Blood Cell Identification – Graded

Case History
This peripheral blood smear is from a 74-year-old woman with partially treated chronic myeloid leukemia. Laboratory data include: WBC = 3.6 x 10E9/L, RBC = 4.08 x 10E12/L; HGB = 10.4 g/dL; HCT = 33.4%; MCV = 82 fL; MCHC = 31.2 g/dL; PLT =177 x 10E9/L; and RDW = 19.8 %.
Absolute neutrophil count 1.5 x 10E9/L. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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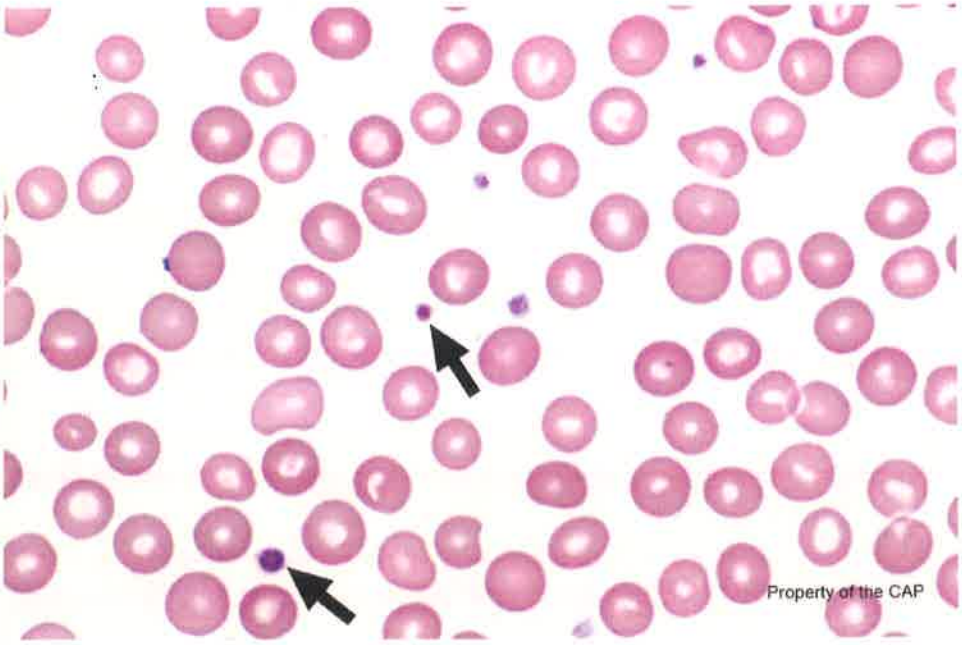
https://documents.cap.org/documents/2020-hematology-clinical-microscopy-glossary.pdf

BCP-21

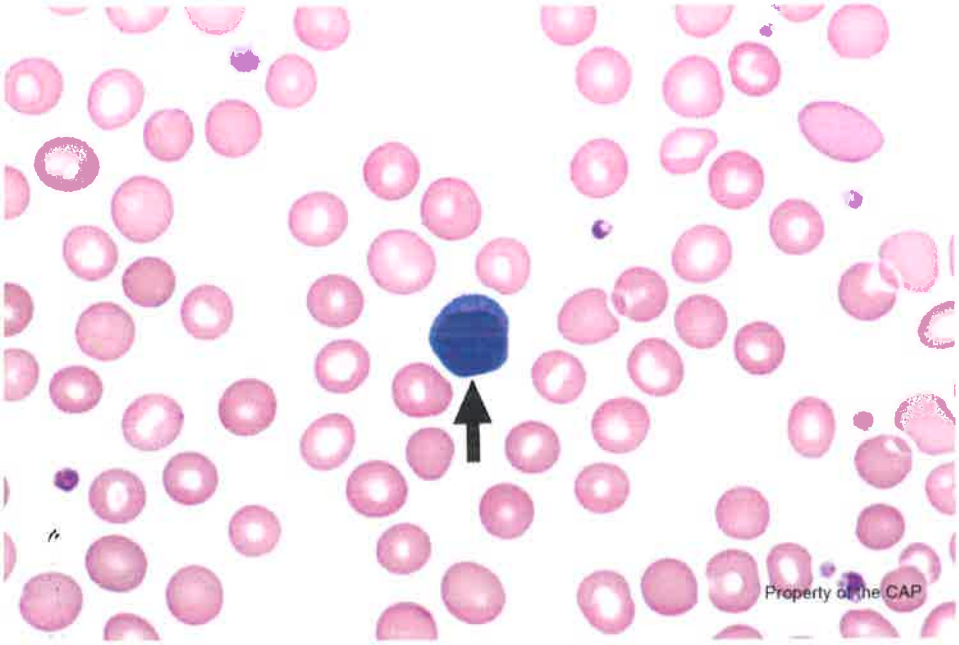


Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Platelet, normal	160	100.0	5573	99.7	Good

The arrowed objects are the normal platelets, as correctly identified by 100.0% of referees and 99.7% 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.

Blood Cell Identification – Graded

BCP-22



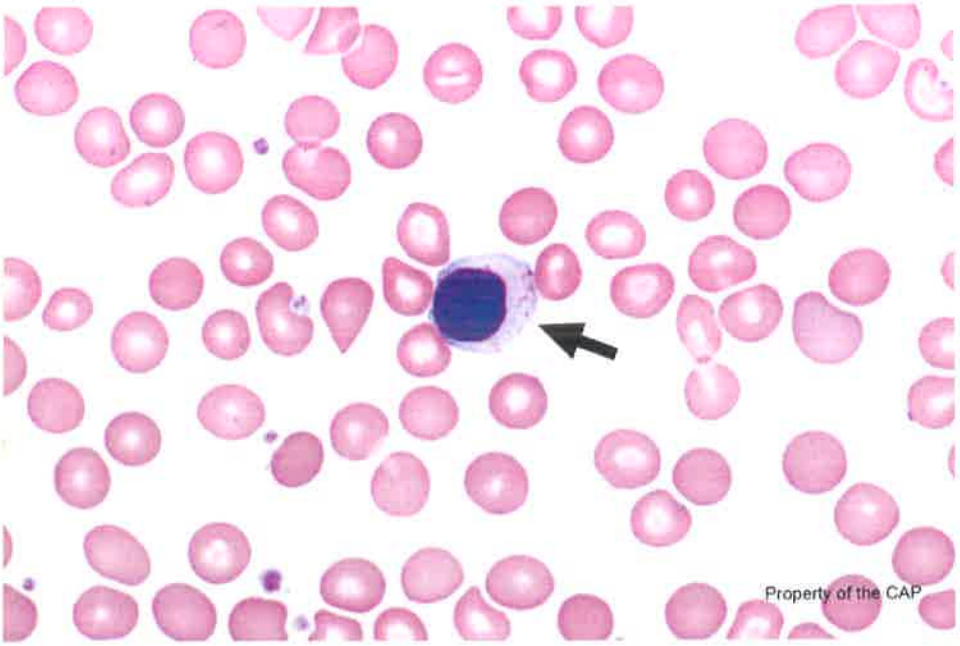
Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Lymphocyte	154	96.3	5339	95.5	Good
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	5	3.1	163	2.9	Unacceptable
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	1	0.6	21	0.4	Unacceptable

The arrowed cell is a normal lymphocyte, as correctly identified by 96.3% of referees and 95.5% of participants. 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. While most normal lymphocytes are fairly homogeneous, they do exhibit a range of normal morphology.

3.1% of referees and 2.9% of participants incorrectly identified the arrowed 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. The cell on the image lacks the key morphologic features of a reactive lymphocyte, such as large size, abundant pale-grey cytoplasm, finely to moderately dispersed chromatin with distinct nucleoli, or plasmacytoid features resembling plasma cells.

Blood Cell Identification – Graded

BCP-23



Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Lymphocyte, large granular	141	88.1	4679	83.7	Good
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	-	-	191	3.4	Acceptable
Lymphocyte	16	10.0	581	10.4	Unacceptable
Neutrophil, myelocyte	1	0.6	40	0.7	Unacceptable

The arrowed cell is a large granular lymphocyte, as correctly identified by 88.1% of referees and 83.7% 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.

10.0% of referees and 10.4% of the participants incorrectly identified the arrowed 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. The identification of "lymphocyte" is not sufficiently specific, and is therefore, considered unacceptable for proficiency testing purposes.

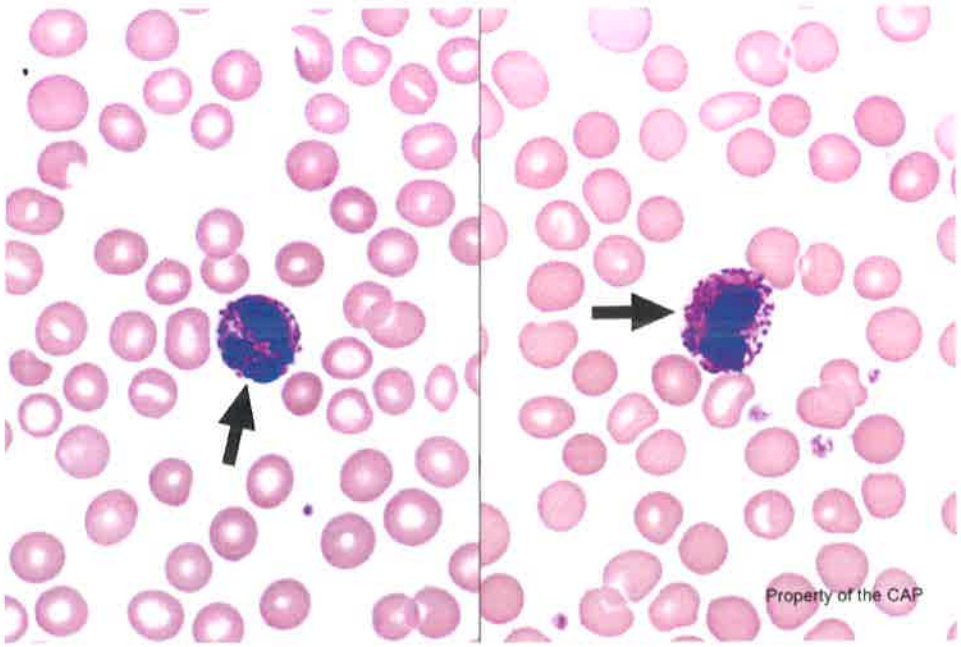
BCP-23, cont'd.

3.4% of the participants identified the arrowed 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. Large granular lymphocytes may resemble a Downey type II cells, the most common type of reactive lymphocytes. However, Downey type II cells have round to oval nuclei, moderately condensed chromatin (giving it a smeared appearance), abundant pale gray-blue cytoplasm and may contain very small granules that are 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.

LGLs may be increased in association with reactive lymphocytes. In some patients who are being treated with specific tyrosine kinase inhibitors (ie, dasatinib), the LGL population can expand to greater numbers as a reactive, self-limited response. Thus, the cell ID 'reactive lymphocyte' was considered acceptable for proficiency testing purposes, though the best response on these photomicrograph challenges is LGL.

Blood Cell Identification – Graded

BCP-24



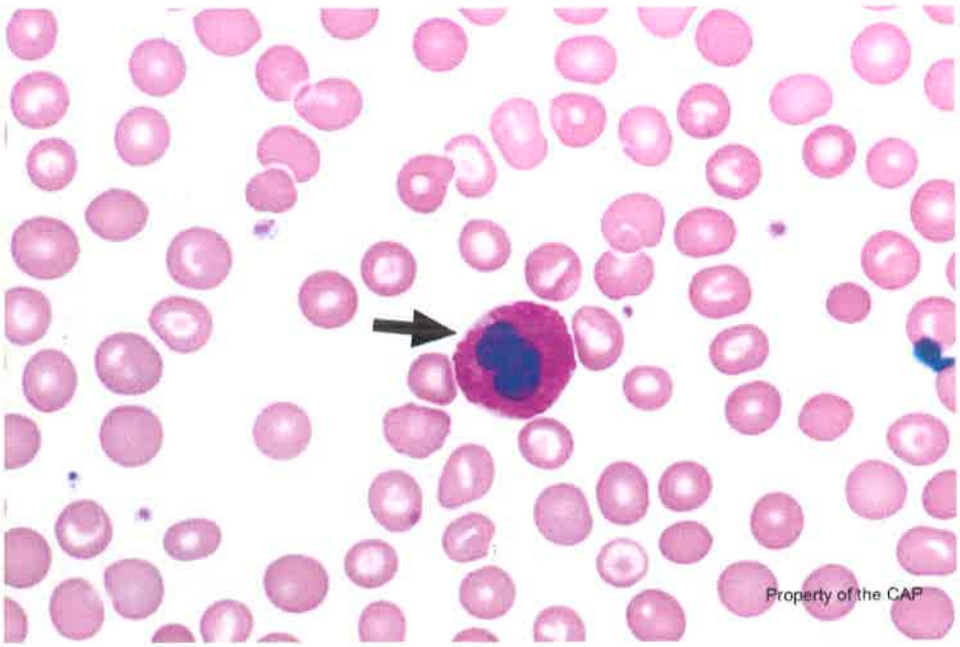
Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Basophil, any stage	155	96.9	5383	96.3	Good
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	4	2.5	189	3.4	Unacceptable
Basophilic stippling (coarse)	1	0.6	8	0.1	Unacceptable

The arrowed cells are basophils, as correctly identified by 96.9% of referees and 96.3% of participants. Basophils have a maturation sequence analogous to neutrophils. All basophils, from the basophilic myelocyte to the mature segmented basophil, are characterized by the presence of numerous coarse and densely stained granules of varying sizes and shapes. The granules are larger than the granules of neutrophils and most are roughly spherical. The granules are typically blue-black, but some may be purple-red when stained using Wright-Giemsa preparations. The granules are unevenly distributed and frequently overlay and obscure the nucleus. Basophils are comparable in size to neutrophils, ie, 10 to 15 µm in diameter, and the nuclear-to-cytoplasm (N:C) ratio ranges from 1:2 to 1:3. Basophilia may be seen in several contexts, including in association with myeloproliferative neoplasms, in hypersensitivity reactions, with hypothyroidism, iron deficiency, and renal disease. Basophil granules can be stained with toluidine blue (resulting in a purple color) to differentiate them from the granules of neutrophils.

2.5% of referees and 3.4% of participants incorrectly identified the arrowed cells as toxic neutrophils. Toxic changes in neutrophils include toxic granulation, toxic vacuolization, and Döhle bodies. Toxic granulation is defined by the presence of large, purple or dark blue cytoplasmic granules in neutrophils, bands, and metamyelocytes. Unlike the granules in basophils, toxic granules are more evenly distributed and usually do not overlay or obscure the nucleus. The arrowed cells do not contain vacuoles or Döhle bodies.

Blood Cell Identification – Graded

BCP-25



Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Eosinophil, any stage	160	100.0	5549	99.2	Good

The arrowed cell is an eosinophil, as correctly identified by 100.0% of referees and 99.2% 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 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 or pictures, however. Due to inherent problems with color rendition on photomicrographs, which is sometimes imperfect, eosinophil granules may appear lighter or darker than on a freshly stained blood film. Discoloration may give the granules a blue, brown, or pink tint. Nonetheless, the uniform, coarse nature of eosinophil granules is characteristic and differs from the smaller, finer granules of neutrophils.

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

Clinical Presentation:

This peripheral blood smear is from a 74-year-old woman with partially treated chronic myeloid leukemia. Laboratory data includes: WBC = 3.6 x 10E9/L; RBC = 4.08 x 10E12/L; HGB = 10.4 g/dL; HCT = 33.4%; MCV = 82 fL; MCHC = 31.2 g/dL; PLT = 177 x 10E9/L; and RDW = 19.8%.
Absolute neutrophil count 1.5 x 10E9/L. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Chronic myeloid leukemia

Chronic myeloid leukemia (CML) is the most common myeloproliferative neoplasm, accounting for approximately 15 - 20% of all adult leukemias. The annual incidence is 1 to 2 cases per 100,000 with a slight male predominance. CML typically occurs at ages 40 to 60 and is discovered incidentally in 20% to 50% of patients by abnormal complete blood count (CBC) results, such as leukocytosis, anemia and/or thrombocytosis, and/or hepatosplenomegaly on physical examination. In other patients, symptoms develop gradually and usually relate to splenomegaly (left upper quadrant discomfort or early satiety), problems from an increased white cell production (bone pain, mild fever, night sweats, weight loss), or anemia (dyspnea, fatigue, pallor).

CML is defined by the presence of the Philadelphia chromosome, a derivative chromosome 22 that harbors the *BCR-ABL1* fusion gene, which usually results from a reciprocal translocation t(9;22)(q34;q11). CML goes through three stages: chronic phase (CML-CP), accelerated phase (CML-AP), and blast phase (CML-BP). CML diagnosis, including the disease phase, is made based on CBC and morphologic findings in peripheral blood and bone marrow in combination with detection of the *BCR-ABL1* translocation by molecular genetic techniques (karyotype, fluorescence in situ hybridization (FISH), or reverse transcription polymerase chain reaction (RT-PCR). The presence of *BCR-ABL1* translocation is required for the CML diagnosis.

Approximately 85% of patients present in CML-CP, and the blood studies typically show leukocytosis that usually exceeds 25 x 10E9/L (median white count of about 170 x 10E9/L), primarily comprising of neutrophils in various stages of maturation, especially myelocytes and mature neutrophils; blasts are rare and account for less than 10%. Basophils are universally increased, and eosinophilia is common. The platelet count is normal or elevated, and in some cases may exceed 1,000 x 10E9/L. Mild anemia is typically present, with 45% to 60% of patients having hemoglobin levels of less than 12.0 g/dL. Rarely patients present in CML-AP or CML-BP; in most cases these phases are encountered as disease progresses due to acquisition of additional genetic abnormalities. Morphologic findings of CML-AP include peripheral blood basophilia of ≥ 20% and 10 - 19% of blasts in peripheral blood or bone marrow. Once the blast count reaches 20%, either in peripheral blood or bone marrow, a diagnosis of CML-BP is made.

CML-CP must be differentiated from many other benign and neoplastic conditions associated with leukocytosis. Leukemoid reaction, a reactive condition, usually presents with white blood cell counts lower than 50 x 10E9/L, toxic granulation and Döhle bodies in neutrophils, and absence of basophilia. Other forms of chronic myeloid neoplasms must also be excluded based on the absence of their clinical, pathologic, and molecular genetic features. Since leukocytosis is a very common CBC finding and the detection of *BCR-ABL1* translocation has significant diagnostic, prognostic, and therapeutic implications, all patients presenting with unexplained increased white blood cell counts should undergo genetic testing to evaluate for *BCR-ABL1*.

The bone marrow morphologic findings mimic peripheral blood and show hypercellularity due to marked granulocytic hyperplasia and eosinophilia. In addition, the majority of cases show increased megakaryocytes with abnormal morphology, such as small hypolobate forms. Because of the excessive hematopoiesis, the number of

cells that eventually die increases, and macrophages containing the lipids from the dead cells may be visible in the bone marrow as sea-blue histiocytes or pseudo-Gaucher cells. Bone marrow findings suggestive of CML-AP are the presence of large clusters or sheets of abnormal small megakaryocytes associated with marked reticulin or collagen fibrosis, along with increased blasts. The blasts are usually myeloid but in about 20% to 30% of cases, they are lymphoid, usually B lymphoblasts.

Development of small molecule tyrosine kinase inhibitors (TKI) targeting the constitutively active *ABL1* tyrosine kinase, as a result of the formation of the *BCR-ABL1* fusion protein, has been one of the most successful therapeutic stories in modern medicine, improving the 10-year survival rate from 20% to 80-90%. The current guidelines recommend the three commercially available TKI (imatinib, dasatinib, and nilotinib) as first-line treatment for CML-CP, with majority of patients demonstrating an excellent response within the first year of treatment. The TKI therapy is usually continued indefinitely as long as it is tolerated, and the treatment milestones are met. Patients should continuously undergo molecular and/or cytogenetic monitoring. Rising levels of *BCR-ABL1* transcripts may be associated with disease progression or development of drug resistance. An alternative TKI or allogeneic stem cell transplant are used in patients who become intolerant to a first-line TKI, show excessive toxicity, treatment failure, or suboptimal response. The prognosis of CML in accelerated or blast phases are dismal, particularly for patients with prior TKI therapy, as many of them develop resistance mutations in *ABL1*. There is a significant relapse rate even after successful treatment with TKI, and these patients should be considered for transplantation.

Olga Pozdnyakova, MD, PhD
Hematology and Clinical Microscopy Committee

References:

1. Glassy EF, ed. *Color Atlas of Hematology: An Illustrated Field Guide Based on Proficiency Testing,* 2nd ed. Peripheral Blood. College of American Pathologists; 2018.
2. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H and Thiele J, eds. *WHO Classification of Tumours of Haematopietic and Lymphoid Tissues*. Revised 4th ed. International Agency for Research on Cancer; 2017.
3. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2018 update on diagnosis, therapy and monitoring. *Am J Hematol.* 2018;93(3):442-459. doi:10.1002/ajh.25011

Blood Cell Identification – Ungraded

Case History
This peripheral blood smear is from a 27-year-old man newly diagnosed with human immunodeficiency virus (HIV) infection. Laboratory data include: WBC = 5.3 x 10E9/L; RBC = 4.73 x 10E12/L; HGB = 14.6 g/dL; HCT = 42.7 %; MCV = 90 fL; MCHC = 34.1 g/dL; PLT = 174 x 10E9/L; and RDW = 12.7 %.
Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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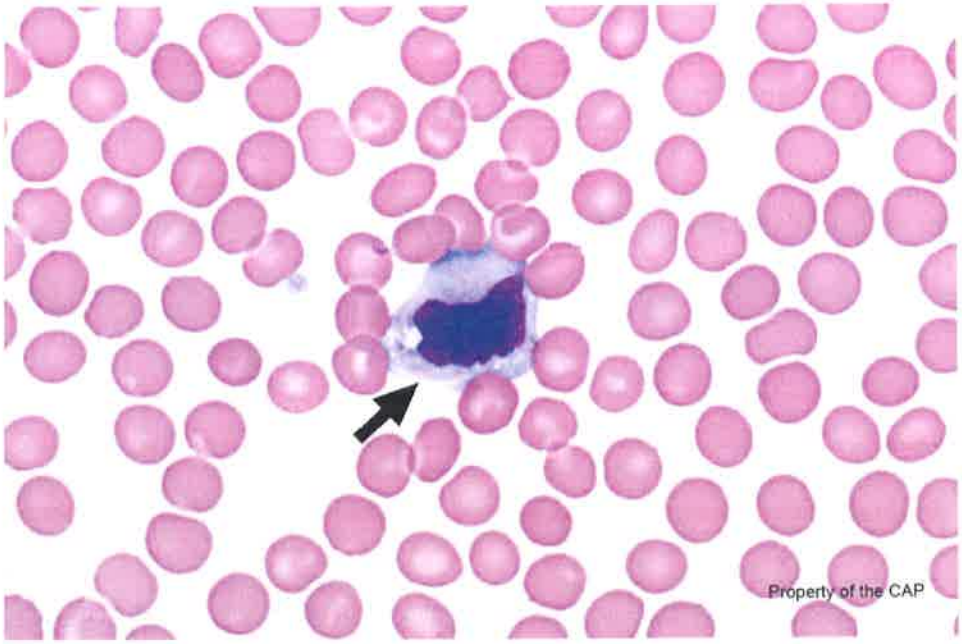
BCP-26



Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Monocyte	152	95.0	5255	95.0	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	7	4.4	192	3.5	Educational
Lymphocyte, large granular	1	0.6	7	0.1	Educational

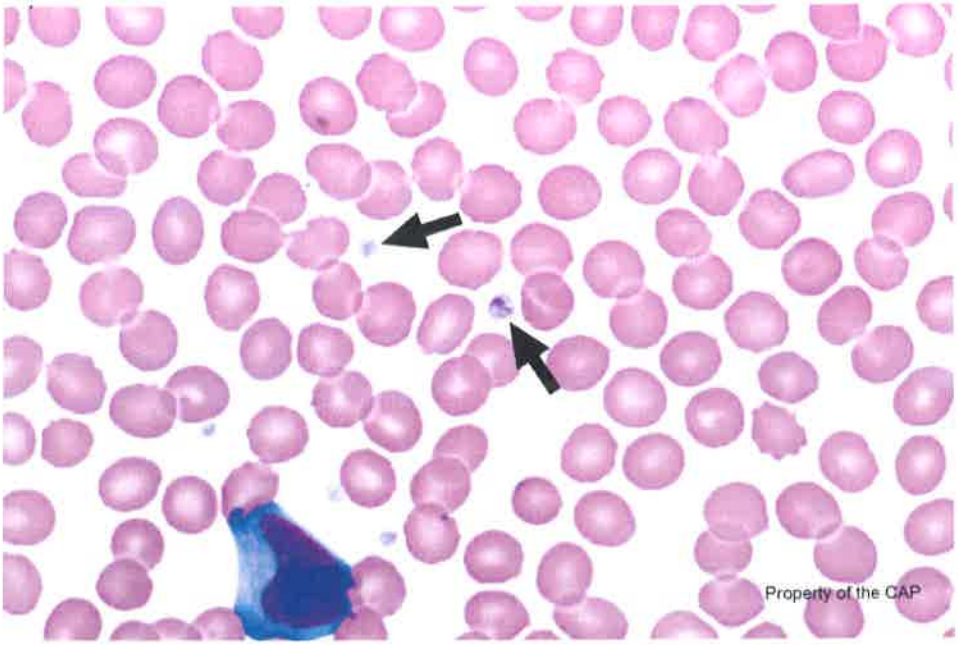
The arrowed cell is a monocyte, as correctly identified by 95.0% of referees and 95.0% of participants. Monocytes are large cells (12 to 20 µm in diameter) with abundant gray or gray-blue cytoplasm that may contain fine, evenly distributed azurophilic granules and/or vacuoles as seen in this example. The nucleus is usually indented or irregular, 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.

The arrowed cell was incorrectly identified as a "lymphocyte, reactive" by 4.4% of referees and 3.5% of participants. Reactive lymphocytes are characterized by their wide range of sizes and shapes and variation in

nuclear features. Their cytoplasm is typically basophilic, and often deeply basophilic, in contrast to the grayer cytoplasm of the arrowed cell. The cytoplasm of the arrowed cell is furled around adjacent erythrocytes and exhibits darker staining at the margin, as is frequently seen in reactive lymphocytes, but the large number of cytoplasmic granules would be unusual for a reactive lymphocyte, as would the observed degree of nuclear irregularity.

Blood Cell Identification – Ungraded

BCP-27



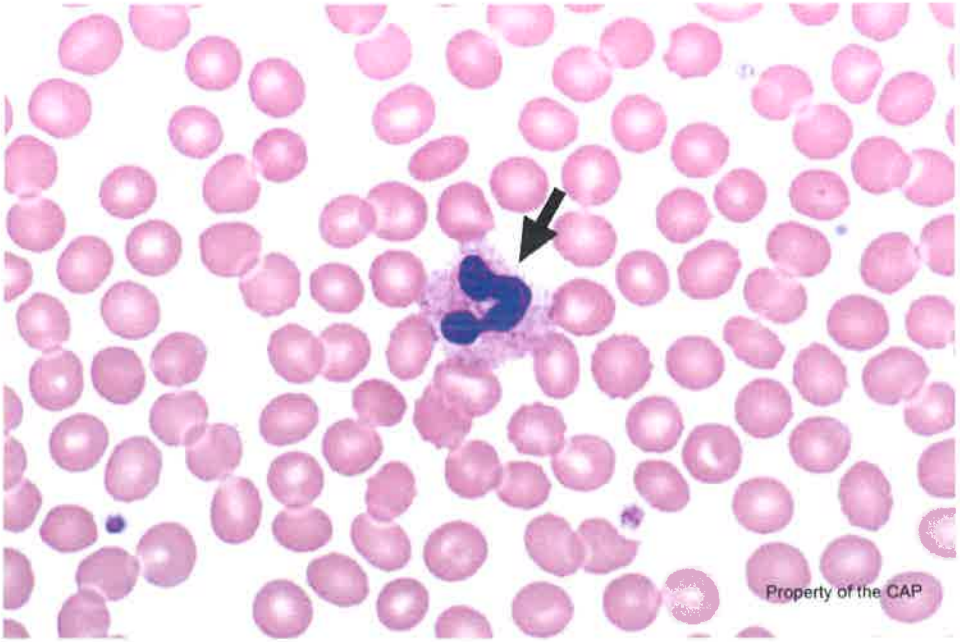
Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Platelet, normal	136	85.0	4347	79.7	Educational
Platelet, hypogranular	22	13.8	1049	19.2	Educational
Platelet, giant (macrothrombocyte)	1	0.6	38	0.7	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.6	1	< 0.1	Educational

The arrowed objects are platelets, as correctly identified by 85.0% of referees and 79.7% of participants. Platelets are small, blue-gray fragments of megakaryocytic cytoplasm typically measuring 1.5 to 3 µm in diameter, though a few smaller platelets and a few large platelets may also be seen in normal blood films. Fine, purple-red granules are distributed throughout the cytoplasm, though they are sometimes aggregated at the center. Platelets vary in shape, but most normal platelets, as seen here, are round or very slightly elliptical. Some have short cytoplasmic projections or ruffled margins.

The arrowed objects were incorrectly identified as "platelets, hypogranular" by 13.8 of referees and 19.2% of participants. Hypogranular platelets either lack granules entirely or have a substantially reduced number of granules in comparison to normal platelets. While the platelet marked by the upper arrow is less granular than the one marked by the lower arrow, it nonetheless features identifiable lighter and darker areas, with some of the darker areas appearing granular on close inspection. The cells of interest are considered normal platelets by the committee.

Blood Cell Identification – Ungraded

BCP-28



Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Neutrophil, segmented or band	151	94.4	5232	96.0	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	8	5.0	177	3.3	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	1	0.6	20	0.4	Educational

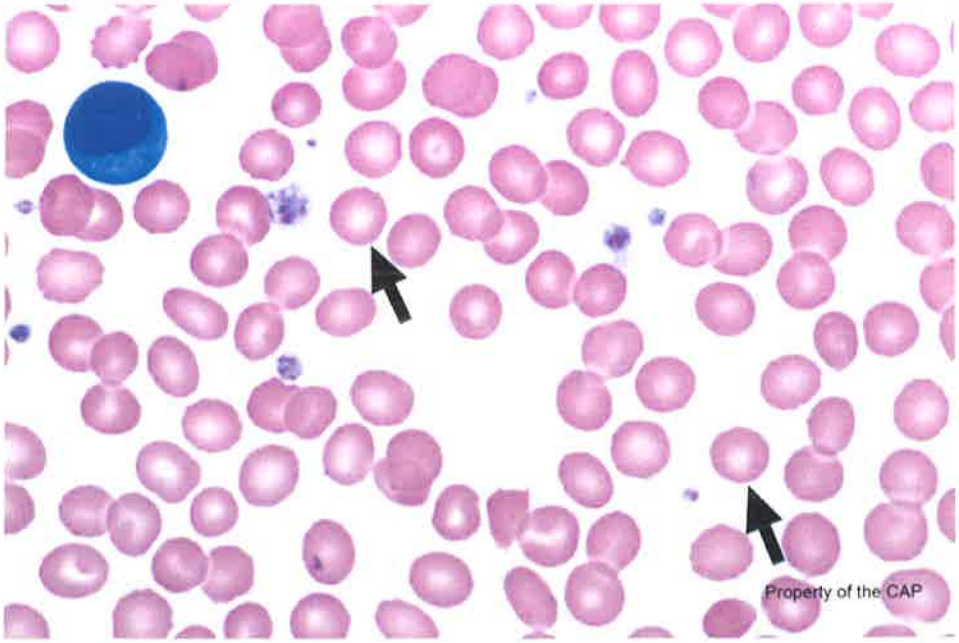
The arrowed cell is a neutrophil, segmented or band, as correctly identified by 94.4% of referees and 96.0% of participants. The segmented neutrophil is the predominant blood leukocyte. Segmented neutrophils and band neutrophils are similar in size (10 to 15 µm in diameter), shape (round to oval), and cytoplasmic appearance (pale pink cytoplasm with specific granules). Their N:C ratio is low (1:3), and their chromatin is highly condensed. The presence of thread-like filaments connecting nuclear segments or lobes is the basis for distinguishing the segmented neutrophil, which normally has three to five lobes, from the band neutrophil, although the CAP strongly advocates for grouping bands with segmented neutrophils in a differential count due to the high intra- and interobserver variability in distinguishing these forms. Thus, distinguishing segmented and band neutrophils is not required for proficiency testing purposes.

5.0% of referees and 3.3% of participants incorrectly identified the arrowed cell as a neutrophil, toxic. 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. 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. Vacuoles within the cytoplasm of cells with toxic granulation or Döhle bodies define toxic vacuolization. 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. There are a few small cytoplasmic vacuoles in the arrowed cell; while these are not accompanied by Döhle bodies, some of the cytoplasmic granules are relatively prominent.

Blood Cell Identification – Ungraded

BCP-29

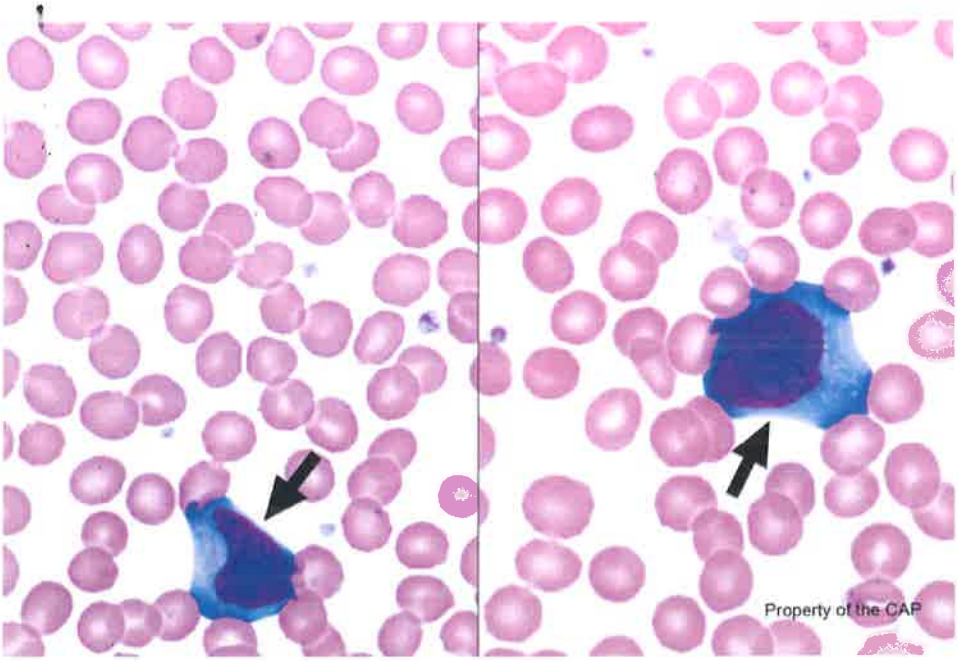


Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Erythrocyte, normal	160	100.0	5391	98.9	Educational

The arrowed cells are erythrocytes, as correctly identified by 100.0% of referees and 98.9% of participants. A normal erythrocyte is a biconcave disc-shaped cell of fairly uniform diameter (6.7 to 7.8 µm) that lacks a nucleus. The erythrocyte contains hemoglobin and stains uniformly pink-red, except in the round zone of central pallor that results from the biconcavity of the cell and occupies approximately one third of the cell's diameter.

Blood Cell Identification – Ungraded

BCP-30



Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	140	87.5	4964	91.0	Educational
Blast cell	15	9.4	267	4.9	Educational
Malignant lymphoid cell (other than blast)	2	1.3	61	1.1	Educational

The arrowed cells are reactive lymphocytes, as correctly identified by 87.5% of referees and 91.0% of participants. Reactive lymphocytes are characterized by a wide range of sizes, shapes, and chromatin patterns. These lymphocytes are reacting to an immune stimulus and are frequently increased in viral illnesses. The arrowed cells, like many reactive lymphocytes, are large cells (15 to 25 µm in diameter) with cytoplasm that partially surrounds adjacent erythrocytes and is darker at the edges. Less common, however, are the immunoblastic morphologic features of these examples, which include moderately dispersed chromatin, variably prominent nucleoli, and deeply basophilic cytoplasm. While the presence of discernible nucleoli in these cells may raise concern for blasts, the presence of a plasmacytoid lymphocyte in the upper left corner of BCP-29 supports an inference that the cells in BCP-30 are part of a reactive lymphoid population exhibiting a spectrum of morphologic appearances.

The arrowed cells were incorrectly identified as "blast cell" by 9.4 % of referees and 4.9% of participants. In contrast to the arrowed cells, blast cells have high nuclear-to- cytoplasmic (N:C) ratios, and abnormal lymphoid cells (eg, lymphoma cells) also would be expected to have higher N:C ratios than those observed here. Additionally, blast cells have fine, lacy or reticular chromatin, in contrast to the more moderately dispersed chromatin in the arrowed cells. The darker-staining, furled cytoplasmic margins of the arrowed cells are a typical feature of reactive lymphocytes and not characteristic of blast cells or of other abnormal cells.

BCP-28, cont'd.

The arrowed cells were incorrectly identified as "malignant lymphoid cell (other than blast)" by 1.3% of referees and 1.1% of participants. In blood smears, it may be difficult to distinguish reactive lymphocytes from lymphoma cells. The most important distinction between these cells is the difference in their nuclear-to- cytoplasmic (N:C) ratios. The N:C ratio is relatively low in the arrowed cells, as would be typical of reactive lymphocytes, while it is often higher in lymphoma cells. In addition, reactive lymphocytes are characterized by their wide range of morphologic appearances within the same blood smear; of note, there is a plasmacytoid lymphocyte in the upper left corner of image BCP-29 that suggests the degree of variation observed in this case. In contrast, while lymphoma cells can exhibit a wide range of morphologic appearances from case to case depending on the lymphoma subtype, any individual case tends to feature a more monotonous population of abnormal cells. The arrowed cells are considered reactive lymphocytes by the committee.

Clinical Presentation:

This peripheral blood smear is from a 27-year-old man with new diagnosis of human immunodeficiency virus (HIV) infection. Laboratory data includes: WBC = 5.3 x 10E9/L; RBC = 4.73 x 10E12/L; HGB = 14.6 g/dL; HCT = 42.7%; MCV = 90 fL; MCHC = 34.1 g/dL; PLT = 174 x 10E9/L; and RDW = 12.7%.
Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Acute / newly diagnosed HIV infection

This case is an example of peripheral blood findings in the setting of newly diagnosed human immunodeficiency virus (HIV) infection. While early HIV infection is asymptomatic in some people, many newly infected individuals experience constitutional symptoms or even a mononucleosis-like syndrome two to four weeks after HIV exposure. The most common symptoms, such as fever, fatigue, sore throat, and enlarged lymph nodes, are nonspecific and may be encountered in other conditions such as: infectious mononucleosis resulting from Epstein-Barr virus (EBV); cytomegalovirus (CMV) or other viral infections; toxoplasmosis; and autoimmune conditions such as systemic lupus erythematosus. The concurrent finding of a maculopapular rash and/or mucocutaneous ulcers with the above symptoms may heighten suspicion of acute HIV infection in a patient with a history compatible with exposure.

While the diagnosis of HIV infection is established using tests such as high sensitivity screening antigen/antibody immunoassays and viral load tests in accordance with diagnostic algorithms, hematologic abnormalities may also be encountered if a sample is received for a complete blood count and differential during acute HIV infection. Thrombocytopenia is commonly encountered, as is lymphopenia, though the characteristic decline in CD4+ T-cells may be counterbalanced initially by an increase in CD8+ T-cells. Even when CD4+ T-cell counts rise after the peak in viral load has passed, the CD4:CD8 ratio generally remains inverted at less than 1. Published reports vary with respect to the likelihood of seeing reactive lymphocytes on review of a blood film during acute HIV, but there is general agreement that such cells are less commonly seen and less numerous in acute HIV than in EBV-associated infectious mononucleosis.

Reactive lymphocytes, which are benign lymphocytes that have been activated by an immune stimulus, are characterized by wide variation in size, shape, and morphologic features even in a single blood film. They are generally large (10 to 25 µm in diameter) with abundant cytoplasm (N:C ratio as low as 1:2). The presence of sizable numbers of reactive lymphocytes is most closely associated with EBV infection (infectious mononucleosis), though they may be seen in a variety of conditions, including those listed above that share non-specific symptoms with acute HIV infection. While the term *atypical lymphocyte* has been used in the past to describe these cells, given the inference drawn when pathologists characterize a lesion as *atypical* (i.e., that malignancy cannot be ruled out), the preferred term is *reactive lymphocyte*.

The variation in morphologic characteristics of reactive lymphocytes has prompted efforts to classify them, with the three-category approach described in the seminal 1923 paper by Downey and McKinlay remaining the best known of these. A comprehensive recitation of the features distinguishing these categories is beyond the scope of this discussion, but the cells presented in cell ID BCP-30 in this case are examples of Downey type III cells. In contrast to the more common Downey type II reactive lymphocytes, the Downey type III cells in this case feature deeply basophilic cytoplasm, moderately dispersed chromatin, and variably prominent nucleoli, which are termed *immunoblastic* features. Cells with these features are the least common among the three Downey types, and their presence is not specific for acute HIV infection or for causes of increased numbers of reactive lymphocytes.

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