Blood Cell Identification – Graded

Case History

This peripheral blood smear is from a one-day old full-term baby boy with trisomy 21. His mother's pregnancy was complicated by polyhydramnios and duodenal atresia. Laboratory data include: WBC = 23.1 x 10E9/L; RBC = 5.01 x 10E12/L; HGB = 18.1 g/dL; HCT = 53.2%; MCV = 106 fL; RDW = 26; and PLT = 775 x 10E9/L. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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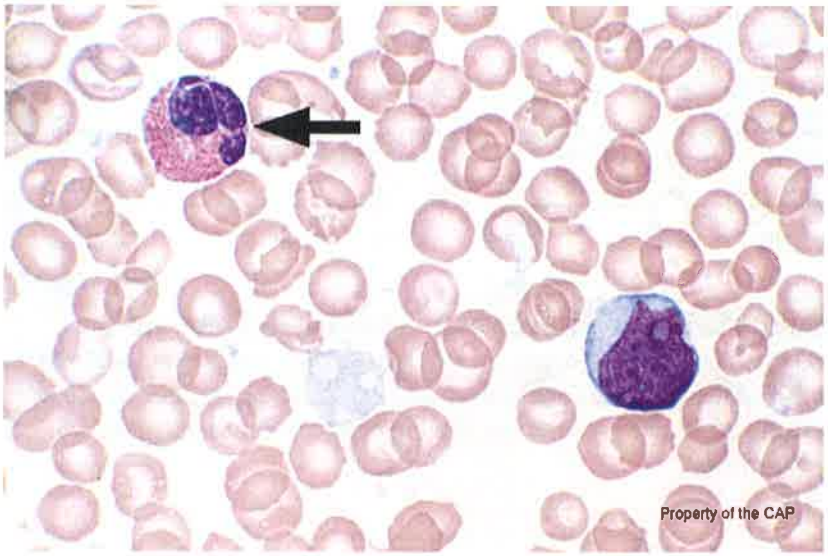
BCP-11

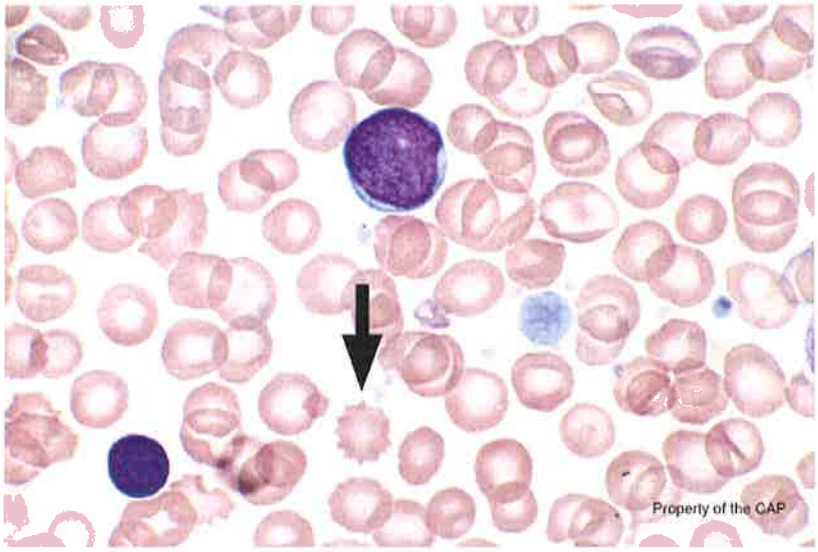


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Eosinophil	81	98.8	4788	99.7	Good
Basophil, any stage	1	1.2	5	0.1	Unacceptable

The arrowed cell is an eosinophil, as correctly identified by 98.8% of referees and 99.7% of participants. Eosinophils are the same size as neutrophils, but are distinguished by coarse red-orange cytoplasmic granules of uniform size. These granules differ from neutrophilic granules, which are smaller, finer, and less refractile. The eosinophil nucleus is typically bilobed, though approximately 20% of eosinophils show three distinct nuclear lobes, as in the depicted cell.

Blood Cell Identification – Graded

BCP-12

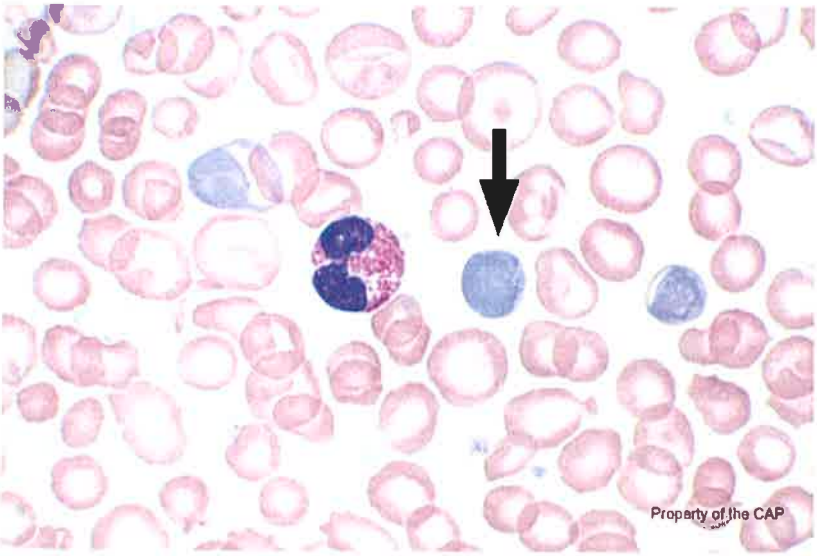


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Echinocyte (burr cell/crenated cell)	81	98.8	4724	98.4	Good
Acanthocyte (spur cell)	1	1.2	69	1.4	Unacceptable

The arrowed cell is an echinocyte, as correctly identified by 98.8% of referees and 98.4% of participants. Echinocytes, also known as burr cells or crenated cells, take their name from the Greek word for sea urchin. Similar to their namesake, these cells have uniform, short, blunt projections that impart a serrated appearance to the cell surface. In contrast, the projections of an acanthocyte are irregularly distributed and variable in size.

Blood Cell Identification – Graded

BCP-13

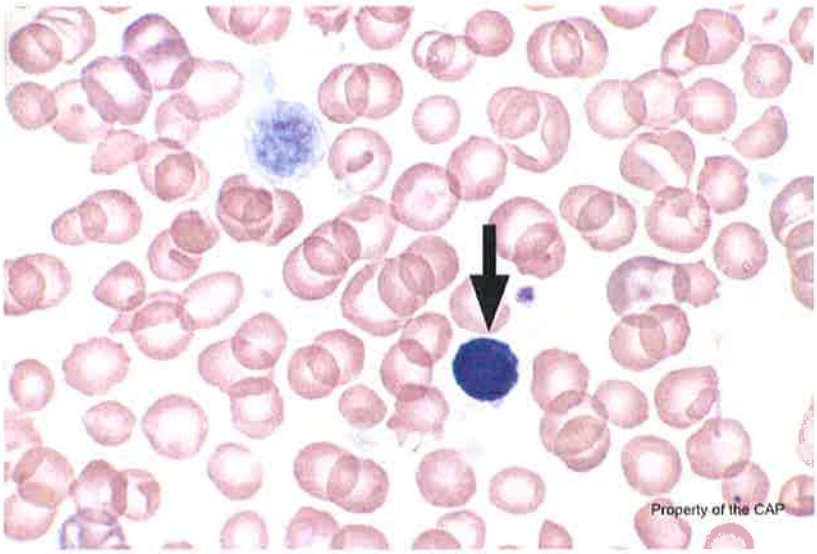


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Polychromatophilic red blood cell	73	89.0	4238	88.3	Good
Lymphocyte	4	4.9	286	6.0	Unacceptable
Blast cell	2	2.4	35	0.7	Unacceptable
Lymphocyte, reactive	1	1.2	27	0.6	Unacceptable
Megakaryocytic cell	1	1.2	22	0.5	Unacceptable
Nucleated red cell, normal or abnormal morphology	1	1.2	47	1.0	Unacceptable

Correctly identified by 89.0% of referees and 88.3% of participants, the polychromatophilic red cell is recognizable by the gray-purple tinge to its cytoplasm. Polychromatophilic red blood cells are also larger than other red blood cells and lack central pallor. These cells represent red cells that have recently been released from the bone marrow and contain increased amounts of RNA, which gives them their characteristic gray-purple staining in a Wright-Geimsa stain. When stained with a supravital stain, these cells may be enumerated as reticulocytes, the final stage of red blood cell maturation.

Blood Cell Identification – Graded

BCP-14

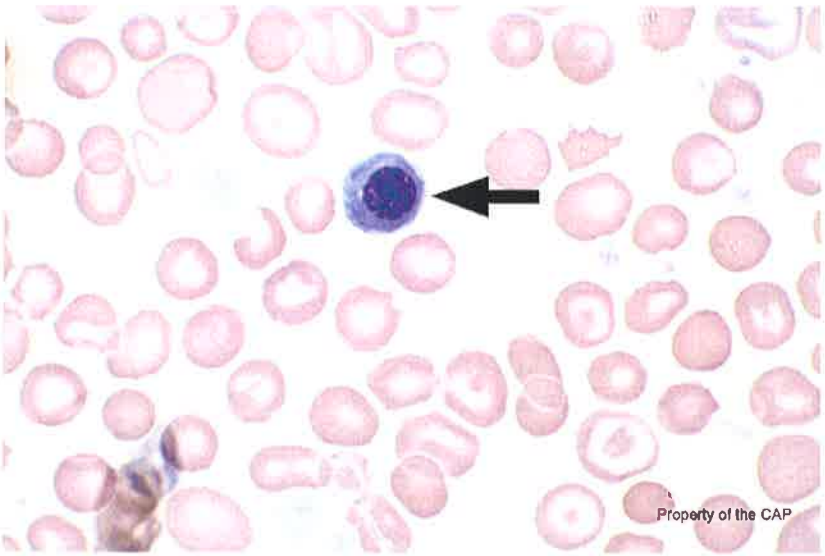


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Lymphocyte	70	85.4	4323	90.1	Good
Megakaryocytic cell	8	9.8	235	4.9	Unacceptable
Nucleated red cell, normal or abnormal morphology	2	2.4	68	1.4	Unacceptable
Basket cell/smudge cell	1	1.2	6	0.1	Unacceptable
Lymphocyte, reactive	1	1.2	84	1.8	Unacceptable

The arrowed cell represents a lymphocyte, as correctly identified by 85.4% of referees and 90.1% of participants. The lymphocyte is a small cell (7 to 15 µm) with rounded nuclear contours, coarse or clumped chromatin, and a scant to modest amount of pale blue cytoplasm that is often agranular.

Blood Cell Identification – Graded

BCP-15



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Nucleated red blood cell	81	98.8	4763	98.6	Good
Immature or abnormal cell, would refer for identification	1	1.2	24	0.5	Unacceptable

The arrowed cell is a nucleated red blood cell (nRBC), as correctly identified by 98.8% of referees and 98.6% of participants. Nucleated red blood cells are not normally present the peripheral blood as the nucleus of erythroid progenitors in the bone marrow is typically extruded prior to entering the circulation. They are recognizable by cytoplasm that shows variable degrees of pink coloration, reflecting the ongoing acquisition of hemoglobin. Their nuclei are small and round with chromatin condensation that varies based on the stage of maturation.

Case Presentation:

This peripheral blood smear is from a one-day old full-term baby boy with trisomy 21. His mother's pregnancy was complicated by polyhydramnios and duodenal atresia. Laboratory data include: WBC = 23.1 x 10E9/L; RBC = 5.01 x 10E12/L; HGB = 18.1 g/dL; HCT = 53.2%; MCV = 106 fL; RDW = 26; and PLT = 775 x 10E9/L. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Discussion: Transient Abnormal Myelopoiesis

The diagnosis in this case is transient abnormal myelopoiesis (TAM), a specific type of disturbed hematopoiesis that occurs in neonates with Down syndrome (DS). DS is caused by trisomy for chromosome 21 and is characterized by a constellation of abnormalities including intellectual disability, characteristic facial features, and cardiac defects. In addition, approximately 10% of infants with DS develop TAM, an unusual myeloid proliferation that in many respects resembles acute leukemia. The peripheral blood smear in cases of TAM shows increased blasts as well as other variable abnormalities including leukocytosis, basophilia, anemia, thrombocytopenia or thrombocytosis. Nucleated red blood cells, giant platelets, and megakaryocyte fragments are also typically present. By immunophenotyping, the blasts are myeloblasts, and often show evidence of megakaryoblastic differentiation. TAM is typically recognized at or shortly after birth, with diagnosis usually made between 3-7 days of age. TAM is also accompanied by a spectrum of possible clinical abnormalities such as ascites, effusions, liver failure and disturbed coagulation, which are responsible for a mortality rate of approximately 20%. However, the most important clinical feature of TAM is also the most unusual: if the patient does not succumb to immediate complications, TAM undergoes spontaneous remission within approximately three months. Despite this outcome, patients who experience TAM remain at higher risk of developing subsequent acute myeloid leukemia.

A key feature of TAM is the presence of mutations affecting the GATA1 gene. GATA1 encodes a transcription factor that interacts with other proteins to control certain aspects of maturation and development of hematopoietic cells in the bone marrow. Patients with TAM show acquired mutations in GATA1 that result in the production of a shortened, or truncated, form of the protein. This mutation is not seen in non-DS-associated acute myeloid leukemias. The exact reason for the close relationship between constitutional trisomy 21 and acquired GATA1 mutations remains unclear, though some data indicate that fetal hematopoiesis is disturbed in the presence of trisomy 21. Likewise, the explanation for the phenomenon of spontaneous remission is uncertain, though some have suggested that it may relate to the completion of the normal transition from fetal to postnatal hematopoiesis. Notably, in patients who develop acute myeloid leukemia after TAM, the later leukemic blasts harbor the same GATA1 mutation that was seen at the earlier stage of TAM. Thus, despite the initial remission, in some patients with TAM the abnormal cell population persists at a low level, acquires additional genetic abnormalities, and returns as an outright acute myeloid leukemia approximately 1 to 3 years later.

From the perspective of the hematology laboratory, several clinical aspects of TAM deserve additional mention. First, although TAM occurs in at least 10% of neonates with DS, the incidence may actually be somewhat higher, as not all patients with DS are screened for TAM. Second, molecular testing for GATA1 mutations is useful in establishing the diagnosis; this abnormality is not expected in reactive circulating blasts which may be seen in some neonates. Finally, there are rare instances in which TAM was the initial presenting feature of an unrecognized case of DS. A high index of suspicion for TAM in the neonatal period is recommended – both to avoid a misdiagnosis of acute leukemia and to trigger cytogenetic analysis for trisomy 21, if indicated.

Most patients with TAM do not need treatment, though low dose chemotherapy may be given in some cases that are clinically aggressive, such as those with very high blast counts or liver disease. Patients should be followed closely for the emergence of subsequent acute myeloid leukemia, which occurs in approximately 20-30% of cases, usually one to three years after resolution of the initial presentation of TAM.

David R. Czuchlewski, MD
Hematology and Clinical Microscopy Resource Committee

References:

1. Bombery M and Vergilio JA. Transient abnormal myelopoiesis in neonates: GATA get the diagnosis. *Arch Pathol Lab Med* 2014; 138: 1302-6.
2. Roy A, Roberts I, Vyas P. Biology and management of transient abnormal myelopoiesis (TAM) in children with Down syndrome. *Semin Fetal Neonatal Med.* 2012; 17: 196-201.
3. Webb D, Roberts I, Vyas P. Haematology of Down syndrome. *Arch Dis Child Fetal Neonatal Ed.* 2007; 92: F503-F507.

Blood Cell Identification – Ungraded

Case History

This peripheral blood smear is from a 52-year-old woman with immune deficiency who is currently on dapsone prophylaxis. Laboratory data include: WBC = 9.4 x 10E9/L; RBC = 2.98 x 10E12/L; HGB = 9.1 g/dL; HCT = 31.5%; MCV = 90 fL; RDW = 20; and PLT = 187 x 10E9/L. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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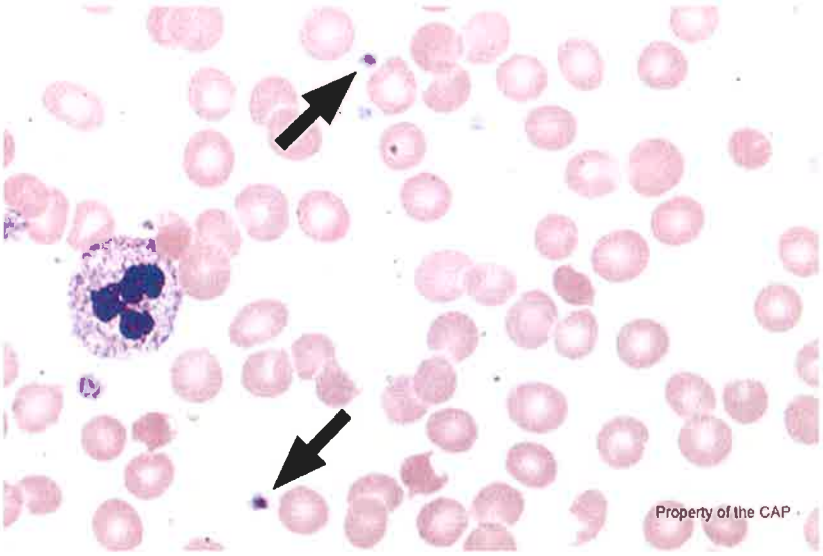
BCP-16

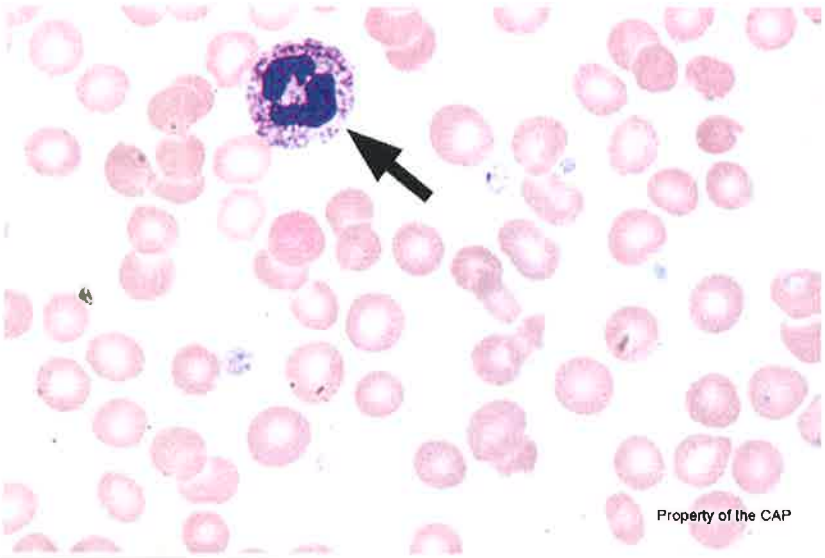


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Platelet	80	98.8	4747	99.8	Educational
Neutrophil, myelocyte	1	1.2	3	0.1	Educational

The arrows point to normal platelets, as correctly identified by 98.8% of referees and 99.8% of participants. Platelets are blue-gray fragments of megakaryocytic cytoplasm that typically measure 1.5 to 3 µm in diameter and contain fine, purple-red granules. Large platelets measure approximately 4 to 7 µm in diameter. The term "giant platelet" is used when the platelet is larger than the size of an average red cell, assuming a normal MCV. All of the arrowed platelets in this image demonstrate normal size and normal cytoplasmic granulation.

Blood Cell Identification – Ungraded

BCP-17

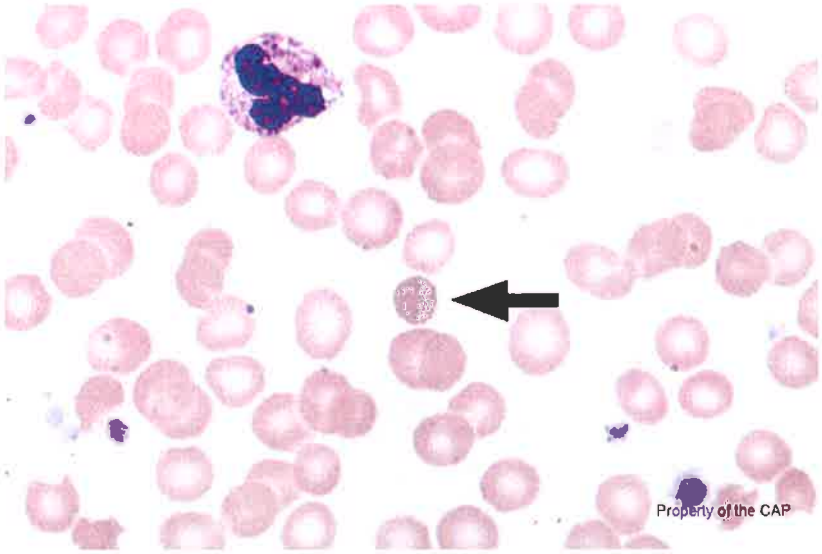


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Neutrophil, Segmented or band/ Neutrophil, toxic	81	100.0	4589	98.6	Educational

The arrowed cell is a segmented neutrophil with mild toxic granulation, as correctly identified as either a neutrophil or toxic neutrophil by 100.0% of referees and 98.6% of participants. Neutrophils are the predominant leukocytes in blood. They demonstrate segmented or lobated nuclei with two to five lobes, condensed chromatin, and pale pink cytoplasm with specific (secondary) granules. In the arrowed example, the cytoplasmic granules are relatively prominent, suggestive of toxic granulation. Neutrophils shown in the other images accompanying this case (15VH-21, -23, and -25) show similar prominent specific granules, and some have small cytoplasmic vacuoles, supporting an interpretation of toxic neutrophils. Döhle bodies, which represent strands of rough endoplasmic reticulum and appear as blue or gray-blue cytoplasmic inclusions, are another feature that may be seen in toxic neutrophils, but are not identified in the neutrophils in this case.

Blood Cell Identification – Ungraded

BCP-18

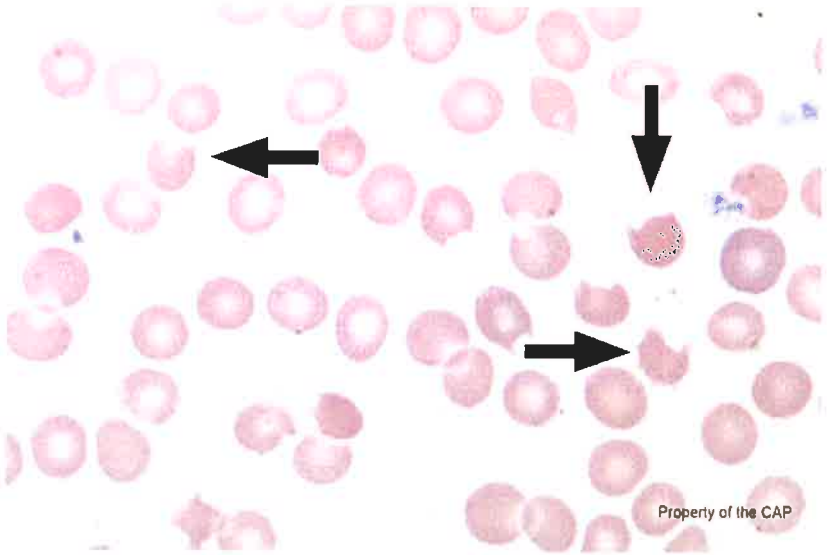


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Spherocyte	77	95.1	4444	95.4	Educational
Microcyte (with increased central pallor)	3	3.7	162	3.5	Educational
Erythrocyte, normal	1	1.2	25	0.5	Educational

The arrowed cell is a spherocyte, as correctly identified by 95.1% of referees and 95.4% of participants. Spherocytes are erythrocytes that are hyperchromic and lack central pallor due to their spherical shape. This contrasts with normal erythrocytes, which have a biconcave shape and visible central pallor on smear preparations. Spherocytes are often smaller than normal erythrocytes and may be very small (ie., microspherocytes, defined as <4 µm in diameter). They form as a consequence of membrane loss, resulting in a decreased ratio of cell surface membrane to cytoplasmic volume. Increased spherocytes are most commonly seen in cases of immune hemolytic anemia and hereditary spherocytosis, but also are typically encountered in smaller numbers in patients with Heinz body anemias where they are associated with bite cells. The background cells in this image include several bite cells, a giant platelet, and two segmented neutrophils with mild toxic change.

Blood Cell Identification – Ungraded

BCP-19



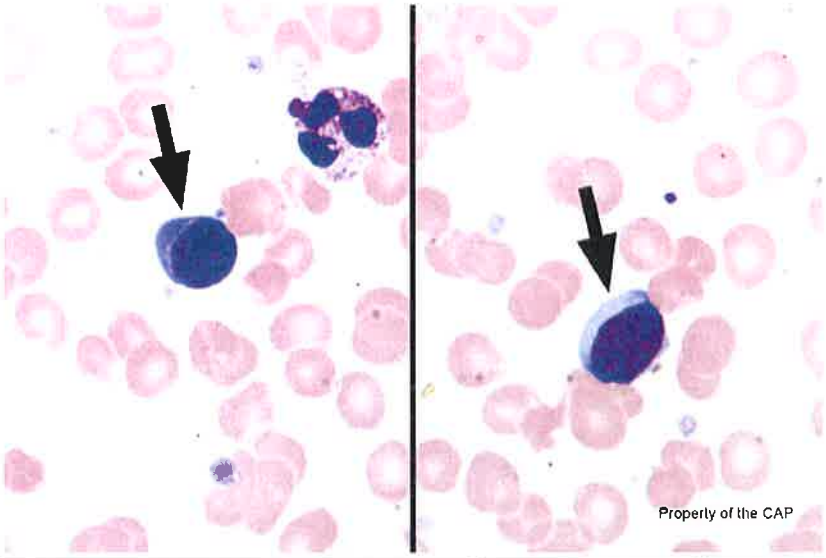
Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Bite cell	32	39.5	1720	36.9	Educational
Fragmented red cell	48	59.3	2867	61.5	Educational
Acanthocyte (spur cell)	1	1.2	52	1.1	Educational

The arrowed cells are bite cells, as correctly identified by 39.5% of referees and 36.9% of participants. Fragmented red cell, as identified by 59.3% of referees and 61.5% of participants, was also deemed acceptable, as further discussed below. Bite cells are red blood cells from which precipitated, denatured masses of hemoglobin (Heinz bodies) have been pitted out by the spleen. Hemoglobin precipitation may result from oxidant injury to hemoglobin by certain drugs (eg., dapsone, as in the current case) or in patients with certain enzyme deficiencies (eg., glucose-6-phosphate dehydrogenase [G6PD] deficiency) who are exposed to an oxidative stress such as a drug, toxin, or infectious agent. Hemoglobin precipitation resulting in Heinz bodies and subsequent bite cells may also be seen due to denaturation of unstable hemoglobin variants.

Irrespective of the etiology, Heinz bodies undergo splenic pitting, which results in a variety of peripheral red blood cell defects ranging from tiny arc-like "nibbles" to large "bites." Red cells with double bites result in the formation of "apple-core" poikilocytes, which are specific for Heinz body anemias. Giant single bites often result in poikilocytes that are morphologically indistinguishable from "helmet" cells (a type of fragmented red cell or schistocyte) seen in microangiopathic hemolytic anemia (MAHA). A major clue that the arrowed cells in this image are bite cells rather than schistocytes is the absence of other fragmented shapes (such as triangulocytes or small, irregular schistocytes) as would typically be encountered in MAHA. However, this distinction may be difficult based on review of only one microscopic field, and would be much more apparent on review of the whole slide. Of note, bite cells are present in all five of the microscopic images provided for this case.

Blood Cell Identification – Ungraded

BCP-20



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Lymphocyte/Reactive lymphocyte	75	92.6	4409	94.8	Educational
Plasma cell	4	4.9	81	1.7	Educational
Nucleated red cell, normal or abnormal morphology	2	2.5	27	0.6	Educational

The arrowed cells are lymphocytes with mild reactive features, as correctly identified by 92.6% of referees and 94.8% of participants. Lymphocytes are small, round to ovoid leukocytes ranging in size from 7 to 15 µm. Most lymphocytes have round to oval nuclei that may be slightly indented or notched. The chromatin is diffusely dense or coarse and clumped. Nucleoli are not visible, although some cells may exhibit a small, pale chromocenter that may be mistaken for a nucleolus. The N:C ratio ranges from 5:1 to 2:1, highlighting the fact that, although many lymphocytes have a thin rim of cytoplasm, some normal lymphocytes have a moderate amount of cytoplasm. The cytoplasm in the two arrowed lymphocytes is more basophilic than is typically encountered in normal lymphocytes and when viewed in conjunction with the moderately abundant cytoplasm, suggests that these lymphocytes may be reactive. The distinction between normal lymphocytes and reactive forms is not always straightforward. Review of the entire slide to evaluate the spectrum of lymphocyte morphologies would likely be very helpful in clarifying whether these lymphocytes are reactive or not.

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Disclosure Statement

The following authors/planners have financial relationships to disclose:
None

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Learning Objectives

Upon completing the reading and answering the learning assessment questions, you should be able to:

1. Identify the major clinical uses of dapsone in immunocompromised patients.
2. List the adverse effects associated with dapsone prophylaxis.
3. Review the morphologic and laboratory diagnosis of dapsone-induced oxidant hemolysis.
4. List the differential diagnostic considerations in oxidant hemolysis and the characteristics that distinguish them.
5. Understand the genetic, clinical, and laboratory manifestations of G6PD deficiency.