EDUCATIONAL COMMENTARY – BLOOD CELL ID: IDENTIFYING MALARIAL PARASITES

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To view the blood cell images in more detail, click on the sample identification numbers underlined in the paragraphs below. This will open a virtual image of the selected cell and the surrounding fields. If the image opens in the same window as the commentary, saving the commentary PDF and opening it outside your browser will allow you to switch between the commentary and the images more easily. Click on this link for the API ImageViewer™ Instructions.

Learning Outcomes

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

- describe morphologic features of normal peripheral blood leukocytes and platelets.
- identify characteristic morphologic findings associated with reactive (atypical, variant) lymphocytes.
- identify malarial parasites and differentiate them from platelets on a Wright-stained peripheral blood smear.

Case Study

A 44 year old female was seen by her physician for fever, headache, and nausea. The CBC results are as follows: WBC=4.6 x 10⁹/L, RBC=4.25 x 10¹²/L, Hgb=11.9 g/dL, Hct=34.2%, MCV=80.5 fL, MCH=28.0 pg, MCHC=34.8 g/dL, RDW=40.7%, Platelet=71 x 10⁹/L.

Educational Commentary

The images presented in this testing event are of blood smears obtained from a patient diagnosed as having malaria caused by Plasmodium falciparum. These photographs represent both normal blood cells and organisms that may be seen in the peripheral blood in this infection.

Image BCI-08 illustrates a segmented neutrophil. Segmented neutrophils characteristically have two to five nuclear lobes connected by thin threads of chromatin. As a mature cell, the chromatin is dense and clumped. The cytoplasm has numerous, small granules that usually stain pink, tan, or violet-pink. The granules in this particular cell are primarily pink.
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Image BCI-09 shows a monocyte. Note the large size of this cell. In fact, monocytes are the largest cell normally seen in the peripheral blood. The nuclei in monocytes may be oval, round, lobulated, or kidney-shaped, as in this example. The chromatin is generally minimally clumped and stains a lighter purple.

However, it is difficult to appreciate these features in this particular cell. The cytoplasm in monocytes is characteristically abundant and blue-gray. Vacuoles may be present as noted here. Sometimes cytoplasmic projections are visible. Typically, the cytoplasm may appear rough or uneven. Small, red-purple, azurophilic granules are often present; note them in this cell.

Author’s notes on participant performance: Some participants identified this cell as a metamyelocyte. Three features should always be reviewed to determine the identity of a cell: size, nuclear characteristics, and cytoplasmic appearance. Note first of all the size of this cell, especially when compared to the adjacent band. Even though a metamyelocyte is an immature cell, its size is still nearly the same as the segmented neutrophil and band cell. Secondly, the nucleus of a metamyelocyte should be indented less than half the width of a hypothetical round nucleus. This nucleus is indented more than this guideline. Finally, though the cytoplasm is unevenly stained (blue, small granules on the left and indistinct, pinkish granules on the right), the large vacuole and underlying bluish cytoplasm on the cell’s right side suggest features of a monocyte. Viewed together, these three characteristics are clues that the cell shown in BCI-09 is a monocyte.

Image BCI-10 is a reactive lymphocyte, also called an atypical or variant lymphocyte. Although reactive lymphocytes are most often associated with viral illnesses, it is not unexpected to see this type of cell in a patient with malaria. Atypical lymphocytes display morphologic features indicating that they are reacting to the malarial invaders as well. Morphology is variable in reactive lymphocytes. However, some general findings distinguish these cells from normal lymphocytes. Although not apparent in this example, reactive lymphocytes may be large. Nuclei in these cells may be elongated, round, oval, lobulated, or indented. Likewise, the chromatin may be fine or
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moderately to coarsely clumped. Many reactive lymphocytes have abundant cytoplasm, so the cell in this image is an exception. It is not unusual to see areas of clearing, as is seen in this cell. The cytoplasm may be gray, pale blue or blue. In areas where the cytoplasm interfaces with adjacent red blood cells (RBCs), it may appear an even darker blue.

Author’s notes on participant performance: Some participants identified this cell as a normal lymphocyte. Evaluating size, nucleus, and cytoplasm with every cell is important, especially when some doubt exists regarding the cell’s identity. However, it is also important to recognize that reactive lymphocytes are pleomorphic. Although this cell is not as large as some reactive lymphocytes and the nucleus is not as fine as in other examples, the deeply basophilic, uneven staining of the cytoplasm is classic for a reactive lymphocyte. Likewise, the nucleus is elongated, a feature distinct from the roundness of a normal lymphocyte. Furthermore, the cytoplasm in a normal lymphocyte is generally a sky blue and not as basophilic as in this cell. The fact that the cell size and nucleus show variability underscores the “definition” of reactive lymph: that is, a cell reacting to an antigenic stimulus and displaying resulting morphologic changes.

Image BCI-11 is a normal platelet. Although slightly enlarged, this cell is not otherwise abnormal. Platelets may be round or oval. They stain blue-gray or light purple. Frequently a central, granular core (granulomere) is surrounded by a clear zone (hyalomere). Note these morphologic features in this platelet.

Author’s notes on participant performance: Some participants reported “giant platelet” for BCI-11. A giant platelet is as large as or larger than a normal RBC. They are larger than 7 µm in diameter and are usually 10 to 20 µm. The platelet shown measures approximately 4 µm. Although this platelet may be considered large, it is not large enough to be considered a giant.
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**Image BCI-12** shows a band neutrophil. Bands are the stage of neutrophil maturation before the segmented neutrophil, so the size is similar to that of the mature neutrophil. A small percentage of band neutrophils can normally be seen in the peripheral blood. Bands are differentiated from segmented neutrophils by their nuclear shape, which may appear as the letters C or U. Sometimes, the shape is compared to a sausage. The nuclear chromatin is dense and clumped. As with the segmented neutrophil, the cytoplasm contains numerous pink, tan, or violet-pink granules.

**Image BCI-13** is the ring form of a malarial parasite. These organisms are most often seen within RBCs. Four species of malaria infect humans: *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium vivax*. These organisms are distinguished from each other by differences in their respective morphology as well as the appearance of the infected RBC. A review of the morphologic features of parasites and RBCs on this peripheral blood smear resulted in a diagnosis of *P. falciparum*.

*Plasmodium knowlesi* is a fifth malarial parasite to infect humans. It is not commonly isolated, but incidence is increasing. It is often misidentified by microscopy as *P. malariae* (morphologically similar/identical) but can be distinguished by PCR-based assays.

Infection with *P. falciparum* is characterized in the peripheral blood by the presence of only the ring trophozoite stage and sometimes gametocytes, which cause the RBCs to appear banana-shaped. Red blood cells with trophozoites are normal in size and shape. Multiple trophozoites within an RBC may be seen in *P. falciparum* infection. Notice the red chromatin dot and delicate blue cytoplasm. The trophozoites in *P. falciparum* are not sprawling, ameboid shapes as may be seen in other *Plasmodium* species. A virtual image of this peripheral blood smear may be viewed using the API ImageViewer™. Other examples of malarial organisms may be seen in the virtual image.
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Image BCI-14, the last image in this test event, shows a platelet superimposed on an RBC. Platelets overlying RBCs may often be mistaken for the ring stage of a malarial organism. A careful review of the stained blood smear is necessary to distinguish platelets from true parasites. Malaria is intracellular and will focus with the red blood cell, whereas platelets focus separately. The granular core of platelets is usually visible, as is the halo or hyalomere; these features also help differentiate platelets from the ring form of malaria. Finally, a review of the entire slide can show platelets extracellular to RBCs. Note similarities in size, shape, and color to the superimposed platelet.

Malaria is an acute infection that occurs after a bite from the female Anopheles mosquito. This mosquito is found worldwide, but disease transmission occurs most often in tropical countries. The severest cases of infection are caused by P. falciparum. The parasite completes a complex life cycle with several stages that infect and mature in RBCs. The RBCs may subsequently have a shortened life span, and a hemolytic anemia may develop. Likewise, thrombocytopenia is sometimes associated with malaria, as is seen in the patient in this case study. This patient had an elevated total bilirubin of 2.4 mg/dL and an elevated lactate dehydrogenase of 330 U/L. These elevated values also indicate that a hemolytic event has occurred.

Summary

Identifying malarial organisms in the peripheral blood is critical in establishing a diagnosis of this infection. It is also important to distinguish organisms from potential lookalikes, such as superimposed platelets. Therefore, a careful and systematic evaluation of the stained peripheral blood smear must always be performed.

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