Blood Cell Identification – Graded

Case History

This peripheral blood smear is from a 48-year-old man with past medical history of neuropathy presenting with an abnormal movement disorder (chorea). Laboratory data include: WBC = 5.2 × 10E9/L; RBC = 4.47 × 10E12/L; HGB = 14.0 g/dL; HCT = 43.0%; MCV = 95.3 fL; PLT = 195 × 10E9/L; and MPV = 9.6 fL. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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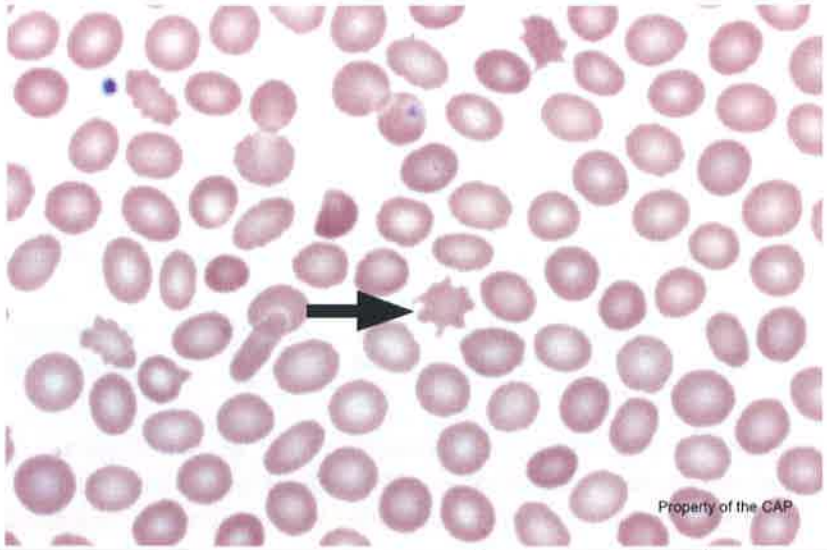
http://www.cap.org/ShowProperty?nodePath=/UCMCon/Contribution%20Folders/WebContent/pdf/hematology-glossary.pdf

BCP-21

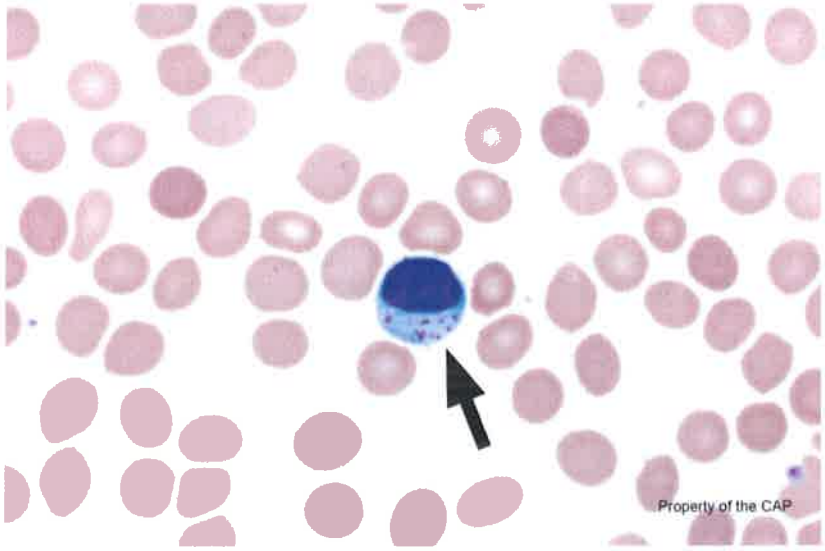


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Acanthocyte (spur cell)	93	82.3	4821	84.5	Good
Echinocyte (burr cell, crenated cell)	20	17.7	848	14.9	Unacceptable

The arrowed object is an acanthocyte (spur cell), as correctly identified by 82.3% of referees and 84.5% of participants. Acanthocytes are red cells with 3 - 20 irregularly distributed sharp spicules often with club or bulb-like ends. In contrast, echinocytes are red cells with 10 - 30 uniformly distributed, short, blunt projections that impart a serrated appearance to the red cell. Acanthocytes are uncommonly identified in otherwise normal blood smears but are more numerous in the setting of abetalipoproteinemia (hereditary acanthocytosis), post-splenectomy, end stage liver disease, anorexia, and McLeod neuroacanthocytosis syndrome.

Blood Cell Identification – Graded

BCP-22



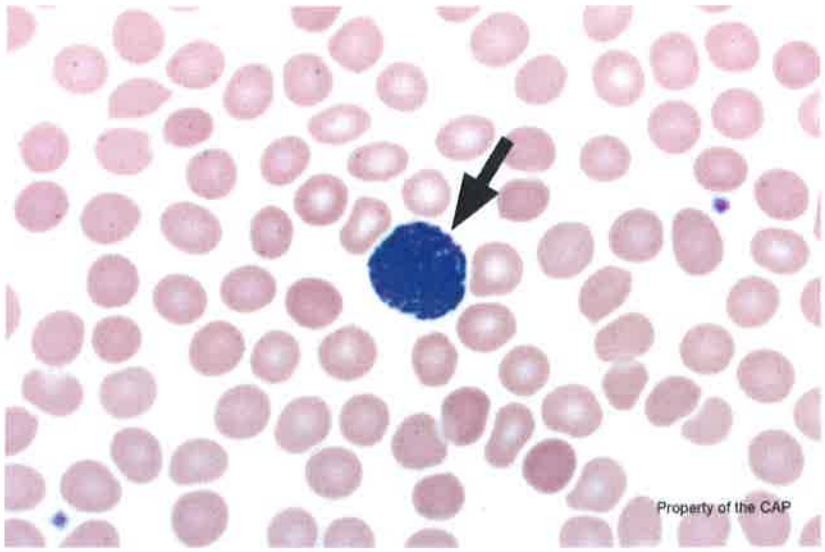
Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Lymphocyte, large granular	92	81.4	4571	80.1	Good
Lymphocyte	20	17.7	997	17.5	Acceptable
Lymphocyte, reactive	1	0.9	46	0.8	Unacceptable

The arrowed object is a large granular lymphocyte, as correctly identified by 81.4% of referees and 80.1% of participants. These cytotoxic T-lymphocytes or natural killer cells are larger than typical circulating lymphocytes, with increased cytoplasm containing several coarse and unevenly distributed azurophilic granules. These can be seen in small numbers of otherwise normal peripheral blood smears or can be significantly increased in the context of infection and autoimmune disease. Malignant proliferations of large granular lymphocytes can also be identified and are typically associated with neutropenia.

17.7% of referees and 17.5% of participants identified the cell as a lymphocyte. While correct, the presence of distinct eosinophilic granules in the cytoplasm make the preferred identification for this cell of large granular lymphocyte rather than the the less specific identification of lymphocyte.

Blood Cell Identification – Graded

BCP-23

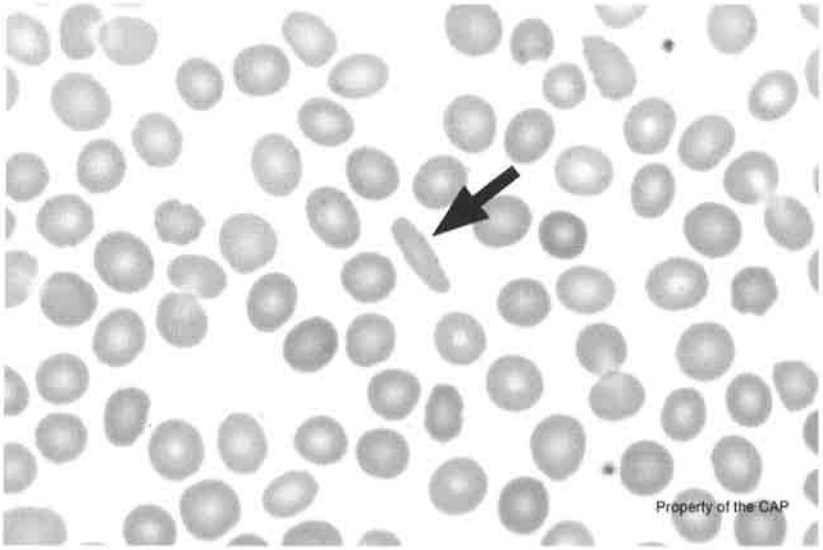


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Basophil, any stage	109	96.5	5544	97.1	Good
Mast cell	3	2.6	118	2.1	Unacceptable
Basophilic stippling (coarse)	1	0.9	16	0.3	Unacceptable

The arrowed object is a basophil, as correctly identified by 96.5% of referees and 97.1% of participants. Basophils are the least common circulating granulocyte, rarely identified in the normal peripheral blood smear. Unlike neutrophils with 3 - 5 lobed nuclei and fine eosinophilic granules, basophils typically have only two prominent nuclear lobes and cytoplasm with numerous dense basophilic granules, often obscuring the nuclear detail. Basophils are an important part of the allergic immune response, and infrequently circulate in appreciable number (typically representing < 0.3% of peripheral leukocytes).

Blood Cell Identification – Graded

BCP-24

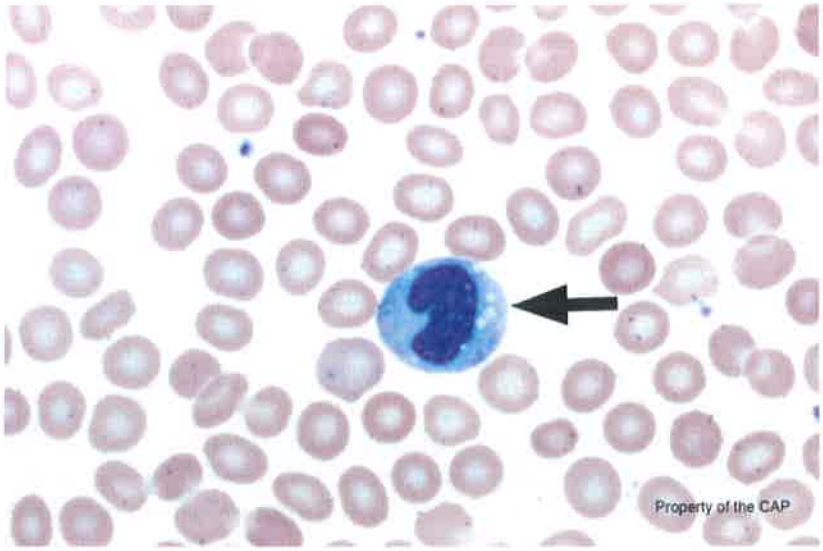


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Ovalocyte (elliptocyte)	112	99.1	5688	99.6	Good
Echinocyte (burr cell, crenated cell)	1	0.9	1	0.4	Unacceptable

The arrowed object is an ovalocyte (elliptocyte), as correctly identified by 99.1% of referees and 99.6% of participants. Ovalocytes are mature, anucleated red blood cells shaped like pencils with blunted ends. Present in small numbers in normal peripheral blood smears, ovalocytes can be significantly increased in the setting of iron deficiency and hereditary elliptocytosis.

Blood Cell Identification – Graded

BCP-25



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Monocyte	110	97.3	5484	96.1	Good
Monocyte, immature (promonocyte, monoblast)	2	1.8	86	1.5	Unacceptable
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	1	0.8	40	0.7	Unacceptable

The arrowed object is a monocyte, as correctly identified by 97.3% of referees and 96.1% of participants. In a normal peripheral blood smear, the monocyte is relatively infrequent, representing up to 10% of peripheral white blood cells. Monocytes are approximately 12 - 20 µm in size with convoluted or folded nucleus and abundant blue-grey cytoplasm. Cytoplasmic vacuolization is common (as seen in the image), and coarse eosinophilic vacuoles can occasionally be appreciated. In contrast to a lymphocyte, the monocyte has a fine and lacy chromatin pattern.

Case Presentation:

This peripheral blood smear is from a 48-year-old man with past medical history of neuropathy presenting with an abnormal movement disorder (chorea). Laboratory data include: WBC = 5.2 × 10E9/L; RBC = 4.47 × 10E12/L; HGB = 14.0 g/dL; HCT = 43.0%; MCV = 95.3 fL; PLT = 195 × 10E9/L; and MPV = 9.6 fL.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: NEUROPATHY – MCLEOD SYNDROME

Neuropathies, diseases affecting the function of peripheral nerves, are commonly encountered in clinical practice. Patients can present with impaired movement, sensation, or other organ dysfunction, but identifying the underlying cause is often difficult, as numerous disorders can affect nerve function. Diagnosis often requires extensive testing, including thorough physical and neuromuscular exam, metabolic and other blood tests, cerebrospinal fluid evaluation, and electrodiagnostic studies. In the developed world, the most frequent cause of peripheral neuropathy is type 2 diabetes mellitus, and testing to establish diagnosis or extent of the damage can be very focused. Careful attention to other presenting symptoms, clinical and family history are key to timely diagnosis and appropriate management.

In this case, the patient's neuropathy is also associated with abnormal involuntary movement disorder known as chorea. While the differential diagnosis of peripheral neuropathy is broad, the constellation of findings including chorea presenting in a man approximately 50 years of age and a blood smear with numerous acanthocytes narrows the diagnostic considerations significantly and underscores the importance of a thorough peripheral blood smear review. Together, the findings are strongly suspicious for McLeod neuroacanthocytosis syndrome.

GENETIC AND CLINICAL PRESENTATION

McLeod syndrome is an X-linked recessive disease caused by mutations of the *XK gene*. The specific function of the expressed protein is not entirely clear, but mutations are associated with weak expression of the Kell red blood cell antigens. Over 30 different mutations have been identified in the *XK gene*, and associate with a variety of neuromuscular findings including myopathy, peripheral neuropathy, muscle atrophy, chorea, and seizure disorders. Cardiac manifestations include arrhythmias and dilated cardiomyopathy.

As the disorder is X-linked, the disease manifests in men, but the reason as to why the presentation is delayed until age 40-50 is not entirely understood. Also, the disease is variably penetrant, while most patients with the mutation are symptomatic, a few individuals show no or minor neuromuscular manifestations.

DIAGNOSIS

Although none of the clinical or conventional laboratory tests are entirely specific, the constellation of findings is often diagnostic.

PERIPHERAL BLOOD EVALUATION

The hematology laboratory plays an important role in establishing the diagnosis of McLeod syndrome, as significant acanthocytosis is an uncommon blood finding. A well-prepared peripheral blood smear from a freshly collected sample confirms numerous acanthocytes (spur cells), with 3 - 20, thin, irregular projections with club-like ends. While acanthocytes can be seen in small numbers in otherwise normal blood smears, true acanthocytosis is rare.

Sequencing of the *XK gene* can be performed but is not necessary to confirm the diagnosis. Along with the McLeod phenotype, that is, Kx-negative red blood cells identified by conventional immunohematology or flow cytometry, the family history and clinical presentations are sufficiently diagnostic.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of acanthocytosis is relatively narrow. In addition to McLeod syndrome, numerous acanthocytes can be seen in the context of abetalipoproteinemia (hereditary acanthocytosis), post-splenectomy, end stage liver disease, and anorexia or severe malnutrition. Further clinical laboratory evaluation can distinguish between these diagnostic considerations. Of note, abetalipoproteinemia is also an inherited genetic disorder associated with dysfunctional absorption of lipid and fat-soluble vitamins. Symptoms can include muscle weakness, poor balance and coordination, and movement disorders, but these typically manifest in childhood along with steatorrhea and failure to thrive.

PROGNOSIS AND THERAPY

Patients with McLeod syndrome presenting in adulthood typically have a life expectancy of 5 - 7 years after diagnosis and related to degree of cardiac or neuromuscular dysfunction. There is no cure for disease, and care is largely supportive, with dopamine antagonists used to treat chorea. Monitoring for progressive cardiac dysfunction is necessary, and medical therapy or cardiac pacemakers may be necessary.

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Hematology and Clinical Microscopy Resource Committee

REFERENCES:

1. Perkins S. Hereditary erythrocyte membrane defects. In: Kjeldsberg CR. *Practical Diagnosis of Hematologic Disorders*. 4th Edition, volume 1. Chicago, IL: ASCP Press; 2006.
2. Glassy EF. *Color Atlas of Hematology: An Illustrated Field Guide Based on Proficiency Testing*. Northfield IL: College of American Pathologists; 1998.
3. Storch A, Kornhass M, Schwarz J. Testing for acanthocytosis A prospective reader-blinded study in movement disorder patients. *J Neurol*. 2005;252:84–90.
4. Danek A, Rubio JP, Rampoldi L, Ho M, Dobson-Stone C, Tison F, Symmans WA, Oechsner M, Kalckreuth W, Watt JM, Corbett AJ, Hamdalla HH, Marshall AG, Sutton I, Dotti MT, Malandrini A, Walker RH, Daniels G, Monaco AP (2001) McLeod neuroacanthocytosis: genotype and phenotype. *Ann Neurol* 50:755–764.

Blood Cell Identification – Ungraded

Case History

This peripheral blood smear is from a 29-year-old man with past medical history of severe burn injury affecting 30% of his total body surface area. Laboratory data include: WBC = 30.1 × 10E9/L; RBC = 2.92 × 10E12/L; HGB = 8.8 g/dL; HCT = 26.1%; MCV = 89.5 fL; MCHC = 33.7 g/dL; PLT = 530 × 10E9/L; and MPV = 9.3 fL. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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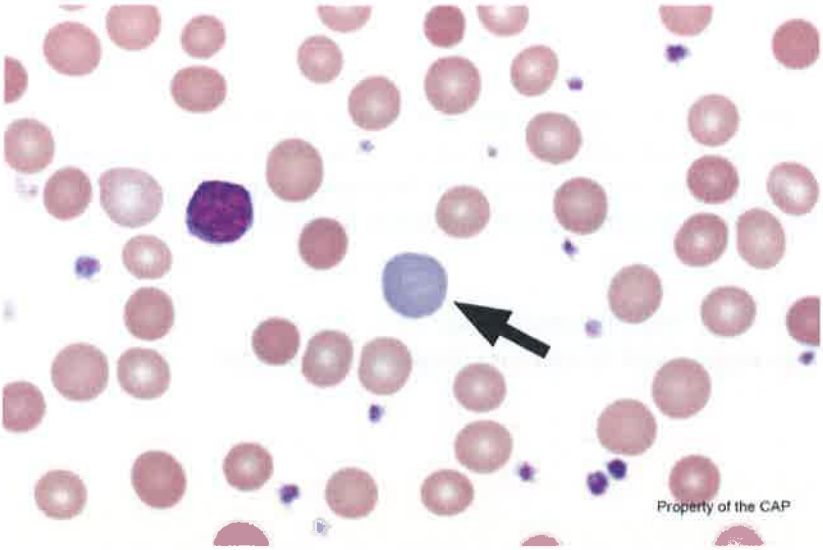
BCP-26

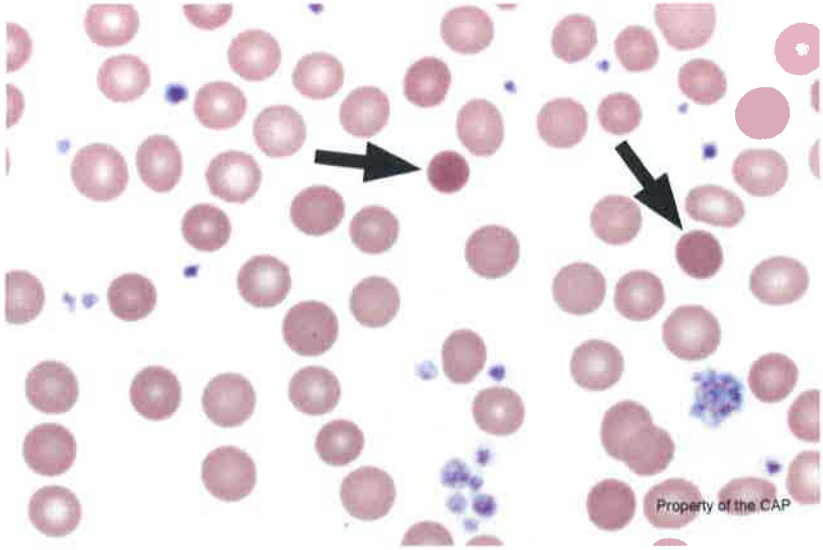


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Polychromatophilic (non-nucleated) red blood cell	110	100.0	5613	99.4	Educational

The arrowed object is a polychromatophilic (non-nucleated) red blood cell, as correctly identified by 100.0% of referees and 99.4% of participants. A polychromatophilic red blood cell is a non-nucleated, round or ovoid red blood cell that represents the final stage of red blood cell maturation after exiting the bone marrow. It is larger than a mature erythrocyte and lacks central pallor. It primarily contains hemoglobin with a small amount of RNA, and thereby stains homogeneously pink-gray or pale purple with Romanowsky or Wright-Giemsa stain. These cells can be stained as reticulocytes and enumerated by using supravital stains, such as new methylene blue. With supravital staining, reticulocytes reveal deep blue granular and/or filamentous structures. This reticulin network is called the "substantia reticulofilamentosa." The amount of precipitated RNA and intensity of polychromasia varies inversely with the age of the reticulocyte. Automated technologies for assessing reticulocytes improve the accuracy and precision of determining reticulocyte numbers.

Blood Cell Identification – Ungraded

BCP-27

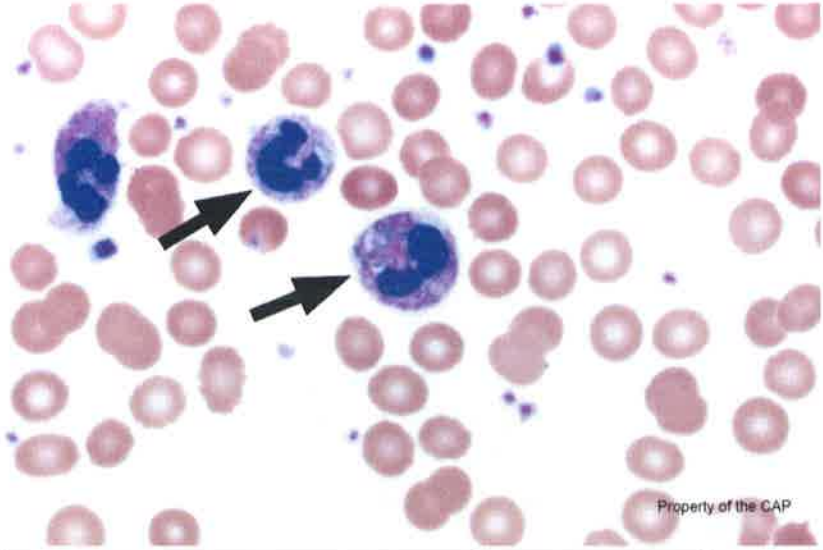


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Spherocyte	109	99.1	5487	98.7	Educational
Erythrocyte, normal	1	0.9	4	0.1	Educational

The arrowed objects are spherocytes, as correctly identified by 99.1% of referees and 98.7% of participants. Spherocytes are identified as densely staining, spherical, or globular red blood cells with normal or slightly reduced volume (ie, normal or low MCV) and increased thickness (more than 3 µm), but with decreased diameter (usually less than 6.5 µm) and usually without central pallor. These cells appear denser than normal RBCs and are commonly found in hereditary spherocytosis and immune hemolytic anemias. Microspherocytes (spherocytes measuring 4 µm or less in diameter) are frequently seen in severe burns or microangiopathies and represent rounded-up fragments of damaged red blood cells.

Blood Cell Identification – Ungraded

BCP-28



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	68	61.8	3589	64.5	Educational
Neutrophil, segmented or band	38	34.5	1748	31.4	Educational
Eosinophil, any stage	3	2.7	189	3.4	Educational
Neutrophil, giant band or giant metamyelocyte	1	0.9	12	0.2	Educational

The arrowed cells are neutrophils with toxic changes, as correctly identified by 61.8% of referees and 64.5% of participants. Toxic changes in neutrophils include toxic granulation, toxic vacuolization, and Döhle bodies. Toxic granulation and Döhle bodies each may be present in an individual cell without the other finding and either change alone is sufficient to designate a neutrophil as toxic. Toxic granulation is defined by the presence of large, purple or dark blue cytoplasmic granules in neutrophils, bands, and metamyelocytes. Vacuoles within the cytoplasm of these same cells define toxic vacuolization. The vacuoles are variable in size and may coalesce, sometimes distorting the neutrophil cytoplasm to form pseudopodia. Ethylenediaminetetraacetic acid (EDTA) blood collection may produce degenerative vacuolization; in this context, only a few, small, punched-out appearing vacuoles may be found. However, as it may be difficult to distinguish toxic from degenerative vacuoles, neutrophil vacuoles should not be labeled as toxic vacuoles unless accompanied by other toxic changes.

Blood Cell Identification – Ungraded

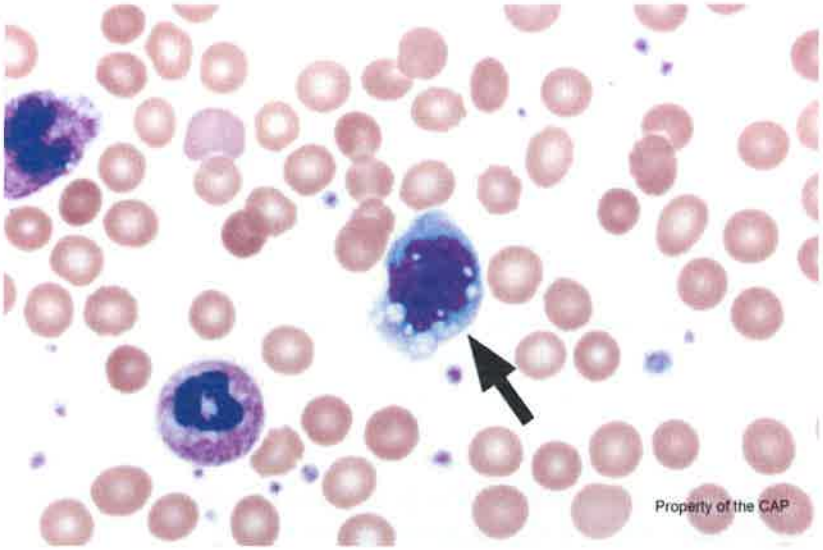
BCP-28 (cont)

34.5 of referees and 31.4% of participants identified these cells as neutrophils, segmented or band. Segmented neutrophils and their immediate precursors, bands, constitute 12% to 25% of the nucleated cells in the bone marrow. Band neutrophils, also known as stabs, constitute 5% to 10% of the nucleated cells in the blood under normal conditions. An increased number of bands may be noted in the blood in a number of physiologic and pathologic states (eg, infectious/inflammatory processes, tissue damage or necrosis, neoplasia, poisoning or intoxication, drug effect, and metabolic abnormalities). The band is round to-oval and 10 to 18 µm in diameter. The N:C ratio is 1:1.5 to 1:2 and the nuclear chromatin is condensed. The nucleus is indented to more than half the distance to the farthest nuclear margin, but the chromatin is not condensed to a single filament (as is the defining feature of the fully mature neutrophil). The nucleus can assume many shapes: it can be band- or sausage-like; S-, C-, or U-shaped; and twisted or folded on itself. The cytoplasm is similar to that of other post-mitotic neutrophils, with specific granules predominating in an otherwise pale cytoplasm.

Although the indicated cells are clearly of the neutrophil lineage, the most specific identification is considered the correct response for the purposes of proficiency testing (see kit instructions) and therefore "neutrophils with toxic changes" would be considered the correct answer.

Blood Cell Identification – Ungraded

BCP-29

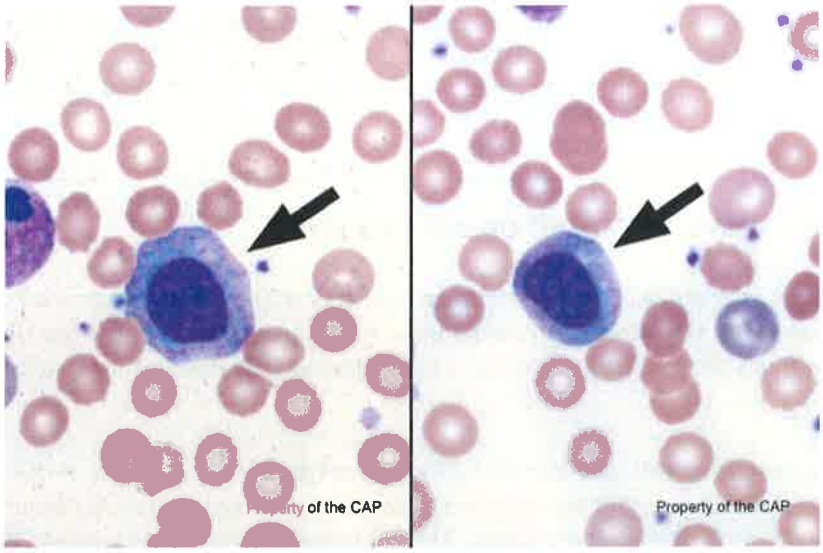


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Monocyte	108	98.2	5336	95.9	Educational
Monocyte, immature (promonocyte, monoblast)	2	1.8	149	2.7	Educational

The arrowed object is a monocyte, as correctly identified by 98.2% of referees and 95.9% of participants. Monocytes are slightly larger than neutrophils, ranging from 12 to 20 µm in diameter. The majority of monocytes are round with smooth edges, but some may have pseudopod-like cytoplasmic extensions. The cytoplasm is abundant, with a gray or gray-blue ground-glass appearance, and may contain vacuoles or fine, evenly distributed azurophilic granules. The nucleus is usually indented, often resembling a three-pointed hat, but it can also be folded or band-like. The chromatin is condensed but is usually less dense than that of a neutrophil or lymphocyte. Nucleoli are generally absent, but occasional monocytes may contain a small, inconspicuous nucleolus. For the purposes of proficiency testing, selection of the response "monocyte, immature (promonocyte, monoblast)" should be reserved for malignant cells in the context of acute monocytic/monoblastic leukemia, acute myelomonocytic leukemia, chronic myelomonocytic leukemia, or myelodysplastic syndromes.

Blood Cell Identification – Ungraded

BCP-30



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Neutrophil, myelocyte	91	82.7	4819	86.7	Educational
Neutrophil, promyleocyte	3	2.7	183	3.3	Educational
Lymphocyte	3	2.7	36	0.7	Educational
Lymphocyte, large granular	2	1.8	89	1.6	Educational
Neutrophil, metamyelocyte	2	1.8	115	2.1	Educational
Monocyte, immature (promonocyte, monoblast)	2	1.8	29	0.5	Educational
Blast cell	1	0.9	20	0.4	Educational
Lymphocyte, reactive	1	0.9	102	1.8	Educational
Neutrophil, promyleocyte, abnormal with/without Auer rod(s)	1	0.9	7	0.1	Educational

The arrowed objects are neutrophils, myelocytes, as correctly identified by 82.7% of referees and 86.7% of participants. The transition from promyelocyte to myelocyte occurs with the end of production of azurophilic (primary) granules and the beginning of production of lilac or pale orange/pink (specific) granules. Myelocytes are usually confined to the marrow where they constitute approximately 10% of the nucleated cells. In pathologic states, myelocytes are seen in blood. The myelocyte is smaller than the earlier precursors, usually 10 to 18 µm. The cells are round-to-oval in shape and have a nuclear-to cytoplasmic ratio of 2:1 to 1:1. The nucleus is slightly eccentric, lacks a nucleolus, and begins to demonstrate chromatin clumping. One side often shows slight flattening. Sometimes a clear space or hof is seen adjacent to the nucleus, indicating the location of the Golgi apparatus. The cytoplasm is relatively more abundant than in earlier precursors and is amphophilic. Both azurophilic and specific granules are present in the cytoplasm with specific granules coming to predominate as maturation progresses.

Case Presentation:

This peripheral blood smear is from a 29-year-old man with past medical history of severe burn injury affecting 30% of his total body surface area. Laboratory data include: WBC = 30.1 × 10E9/L; RBC = 2.92 × 10E12/L; HGB = 8.8 g/dL; HCT = 26.1%; MCV = 89.5 fL; MCHC = 33.7 g/dL; PLT = 530 × 10E9/L; and MPV = 9.3 fL.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: HEMATOLOGIC MANIFESTATIONS OF BURN INJURY

Worldwide, over 60 million injuries per year are related to exposure to heat and/ or fire, with approximately 2.9 million patients requiring hospitalization. Burn injuries are therefore the 4th leading cause of injury. In the United States approximately 500,000 individuals per year have burn injuries severe enough to necessitate medical attention.

The principal hematologic manifestation in patients with thermal injury is hemolytic anemia. The risk of hemolytic anemia appears to be related to the percentage of the skin affected by the burn, and most commonly occurs in patients having burns involving at least 15% of body surface area. The hemolytic episode peaks between 24 - 48 hours after the burn and may result in destruction of up to 30% of the circulating red blood cell mass. The resultant anemia is similar to intravascular hemolytic anemias of other etiologies. Red blood cell morphologic changes are the direct result of the heating of red blood cells to
> 47^{0}C, and include schistocytes, spherocytes, and echinocytes. As in other forms of hemolytic anemia with spherocyte production, the presence of microspherocytes may result in a spuriously elevated platelet count, as noted in this patient. The damaged erythrocytes are removed from circulation by the spleen and to a lesser extent the liver. During convalescence, patients are at increased risk for development of an anemia of chronic disease.

The morphologic differential diagnosis of hemolytic anemia related to thermal injury includes any anemia with the associated red blood cell changes described above. In addition, two entities deserve special consideration. Blood products warmed in microwave ovens or inline blood warmers may develop similar red blood cell changes to those described in burn patients. This is not surprising, since the red blood cell morphologic changes induced by blood warmers are also caused by direct thermal damage to red blood cell membranes. Adherence to manufacturers' guidelines minimizes the risk of thermal related injuries to blood products.

Hereditary pyropoikilocytosis is an autosomal recessive inherited condition resulting in severe hemolytic anemia in affected individuals. The pathophysiology of the condition is related to defects in production of α-spectrin, a normal component of the red blood cell membrane. The morphologic features of peripheral blood from affected individuals simulate those of burn patients, with prominent poikilocytosis, red cell fragmentation, and spherocytosis.

In summary, striking red blood cell morphologic changes can be encountered in patients with clinically significant thermal injuries. However, the morphologic features are not specific and clinical correlation is necessary to exclude other conditions resulting in red blood cell hemolysis.

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Hematology and Clinical Microscopy Resource Committee

REFERENCES:

1. GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; 388(10053); 1459-1544.
2. Greer JP, Arber DA, Glader B, List AF, Means RT, Paraskevas F, and Rodgers GM, eds. *Wintrobe's Clinical Hematology.* 13th ed. Philadelphia, PA: Wolters Kluwer; 2014.

COLLEGE of AMERICAN PATHOLOGISTS

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Participant	Date	Participant	Date

Director (or Designee) Signature - I have verified that the individuals listed above have successfully participated in this activity. Date

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