Educational commentary is provided for participants enrolled in program #259- Advanced Blood Cell Identification. This virtual blood cell identification program includes case studies with more difficult challenges. 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

After completion of this exercise, participants will be able to:

- Identify abnormalities in erythrocyte morphology associated with thalassemia.
- Compare and contrast the morphology of nucleated red blood cells and normal lymphocytes.

Case Study

A 7 month old male was seen by his physician for fever, irritability, and failure to thrive. The CBC results are as follows: WBC=8.3 x 10⁹/L, RBC=5.43 x 10¹²/L, Hgb=9.1 g/dL, Hct=27.3%, MCV=65.4 fL, MCH=20.9 pg, MCHC=32.2 g/dL, RDW=17.7 %, Platelet=310 x 10⁹/L.

Educational Commentary

The baby whose virtual peripheral blood smear was evaluated in this testing event presented with a microcytic, hypochromic anemia as indicated by the low hemoglobin, hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) values. The patient was diagnosed with homozygous beta thalassemia (beta thalassemia major). The cells annotated for discussion primarily represent variations in RBC morphology associated with this condition.

The cell identified in ABI-08 is a target cell, also called a codocyte, derived from the Greek word for "hat." This round red blood cell has a central, dense area of hemoglobin surrounded by a clear area and then a final rim of more hemoglobin. Codocytes circulate shaped as bells or Mexican hats and acquire their target appearance when flattened and dried on a glass slide. Three basic mechanisms can result in target cell formation, but the primary two causes relate to an increase in RBC membrane surface relative to the hemoglobin content. In liver disease, for example, excess cholesterol and phospholipid accumulate on the erythrocyte membrane, resulting in more membrane surface for the amount of hemoglobin present and subsequent target cell formation. Other conditions, such as iron deficiency anemia, hemoglobinopathies like sickle cell anemia, and thalassemia are associated with a decreased hemoglobin content while the same membrane surface area is maintained. Again, the consequence is codocyte formation. The third mechanism that can result in target cell production is artifactual and is seen if a wet peripheral blood smear is blown dry instead of air dried during preparation.
ADVANCED BLOOD CELL ID: IDENTIFYING SELECTED ABNORMALITIES IN RED BLOOD CELLS (cont.)

The cell selected for **ABI-09** demonstrates polychromasia or polychromatophilia in a red blood cell. Polychromatophilic RBCs represent reticulocytes, the stage of erythrocyte maturation just prior to the mature red blood cell. Reticulocytes have extruded their nuclei, but retain some ribonucleic acid (RNA) that allows the cell to appear a blue-gray or bluish when Wright stained. Reticulocytes typically mature for about 48 hours in the bone marrow and another 24 hours once released into the peripheral blood. The presence of polychromasia indicates accelerated bone marrow response to a peripheral blood anemia. The appearance of polychromatophilic cells in the peripheral blood of this patient is expected given the diagnosis of thalassemia. A scan of the virtual slide shows several polychromatophilic erythrocytes. Note that the cell in ABI-09 is slightly larger than many of the surrounding RBCs. It is common to see polychromatophilic cells that are larger as they represent immature erythrocytes. Also note that this particular cell may be a codocyte. Though a classic central dense area is not evident, the remnant of a white area and rim of hemoglobin are still visible.

The cell annotated in **ABI-10** is an erythrocyte with a Howell-Jolly body inclusion. RBCs should have no inclusions, so the presence of a Howell-Jolly body is indicative of an anemic process. Howell-Jolly bodies are generally small, but are variable in size. They are round and stain purple or purple-blue, sometimes almost appearing black, as in this case. Howell-Jolly bodies are also usually located near the periphery of the cell as single inclusions. This particular inclusion is uncharacteristically more centrally positioned in the red blood cell. Sometimes, multiple Howell-Jolly bodies can be seen in severe anemias. There are two basic mechanisms that result in Howell-Jolly formation. A chromosome may separate from the mitotic spindle during cell division and remain in the cell after the nucleus is extruded. Or, the erythrocyte nucleus may fragment abnormally during expulsion, leaving a piece of deoxyribonucleic acid (DNA) behind. Although Howell-Jolly bodies are more often associated with megaloblastic anemias, severe hemolytic anemias, and congenital dyserythropoietic anemias, it is not surprising to also see them in the peripheral blood of this patient with thalassemia. In thalassemia, overall erythropoiesis is unbalanced, so any RBC morphologic abnormality may be seen. In addition, it should be noted that the spleen normally removes red cell inclusions such as Howell-Jolly bodies. However, if the spleen is absent, dysfunctional, or overwhelmed, Howell-Jolly bodies (and other inclusions) may be increased in the peripheral blood. Patients with homozygous beta thalassemia often have massively enlarged and less functional spleens because abnormal erythrocytes are sequestered.

The cell identified in **ABI-11** is the first of two nucleated red blood cells (NRBCs). A scan of the virtual slide shows numerous other NRBCs similar in morphology to this cell. Nucleated erythrocytes are immature and still retain their nuclei. They are normally only present in the bone marrow, though a few may be seen in the peripheral blood of an infant. Although the patient in this testing event is an infant, the numbers of NRBCs present on the virtual slide is far too many for a typical infant. An increase in nucleated red blood cells, whether in an infant or adult, can be indicative of abnormal or accelerated
erythropoiesis, though this erythropoiesis is often ineffective. Again, the appearance of NRBCs in the peripheral blood of a thalassemia patient is not unexpected. This nucleated erythrocyte is small. The nucleus is round and the nuclear chromatin is dense and clumped. The clearing area in the nucleus of this particular cell is not characteristic and may represent parachromatin or some artifact exposed during smear preparation. The cytoplasm is pink, reflecting the presence of hemoglobin synthesized by the cell. Usually only later maturation stages of NRBCs are seen in the peripheral blood and the nucleated red blood cell selected for ABI-11 is typical of those visible. Note that the maturation stage for nucleated erythrocytes does not need to be identified, but the number of cells (per 100 white blood cells) should be counted and reported.

The second NRBC is identified for ABI-12. This nucleated RBC is an earlier maturation stage than the cell in ABI-11. It is a slightly larger cell with a big, round nucleus. While the nuclear chromatin is essentially condensed and clumped, areas of lighter purple parachromatin are evident. As with the cell in ABI-11, the nucleus is eccentrically located, a feature commonly seen in NRBCs. Note the perinuclear halo, which is also often seen in this stage of NRBC. The cytoplasm is blue-gray as some hemoglobin has accumulated, though not the full complement that will be produced by the cell after maturation is complete.

The cell chosen for ABI-13 is a normal lymphocyte. Lymphocytes vary in size; this is an example of a medium-sized cell. Nuclei in normal lymphocytes are usually round, oval or slightly indented. The dense and clumped chromatin is characteristic. Likewise, the rim of blue cytoplasm is a common feature.

Sometimes, small and medium-sized lymphocytes may be confused with NRBCs, especially less mature stages such as that seen in ABI-12. The cells in ABI-12 and ABI-13 differ enough in size, with the lymphocyte appearing larger, that size should not be a factor complicating identification of these cells. However, cell size is not always the most reliable characteristic to help differentiate these cells. Slight differences in nuclear chromatin and cytoplasmic color can provide clues to the type of cell observed.

The chromatin in the NRBC is clumped and condensed, but areas of parachromatin are visible. The nuclear chromatin in the lymphocyte is also clumped and dense, but the areas of parachromatin are less evident; the stain color is also a slightly lighter shade of purple. The cytoplasm in the nucleated red blood cell is a dull blue or blue-gray with a more obvious perinuclear halo. The cytoplasm in the lymphocyte is blue. It is always important to assess several morphologic characteristics when identifying peripheral blood cells.

The last cell selected for commentary, ABI-14, is a spherocyte. Spherocytes are smaller than normal erythrocytes, are dense, and have no area of central pallor. Spherocytes have lost cellular membrane and have a decreased surface to volume ratio. This is in contrast to target cells, as seen in ABI-08, which have increased surface membrane relative to their volume. Spherocytes have reduced surface area, but
retain the same volume of cytoplasm, resulting in small, densely compact cells. Spherocytes are most often associated with hereditary spherocytosis, immune-mediated hemolytic anemias, and microangiopathic hemolytic anemias. However, the appearance of spherocytes is not surprising in a case of homozygous beta thalassemia. This condition results in excess alpha hemoglobin chains, which can precipitate in the red blood cells. These deposits of alpha chains can damage the RBCs’ membranes. Although rare in this virtual slide, schistocytes can often appear in the peripheral blood of a patient with beta thalassemia major. If damaged RBC membranes are able to reseal, spherocytes can form. It is just possible that in this particular infant, spherocytes are more prominent than schistocytes.

**Homozygous Beta Thalassemia (Beta Thalassemia Major)**

Homozygous beta thalassemia is an inherited condition in which the quantity of hemoglobin is decreased. Hemoglobin normally consists of two pairs of unlike globin chains, alpha and beta. In beta thalassemia, beta globin chain synthesis is absent or decreased, depending on the number of abnormal genes inherited. In severe cases of homozygous beta thalassemia, no beta chain production occurs. This disorder becomes apparent in infancy or early childhood. Children have a serious microcytic, hypochromic anemia. Again, note the decreased hemoglobin, hematocrit, MCV, and MCH values as previously discussed for this case study patient. In addition, abnormal RBC morphologies also include shape changes and the presence of inclusions, such as the target cell and Howell-Jolly body identified in the peripheral blood of the infant presented in this testing event. Another noteworthy finding in beta thalassemia is an elevated or normal red blood cell count, even when the hemoglobin and hematocrit are decreased. Hemoglobin electrophoretic procedures are used to confirm the diagnosis of homozygous beta thalassemia. Increased hemoglobin F levels, normal or slightly elevated hemoglobin A2 values, and absent or decreased levels of normal hemoglobin A reflect the decreased production of normal beta globin chains and the respective pairings of gamma or delta chains with excess alpha chains. Hemoglobin F consists of two gamma chains and two alpha chains whereas hemoglobin A2 is comprised of two delta globin chains and two alpha chains.

**Summary**

This advanced blood cell identification testing event emphasizes the variety of erythrocyte abnormalities that can be seen in homozygous beta thalassemia. A codocyte, polychromasia, NRBCs, and a spherocyte are only some of the red blood cell morphologic variations selected for discussion. Laboratory professionals contribute important information to the diagnosis of homozygous beta thalassemia by recognizing and reporting significant peripheral blood changes in erythrocytes.
References


