EDUCATIONAL COMMENTARY – BLOOD CELL IDENTIFICATION

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

- describe morphologic features of normal peripheral blood leukocytes and platelets.
- identify morphologic abnormalities in erythrocytes associated with certain hemoglobinopathies and thalassemias.
- discuss morphologic characteristics of reactive lymphocytes.

A 2-year-old boy was diagnosed with combined qualitative and quantitative hemoglobin defects. He was heterozygous for HbE and also had alpha thalassemia. The images for review represent normal leukocytes, but several abnormal shape changes in RBCs may be seen in the peripheral blood in these conditions.

Two schistocytes, or fragmented erythrocytes, are identified by arrows in photograph BCI-08. Note the irregular shape to these cells. The size of schistocytes may vary, although they are often small as in these examples. Schistocytes are usually caused by some type of mechanical damage or trauma, such as may result from a microangiopathic hemolytic anemia or severe burns. However, disruptions in hemoglobin production, as diagnosed in this patient, can also result in cells that are more easily fractured. Schistocytes have decreased survival in the peripheral circulation and contributed to the anemia present in this patient.

BCI-09 shows a normal basophil. Basophils characteristically have deep purple or blue-black cytoplasmic granules. These granules are often large, round, and numerous. They frequently obscure the nucleus. Basophilic granules are water soluble and may be washed away during the staining process. In such cases, clear or light areas will be seen in the cytoplasm.
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BCI-10 shows target cells, also called codocytes. Target cells are erythrocytes that have a central dense area of hemoglobin surrounded by a white circle, and then a final rim of hemoglobin. These cells actually circulate in the peripheral blood in a bell-shape, or as a “Mexican hat.” They become “targeted” when flattened and dried on a glass slide. Codocytes have excess membrane for their surface area. Two primary mechanisms result in target cell formation: excessive lipid accumulates on the membrane, or the hemoglobin content of the cell is decreased. Disorders, such as liver disease, can result in increased cholesterol and phospholipid depositing on the cell membrane. Decreased hemoglobin content can occur in iron deficiency anemia but is more often seen in thalassemias and hemoglobinopathies. Additionally, target cells can appear artifically in the process of smear preparation in a very humid environment or if a wet smear is blown dry rather than air-dried.

BCI-11 shows ovalocytes, also called elliptocytes. They vary in shape from ovals to cigar-shaped, rodlike structures. Classic elliptocytes have sides that are almost parallel, blunt or rounded ends, and a prominent area of central pallor. Ovalocytes may even be seen occasionally in the peripheral blood of a normal patient but are always present in numbers <1%. Elliptocytes may sometimes be seen in patients with iron deficiency anemia, and they are traditionally called “pencil” cells. Large, oval macrocytes are associated with megaloblastic anemias. These cells are also characteristically identified in hereditary elliptocytosis, which results because of abnormalities in RBC membrane proteins. In this inherited condition, <25% of the cells will be ovalocytes. It is also not unusual to see ovalocytes in thalassemias, as in this case example. Although the exact mechanism of ovalocyte formation is not known in these conditions, they represent morphologic changes in erythrocytes resulting from unbalanced hemoglobin synthesis.
BCI-12 illustrates a normal segmented neutrophil. Neutrophils characteristically have 2 to 5 nuclear lobes connected by only thin strands of chromatin. The cellular chromatin is densely clumped and coarse. The cytoplasm of neutrophils contains numerous small pink, violet, or tan granules.

The arrow in BCI-13 identifies a platelet that is superimposed on an erythrocyte. When viewing peripheral blood smears, superimposed platelets or artifacts must be distinguished from RBC inclusions. Any cell or artifact that is not part of an erythrocyte will lie in a different focal plane than the cell. Several morphologic features distinguish the platelet from any possible RBC inclusion. Platelets are actually fragments of cytoplasm from bone marrow megakaryocytes. They are characteristically small and granular. Platelets stain blue-gray or light purple and are usually round or oval in shape. Using the other images provided can also help in identifying this cell as a platelet. Note that other images have platelets with similar size, shape, color, and granularity to the platelet in BCI-13.

Photograph BCI-14 shows a reactive lymphocyte. Other terms sometimes used to describe this kind of cell are “variant” and “atypical.” Reactive lymphocytes are characterized by a wide variety of morphologic appearances on a blood smear. In fact, there are no “typical” atypical lymphocytes. This diversity in cellular features reflects the heterogeneity of immune responses. Reactive lymphocytes are most often associated with viral illnesses. Although these cells display many different characteristics, some generalizations can be made. Reactive lymphocytes are often large cells with abundant cytoplasm.

The cytoplasm may stain gray, pale blue, or a very deep blue, and may stain unevenly with peripheral or radial basophilia. The cytoplasm may also have azurophilic granules or, as in this example, vacuoles. Likewise, it is not unusual to see the cytoplasmic margin of the cell indented by surrounding erythrocytes.
Although the nucleus of the cell shown in this image is round to slightly oval, nuclei in reactive lymphocytes are variable in shape. Nuclei may be indented, folded, or lobular. The nuclear chromatin is frequently more fine and open than what is seen normally in lymphocytes, and areas of parachromatin may be more prominent. The only WBC shown in photograph BCI-13 is a normal lymphocyte. Compare the cell size, cytoplasmic characteristics, and nuclear features of the cell in BCI-13 with the reactive lymphocyte in BCI-14.

**Disorders of Hemoglobin**

The patient had been diagnosed with inherited abnormalities that impair the structure and quantity of hemoglobin. Normal hemoglobin contains 2 pairs of unlike polypeptide chains, designated alpha and beta, and is termed HbA ($\alpha_2\beta_2$). Altogether 4 types of globin chains are produced during infancy and into adulthood: alpha, beta, gamma, and delta.

Each globin chain consists of a specific arrangement and number of amino acids. An individual with HbE has an inappropriate amino acid substitution on the beta chain. This results in a form of hemoglobin that is less soluble and slightly unstable with oxidant stress. HbE is a common abnormality and is especially prevalent in Southeast Asian populations. However, the patient in the case example has a defect in only 1 of his beta chain genes and is considered heterozygous for HbE. He has inherited 1 normal beta gene and is able to produce some normal HbA.

HbE may also be seen combined with alpha thalassemia, as in this patient. Thalassemias result when the quantity of a particular globin chain is decreased. Alpha thalassemia represents a decreased production in alpha chains, whereas beta thalassemia is impaired production of beta chains. Alpha thalassemias are also frequently seen in Southeast Asian populations.

Alpha chain synthesis is more complex than beta chain formation. There are normally 4 alpha chain genes (2 inherited from each parent) that code for the production of alpha chains. Alpha thalassemia occurs when 1 or more of these genes are deleted. The clinical severity is directly related to the number of deleted alpha genes. The Table summarizes the number of functional alpha chain genes and the associated clinical conditions.
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TABLE. Clinical Conditions by Number of Alpha Chain Genes.

<table>
<thead>
<tr>
<th>No. of Functional Alpha Chain Genes</th>
<th>Clinical Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 (αα/αα)</td>
<td>Normal</td>
</tr>
<tr>
<td>3 (-α/αα)</td>
<td>Silent carrier</td>
</tr>
<tr>
<td>2 (-α/-α or --/αα)</td>
<td>Heterozygous α-thalassemia</td>
</tr>
<tr>
<td>1 (-α/--</td>
<td>HbH disease</td>
</tr>
<tr>
<td>0 (--/--</td>
<td>Hydrops fetalis</td>
</tr>
</tbody>
</table>

- Indicates deleted chain.

The spectrum of clinical severity seen in alpha thalassemia varies from no disease (silent carrier) to a condition incompatible with life, hydrops fetalis. Because no normal alpha chains are produced in hydrops fetalis, hemoglobin is formed using other available chains, including gamma. Abnormal tetramers of gamma chains can result, producing Hb Bart’s (4 gamma chains). Hb Bart’s has a very high oxygen affinity and does not effectively release oxygen to the fetus.

In patients with a 3-gene deletion in alpha chains, excess beta chains can form tetramers called HbH. However, beta chain production occurs later in infancy than gamma chain synthesis. Therefore, even in a patient with a deletion of 3 alpha chains, Hb Bart’s as well as HbH can be detected at birth. Hemoglobin variants can be identified using electrophoresis. Hemoglobin electrophoresis in this patient indicated that he had some normal HbA (72.4%), but also HbE (15.8%), fetal hemoglobin (HbF, 8.1%), and Hb Bart’s (3.7%). No HbH was detected.

Summary
The peripheral blood morphology in this young child also reflects his abnormal hemoglobin synthesis. Both HbE and alpha thalassemia are associated with microcytosis, hypochromia, and target cells. The additional abnormal RBC morphologies are not unexpected given the chronic hemolytic anemia that developed in this patient.

Hemoglobin synthesis is a complex and balanced process. Hemoglobin defects such as HbE and alpha thalassemia result in structural and quantitative changes that are associated with a variety of clinical and hematologic abnormalities.

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