EDUCATIONAL COMMENTARY – MORPHOLOGIC FEATURES OF NORMAL AND ABNORMAL LYMPHOCYTES

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

- delineate the morphologic characteristics of normal peripheral blood leukocytes and platelets.
- describe morphologic features unique to echinocytes.
- compare and contrast normal lymphocytes, reactive lymphocytes, and malignant lymphocytes, such as Sézary cells.

A CBC count with differential was requested for a 53-year-old man with a history of lymphoma. Laboratory findings included: a WBC count of 13,100/µL (13.1 ×10⁹/L), a hemoglobin level of 12.3 g/dL (123 g/L), a hematocrit of 34%, a mean corpuscular volume of 101 fl, a mean corpuscular hemoglobin of 36 pg, a mean corpuscular hemoglobin concentration of 35.6 g/dL (356 g/L), a RBC distribution width of 15.3%, and a platelet count of 137 ×10³/µL (×10⁹/L).

The images for review represent both normal and abnormal cells that may be seen in the peripheral blood of a patient with lymphoma.

Image BCI-15 depicts an echinocyte, also called a crenated or burr cell. These cells are usually about the same size as normal erythrocytes. The projections are evenly spaced around the cell surface. They are short, rounded, or slightly pointed. Echinocytes have anywhere from 10 to 30 projections. An area of central pallor is still visible. Echinocytes are most often seen on the peripheral blood smear as artifacts of preparation. Blood smears that are too thick, made from old blood, or allowed to air dry too slowly can cause echinocytes to appear. In addition, RBCs are very sensitive to environmental changes, and many physiological conditions induce the formation of echinocytes. For instance, an increase in the pH on the glass slide often transforms erythrocytes into echinocytes.

Echinocytes are occasionally seen in the blood smear of patients with severe renal disease (uremia), liver disease, pyruvate kinase deficiency, burns, or other conditions. The echinocytes evident in this photograph are most likely a result of smear preparation.
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Image BCI-16 shows a normal basophil. Basophils are characterized by the presence of blue-black or deep purple cytoplasmic granules. These granules are generally numerous, round, and large. In fact, the granules are often so prominent that they obscure the nucleus. Note that basophilic granules are water soluble and sometimes wash away during the staining process, leaving a clear or light area in the cytoplasm.

Two normal platelets are identified in Image BCI-17. Platelets are generally 1 to 4 µm in diameter. While variable in shape, typically they are round or oval. Platelets stain purple or blue-gray and often appear grainy. They are technically not cells because they have no nuclei. However, they originate from nucleated cells in the bone marrow (called megakaryocytes) and represent fragments of cytoplasm from these cells.

Image BCI-18 shows a normal lymphocyte. This cell is an example of a small lymphocyte. However, lymphocytes are variable in size. In small lymphocytes the nuclei are relatively large when compared with the scanty amount of pale or moderately blue cytoplasm. In fact, the cytoplasm in this cell is barely visible. No vacuoles or granules are generally present in the cytoplasm. However, note that in normal lymphocytes that are medium-sized, a few azurophilic granules may be seen. Nuclei in lymphocytes are generally round, oval, or only slightly indented. The nuclear chromatin is clumped, dense, and stains a deep purple. Although not visible in this example, nucleoli are sometimes present in lymphocytes.
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Image BCI-19 depicts an eosinophil. Eosinophils are characterized by the presence of numerous red-orange cytoplasmic granules. These granules are also fairly large and uniform in size and shape. The nucleus of mature eosinophils is frequently bilobed. The nuclear chromatin is dense, clumped, and stains a dark purple.

The cells in Images BCI-20 and BCI-21 are both malignant lymphocytes called Sézary cells. Sézary cells are variable in morphology. Their size may be just slightly larger than a normal lymphocyte, as represented in Image BCI-18. Note that the Sézary cell in Image BCI-20 is about the same size as the cell in Image BCI-18. Sometimes Sézary cells may be much larger, even twice the size of a normal lymphocyte, as represented in Image BCI-21. Sézary cells are generally round to oval in shape but also may be irregular. Nuclei in Sézary cells are distinctive. Convolutions or folds are often apparent. The nuclei may also appear cerebriform. In larger Sézary cells, the nucleus may even resemble a cluster of berries, as in Image BCI-21. Nucleoli are usually not visible, and the chromatin stains dark and frequently hyperchromatic (overpigmented). The cytoplasm is generally scanty, pale blue, and lacks granules.

Sézary cells may sometimes be mistaken for reactive lymphocytes. It is important to morphologically distinguish these cells because Sézary cells are associated with a malignant lymphoma, whereas reactive lymphocytes are seen most often in benign viral disorders. A key differentiating feature of reactive lymphocytes is the wide variability in morphologies that are possible. Reactive lymphocytes may be small, medium, or large. Their nuclear shape can vary from round or oval to indented or lobulated. Nucleoli may or may not be visible. The nuclear chromatin is also variable, clumped and dense in some cells, but more brainlike and open in others. The cytoplasm in reactive lymphocytes is frequently more
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abundant than in normal lymphocytes and “sprawls” around adjacent RBCs. Color ranges are frequent as well. The cytoplasm may be a blue-gray or stain a deep blue. Sometimes peripheral basophilia is evident where the cell interfaces with erythrocytes. Occasionally, cytoplasmic vacuoles or azurophilic granules are evident. The Table compares normal lymphocytes, reactive lymphocytes, and Sézary cells.

TABLE. Morphologic Comparison of Lymphocytes.

<table>
<thead>
<tr>
<th>Cell Feature</th>
<th>Normal Lymphocyte</th>
<th>Reactive Lymphocyte</th>
<th>Sézary Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>Small to medium</td>
<td>Small to large</td>
<td>Small to large</td>
</tr>
<tr>
<td>Shape</td>
<td>Usually round</td>
<td>Round, oval, often irregular or “sprawling”</td>
<td>Usually round</td>
</tr>
<tr>
<td>Nuclear shape</td>
<td>Round, oval, slightly indented</td>
<td>Round, oval, indented, lobulated</td>
<td>Convoluted, folded, cerebriform, berry clusters</td>
</tr>
<tr>
<td>Chromatin</td>
<td>Dense, clumped</td>
<td>Dense, clumped, open, brain-like</td>
<td>Clumped, often hyperchromatic</td>
</tr>
<tr>
<td>Cytoplasm amount</td>
<td>Usually scant, sometimes moderate</td>
<td>Moderate to abundant</td>
<td>Scant</td>
</tr>
<tr>
<td>Cytoplasm color</td>
<td>Pale to moderately basophilic</td>
<td>Pale to deeply basophilic</td>
<td>Pale blue or blue-gray</td>
</tr>
<tr>
<td>Cytoplasmic inclusions</td>
<td>Sometimes few azurophilic granules</td>
<td>Sometimes vacuoles and azurophilic granules</td>
<td>None</td>
</tr>
</tbody>
</table>

Cutaneous T-Cell Lymphoma
This patient was diagnosed with lymphoma, a malignant proliferation of lymphocytes most often manifested in lymphoid tissue. Sometimes, however, and as demonstrated in this case, bone marrow and peripheral blood involvement occur. Classification of lymphomas includes delineation of lymphocyte subset, either T or B cell. T cells are further categorized as T-helper cells or T-suppressor cells. The classification of lymphomas is important in defining treatment protocols and prognosis.

The presence of Sézary cells is a typical finding in a cutaneous T-cell lymphoma, sometimes also called mycosis fungoides. This malignancy primarily affects the skin, but it is not uncommon to see the abnormal cells in lymph nodes, bone marrow, and peripheral blood. Immunophenotyping indicates that Sézary cells are mature memory helper T cells, positive for CD2, CD3, CD4, and CD5, but usually negative for CD7. Flow cytometry performed on the peripheral blood from this patient showed a pattern consistent with a T-helper cell phenotype (dim reactions for CD2 and CD3, positive for CD4 and CD5, and negative for CD7 and CD8).
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Summary
A careful review of peripheral blood smears is often critical in the initial assessment of suspected hematologic abnormalities. However, immunophenotyping is important to establish a diagnosis in many instances because morphology alone is not sufficient to classify disorders. In the case example, the morphology of lymphocytes is not specific enough to distinguish B cells from T cells or helper T cells from suppressor T cells. Separating malignant lymphocytes from benign or reactive cells by morphology is also difficult. Morphology is useful in indicating that an abnormality may be present. For example, it is unusual to see peripheral blood lymphocytes with convoluted or folded nuclei, and this finding suggests that additional testing is necessary.

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