are larger than bacteria. They are variable in shape but are usually round or oval. Platelets often appear granular. They stain a lighter purple or blue-gray when compared with bacteria.

This patient had a severe infection that resulted in septicemia. The WBC was only $6.5 \times 10^9$/L. A patient who was adequately fighting the infection would have a higher leukocyte count. The presence of immature neutrophils and extracellular and intracellular bacteria are associated with pneumonia. Blood cultures identified the causative organism as *Streptococcus pneumoniae*. The patient succumbed to the overwhelming infection.

**Reference**


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