Blood Cell Identification – Graded

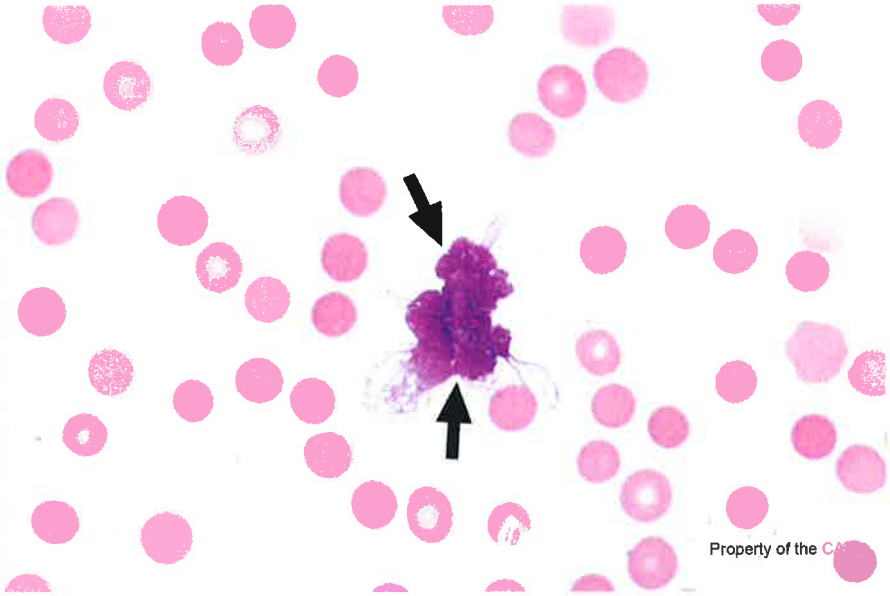
Case History

This peripheral blood smear is from a 21-year-old man who was recently diagnosed with B-lymphoblastic leukemia (WBC at diagnosis 233.0 x 10E9/L) and is on day 5 of initial chemotherapy. Laboratory data include: WBC = 14.2 x 10E9/L; RBC = 2.92 x 10E12/L; HGB = 8.5 g/dL; HCT = 23.9%; MCV = 82 fL; MCHC = 35.5 g/dL; PLT = 12 x 10E9/L; and RDW = 16%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

To access the online Hematology Glossary, please click the hyperlink below:
https://documents.cap.org/documents/2021-hematology-and-clinical-microscopy-glossary.pdf

BCP-21

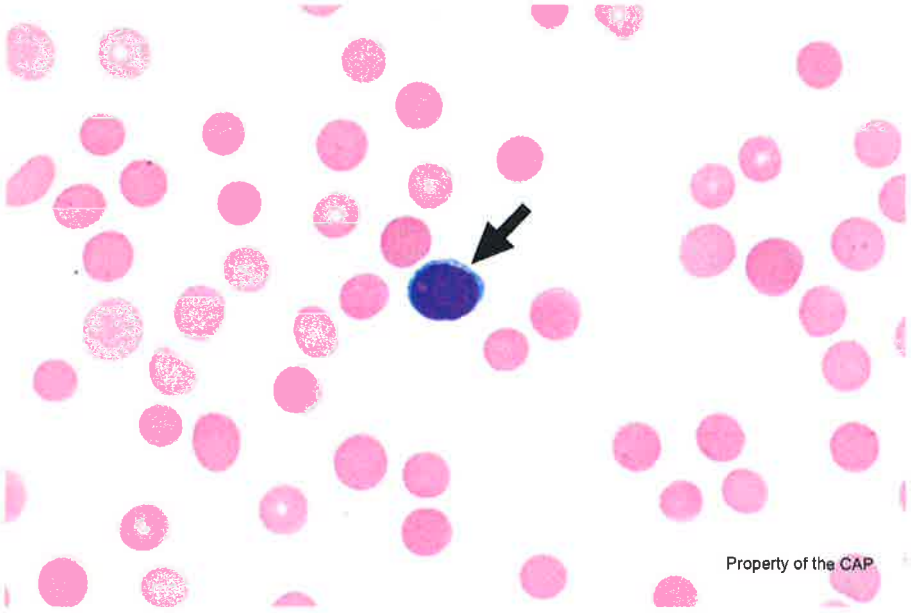


Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Basket cell/smudge cell	174	98.9	5482	98.7	Good
Neutrophil necrobiosis (degenerated neutrophil)	1	0.6	13	0.2	Unaccpetable
Stain precipitate	1	0.6	40	0.7	Unacceptable

The arrowed cell is a basket cell or smudge cell, as correctly identified by 98.9% of referees and 98.7% of participants. Basket or smudge cells are most commonly associated with cells that are fragile and easily damaged in the process of making a peripheral blood smear. The nucleus may either be a non-descript chromatin mass or have chromatin strands spreading out from a condensed nuclear remnant, giving the appearance of a basket. Cytoplasm is either absent or indistinct. Smudge cells are usually lymphocytes, but there is no recognizable cytoplasm to give a clue to the origin of the cell. They are seen most commonly in disorders characterized by lymphocyte fragility, such as infectious mononucleosis and chronic lymphocytic leukemia. Basket cells should not be confused with necrobiotic neutrophils, which have enough cytoplasm to allow the cell to be classified.

Blood Cell Identification – Graded

BCP-22

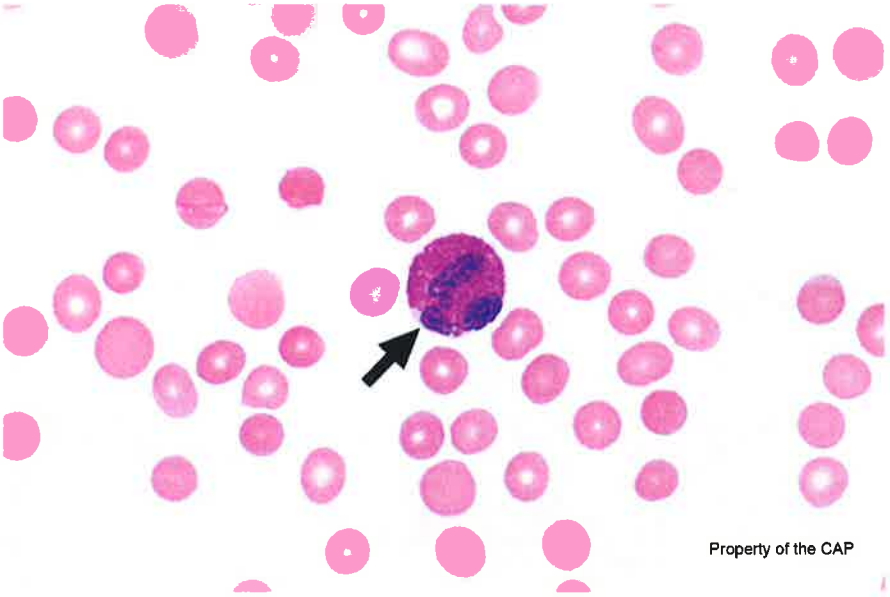


Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Lymphocyte	176	100.0	5508	99.2	Good

The arrowed cell is a normal lymphocyte, as correctly identified by 100.0% of referees and 99.2% 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.

Blood Cell Identification – Graded

BCP-23

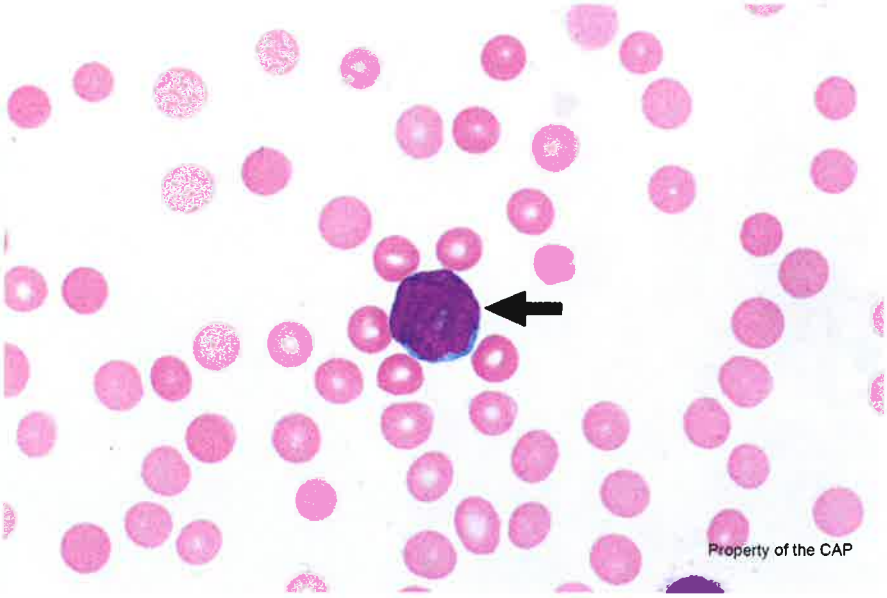


Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Eosinophil, any stage	176	100.0	5540	99.8	Good

The arrowed cell is a mature 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 N:C ratio ranges from 1:3 for mature forms to 2:1 for immature forms. The eosinophil cytoplasm is 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. 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 (as seen in the photomicrograph). 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.

Blood Cell Identification – Graded

BCP-24



Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Blast cell	133	75.6	4242	76.3	Non-Consensus
Malignant lymphoid cell (other than blast)	15	8.5	544	9.8	Non-Consensus
Immature or abnormal cell, would refer for identification	14	8.0	267	4.8	Non-Consensus
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	12	6.8	367	6.6	Non-Consensus
Lymphocyte	1	0.6	79	1.4	Non-Consensus
Neutrophil, metamyelocyte	1	0.6	1	0.0	Non-Consensus

The arrowed cell is a blast, as correctly identified by 75.6% of referees and 76.3% of participants. Blasts are divided into myeloid (myeloblast) and lymphoid (lymphoblast) lineages. Myeloblasts are usually fairly large, 15 to 20 µm in diameter, with a high N:C ratio, usually 7:1 to 5:1, and typically basophilic cytoplasm. Myeloblasts may occasionally be smaller, similar to the size of a mature myeloid cell. The cell and nucleus are usually round, although irregularly shaped or folded nuclei may be present. The myeloblast nucleus has a characteristically finely reticulated chromatin pattern with distinct nucleoli present. Leukemic myeloblasts may also exhibit a few delicate granules and/or Auer rods, which are absent in the cell on the image.

Lymphoblasts are round to oval cells that range in size from 10 to 20 µm. The N:C ratio varies from 7:1 to 4:1. Morphologically, lymphoblasts are variable in appearance, at times within a single case. At one end of the spectrum are small lymphoblasts (previously called L1 subtype) with dense but not clumped chromatin, inconspicuous or absent nucleoli, and extremely scanty cytoplasm. At the other end are large lymphoblasts (previously called L2 subtype) with finely dispersed chromatin, variable numbers of distinct nucleoli, and moderate

amounts of cytoplasm, closely resembling myeloblasts. The nuclear contours of lymphoblasts range from round to convoluted. The cytoplasm is typically slightly to moderately basophilic and is usually agranular. Auer rods are absent.

Distinguishing one type of abnormal blast cell from another, especially in the absence of Auer rods, is not always possible using Wright-Giemsa stains alone. Additional testing such as cytochemical staining (eg, using myeloperoxidase or Sudan black for myeloblasts), or immunophenotyping by flow cytometry may be required to further define the lineage of a given blast population. For purposes of proficiency testing, one should identify individual cells exhibiting this immature type of morphology as blast cells.

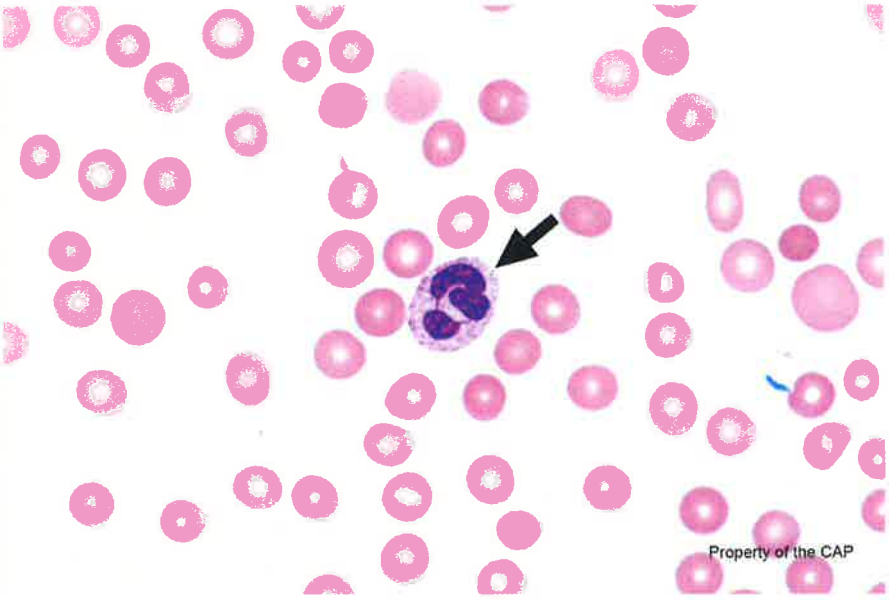
8.5% of referees and 9.8% of participants incorrectly identified the arrowed cell as a malignant lymphoid cell. While malignant lymphoid cells can be difficult to distinguish from blasts by morphology alone, in the context of a recently diagnosed B lymphoblastic leukemia, the abnormal cell shown is best classified as a blast and not a malignant lymphoid cell.

6.8% of referees and 6.6% of participants incorrectly identified the arrowed cell as a lymphocyte, reactive (includes plasmacytoid and immunoblastic forms). Reactive lymphocytes typically have a lower nucleus-to-cytoplasm ratio than blasts, due to the presence of more abundant cytoplasm. The high nucleus-to-cytoplasm ratio and irregular nuclear contours of the arrowed cell are indicative of a blast and not a lymphocyte, reactive.

0.6% of referees and 1.4% of participants incorrectly identified the arrowed cell as a lymphocyte. Lymphocytes are typically smaller than blasts, ranging from 7 - 15 µm with round to oval nuclei, condensed chromatin, and lack nucleoli. The cell shown in this example is larger with more dispersed chromatin, and prominent nucleoli, with irregular nuclear contours, consistent with a blast.

Blood Cell Identification – Graded

BCP-25



Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Neutrophil, segmented or band	173	98.3	5449	98.1	Good
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	2	1.1	79	1.4	Unacceptable
Neutrophil with hypersegmented nucleus	1	0.6	4	0.1	Unacceptable

The arrowed cell is a segmented neutrophil, as correctly identified by 98.3% of referees and 98.1% of participants. Segmented neutrophils and their immediate precursors, bands, constitute 12% to 25% of the nucleated cells in the bone marrow. The band is round to oval and 10 to 18 µm in diameter. The nuclear-to-cytoplasmic 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 neutrophilic cells, with specific granules predominating in the pale cytoplasm. The segmented neutrophil, the most mature cell of the myeloid series and the predominant white blood cell in blood, mimics its immediate precursors in size (10 to 15 µm), shape (round to oval), and cytoplasmic appearance (pale pink cytoplasm with specific granules). The N:C ratio is 1:3, the most mature of any cell in the neutrophilic series, and the nuclear chromatin is highly condensed. The nucleus is segmented or lobated (three to five lobes normally). The lobes are connected by a thin filament that contains no internal chromatin, giving it the appearance of a solid, dark, thread-like line. The presence of these thread-like filaments is the basis for distinguishing the segmented neutrophil from its precursor, the band neutrophil. However, in repeated proficiency testing studies, it has not been possible to achieve consistent differentiation between bands and segmented neutrophils. Therefore, for the purposes of proficiency testing, it is not required that they be differentiated (for a detailed guideline for the differentiation of segmented and band neutrophils, see Glassy, 2018; for other reading related to the clinical utility of band counts, see Cornbleet, 2002).

BCP-25, cont'd

1.1% of referees and 1.4% of participants incorrectly identified the arrowed cell as neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization). Toxic changes to neutrophils include toxic granulation, toxic vacuolization, and Döhle bodies. Toxic granulation is 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. Döhle bodies appear as single or multiple blue or gray-blue inclusions of variable size (0.1 to 5.0 μm) and shape (round, elongated, or crescent shaped) in the cytoplasm of neutrophils, bands, or metamyelocytes. They are often found in the periphery of the cytoplasm, near the cell membrane. The arrowed cell in the image demonstrates pale pink cytoplasm with specific granules, consistent with a neutrophil. Importantly, this cell lacks the large purple or dark blue cytoplasmic granules and blue to blue-gray inclusions to suggest toxic changes.

Clinical Presentation:

This peripheral blood smear is from a 21-year-old man who was recently diagnosed with B-lymphoblastic leukemia (WBC at diagnosis 233 x 10E9/L) and is on day 5 of initial chemotherapy. Laboratory data include: WBC = 14.2 x 10E9/L; RBC = 2.92 x 10E12/L; HGB = 8.5 g/dL; HCT = 23.9%; MCV = 82 fL; MCHC = 35.5 g/dL; PLT = 12 x 10E9/L; and RDW = 16%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case discussion: B-lymphoblastic leukemia/lymphoma currently being treated

B-lymphoblastic leukemia/lymphoma (B-ALL/LBL) is a neoplasm of precursor B-cells, typically composed of small to medium-sized blasts with scant cytoplasm, moderately condensed to dispersed chromatin, and inconspicuous nucleoli. In B-ALL/LBL, the B-cell lineage is established by flow cytometric analysis or immunohistochemistry. When the disease involves only the peripheral blood and bone marrow, it is considered B-ALL, while primarily nodal or extranodal involvement is diagnosed as B-LBL. In patients with a mass lesion and bone marrow involvement, the distinction between leukemia and lymphoma is arbitrary, though >25% blasts in the marrow is typically used to define leukemia in many treatment protocols. Although a minimum blast count is not as well defined as in acute myeloid leukemia, one should avoid a lymphoblastic leukemia diagnosis with less than 20% marrow lymphoblasts.

Patients with B-ALL often present with evidence of bone marrow dysfunction (anemia, thrombocytopenia, and/or neutropenia). The leukocyte count is extremely variable and can be decreased, normal or increased. Additional findings, such as bone pain, lymphadenopathy, splenomegaly, and/or hepatomegaly are common. Patients with B-LBL usually present with a mass lesion and localized symptoms. CNS and testicular involvement are relatively common, particularly in the post-therapy setting. B-ALL is diagnosed predominantly in childhood, with 75% of cases occurring in children under 6 years of age. However, the disease also occurs in older patients.

Immunophenotypic analysis, either by flow cytometry or immunohistochemistry, is required in all patients with suspicion for acute lymphoblastic leukemia to confirm blast lineage. By definition, B-lymphoblasts are committed to the B-cell lineage and are almost always positive for the B-cell markers CD19, CD79a, and cytoplasmic CD22. Except for CD19, none of the individual markers are B-cell lineage specific, and a combination of multiple B-cell associated markers is usually required. Most cases also express surface CD22, CD24, and PAX5. In addition, the presence of TdT, CD34, CD10 and the absence of surface immunoglobulin light chains confirm immature/precursor stage of B cells. CD45 may be weakly expressed or even absent. Occasionally, myeloid markers such as CD13 and CD33 may be expressed. Importantly, the presence of myeloid antigens does not exclude the diagnosis of B-ALL/LBL, however, in cases that express myeloperoxidase (MPO), acute myeloid leukemia (AML) and B/myeloid mixed-phenotype acute leukemia must be excluded. Although not routinely performed, periodic acid-Schiff cytochemical stain reveals "block-like", "chunky" cytoplasmic granules in lymphoblasts on peripheral blood or bone marrow aspirate smears, while MPO is negative.

Cytogenetic and molecular abnormalities are identified in most cases of B-ALL/LBL and are important for determining the patient's prognosis and treatment. In addition, some of the genetic abnormalities have a distinct immunophenotype, such as the presence of CD13 and/or CD33 in B-ALL with *BCR-ABL1* translocation or the characteristic absence of CD10 expression and presence of CD15 expression in B-ALL with *KMT2A*

rearrangement. Many cases of B-ALL/LBL can be categorized based on these abnormalities, under B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities. Below are the specific diagnostic entities:

- B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2); *BCR-ABL1*
- B-lymphoblastic leukemia/lymphoma with t(v;11q23.3); *KMT2A*-rearranged
- B-lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
- B-lymphoblastic leukaemia/lymphoma with t(5;14)(q31.1;q32.1); *IGH-IL3*
- B-lymphoblastic leukemia/lymphoma with hyperdiploidy
- B-lymphoblastic leukemia/lymphoma with hypodiploidy
- B-lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3); *TCF3-PBX1*
- B-lymphoblastic leukemia/lymphoma, *BCR-ABL1*-like
- B-lymphoblastic leukemia/lymphoma with *iAMP21*

The features associated with poor prognosis include age (≤1 year or ≥10 years), WBC count ≥50K/µL, central nervous system or testicular involvement, male gender, the presence of *KMT2A* rearrangement, *iAMP21* amplification, *BCR-ABL1* translocation, hypodiploidy, and slow response to therapy, including the presence of measurable residual disease after induction therapy.

In pediatric patients, high-intensity chemotherapy has been very effective and shown high cure rates, while adults have a poorer prognosis. Recently, there has been increased usage of targeted therapies, especially in patients with relapsed or refractory disease. The use of tyrosine kinase inhibitors in *BCR-ABL1*-positive B-ALL/LBL has improved outcomes for these patients. Use of immunotherapies targeting CD19, CD20, or CD22-positive cells have also become more prevalent.

Jonathan Galeotti, MD
Olga Pozdnyakova, MD, PhD
Hematology and Clinical Microscopy Committee

References:

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Revised 4th Edition, Volume 2. IARC; 2017.
2. Glassy EF. *Color Atlas of Hematology: An Illustrated Field Guide Based on Proficiency Testing*. College of American Pathologists; 2018.
3. Arber DA, Borowitz MJ, Cessna M, et al. Initial Diagnostic Workup of Acute Leukemia: Guideline from the College of American Pathologists and the American Society of Hematology. *Archives of Pathology and Laboratory Medicine*. 2017;141:1342-1393.
4. Jaffe ES, Harris NL, Arber DA, Campo E, and Quintanilla-Martinez L. *Hematopathology*. 2nd Edition. Elsevier - Health Sciences Division; 2016.

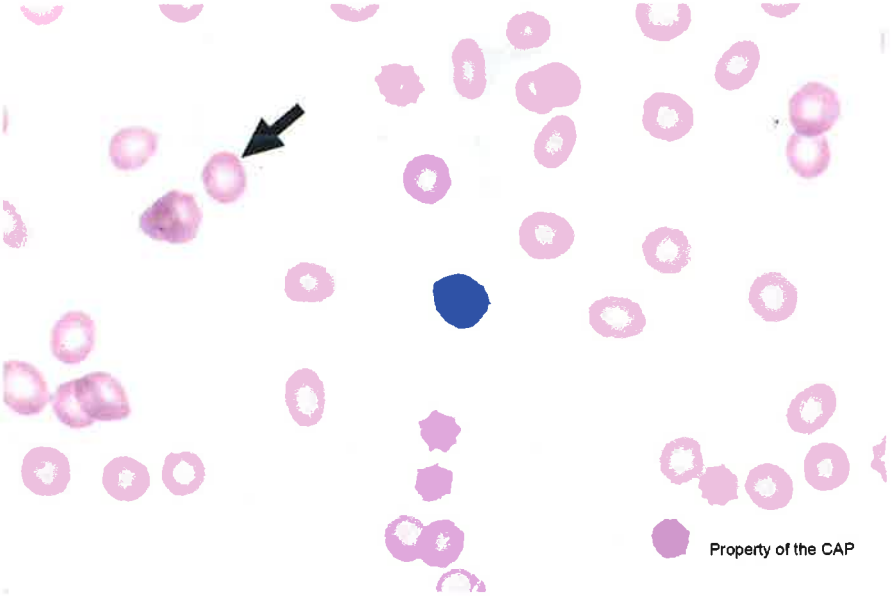
Blood Cell Identification – Ungraded

Case History
This peripheral blood smear is from a 46-year-old man with HIV/AIDS, who presents with severe abdominal pain, pancytopenia, and hepatosplenomegaly. Laboratory data include: WBC = 3.0 x 10E9/L; RBC = 0.90 x 10E12/L; HGB = 2.6 g/dL; HCT = 9.0%; MCV = 87 fL; MCHC = 29.2 g/dL; PLT = 20 x 10E9/L; and RDW = 19%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

To access the online Hematology Glossary, please click the hyperlink below:
https://documents.cap.org/documents/2021-hematology-and-clinical-microscopy-glossary.pdf

BCP-26



Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Erythrocyte, normal	168	95.5	5098	92.8	Educational
Microcyte (with increased central pallor)	8	4.5	328	6.0	Educational

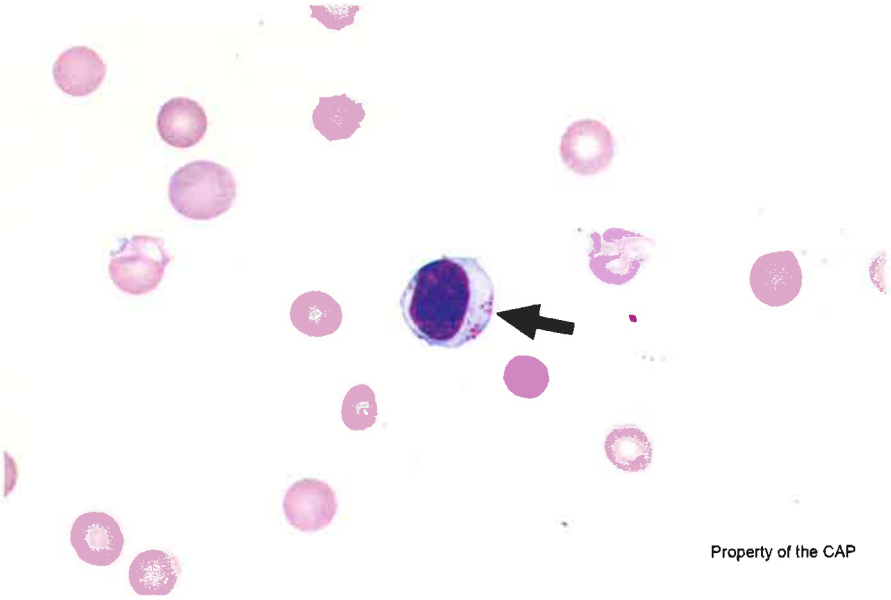
The arrowed cell is a normal erythrocyte, as correctly identified by 95.5% of referees and 92.8% of participants. An erythrocyte is a mature, non-nucleated biconcave disc-shaped cell of fairly uniform diameter (6.7 to 7.8 µm) with a uniform round area of central pallor. It contains hemoglobin and stains uniformly pink red. The zone of central pallor is due to the biconcavity of the cell and occupies approximately one third (2 to 3 µm) of the cell diameter. Normal erythrocytes circulate in the peripheral blood for approximately 120 days before they undergo catabolism or destruction in the spleen.

BCP-26, cont'd

The arrowed cell was incorrectly identified as a microcyte with increased central pallor by 4.5% of referees and 6.0% of participants. Microcytes are smaller than normal red blood cells, measuring less than 6 µm in diameter and less than 80 fL in volume. On the blood film, they generally appear smaller than the nucleus of a small lymphocyte (a lymphocyte is included in the image for comparison). On a peripheral blood film, microcytes retain central pallor, appearing either normochromic or hypochromic. RBCs are considered hypochromic when central pallor exceeds 50% of cell diameter.

Blood Cell Identification – Ungraded

BCP-27



Property of the CAP

Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Lymphocyte, large granular	162	92.0	4888	90.2	Educational
Lymphocyte	5	2.8	218	4.0	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	3	1.7	168	3.1	Educational
Leukocyte containing Alder (Alder-Reilly) anomaly inclusion(s)	3	1.7	31	0.6	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	1	0.6	4	0.1	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	1	0.6	1	0.0	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	1	0.6	14	0.3	Educational

The arrowed cell is a large granular lymphocyte, as correctly identified by 92.0% of referees and 90.2% 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.

BCP-27, cont'd

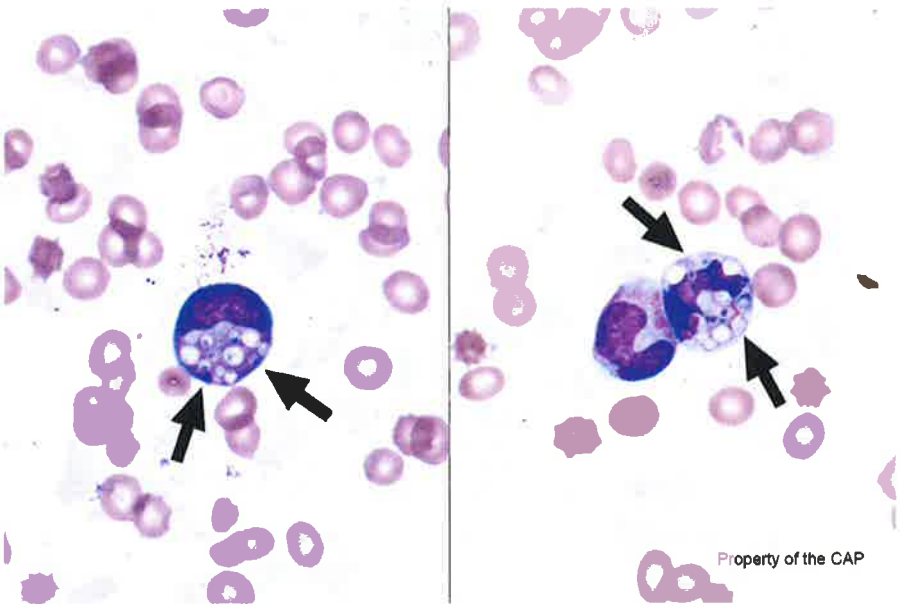
The arrowed cell was incorrectly identified as a lymphocyte by 2.8% of referees and 4.0% of participants. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 µm with an N:C ratio ranging from 5:1 to 2:1. Most lymphocytes have round to oval nuclei that may be slightly indented or 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.

The arrowed cell was incorrectly identified as a lymphocyte, reactive (includes plasmacytoid and immunoblastic forms) by 1.7% of referees and 3.1% of participants. 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, unlike the arrowed cell that contains coarse azurophilic granules.

The arrowed cell was incorrectly identified as a leukocyte containing Alder (Alder-Reilly) anomaly inclusion(s) by 1.7% of referees and 0.6% of participants. Alder-Reilly anomaly inclusions are large, purple, or purplish black, coarse, azurophilic granules resembling the primary granules of promyelocytes. They are seen in the cytoplasm of virtually all mature leukocytes and, occasionally, in their precursors. At times, clear zones or halos surround the granules. The prominent granulation in lymphocytes and monocytes distinguishes these inclusions from toxic granulation, which only occurs in neutrophils.

Blood Cell Identification – Ungraded

BCP-28



Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Leukocyte with intracellular fungi	109	61.9	3009	55.5	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	22	12.5	815	15.0	Educational
Leukocyte with intracellular bacteria	12	6.8	666	12.3	Educational
Immature or abnormal cell, would refer for identification	9	5.1	178	3.3	Educational
Monocyte	6	3.4	202	3.7	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	5	2.8	194	3.6	Educational
Leukocyte with intracellular *Anaplasma/Ehrlichia*	3	1.7	129	2.4	Educational
Parasite(s) seen, referred for definitive identification	3	1.7	48	0.9	Educational
Neutrophil necrobiosis (degenerated neutrophil)	2	1.1	25	0.5	Educational
Leukocyte containing Alder (Alder-Reilly) anomaly inclusion(s)	1	0.6	6	0.1	Educational
Leukocyte containing Chediak-Higashi anomaly inclusion(s)	1	0.6	20	0.4	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.6	24	0.4	Educational
Malignant lymphoid cell (other than blast)	1	0.6	33	0.6	Educational
Protozoa (non-malarial)	1	0.6	7	0.1	Educational

BCP-28, cont'd

The arrowed cells are leukocytes with phagocytosed fungi, as correctly identified by 61.9% of referees and 55.5% of participants. Fungi are only rarely visualized in peripheral blood. When present, the fungi are usually seen within the cytoplasm of monocytes, macrophages, or neutrophils. Phagocytized fungi are usually localized within a vacuole that forms a clear halo around the organism. Usually, the number of organisms present is scant. Clinical history and blood cultures are very important in making the appropriate identification. In this case, leukocytes contain 2 to 4 µm budding yeast forms of Histoplasma capsulatum. Although other fungi can be grown from blood cultures and therefore are present in the circulation, the level of fungemia is so low that they are virtually never visualized on a blood film. Intracellular fungi can be confused with precipitated stain overlying a leukocyte, large toxic granules, Dohle bodies, or large bacterial cocci.

The arrowed cells were incorrectly identified as a neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization) by 12.5% of referees and 15.0% of participants. Toxic granulation is defined by the presence of large, purple or dark blue cytoplasmic granules in neutrophils, bands, and metamyelocytes (monocytes, as seen in the image, do not demonstrate toxic granulation). Vacuoles within the cytoplasm of these same cells define toxic vacuolization. The vacuoles are variable in size (unlike in the image) and may coalesce, sometimes distorting the neutrophil cytoplasm to form pseudopodia. 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

The arrowed cells were incorrectly identified as a leukocyte with intracellular bacteria by 6.8% of referees and 12.3% of participants. It is very unusual to see bacteria on a routine blood film. This finding usually represents an overwhelming infection. When present, the bacteria may be ingested by neutrophils or monocytes and can be seen within the cytoplasm of these cells. Although leukocytes with phagocytized bacteria are rare in the blood film; they are commonly seen in infected body fluids. When present within neutrophils, bacteria can be difficult to distinguish from toxic granulation. However, toxic granulation tends to involve nearly all of the cytoplasm of the neutrophil, whereas engulfed bacteria are usually few in number. In addition, bacteria are typically larger than toxic granules, measuring around 1 µm in size, and are more defined in shape, ranging from cocci to bacilli and arranged singly, as diplococci, in clusters or in chains. They can be accentuated and confirmed with a Gram stain.

The arrowed cells were incorrectly identified as a monocyte by 3.4% of referees and 3.7% of participants. While one of the imaged cells is a monocyte, it is not normal and contains phagocytosed fungi.

The arrowed cells were incorrectly identified as plasma cell, morphologically mature/immature/containing inclusions (eg, Dutcher bodies, Russell body) by 2.8% of referees and 3.6% of participants. Plasma cells represent terminally differentiated B-lymphocytes are rarely seen in normal peripheral blood. They range in size from 10 to 20 µm, and they are often oval shaped with relatively abundant cytoplasm and eccentrically located nuclei. The N:C ratio is 1:2. Their nuclei are usually round to ovoid with prominently coarse and clumped chromatin that is often arranged in a cartwheel-like or clock-face pattern. The cytoplasm stains gray blue to deeply basophilic. A prominent hof or perinuclear zone of pale or lighter staining cytoplasm is typically seen adjacent to one side of the nucleus. This area corresponds to the Golgi zone, which is prominent in cells that produce large amounts of protein, such as immunoglobulin in the case of plasma cells. Cytoplasmic granules are absent, and scattered vacuoles of varying size may be seen.

BCP-28, cont'd

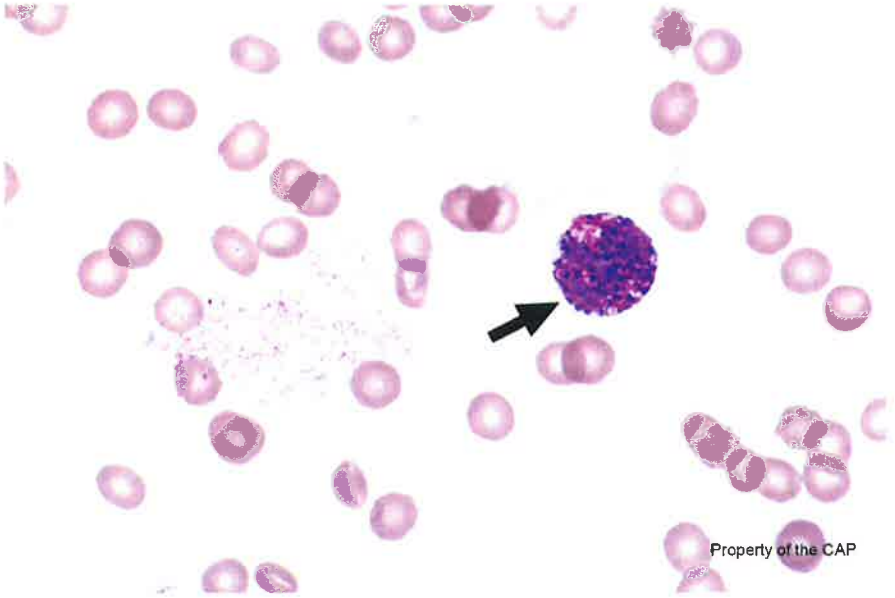
The arrowed cells were incorrectly identified as leukocytes with intracellular *Anaplasma/Erlichia* by 1.7% of referees and 2.4% of participants. On Wright-stained preparations, Anaplasma species appear as round, dark purple-stained dots or clusters of dots (morulae) in the cytoplasm of either neutrophils (*A. phagocytophilium*) or monocytes and macrophages (*A. chafeensis*). They usually do not fill in the entire cytoplasm.

The arrowed cells were incorrectly identified as parasites seen, refer identification by 1.7% of referees and 0.9% of participants. Plasmodium (malaria) and Babesia infections can be seen as parasites on blood smears within red blood cells (and not leukocytes).

The arrowed cells were incorrectly identified as neutrophil necrobiosis (degenerating neutrophil) by 1.1% of referees and 0.5% of participants. Neutrophil necrobiosis is a common phenomenon that can be seen both in normal individuals and in patients with a variety of medical conditions (including infections, in association with inflammatory disorders, and in malignancies). It is a non-diagnostic and non-specific finding. Degenerated neutrophils are generally easily identified because they resemble normal segmented neutrophils: they are round to oval cells ranging from 10 to 15 µm in diameter and their N:C ratio is 1:3 or less. The major distinguishing feature is that the nucleus shows karyorrhexis and/or pyknosis. These changes are appreciated when a cell with neutrophilic granules (pale pink cytoplasm with fine lilac granules) contains multiple, unconnected nuclear lobes (karyorrhexis) or a single, dark, round to oval nucleus (pyknosis). The chromatin pattern in these karyorrhexic or pyknotic states is also characteristic: dense and homogeneous, without visible parachromatin or nucleoli. The nuclear lobes may fragment into numerous small particles of varying size that can resemble microorganisms such as bacteria or fungi. Also, the nuclear outlines may become indistinct and blurred. As cellular degeneration continues, the cytoplasm will become hypogranulated, then agranular, and the cytoplasmic borders may become frayed and indistinct.

Blood Cell Identification – Ungraded

BCP-29



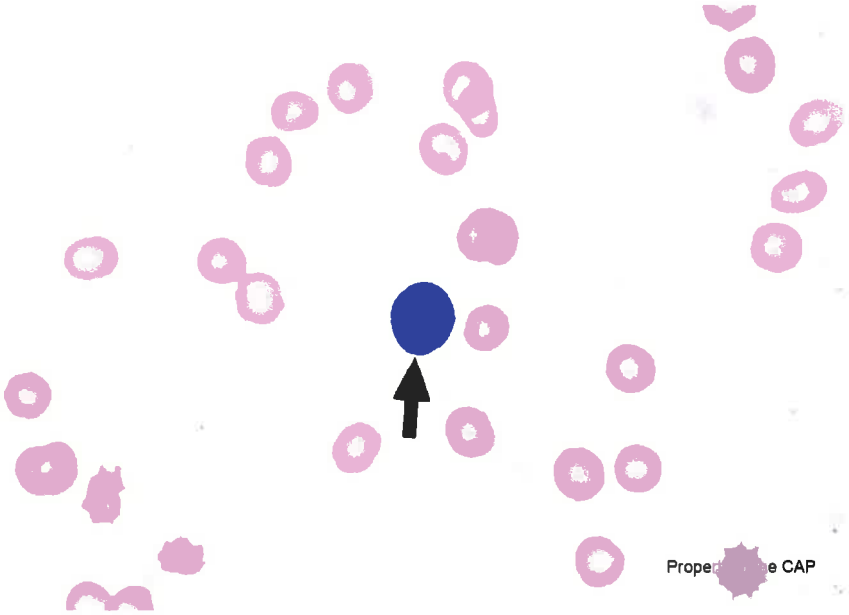
Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Basophil, any stage	173	98.3	5324	98.2	Educational
Mast cell	2	1.1	19	0.3	Educational
Leukocyte with intracellular fungi	1	0.6	13	0.2	Educational

The arrowed cell is a basophil, as correctly identified by 98.3% of referees and 98.2% of participants. Basophils have a maturation sequence analogous to neutrophils. At the myelocyte stage, when specific granules begin to develop, basophil precursors can be identified. 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.

The arrowed cell was incorrectly identified as a mast cell by 1.1% of referees and 0.3% of participants. The mast cell is a large (ie, 15 to 30 µm in diameter) round or elliptical cell with a small, round nucleus and abundant cytoplasm filled with black, bluish-black, or reddish-purple metachromatic granules. Normal mast cells are differentiated from blood basophils by the fact that they are larger (often twice the size of blood basophils), have more abundant cytoplasm, and have round rather than segmented nuclei. The cytoplasmic granules are smaller, more numerous, more uniform in appearance, and less water-extractable than basophil cytoplasmic granules.

Blood Cell Identification – Ungraded

BCP-30



Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Lymphocyte	176	100.0	5324	98.2	Educational

The arrowed cell is a normal lymphocyte, as correctly identified by 100.0% of referees and 98.2% 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.

Clinical Presentation:

This peripheral blood smear is from a 46-year-old man with HIV/AIDS, who presents with severe abdominal pain, pancytopenia, and hepatosplenomegaly. Laboratory data include: WBC = 3.0 × 10E9/L; RBC = 0.90 × 10E12/L; HGB = 2.6 g/dL; HCT = 9.0 %; MCV = 87 fL; MCHC = 29.2 g/dL; PLT = 20 × 10E9/L; and RDW = 19 %.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case discussion: Disseminated histoplasmosis

Histoplasma capsulatum var. capsulatum is the most widely distributed of the endemic mycoses, being present in many parts of the world. In North America, *H. capsulatum* is endemic to the Mississippi and Ohio River valleys but is also present in other areas. The fungus is associated with bat and bird guano, nitrogen-rich substrates that support fungal growth.

The clinical manifestation of *H. capsulatum* infection depends on the degree of the exposure and the immune status of the host, ranging from an asymptomatic infectious process to disseminated life-threatening disease. Exposure to low concentrations of spores from the environment in a normal host is typically asymptomatic, while immunocompromised individuals, particularly those with advanced HIV disease and those receiving tumor necrosis factor inhibitors, are at risk for disseminated histoplasmosis. Individuals at the extremes of age who do not have a recognized immunosuppressive condition but may have an immune system that is incompletely developed or is diminished by age are also at a higher risk for disseminated infection, however, the mechanism for this group of patients is not completely understood.

Disseminated histoplasmosis may affect any system with the hallmark of disease being an oropharyngeal ulcer, which may cause hoarseness, dysphagia, or a painful lesion on the tongue or gingiva. Infection of the reticuloendothelial system results in lymphadenopathy, hepatosplenomegaly and/or thrombocytopenia. Central nervous system infection may manifest as chronic meningitis, intracerebral granulomas, or both. Destruction of the adrenal cortex by the granulomatous process may be sufficiently extensive to cause hormonal insufficiency. Endovascular infection includes endocarditis with large, bulky vegetations. Any part of the gastrointestinal tract may be affected, and ulcerating lesions may suggest a neoplasm macroscopically.

H. capsulatum is a facultative intracellular pathogen and found predominantly in macrophages and monocytes. In affected tissues, pathologic lesions consist of collections of infected macrophages, non-necrotizing granulomas, or necrotizing granulomas and intracellular organisms (2-4 μm yeast forms with narrow based budding) are visible by H&E staining (with PAS and GMS stains being more sensitive). In the profoundly immunocompromised patients *H. capsulatum* are found in circulating monocytes and neutrophils on peripheral blood smears using Wright-Giemsa stain, as seen in our case. Phagocytosed fungi appear in cytoplasm as round forms with clearing around the yeast giving an appearance of a cell wall. However, this clearing represents an artefact due to the poorly staining yeast wall and retraction of its cytoplasm during fixation.

The diagnosis of Histoplasma infections requires a combination of culture, cyto- and histopathology, serology, and antigen testing. A urinary antigen test is the most sensitive in active and disseminated disease. However, this test is known to have some cross-reactivity in patients with blastomycosis. Culture, including blood culture, is generally required for disease confirmation.

The prognosis for patients with histoplasmosis depends on status of their immune system, as well as the presence of comorbid conditions. Patients with disseminated disease require systemic antifungal treatment and supportive care. Patients with endocarditis require surgical valve replacement in conjunction with antifungal therapy.

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. Procop Gary W and Pritt Bobbi. *Pathology of Infectious Diseases: A volume in the Series: Foundations in Diagnostic Pathology*, 1st ed. Saunders; 2014.
3. McPherson Richard A and. Pincus Matthew R. *Henry's Clinical Diagnosis and Management by Laboratory Methods,* 23rd ed. Elsevier; 2016.