Blood Cell Identification – Graded

Case History
This peripheral blood smear is from a 55-year-old woman with advanced squamous cell carcinoma presenting with fatigue and dark urine. Laboratory data includes: WBC = 5.1 × 10E9/L; RBC = 1.75 × 10E12/L; HGB = 5.9 g/dL; HCT = 16.9%; PLT = 5 × 10E9/L; MCV = 98 fL; and RDW = 16%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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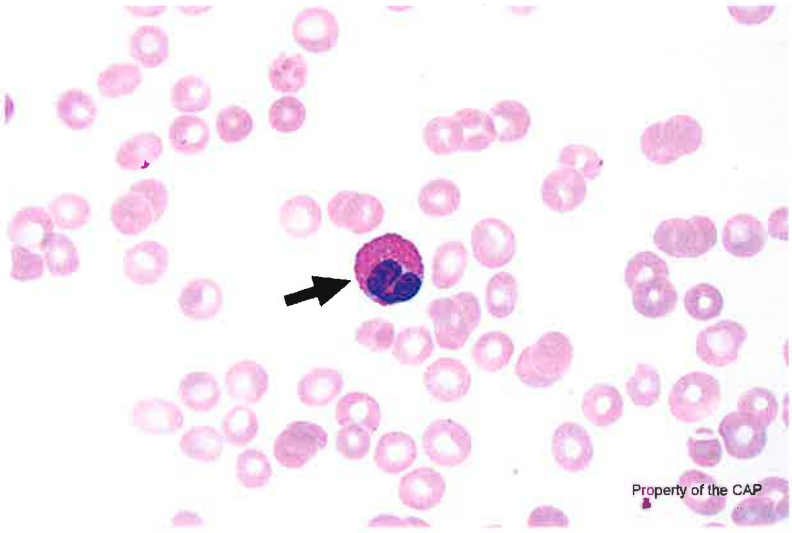
BCP-11



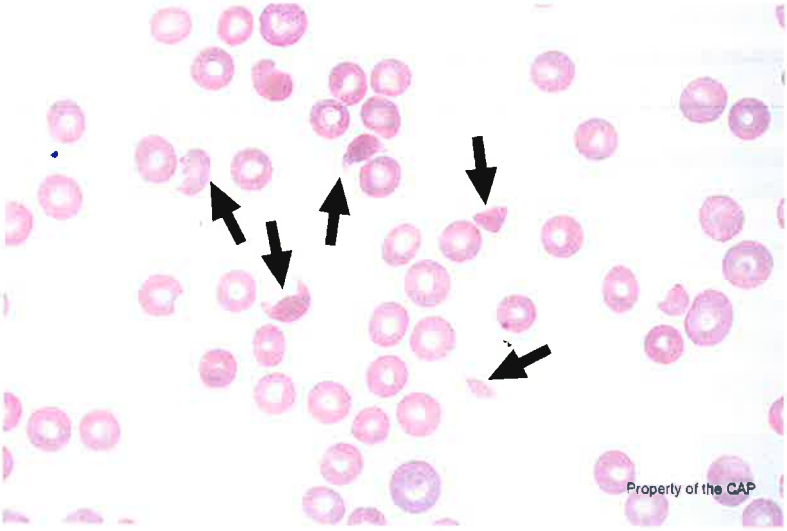
Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Eosinophil, any stage	183	100.0	5497	99.8	Good

The arrowed cell is an eosinophil, as correctly identified by 100.0% of referees and 99.8% of participants. Eosinophils are round-to-oval leukocytes that are recognizable by their characteristic coarse, orange-red granulation. They are comparable in size to neutrophils, ie, 10 to 15 µm in diameter in their mature forms, and 10 to 18 µm in diameter in immature forms. The eosinophil nuclear:cytoplasmic (N:C) ratio ranges from 1:3 for mature forms to 2:1 for immature forms. The eosinophil cytoplasm is generally evenly filled with numerous coarse, orange-red granules of uniform size. These granules rarely overlie the nucleus and are refractile by light microscopy due to their crystalline structure. This refractile appearance is not apparent in photomicrographs. Due to inherent problems with color rendition on photomicrographs, eosinophil granules may appear lighter or darker in blood films that are not freshly made. Discoloration may give the granules a blue, brown, or pink tint. The uniform, coarse eosinophil granules are characteristic and differs from the smaller, finer granules of neutrophils. Occasionally, eosinophils can become degranulate, with only a few orange-red granules remaining visible within the faint pink cytoplasm. In the most

mature eosinophil forms, the nucleus segments into two or more lobes connected by a thin filament. About 80% of segmented eosinophils will have the classic bi-lobed appearance. Typically, these lobes are of equal size and round to ovoid with dense, compact chromatin. The remainder of segmented eosinophils will have three lobes and an occasional cell will have four to five lobes.

Blood Cell Identification – Graded

BCP-12

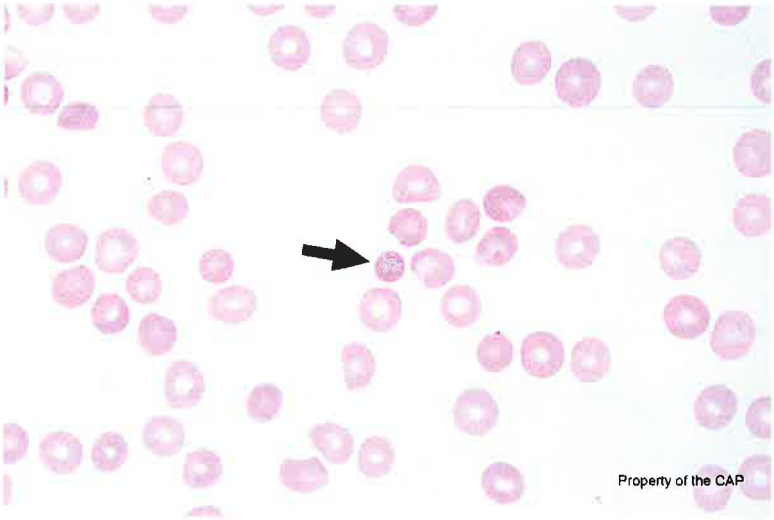


Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	181	98.8	5399	98.1	Good
Acanthocyte (spur cell)	1	0.6	16	0.3	Unacceptable
Bite cell (degmacyte)	1	0.6	70	1.3	Unacceptable

The arrowed cells are fragmented red blood cells, as correctly identified by 98.8% of referees and 98.1% of participants. Fragmented red blood cells are red blood cells that have undergone rips and tears when draped over fibrin strands or have suffered buffeting against unyielding structures in the macrocirculation. Fragments reseal by fusion of the opposing ends and persist in the circulation, presumably for a short time. Fragmented red blood cells include helmet cells, keratocytes (horn cells), triangulocytes and a more inclusive term, schistocytes. A zone of central pallor is rarely present in fragmented red blood cells. Occasional spherocytes are almost invariably present in association with fragmented red blood cells. These spherocytes are the result of rounding up of red blood cell fragments. Fragmented red blood cells are seen in microangiopathic hemolytic anemia including disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), and in patients with severe burns, prosthetic cardiac valves or severe valvular stenosis, malignant hypertension, and other mechanical trauma to the cell (eg, march hemoglobinuria, marathon running). When present in large numbers, they may cause the mean corpuscular volume (MCV) to fall into the microcytic range or interfere with platelet enumeration.

Blood Cell Identification – Graded

BCP-13



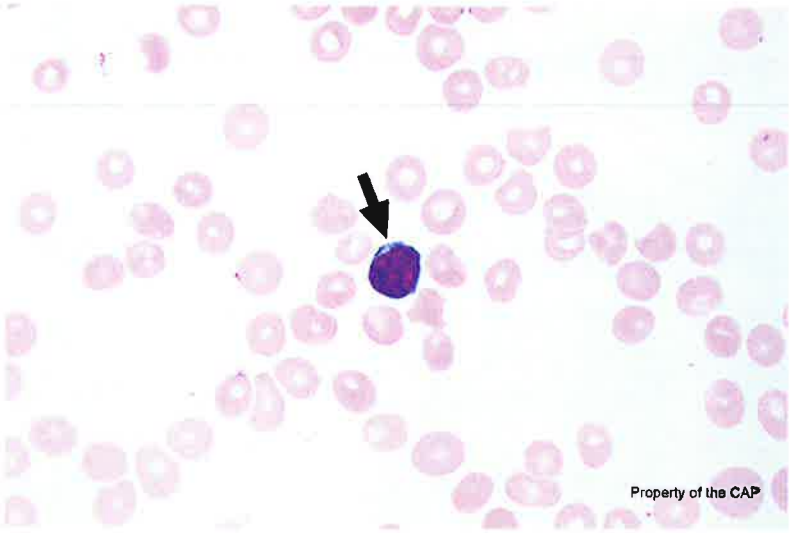
Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Spherocyte	178	97.3	5342	97.0	Good
Microcyte (with increased central pallor)	4	2.1	126	2.3	Unacceptable
Erythrocyte, normal	1	0.6	9	0.2	Unacceptable

The arrowed cell is a spherocyte, as correctly identified by 97.3% of referees and 97.0% of participants. Spherocytes are densely staining, spherical, or globular red blood cells with normal or slightly reduced volume (ie, normal or low MCV) and increased thickness (> 3 µm), but with decreased diameter (usually < 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, including hemolytic anemia, and represent rounded-up fragments of red blood cells.

The arrowed cell was incorrectly identified as a microcyte (with increased central pallor) by 2.1% of referees and 2.3% of participants. Microcytes are smaller than normal red blood cells, measuring < 6 µm in diameter and MCV values < 80 fL in volume. On the blood film, they generally appear smaller than the nucleus of a small lymphocyte. On a peripheral blood film, microcytes retain central pallor, appearing either normochromic or hypochromic. Microcytic RBCs are considered hypochromic when central pallor exceeds 50% of cell diameter. The arrowed cell has no central pallor and the MCV for this case is 98 fL.

Blood Cell Identification – Graded

BCP-14

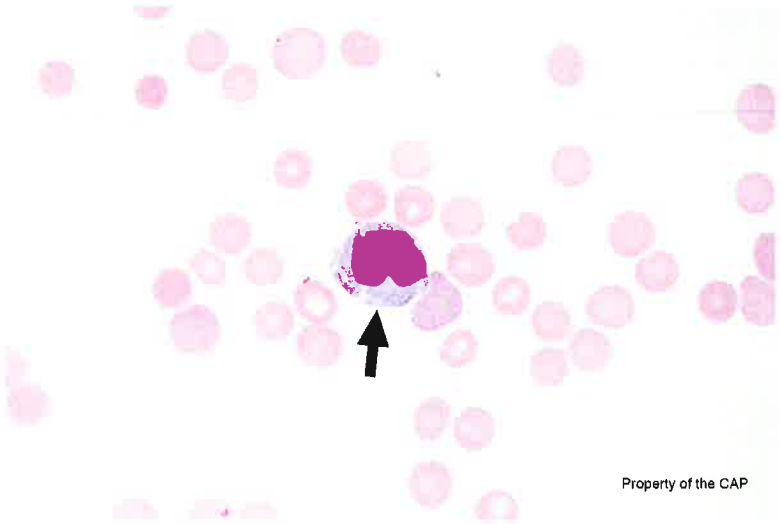


Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Lymphocyte	182	99.5	5411	98.3	Good
Blast cell	1	0.5	33	0.6	Unacceptable

The arrowed cell is a lymphocyte, correctly identified by 99.5% of referees and 98.3% of participants. While most normal lymphocytes are fairly homogeneous, they have a range of normal morphology. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 µm with an N:C ratio ranging from 5:1 to 2:1. 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. Most lymphocytes have a scant amount of pale blue to moderately basophilic, agranular cytoplasm. Occasionally, the edges may be slightly frayed or pointed due to artifacts induced during smear preparation. Occasional lymphocytes will have a small clear zone, or hof, in the cytoplasm adjacent to one side of the nucleus.

Blood Cell Identification – Graded

BCP-15



Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Monocyte	175	95.6	5314	96.5	Good
Monocyte, immature (promonocyte, monoblast)	5	2.7	69	1.3	Unacceptable
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	2	1.1	10	0.2	Unacceptable
Neutrophil, metamyelocyte	1	0.6	75	1.4	Unacceptable

The arrowed cell is a monocyte, as correctly identified by 95.6% of referees and 96.5% of participants. Monocytes are slightly larger than neutrophils, 12 to 20 µm in diameter. The majority of monocytes are round with smooth edges, but some 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 N:C ratio is 4:1 to 2:1. The nucleus is usually indented, often resembling a three-pointed hat, but it can also be folded, band-like, or kidney-shaped. The chromatin is condensed but less dense than that of a neutrophil or lymphocyte. Nucleoli are generally absent, but occasional monocytes may contain a small inconspicuous nucleolus.

2.7% of referees and 1.3% of participants selected monocyte, immature (promonocyte, monoblast). 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. While immature monocytes may be normally identified in marrow aspirates, they are generally inconspicuous and are not present in non-leukemic peripheral blood. The malignant monoblast is a large cell, usually 15 to 25 µm in diameter. It has relatively more cytoplasm than a myeloblast with the N:C ratio ranging from 7:1 to 3:1. The monoblast nucleus is round or oval and has finely dispersed chromatin and distinct nucleoli. The cytoplasm is blue to gray-blue and may contain small, scattered azurophilic granules. Some monoblasts cannot be distinguished morphologically from other blast forms; in these instances, additional tests (eg, cytochemistry and/or flow cytometry) are required to accurately assign blast lineage. Promonocytes have nuclear and cytoplasmic characteristics that are between those of

monoblasts and mature monocytes. They are generally larger than mature monocytes, but they have similar-appearing gray-blue cytoplasm that often contains uniformly distributed, fine azurophilic granules. Cytoplasmic vacuolization is not a typical feature. The nuclei show varying degrees of lobulation, usually characterized by delicate folding or creasing of the nuclear membrane, in contrast to the rounder nuclear profile of monoblasts. Nucleoli are present but may not be as distinct as in monoblasts.

1.1% of referees and 0.2% of participants answered lymphocyte, reactive. The key distinguishing feature of reactive lymphocytes is their wide range of cellular sizes and shapes, as well as nuclear sizes, shapes, and chromatin patterns. These lymphocytes are reacting to an immune stimulus and are frequently increased in viral illnesses. The classic example is infectious mononucleosis (acute Epstein-Barr virus infection). Reactive lymphocytes can also be found in a variety of other viral infections (including cytomegalovirus, adenovirus, or acute HIV infection) protozoal infections (such as toxoplasmosis), some drug reactions, connective tissue diseases, and after major stress to the body's immune system. A variety of reactive lymphocyte forms have been described and they are often seen concurrently in the same blood film. These round to ovoid to irregular cells range from 10 to 25 µm in size with an N:C ratio that varies from 3:1 to 1:2.

The most common type of reactive lymphocyte resembles a larger lymphocyte and corresponds to a Downey type II cell. These cells have round to oval nuclei, moderately condensed chromatin (giving it a smeared appearance), and absent or indistinct nucleoli. They contain abundant pale gray-blue cytoplasm. Granules, if present, are usually small and few in number. Frequently, these reactive lymphocytes have an amoeboid cytoplasm that partially surrounds adjacent red cells and has a darker- staining, furled margin. Basophilia radiating out from the nucleus may also be present.

Immunoblasts and immunoblastic-like reactive lymphocytes are large cells (15 to 20 µm) with round to oval nuclei. They have finely to moderately dispersed chromatin with abundant parachromatin and one or more prominent nucleoli. These may resemble lymphoma cells or blasts. Their cytoplasm is moderately abundant and stains deeply basophilic. The N:C ratio is high (3:1 to 2:1). These reactive lymphocytes correspond to Downey type III cells.

Another type of reactive lymphocyte is referred to as a Downey I cell. These cells are rare. These cells possess scant to moderate amounts of basophilic cytoplasm. The nuclei often appear indented, folded, or lobulated. The chromatin is condensed. A few small vacuoles may be present. Granules may also be apparent.

Plasmacytoid lymphocytes resemble plasma cells and are intermediate in size (10 to 20 µm) and round to oblong in shape. They have round nuclei that are centrally placed or slightly eccentric. The chromatin is slightly to moderately coarse and forms small dense masses or a meshwork of strands resembling that of plasma cells. Nucleoli are generally not visible, but some cells may have one or two small irregular nucleoli. The cytoplasm is moderately abundant, homogeneous, and light blue to deep slate-blue, and it may show a perinuclear clear zone, or hof.

0.6% of referees and 1.4% of participants answered neutrophil, metamyelocyte. Metamyelocytes are the first of the postmitotic myeloid precursors. They constitute 15% to 20% of nucleated cells in the bone marrow and may be seen in the blood in pathologic states and in response to stress. They are approximately 10 to 18 µm in diameter. They are round to oval with a N:C ratio of 1.5:1 to 1:1. The nuclear chromatin is condensed, and the nucleus is indented to less than half of the maximal nuclear diameter (ie, the indentation is smaller than half of the distance to the farthest nuclear margin). The cytoplasm is amphophilic containing rare azurophilic or purple (primary) granules and many fine lilac or pale orange/pink specific granules.

Clinical Presentation:

This peripheral blood smear is from a 55-year-old woman with advanced squamous cell carcinoma presenting with fatigue and dark urine. Laboratory data includes: WBC = 5.1 × 10E9/L; RBC = 1.75 × 10E12/L; HGB = 5.9 g/dL; HCT = 16.9%; PLT = 5 × 10E9/L; MCV = 98 fL; and RDW = 16%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Microangiopathic hemolytic anemia

The patient's peripheral blood smear, together with the clinical signs and symptoms, are characteristic of microangiopathic hemolytic anemia (MAHA). MAHA is a clinical term used to describe the result of a heterogeneous group of processes that shear red blood cells, causing them to fragment inside blood vessels (intravascular hemolysis). Review of the peripheral blood smear shows characteristic findings in MAHA. The shape of normal healthy red blood cells is described as a biconcave disc, a flattened sphere with central indentation on both sides, giving the appearance of central pallor on peripheral blood smear preparations. Another name for fragmented red blood cells is schistocytes. Helmet cells, keratocytes (horn cells), and triangulocytes are descriptive terms for types of schistocytes. A few spherocytes are also almost always seen in MAHA, as they may be produced by red blood cell shearing and subsequent membrane closure. Other laboratory features of MAHA include consequences of the red blood cell fragmentation resulting in anemia: increased reticulocyte count, elevated lactate dehydrogenase (LDH), decreased haptoglobin, and increased unconjugated bilirubin.

There are many causes of MAHA. A subset of MAHA is due to intravascular microthrombi, or clots, that shear the red blood cells. These are termed thrombotic microangiopathy (TMA) and include disseminated intravascular coagulation (DIC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP), among others. In addition to features of MAHA, TMA also shows thrombocytopenia, since platelets are consumed as a component of the microthrombi. Underlying causes of isolated MAHA with or without thrombocytopenia include advanced carcinoma as in this case, plasma cell myeloma (multiple myeloma), lymphoma, systemic infection, drug-induced processes, autoimmune diseases including lupus (systemic lupus erythematosus (SLE)), mechanical or calcified heart values, and severe high blood pressure (termed malignant hypertension). Each of these conditions leads to mechanical stress on red blood cells as they travel through the blood stream. The proposed mechanisms of red blood cell fragmentation are complex in advanced cancers, and include bone marrow involvement by metastatic cancer and/or micrometastases in circulation, each of which could collide with red blood cells and disrupt their membranes.

The clinical manifestations of MAHA are variably acute and severe, and depend on the underlying cause. Free plasma hemoglobin and microthrombi in cases of TMA can cause dark urine and clog the kidney's ability to filter blood, respectively, leading to renal failure which may not be reversible. Anemia may also be severe, but red blood cell transfusions alone may "add fuel to the fire" and worsen the hemolysis. Although severe MAHA may be transient, correction of the underlying causative condition, if possible, is the only way to cure MAHA. For example, temporarily or permanently stopping a suspected offending drug may work. In the absence of this possibility, for example, in a patient with incurable metastatic cancer, repetitive plasma exchange transfusion to temporarily remove the causative factors in the patient's blood is often necessary.

Your expertise in the Hematology laboratory in correctly classifying fragmented red blood cells, spherocytes, and reticulocytes may facilitate a diagnosis of MAHA and help save a patient's life!

Alexandra E. Kovach MD
Hematology and Clinical Microscopy Committee

References:

1. George JN, Nester CM. Syndromes of thrombotic microangiopathy. *N Engl J Med*. 2014;371(7):654-66. PMID: 25119611. doi: 10.1056/NEJMra1312353.
2. Thomas MR, Scully M. How I treat microangiopathic hemolytic anemia in patients with cancer. *Blood*. 2021;137(10):1310-7. PMID: 33512445. doi: 10.1182/blood.2019003810.
3. Lachner K, Obermeier HL. Cancer-related microangiopathic hemolytic anemia: clinical and laboratory features in 168 reported cases. *Medicine (Baltimore)*. 2021;91(4):195-205. PMID: 22732949. doi: 10.1097/MD.06013e3182603598.

Blood Cell Identification – Ungraded

Case History

This peripheral blood smear is from an 80-year-old man from the northeastern US with a tick bite. Laboratory data includes: WBC = 8.1 x 10E9/L; RBC = 4.51 x 10E12/L; HGB = 13.5 g/dL; HCT = 41.1%; MCV = 88 fL; PLT = 50 x 10E9/L; and RDW = 14%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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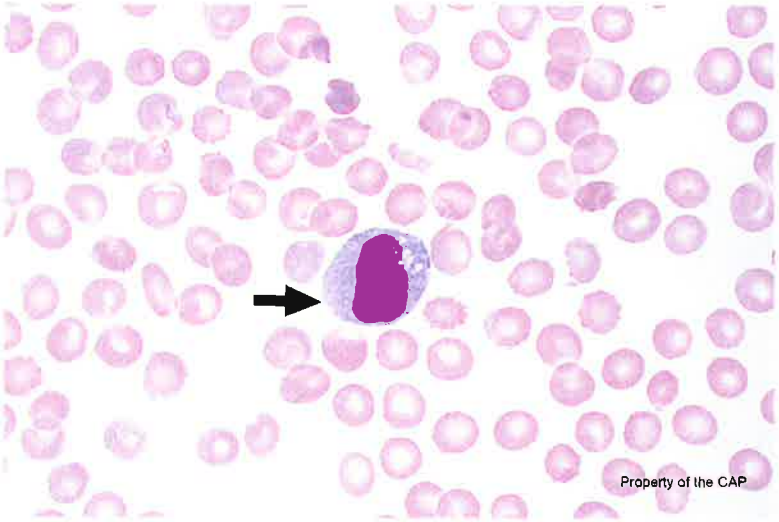
BCP-16



Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Monocyte	162	89.0	4796	88.0	Educational
Monocyte, immature (promonocyte, monoblast)	10	5.5	405	7.4	Educational
Neutrophil, myelocyte	4	2.2	58	1.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	3	1.6	82	1.5	Educational
Immature or abnormal cell, would refer for identification	2	1.1	35	0.6	Educational
Blast cell	1	0.6	5	0.1	Educational

The arrowed cell is a normal monocyte, as correctly identified by 89.0% of referees and 88.0% 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 N:C ratio ranges from 4:1 to 2:1. 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.

BCP-16, cont'd

5.5% of referees and 7.4% of participants incorrectly identified the cell as an immature monocyte (promonocytes or monoblast). The monoblast is a large cell, usually 15 to 25 µm in diameter. It has relatively more cytoplasm than a myeloblast with the N:C ratio ranging from 7:1 to 3:1. The monoblast nucleus is round or oval and has finely dispersed chromatin and distinct nucleoli. The cytoplasm is blue to gray-blue and may contain small, scattered azurophilic granules. Promonocytes have nuclear and cytoplasmic characteristics that are between those of monoblasts and mature monocytes. They are generally larger than mature monocytes, but they have similar-appearing gray-blue cytoplasm that often contains uniformly distributed, fine azurophilic granules. Cytoplasmic vacuolization is not a typical feature. The nuclei show varying degrees of lobulation, usually characterized by delicate folding or creasing of the nuclear membrane, in contrast to the rounder nuclear profile of monoblasts. Nucleoli are present but may not be as distinct as in monoblasts.

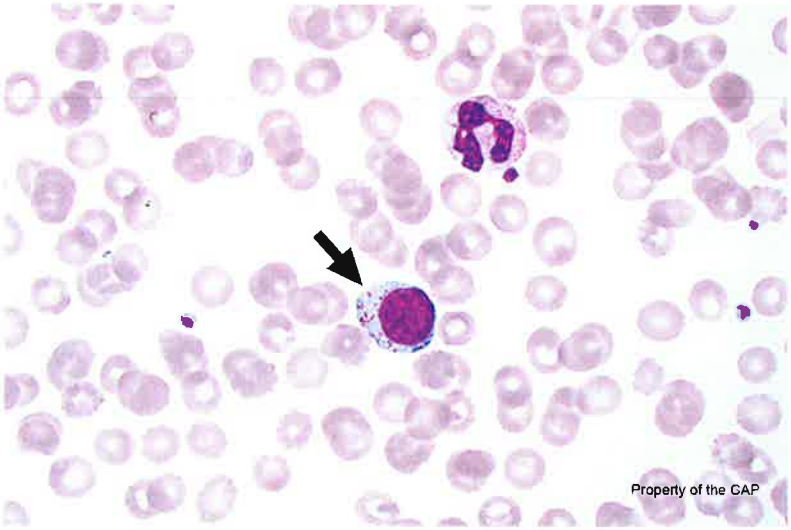
2.2% of referees and 1.1% of participants incorrectly identified the cell as a neutrophil, myelocyte. The myelocyte is smaller than the earlier precursors, usually 10 to 18 µm. The cells are round to oval in shape and have a nuclear-to- cytoplasmic ratio of 2:1 to 1:1. The nucleus is slightly eccentric, 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.

1.6% of referees and 1.5% of participants incorrectly identified the cell as a reactive lymphocyte. The key distinguishing feature of reactive lymphocytes is their wide range of cellular sizes and shapes, as well as nuclear sizes, shapes, and chromatin patterns. These lymphocytes are reacting to an immune stimulus and are frequently increased in viral illnesses. The classic example is infectious mononucleosis (acute Epstein-Barr virus infection). Reactive lymphocytes can also be found in a variety of other viral infections (including cytomegalovirus, adenovirus, or acute HIV infection) protozoal infections (such as toxoplasmosis), some drug reactions, connective tissue diseases, and after major stress to the body's immune system. A variety of reactive lymphocyte forms have been described and they are often seen concurrently in the same blood film. These round to ovoid to irregular cells range from 10 to 25 µm in size with an N:C ratio that varies from 3:1 to 1:2.

1.1% of referees and 0.6% of participants incorrectly identified the cell as an abnormal cell, rather than a normal monocyte. See explanation above.

Blood Cell Identification – Ungraded

BCP-17



Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Lymphocyte, large granular	172	94.4	4992	92.8	Educational
Lymphocyte	4	2.2	207	3.9	Educational
Leukocyte with intracellular *Anaplasma/Ehrlichia*	2	1.1	10	0.2	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	2	1.1	84	1.6	Educational
Basophil, any stage	1	0.6	5	0.1	Educational
Immature or abnormal cell, would refer for identification	1	0.6	12	0.2	Educational

The arrowed cell is a large granular lymphocyte, as correctly identified by 94.4% of referees and 92.8% of participants. Large granular lymphocytes are medium to large cells with round nuclei, dense chromatin, and no visible nucleoli. The cytoplasm is moderate to abundant, clear, and lightly basophilic, and contains several variable coarse, unevenly distributed, small azurophilic granules. These cells are found in small numbers in blood smears from normal individuals, but they may be increased in association with reactive lymphocytes. Cell surface marker studies show that these cells are natural killer cells or suppressor/cytotoxic T-lymphocytes.

2.2% of referees and 3.9% of participants incorrectly identified the cell as a lymphocyte. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 µm with an N:C ratio ranging from 5:1 to 2:1. 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. Most lymphocytes have a scant amount of pale blue to moderately basophilic, agranular cytoplasm. Occasionally, the edges may be slightly frayed or pointed due to artifacts induced during smear preparation. Occasional lymphocytes will have a small clear zone, or hof, adjacent to one side of the nucleus.

1.1% of referees and 1.6% of participants incorrectly identified the cell as a reactive lymphocyte. The key distinguishing feature of reactive lymphocytes is their wide range of cellular sizes and shapes, as well as nuclear

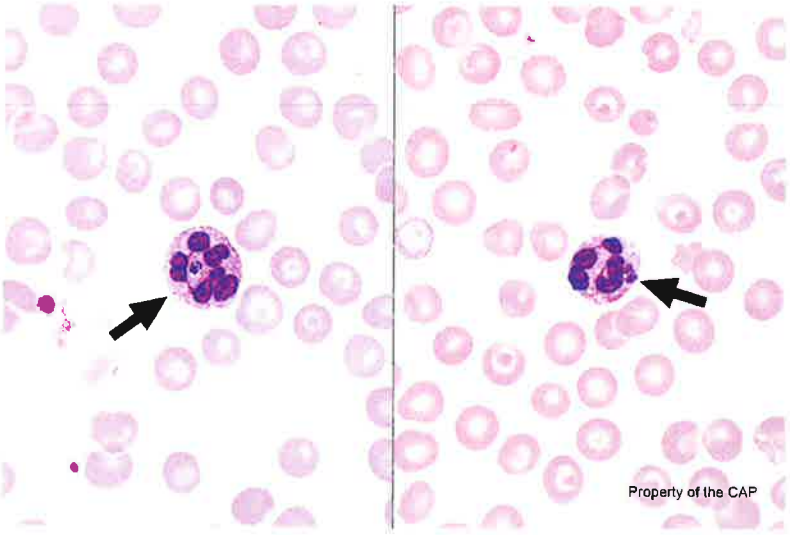
BCP-17, cont'd

sizes, shapes, and chromatin patterns. These lymphocytes are reacting to an immune stimulus and are frequently increased in viral illnesses. The classic example is infectious mononucleosis (acute Epstein-Barr virus infection). Reactive lymphocytes can also be found in a variety of other viral infections (including cytomegalovirus, adenovirus, or acute HIV infection) protozoal infections (such as toxoplasmosis), some drug reactions, connective tissue diseases, and after major stress to the body's immune system. A variety of reactive lymphocyte forms have been described and they are often seen concurrently in the same blood film. These round to ovoid to irregular cells range from 10 to 25 µm in size with an N:C ratio that varies from 3:1 to 1:2.

1.1% of referees and 0.2% of participants incorrectly identified the cell as a leukocyte with intracellular *Anaplasma/Ehrlichia*. Please see the explanation for BCP-18.

Blood Cell Identification – Ungraded

BCP-18



Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Neutrophil with hypersegmented nucleus	125	68.7	3671	68.2	Educational
Leukocyte with intracellular *Anaplasma/Ehrlichia*	42	23.1	1345	25.0	Educational
Neutrophil, segmented or band	8	4.4	185	3.4	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	3	1.5	69	1.3	Educational
Immature or abnormal cell, would refer for identification	2	1.1	28	0.5	Educational
Eosinophil, any stage	1	0.6	4	0.1	Educational
Parasite(s) seen, referred for definitive identification	1	0.6	31	0.6	Educational

The arrowed cells are neutrophils with intracellular/intracytoplasmic *Anaplasma/Ehrlichia*, as correctly identified by 23.1% of referees and 25.0% of participants. Recognized as an arthropod-borne infectious agent in humans, members of the genus *Anaplasma* (previously *Ehrlichia*) are small, Gram-negative, obligate intracellular organisms currently classified as rickettsiae. On Wright-stained preparations, *Anaplasma* species appear as round, dark purple-stained dots or clusters of dots (morulae) in the cytoplasm of either neutrophils (*A. phagocytophilium*) or monocytes and macrophages (*A. chafeensis*). The morulae are microcolonies of organisms.

The arrowed cells were incorrectly identified as neutrophils with hypersegmented nucleus by 68.7% of referees and 68.2% of participants. Although one of these cells depicted shows more than six nuclear lobes, both demonstrate cytoplasmic morulae and thus, the more specific and correct response is "leukocyte with intracellular *Anaplasma/Ehrlichia*."

A hypersegmented neutrophil necessitates that the neutrophil demonstrates six or more nuclear lobes. Hypersegmented neutrophils are uncommon unless there is megaloblastic hematopoiesis which occurs when DNA synthesis is impaired. Such conditions include deficiency of cofactors for nucleotide synthesis, such as

BCP-18, cont'd

vitamin B12 and folate, and cases in which patients are receiving a nucleotide analog drug (such as 6-mercaptopurine) or nuclear cofactor blocking agents (such as methotrexate) for the treatment of neoplastic or rheumatologic conditions. Hypersegmented neutrophils may also be seen in sepsis, renal disease, and myeloproliferative neoplasms.

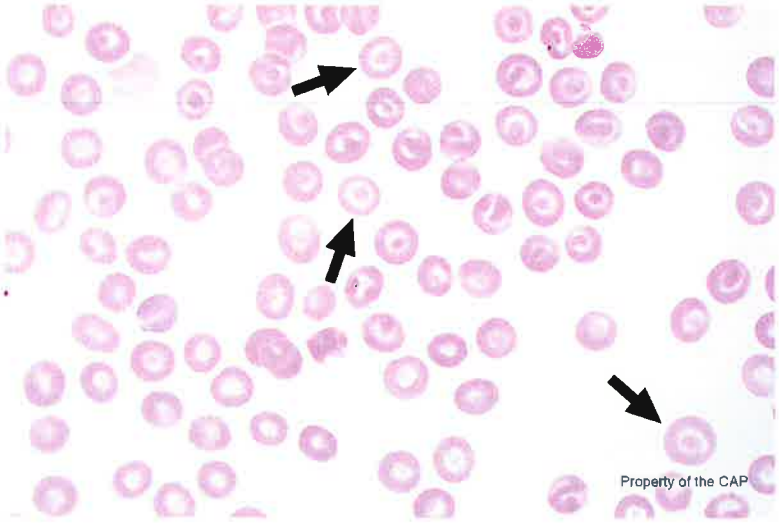
The arrowed cells were incorrectly identified as neutrophils, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization by 1.5% of referees and 1.3% of participants. Despite some toxic changes, there are microcolonies of organisms (morulae) in the cytoplasm of the neutrophils, thus the more specific and intended response is "leukocyte with intracellular *Anaplasma/Ehrlichia*." 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. 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. Döhle bodies appear as single or multiple blue or gray-blue inclusions of variable size (0.1 to 5.0 µm) and shape (round or elongated or crescent shaped) in the cytoplasm of neutrophils, bands, or metamyelocytes. They are often found at the periphery of the cytoplasm, near the cell membrane. These inclusions represent parallel strands of rough endoplasmic reticulum. Toxic changes result from the action of cytokines released in response to infection, burns, trauma, and granulocyte colony stimulating factor (G-CSF), and they indicate a shortened maturation time and activation of post-mitotic neutrophil precursors.

4.4% of referees and 3.4% of participants correctly identified the cell as a neutrophil (segmented or band) but failed to identify intracellular/intracytoplasmic *Anaplasma* sp.

1.1% of referees and 0.5% of participants identified the cell as an immature/abnormal cell, which is an acceptable answer for laboratories that always refer abnormal cell identification to an outside laboratory.

Blood Cell Identification – Ungraded

BCP-19

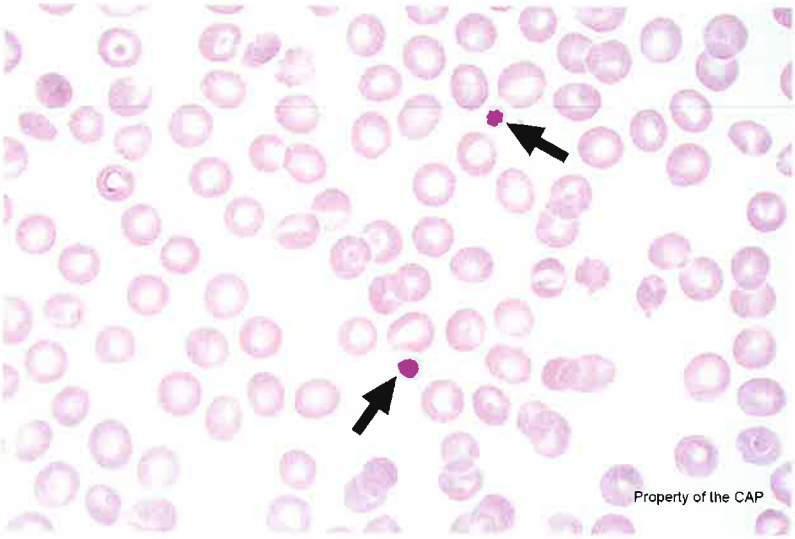


Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Target cell (codocyte)	182	100.0	5362	99.7	Educational

The arrowed cells are target cells (codocytes), as correctly identified by 100.0% of referees and 99.7% of participants. Target cells are thin red blood cells with an increased surface membrane-to-volume ratio. They are often flattened out on the smears and may appear macrocytic. Target cells are believed to arise from disturbances in red blood cell membrane cholesterol and lecithin content or decreased cytoplasmic hemoglobin content. Target cells are characterized by a central hemoglobinized area within the surrounding area of pallor, which in turn is surrounded by a peripheral hemoglobinized zone giving target cells the appearance of a sombrero or a bull's-eye. Target cells associated with hemoglobin C may have a slightly reduced or normal MCV, whereas those associated with hemoglobin E disorders or hemoglobin H disease exhibit microcytosis of varying degree. Target cells are usually seen in thalassemias, iron deficiency anemia, following splenectomy or in patients who are jaundiced or who have chronic liver disease; in the latter two conditions, the MCV may be normal or increased. Target cells may also appear as artifacts from slow drying the slides in a humid environment or from specimens anticoagulated with excessive EDTA. The drying artifact results in the presence of numerous target cells in some fields, but none or few in other fields.

Blood Cell Identification – Ungraded

BCP-20



Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Platelet, normal	177	97.3	5215	96.9	Educational
Platelet, giant (macrothrombocyte)	5	2.7	145	2.7	Educational

The arrowed cells are normal platelets, as correctly identified by 97.3% of referees and 96.9% of participants. Platelets, also known as thrombocytes, are small, blue-gray fragments of megakaryocytic cytoplasm. Most measure 1.5 to 3 µm in diameter. A few small platelets, less than 1.5 µm in diameter, and a few large platelets, 4 to 7 µm in diameter, may also be seen in normal blood films. Fine, purple-red granules are dispersed throughout the cytoplasm or are sometimes aggregated at the center. These granules are platelet alpha granules. Platelet delta granules (or dense granules) are not visible on light microscopy. Platelets may be variable in shape, but most normal platelets are round or very slightly elliptical. Some have short cytoplasmic projections or ruffled margins. They are typically single but may form aggregates, particularly in fresh (fingerstick) preparations.

2.7% of referees and 2.7% of participants incorrectly identified the cells the giant platelets. Giant platelets are larger than 7 µm, usually measuring 10 to 20 µm in diameter. For proficiency testing purposes, the term giant platelet is used when the platelet is larger than the size of the average red blood cell in the field, assuming a normal MCV. The periphery of the giant platelet may be round, scalloped, or stellate. The cytoplasm may contain a normal complement of fine azurophilic granules, or the granules may fuse into giant forms. Giant platelets are a rare finding in normal peripheral blood, but may be seen in many different reactive, neoplastic, and inherited conditions.

Actions Laboratories Should Take when a PT Result is Not Graded

The CAP uses exception reason codes that signify the proficiency testing (PT) for an analyte has not been graded. The exception reason code is located on the evaluation report in brackets to the right of the result. Your laboratory must identify all analytes with an exception reason code, review, and document the acceptability of performance as outlined below and retain documentation of review for at least 2 years. The actions laboratories should take include, but are not limited to:

Code	***Exception Reason Code Description***	***Action Required***
11	Unable to analyze	Document why the specimens were not analyzed (eg, instrument not functioning or reagents not available). Perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
20	Response was not formally graded due to insufficient peer group data. Please see the participant summary for additional information.	Applies to a response that is not formally evaluated when a peer group is not established due to fewer than 10 laboratories reporting. Document that the laboratory performed a self-evaluation using the data presented in the participant summary and compared its results to a similar method, all method, all participant statistics, or data tables for groups of 3-9 laboratories, if provided. Perform and document the corrective action of any unacceptable results. If self-evaluation is not possible, it is up to the laboratory director/designee to determine an alternative performance assessment.
21	Specimen problem	Document that the laboratory has reviewed the proper statistics supplied in the participant summary. Perform and document alternative assessment for the period that commercial PT was not tested to the same level and extent that would have been tested. Credit is not awarded in these cases.
22	Result is outside the method/ instrument reportable range	Document the comparison of results to the proper statistics supplied in the participant summary. Verify detection limits. Perform and document the corrective action of any unacceptable results.
24	Incorrect response due to failure to provide a valid response code	Document the laboratory's self-evaluation against the proper statistics and evaluation criteria supplied in the participant summary. Perform and document the corrective action of any unacceptable results. Document corrective action to prevent future failures.
25	Inappropriate use of antimicrobial	Document the investigation of the results as if they were unacceptable and review the proper reference documents to gain knowledge of the reason your response is not appropriate.
26	Educational challenge	Review participant summary for comparative results and document performance accordingly. Evaluation criteria are not established for educational challenges. Laboratories should determine their own evaluation criteria approved by their laboratory director for self-evaluation.
27,31	Lack of participant or referee consensus	Document that the laboratory performed a self-evaluation and compared its results to the intended response when provided in the participant summary. If comparison is not available, perform and document alternative assessment (ie, split samples) for the period that commercial PT reached non-consensus to the same level and extent that would have been tested.
28	Response qualified with a greater than or less than sign; unable to quantitate	Applies to a response that is not formally evaluated when a less than or greater than sign is reported. Document that the laboratory performed a self-evaluation and compared its results to the proper statistics supplied in the participant summary. Verify detection limits. Perform and document the corrective action of any unacceptable results.
30	Scientific committee decision	Applies to a response that is not penalized based on scientific committee decision. Document that the laboratory has reviewed the proper statistics supplied in the participant summary.

Actions Laboratories Should Take when a PT Result is Not Graded

The CAP uses exception reason codes that signify the proficiency testing (PT) for an analyte has not been graded. The exception reason code is located on the evaluation report in brackets to the right of the result. Your laboratory must identify all analytes with an exception reason code, review and document the acceptability of performance as outlined below and retain documentation of review for at least 2 years. The actions laboratories should take include but are not limited to:

Code	***Exception Reason Code Description***	***Action Required***
33	Specimen determined to be unsatisfactory after contacting the CAP	Document that the laboratory has contacted the CAP and no replacements specimens were available. Perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
40 41	Results for this kit were not received. Results for this kit were received past the evaluation cut-off date.	Document why results were not received, corrective action to prevent recurrence and the laboratory's self-evaluation of the results by comparing results to the proper statistics and evaluation criteria supplied in the participant summary. If PT specimens were not analyzed, perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
42	No credit assigned due to absence of response	The participant summary indicates which tests are graded (see evaluation criteria) and which tests are not evaluated/educational. Updates to grading will also be noted. If a test is educational, the laboratory is not penalized for leaving a result(s) blank. If a test is graded (regulated and non-regulated analytes) and your laboratory performs that test, results cannot be left blank. The laboratory is required to submit results for **all** challenges within that test or use an appropriate exception code or indicate test not performed/not applicable/not indicated. Exceptions may be noted in the kit instructions and/or the result form. Document corrective actions to prevent future failures.
44	This drug is not included in our test menu. Use of this code counts as a correct response.	Verify that the drug is not tested on patient samples and document to ensure proper future reporting.
45	Antimicrobial agent is likely ineffective for this organism or site of infection	Document that the laboratory performed a self-evaluation of written protocols and practices for routine reporting of antimicrobial susceptibility reports to patient medical records. Document that routine reporting of this result to clinicians for patient care is compliant with specific recommendations of relevant medical staff and committees (eg, infectious diseases, pharmacy and therapeutics, infection control).
77	Improper use of the exception code for this mailing	Document the identification of the correct code to use for future mailings.
91	There was an insufficient number of contributing challenges to establish a composite grade.	Document the investigation of the result as if it were an unacceptable result. Perform and document the corrective action if required.
35, 43, 46, 88, 92	Various codes	No action required.

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FH (1-4, 9-10, 13,16)-B 2022: Human Anaplasmosis

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Disclosure Statement

Below you will find the financial disclosure relationships for anyone who was able to affect the content of this educational activity.

The CAP mitigates all the relevant financial relationships listed for these individuals.

The following authors/planners have no financial relationships to disclose:
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The following authors/planners have financial relationships to disclose:
Olga Pozdnyakova, MD, PhD, FCAP: Scopio, Consultant; Hoffman La Roche, Consultant

The following In-Kind Support has been received for this activity:
None

The following Commercial Support has been received for this activity:
None

Learning Objectives

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

1. Describe the diagnostic criteria of human granulocytic anaplasmosis (HGA).
2. Identify the clinical, morphologic, and common laboratory features of HGA.
3. Recognize the arthropod vector, life cycle, geographic predilection, and etiologic agent of HGA.
4. Understand the rationale for treatment in symptomatic patients.

FH (1-4, 9-10, 13, 16)-B 2022: Human Anaplasmosis

Case Presentation

This peripheral blood smear is from an 80-year-old man from the northeastern US with a tick bite. Laboratory data includes: WBC = 8.1 x 10E9/L; RBC = 4.51 x 10E12/L; HGB = 13.5 g/dL; HCT = 41.1%; MCV = 88 fl, PLT = 50 x 10E9/L; and RDW = 14%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

INTRODUCTION

Human anaplasmosis, or human granulocytic anaplasmosis (HGA), is a tick-borne rickettsial disease that often presents as nonspecific acute febrile illness. The dominant etiologic agent of human granulocytic anaplasmosis (HGA is *Anaplasma phagocytophilum*, which is transmitted most commonly by the deer tick (*Ixodes scapularis* in the eastern and midwestern US) or western deer tick (*Ixodes pacificus* in the western US).

A. phagocytophilum infects human white blood cells (WBC), namely neutrophilic granulocytes, causing fever and cytopenia in human hosts. Severe illness and death are seen most commonly with older patients and those with some immune suppression, though delayed treatment in young, otherwise healthy patients has resulted in protracted clinical course and fatal outcomes.

HGA is a nationally notifiable disease in the US and requires reporting to state and local health departments. Although reported cases of HGA have increased since the beginning of the 21st century, the death rate from HGA has remained low at less than 1%.

EPIDEMIOLOGY

HGA was initially discovered in Michigan and Wisconsin in the early 1990s and nearly indistinguishable from human monocytic ehrlichiosis (HME) save for the presence of characteristic morulae in granulocytes rather than monocytes. Given the clinical and morphologic similarities, the bacterium was initially placed into the genus ehrlichia and named *Ehrlichia phagocytophilum* with the resultant disease termed human granulocytic ehrlichiosis. Subsequent taxonomic changes would eventually place this organism into the *Anaplasma* genus and thus assume the designation human granulocytic anaplasmosis (HGA).

Ticks, most commonly *I. scapularis,* are responsible for spreading and infecting individuals with *A. phagocytophilum.* These ticks are relatively ubiquitous in wooded and grassy areas, particularly in the upper midwestern and northeastern US (namely, Minnesota, Wisconsin, and Rhode Island). The geographic range of *I. scapularis* has been expanding to other areas in the US. The ticks may be active, and thus infectious, year-round; however, the majority of reported infections occur largely in the summer months (May through August) with two peaks in June and July and a second, albeit smaller, peak in October.

A. phagocytophilum has been reported in several European (Slovenia, Netherlands, Spain, Sweden, Norway, Croatia, Poland) and Asian (China, Korea, Japan) countries, as well as Russia, though tick vectors vary depending on the location.

PATHOGENESIS

Transmission of *A. phagocytophilum* occurs most commonly by the deer tick (or black-legged tick), *I. scapularis* in the eastern and midwestern US and less commonly by the western deer tick, *I. pacificus* in the western US. Typical animal reservoirs for *A. phagocytophilum* are wild rodents such as squirrels and mice. Humans are often incidental hosts becoming infected during vocational activities in endemic areas or contact with household pets.

Anaplasma sp. are obligate intracellular Gram-negative bacteria, which preferentially infect human granulocytes. During replication within host WBC cytoplasm, the bacteria multiply in membrane-bound vacuoles and form microcolonies called *morulae* (from Latin, for mulberry). The lack of typical cell membrane components (pathogens-associated molecular patterns [PAMPs] and lipopolysaccharides) allows the bacteria to evade the host innate immune defense and enter the host cell for replication.

HGA has some capacity for immune suppression in infected individuals though the mechanisms for this phenomenon are not entirely understood. Secondary opportunistic infections and organ failures have been reported in HGA.

Rarely, HGA has been reported to be transmitted through blood products. Transmission of rickettsial disease through solid organ transplantation has also been described, albeit even more rarely.

CLINICAL FEATURES

Symptoms of HGA typically occur approximately 1 - 2 weeks after a tick bite, though tick bites are often painless and go unnoticed by their victim. Early symptoms of HGA are often nonspecific, mimicking viral illnesses (fever, chills, headache, body aches) in many cases. Nausea, vomiting, and loss of appetite may also occur early in the disease. Rash and nervous system involvement have rarely been reported in HGA and their presence should prompt further evaluation of other tickborne diseases (see Table 1). Severe, or late-stage illness can include respiratory failure, bleeding disorders, organ failure, and rarely death. Delay in antibiotic therapy, older age, and weakened immune systems are some risk factors that may lead one to severe disease or death.

Patients present with a variety of laboratory findings with leukopenia (often accompanied by a left shift), thrombocytopenia, and elevated plasma levels of aminotransferases (transaminases), lactate dehydrogenase, and alkaline phosphatase being the most common. Thrombocytopenia is observed more often than leukopenia. Leukopenia in patients with HGA can be caused by lymphopenia or neutropenia. Lymphopenia tends to occur in the early stages of infection, followed by lymphocytosis. In contrast, the initial neutrophil count in patients with HGA is inversely related to the duration of symptoms before treatment is begun. Anemia and an elevated plasma creatinine concentration also may be seen.

DIAGNOSIS

Making a diagnosis of HGA is often difficult and requires a high index of suspicion and thorough clinical and laboratory investigation and integration of the findings. As mentioned, patients often present with fever of unknown origin and non-specific viral illness-like symptoms. Clinical history should include questions about potential tick exposure including recreational activities, occupational exposure, travel to endemic areas, and contact with pets. Although helpful, the absence of a known tick bite should not dissuade the provider in considering HGA. Seasonality is also important as ticks are most active in the summer months and thus most apt to transmit disease during this time. Warmer climates may see year-round infections, and HGA has been reported during every month of the year.

Patients should receive a complete blood count with manual WBC differential and chemistry panel. Although the majority of patients present with some degree of laboratory abnormalities, it is important to remember than normal laboratory findings do not rule out the possibility of HGA.

Manual WBC differential count should always be performed if HGA is suspected, since HGA produces characteristic morulae in the cytoplasm of granulocytes (typically neutrophils), which can only be detected on a careful peripheral blood smear examination. These are most likely to be detected within the first week of the illness. The proportion of infected granulocytes may be quite low and thus a buffy-coat or concentrated blood film may increase the sensitivity of examination. The presence of morulae in a particular cell type, however, does not differentiate HGA from HME. Also, various other cytoplasmic inclusions may look similar to the morulae seen in HGA. Other peripheral blood morphologic findings could include the presence of atypical lymphocytes, toxic granulation and/or cytoplasmic vacuolization in granulocytes, along with the left shift.

Occasionally, the presence of cytopenia may prompt investigation for a primary marrow process with resultant bone marrow biopsy. Immunohistochemical studies for anaplasmosis can be used in revealing the causative agent.

Indirect immunofluorescence antibody (IFA) assays for immunoglobulin G (IgG) using *A. phagocytophilum* antigen are the gold standard for confirmation of infection. IgG IFA assays should be performed on two separate paired serum samples collected 2 - 4 weeks apart (acute and convalescent sera) and demonstrate evidence of a fourfold seroconversion. Notably, antibodies to other rickettsial organisms may cross-react with *A. phagocytophilum.* IgM antibodies, although available through some reference laboratories, lack specificity, may remain elevated for months to years after infection, and are not recommended for disease confirmation. Despite being the gold standard, the clinical utility of IFA in the diagnosis of HGA is less than optimal since two temporally distinct samples are required and a negative result does not preclude the diagnosis.

Polymerase chain reaction (PCR) for *A. phagocytophilum* is highly specific with variable sensitivity depending on the stage of illness, being most sensitive in the first week of the illness or in the setting of severe disease. Blood samples for PCR testing should be obtained prior to initiating antibiotic therapy as treatment may produce false negative results. A negative PCR result does not rule out the diagnosis.

Given significant caveats to available laboratory testing, high clinical suspicion and supportive epidemiologic information should drive administration of empiric antibiotic therapy. Treatment should not be delayed while awaiting diagnostic testing results or if initial testing is not supportive of the diagnosis.

Differential diagnosis

Fever, headache, and myalgias are common presenting symptoms among all tickborne rickettsial disease in humans, as well as many other reactive, infectious, and neoplastic conditions. Patients with unusual presentations should be extensively evaluated for other tick-borne diseases (**Table 1**).

I. scapularis is also known to transmit *Borrelia burgdorferi* (the causative agent of Lyme disease), *Babesia microti* (the primary cause of human babesiosis), among others. Any of these may be seen as a coinfection with HGA and should be considered in patients who do not respond promptly to appropriate antibiotic treatment or in patients with unusual presentations (eg, presence of a rash).

E. ewingii ehrlichiosis may demonstrate the characteristic morulae in granulocytes similar to HGA, but the clinical presentation and geographic location more closely resembles HME.

International travel should prompt more inclusive investigation of non-*Anaplasma* tickborne diseases that may present similarly.

Less common, but emerging tickborne/transmitted human pathogens that should also be included in the differential are *Borrelia miyamotoi*, *Borrelia mayonii*, deer tick virus, and *Ehrlichia muris*-like agent, among others, which may cause undifferentiated fever and viral illness-like symptoms in patients (**Table 2**).