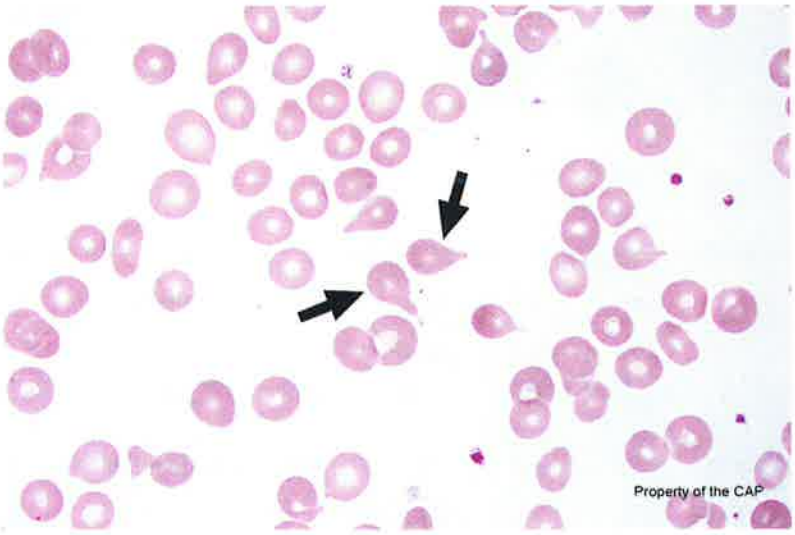
Blood Cell Identification – Graded

Case History
This peripheral blood smear is from an 85-year-old man with past medical history of primary myelofibrosis presenting with worsening fatigue and intermittent dizziness. Laboratory data includes: WBC = 9.9 × 10E9/L; RBC = 1.77 × 10E12/L; HGB = 6.1 g/dL; HCT = 17.7%; PLT = 108 × 10E9/L; MCV = 100 fL; and RDW = 22%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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

BCP-21

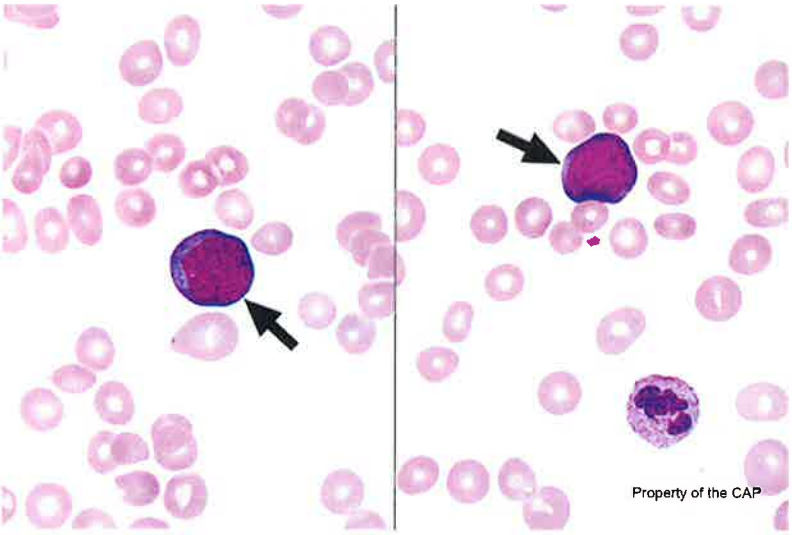


Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Teardrop cell (dacrocyte)	172	99.4	5343	99.7	Good
Target cell (codocyte)	1	0.6	14	0.3	Unacceptable

The arrowed cells are teardrop cells (dacrocytes), as correctly identified by 99.4% of referees and 99.7% of participants. Teardrop cells are red blood cells appearing in the shape of a teardrop or a pear with a single, short or long, often blunted or rounded end. These are commonly seen in patients with bone marrow fibrosis, but may also be seen in pernicious anemia, anemia of renal disease, hemolytic anemias, and other forms of severe anemia. These cells are often associated with an abnormal spleen or bone marrow. Bone marrow infiltration with hematologic and non-hematologic malignancies may also be accompanied by dacrocytosis. Teardrop cells may be seen as an artifact of slide preparation; such dacrocytes are usually easily recognized due to the fact that their "tails" all point in the same direction.

Blood Cell Identification – Graded

BCP-22



Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Blast cell	127	73.4	4035	75.3	Good
Immature or abnormal cell, would refer for identification	17	9.8	306	5.7	Good
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	8	4.6	207	3.9	Unacceptable
Lymphocyte	7	4.0	219	4.1	Unacceptable
Neutrophil, promyelocyte	6	3.5	226	4.2	Unacceptable
Lymphocyte, large granular	4	2.3	114	2.1	Unacceptable
Myeloblast with Auer rod	2	1.2	18	0.3	Unacceptable
Malignant lymphoid cell (other than blast)	1	0.6	113	2.1	Unacceptable
Neutrophil, promyelocyte, abnormal with/without Auer rod(s)	1	0.6	34	0.6	Unacceptable

The arrowed cells are blast cells, as correctly identified by 73.4% of referees and 75.3% of participants. 9.8% of referees and 5.7% of participants identified the arrowed cells as immature or abnormal cells, would refer. This is considered to be a correct response. A blast is a large, round-to-oval cell, 10 to 20 µm in diameter. In the blood film, the cell may appear flattened or compressed by adjacent red blood cells. The nuclear-to-cytoplasmic ratio is high, varying from 7:1 to 5:1. The blast often has a round to oval nucleus, but sometimes it is indented or folded. The blast cell has fine, lacy or reticular chromatin. One or more prominent nucleoli may be seen. The cytoplasm is variably basophilic and typically agranular. The morphologic features of a blast cell do not permit determination of the cell lineage, ie, myeloblast versus lymphoblast. The one exception is the presence of Auer rods, which are diagnostic of myeloid lineage (ie, "myeloblast"). Other cells may have the appearance of a blast, including some lymphoma cells. In the absence of Auer rods, immunophenotyping by flow cytometry, immunohistochemistry on tissue sections, or, less commonly, cytochemical staining (eg, peroxidase or Sudan black) is required to determine the lineage of a given blast cell.

BCP-22, cont'd

As blasts are quite variable in appearance, it is often impossible to correctly classify an individual cell based on the morphology alone. Blasts may rarely be morphologically indistinguishable from lymphoma cells. For identification purposes, one should classify individual cells exhibiting this type of morphology as blast cells when additional confirmatory information is unavailable.

4.6% of referees and 3.9% of participants identified the arrowed cells as reactive lymphocytes. Reactive lymphocytes show a range of cellular shapes, sizes and chromatin patterns. The most common type of reactive lymphocyte (Downey type II) are larger lymphocytes with round to oval nuclei, moderately condensed chromatin, and abundant blue-gray cytoplasm. Immunoblasts and immunoblastic-like reactive lymphocytes (Downey type III cells) are large cells with deeply basophilic cytoplasm and round to oval nuclei with moderately to finely dispersed chromatin and abundant parachromatin. The arrowed cells have more homogeneously open chromatin, a higher N:C ratio, and less basophilic cytoplasm than is typical of Downey type III cells.

4.0% of referees and 4.1% of participants identified the arrowed cells as lymphocytes. Normal lymphocytes are small, round to ovoid cells with high N:C ratios and diffusely dense or coarsely clumped chromatin. The arrowed cells are larger and have more open chromatin than lymphocytes.

3.5% of referees and 4.2% of participants identified the arrowed cells as promyelocytes. Promyelocytes are large (12-24 µm) round to oval cells with fine chromatin, basophilic cytoplasm, and multiple distinct azurophilic (primary) granules; a paranuclear hof may be present. The arrowed cells lack primary granules and have a higher N:C ratio than promyelocytes.

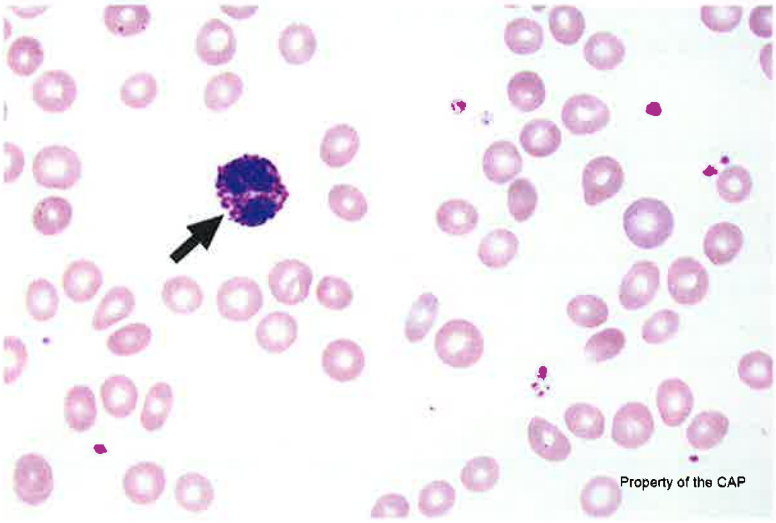
2.3% of referees and 2.1% of participants identified the arrowed cells as large granular lymphocytes. Large granular lymphocytes are medium to large cells with round nuclei, dense chromatin, and no visible nucleoli. The cytoplasm is moderate to abundant, lightly basophilic, and contains several coarse, unevenly distributed, small azurophilic granules. The arrowed cells have a higher N:C ratio and more open chromatin than large granular lymphocytes, and lack coarse azurophilic granules.

1.2% of referees and 0.3% of participants identified the arrowed cells as myeloblasts with Auer rod(s). Auer rods are pink or red, rod-shaped cytoplasmic inclusions representing crystallization of azurophilic (primary) granules. Auer rods are seen in neoplastic early myeloid forms and occasionally in early monocytic forms in patients with high-grade myelodysplastic syndromes and myeloid leukemia. Auer rods are not present in the arrowed cells.

0.6% of referees and 2.1% of participants identified the arrowed cells as malignant lymphoid cells other than blasts. Lymphoma cells can exhibit a variety of appearances based on the lymphoma subtype. Cell size ranges from 8-30 µm, and the N:C ratio varies from 7:1 to 3:1. It is critical to obtain an accurate clinical history, since knowledge of a previous diagnosis of lymphoma greatly aids in the identification of these cells. Some types of lymphoma cells, including large cell lymphoma, Burkitt lymphoma, and blastoid mantle cell lymphoma, may be difficult to distinguish from blasts, and immunophenotyping studies may be required to make the correct diagnosis. In this case, the clinical history of primary myelofibrosis provides an important clue, as leukoerythroblastosis and circulating blasts may be present in this clinical setting.

Blood Cell Identification – Graded

BCP-23



Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Basophil, any stage	164	94.8	5214	97.3	Good
Eosinophil, any stage	8	4.6	104	1.9	Unacceptable
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	1	0.6	25	0.5	Unacceptable

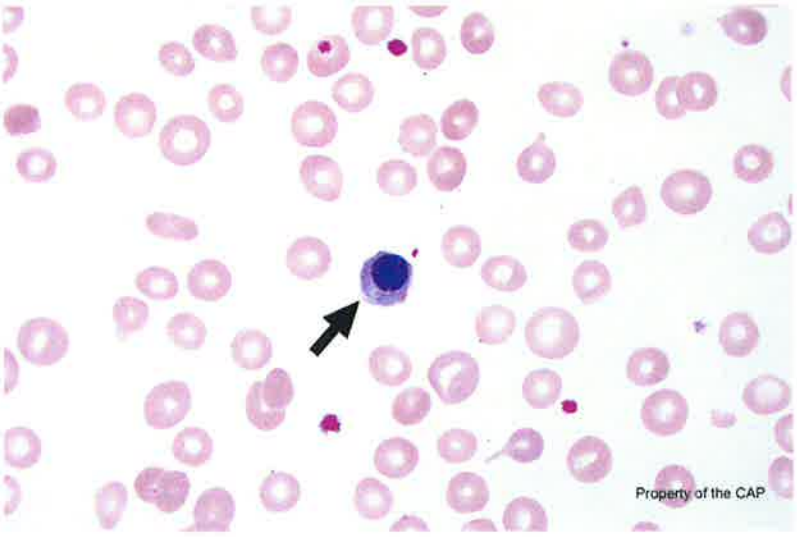
The arrowed cell is a basophil, as correctly identified by 94.8% of referees and 97.3% of participants. Basophils have a maturation sequence analogous to neutrophils. At the myelocyte stage, when specific granules begin to develop, basophil precursors can be identified. All basophils, from the basophilic 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.

While toxic neutrophils have prominent cytoplasmic granules, neutrophilic toxic granules are not as large and coarse as basophilic granules, and do not obscure the nucleus. Toxic granules in neutrophils are accompanied by other toxic changes, including Döhle bodies and cytoplasmic vacuolization.

4.6% of referees and 1.9% of participants identified the arrowed cells as eosinophils, any stage. Eosinophils are similar in size to basophils, but contain numerous coarse, orange-red cytoplasmic granules of uniform size. The granules in the arrowed cell are the blue-black to purple cytoplasmic granules typical of basophils.

Blood Cell Identification – Graded

BCP-24

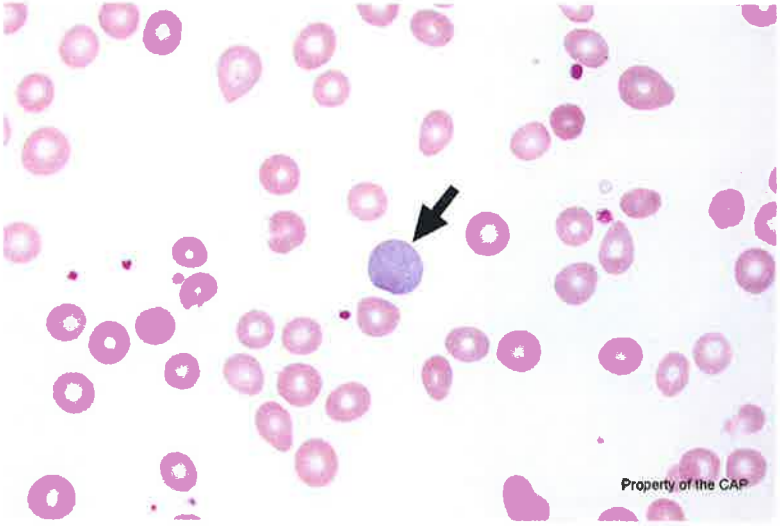


Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Nucleated red blood cell, normal or abnormal morphology	172	99.4	5292	98.7	Good
Immature or abnormal cell, would refer for identification	1	0.6	16	0.3	Unacceptable

The arrowed cell is a nucleated red blood cell, as correctly identified by 99.4% of referees and 98.7% of participants. The term nucleated red blood cell (nRBC) is used to state the presence of normoblasts in the peripheral blood and includes all normoblasts regardless of the stage of maturation. Typically, the circulating nucleated red blood cell is at the orthochromic stage of differentiation. Both megaloblastic and dysplastic changes can be seen in these circulating red blood cells, reflecting simultaneous erythroid maturation abnormalities present in the bone marrow. Caution should be used in classifying a circulating nucleated red blood cell as dysplastic on the basis of abnormal nuclear shape (lobated or fragmented), as these changes may occur during their egress from the marrow space and may not be present in the maturing erythroid precursors present in the marrow. For the purposes of proficiency testing, it is adequate to identify a cell as a nucleated red blood cell when it is present in the peripheral blood, be it normal or abnormal (ie, exhibits megaloblastic or dysplastic changes).

Blood Cell Identification – Graded

BCP-25



Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Polychromatophilic (non-nucleated) red blood cell	171	98.8	5248	97.9	Good
Basophilic stippling (coarse)	1	0.6	41	0.8	Unacceptable
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	1	0.6	39	0.7	Unacceptable

The arrowed cell is a polychromatophilic (non-nucleated) red blood cell, as correctly identified by 98.8% of referees and 97.9% of participants. A polychromatophilic red blood cell is a non-nucleated, round or ovoid red blood cell that represents the final stage of red blood cell maturation after exiting the bone marrow. It is larger than a mature erythrocyte and usually lacks central pallor. It primarily contains hemoglobin with a small amount of RNA, and thereby stains pale purple to pink-gray with Romanowsky or Wright-Giemsa stain. These cells can be stained as reticulocytes and enumerated by using supravital stains, such as new methylene blue. With supravital staining, reticulocytes reveal deep blue granular and/or filamentous structures. This reticulin network is called the "substantia reticulofilamentosa." The amount of precipitated RNA and intensity of polychromasia varies inversely with the age of the reticulocyte. The intensity of the polychromasia will vary with the amount of RNA and the age of the cell, with younger cells (ie, earlier polychromatophilic red cells) appearing more purple or blue and relatively more mature cells (ie, later polychromatophilic red cells) appearing more pink-gray. Automated technologies for assessing reticulocytes improve the accuracy and precision of determining reticulocyte numbers.

The arrowed cell shows polychromasia; it appears more bluish-purple in color than the surrounding mature erythrocytes. The presence of polychromasia distinguishes polychromatophilic red blood cells from oval macrocytes, which are large oval cells that lack significant polychromasia. If polychromasia is readily identified, the term polychromatophilic red blood cell is preferred for proficiency testing purposes.

Clinical Presentation:

This peripheral blood smear is from an 85-year-old man with a past medical history of primary myelofibrosis presenting with worsening fatigue and intermittent dizziness. Laboratory data includes: WBC = 9.9 × 10E9/L; RBC = 1.77 × 10E12/L; HGB = 6.1 g/dL; HCT = 17.7%; PLT = 108 × 10E9/L; MCV = 100 fL; and RDW = 22%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: PRIMARY MYELOFIBROSIS

Primary myelofibrosis (PMF) is one of the *BCR::ABL1*-negative chronic myeloproliferative neoplasms; other diseases in this category include polycythemia vera (PV) and essential thrombocythemia (ET). PMF is a clonal disorder characterized by proliferation of granulocytes and atypical megakaryocytes in the bone marrow. Over time, PMF is associated with development of progressive bone marrow fibrosis and extramedullary hematopoiesis in the spleen and liver. Among the *BCR::ABL1*-negative myeloproliferative neoplasms, PMF has the highest risk of progression to acute myeloid leukemia (AML).

Approximately 90% of cases of PMF are associated with driver mutations in the *JAK2* (50 - 60% of cases), *CALR*, or *MPL* genes, resulting in activation of JAK-STAT targets. JAK-STAT signaling is involved in control of a number of cellular pathways, including the response of hematopoietic cells to stimulation by hematopoietic growth factors. Disruption of this pathway leads to cell proliferation in the absence of normal growth factor stimulation. A minority of PMF cases are "triple-negative," that is, lacking detectable mutations in *JAK2*, *CALR*, or *MPL*. These cases may harbor other genetic or cytogenetic abnormalities.

In pre-fibrotic (early) PMF, the bone marrow is hypercellular for age, with proliferation of atypical megakaryocytes and granulocytes and absent or minimal reticulin fibrosis. Megakaryocytes may be enlarged or small, with variations in the nuclear:cytoplasmic (N:C) ratio, abnormal chromatin clumping, and abnormally lobulated "cloud-like" nuclei; clusters of megakaryocytes are often present in trephine core biopsy sections. Peripheral blood findings at this stage may include anemia, neutrophilia, and thrombocytosis. As in other myeloproliferative neoplasms, basophilia may be present.

Progressive deposition of reticulin and/or collagen fibrosis in the bone marrow leads to development of overt (fibrotic) PMF. In later stages, the bone marrow may be extensively replaced by fibrosis, and osteosclerosis may be present. Peripheral blood smears from patients with overt PMF often have leukoerythroblastic features, characterized by the presence of nucleated red blood cells and immature granulocytes. Circulating blasts and/or micromegakaryocytes and/or megakaryocyte fragments may be present. Dacrocytes, or teardrop-shaped red blood cells, are often present in the setting of bone marrow fibrosis. The white blood cell and platelet counts may be increased or decreased.

In this case, the CBC shows anemia and thrombocytopenia. Review of the peripheral blood smear shows evidence of leukoerythroblastosis, including circulating nucleated red blood cells and blasts. Dacrocytes and basophils are also present. Given the patient's reported history, these findings are consistent with fibrotic-stage PMF.

The clinical presentation of PMF varies with the stage of disease. Splenomegaly is present in approximately 90% of patients and may be massive in overt disease. Hepatomegaly is seen in approximately 50% of patients. Constitutional symptoms such as fever, night sweats and weight loss may be present. Increased cell turnover

leads to elevated lactate dehydrogenase (LDH) and uric acid levels, which may lead to renal stones and gouty arthritis.

The prognosis of PMF depends on the stage at diagnosis and other risk factors, including patient age, white blood cell count, blood transfusion dependence status, and cytogenetic abnormalities. Median survival ranges from < 2 years for high-risk patients to 15 years for low-risk patients. Treatment depends on the patient's symptoms and risk stratification. Hydroxyurea or JAK inhibitor therapy may be used to treat symptomatic splenomegaly. High-risk, transplant-eligible patients may be treated with allogeneic hematopoietic stem cell (bone marrow) transplant.

Alexandra E. Kovach MD
Hematology and Clinical Microscopy Committee

References:

1. Dunbar AJ, Rampal RK, Levine R. Leukemia secondary to myeloproliferative neoplasms. *Blood.* 2020;136 (1):61–70.
2. Greenfield G, McMullin MF, and Mills K. Molecular pathogenesis of the myeloproliferative neoplasms. *J Hematol Oncol.* 2021;14(1):103.
3. Swerdlow SH, Campo E, Harris NL, et al, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* (Revised 4th edition). IARC: 2017.
4. Tefferi, A. Primary myelofibrosis: 2021 update on diagnosis, risk-stratification and management. *Am J Hematol.* 2021;96(1):145-162.

Blood Cell Identification – Ungraded

Case History

This peripheral blood smear is from a 48-year-old man presenting with a lump in his left groin. Laboratory data includes: WBC = 6.6 × 10E9/L; RBC = 4.52 × 10E12/L; HGB = 13.5 g/dL; HCT = 40.2%; PLT = 151 × 10E9/L; MCV = 89 fL; and RDW = 14%. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

To access the online Hematology Glossary, please click the hyperlink below:
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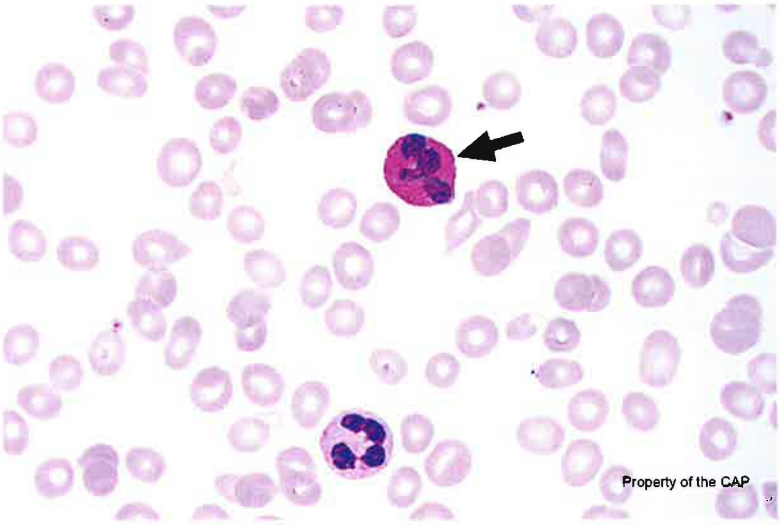
BCP-26

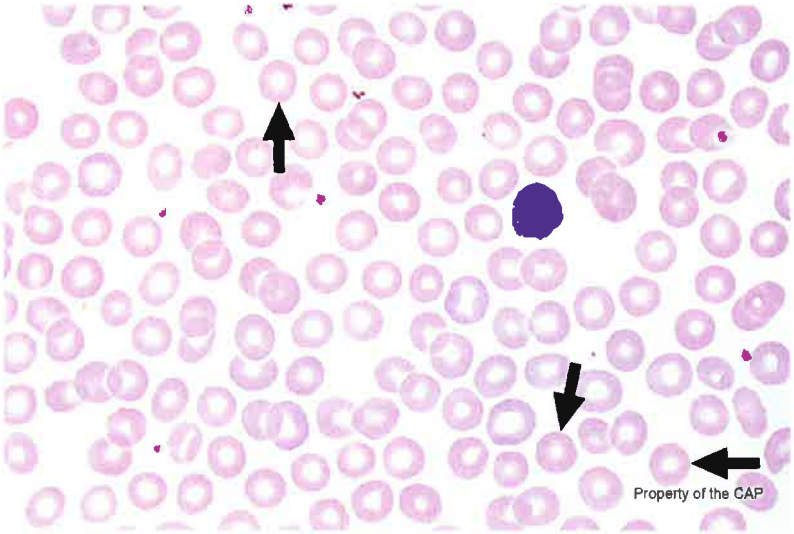


Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Eosinophil, any stage	171	98.8	5284	99.7	Educational
Platelet, hypogranular	1	0.6	1	0.0	Educational
Teardrop cell (dacrocyte)	1	0.6	2	0.0	Educational

The arrowed cell is an eosinophil, as correctly identified by 98.8% of referees and 99.7% 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. 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. 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. The remainder of segmented eosinophils will have three lobes and an occasional cell will exhibit four to five lobes.

Blood Cell Identification – Ungraded

BCP-27



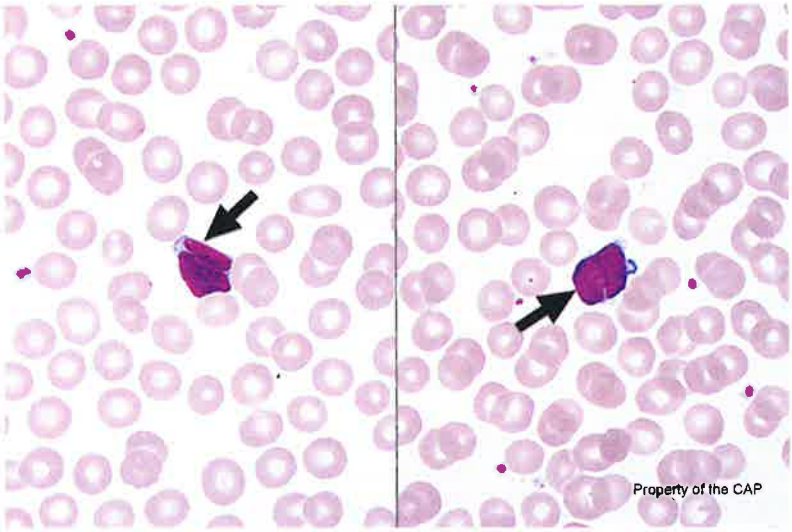
Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Erythrocyte, normal	169	97.7	5104	97.6	Educational
Microcyte (with increased central pallor)	3	1.7	104	2.0	Educational
Blast cell	1	0.6	3	0.1	Educational

The arrowed cells are normal erythrocytes, as correctly identified by 97.7% of referees and 97.6% 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.

1.7% of referees and 2.0% of participants incorrectly identified the arrowed cells as microcytes with increased central pallor. 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. When there is little or no variation in RBC size, morphology is less reliable than instrument generated MCVs in determining if microcytosis is present. 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. Although other poikilocytes, such as spherocytes and fragmented red blood cells, can be very small in size, these red blood cells lack central pallor and should be specifically identified rather than classified as "microcytes." Microcytes commonly are seen in iron deficiency anemia, thalassemia, lead poisoning and some cases of anemia of chronic disease.

Blood Cell Identification – Ungraded

BCP-28



Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Malignant lymphoid cell (other than blast)	86	49.7	2619	50.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	50	28.9	1625	31.1	Educational
Immature or abnormal cell, would refer for identification	13	7.5	217	4.2	Educational
Lymphocyte	11	6.4	358	6.8	Educational
Monocyte	6	3.5	207	4.0	Educational
Basket cell/smudge cell	1	0.6	40	0.8	Educational
Basophil, any stage	1	0.6	2	0.0	Educational
Blast cell	1	0.6	47	0.9	Educational
Hemoglobin C crystal	1	0.6	1	0.0	Educational
Mitotic figure	1	0.6	4	0.1	Educational
Myeloblast with Auer rod	1	0.6	17	0.3	Educational
Neutrophil, segmented or band	1	0.6	2	0.0	Educational

The arrowed cells are malignant lymphoid cells, as correctly identified by 49.7% of referees and 50.1% of participants. 7.5% of referees and 4.2% of participants identified the arrowed cells as immature, would refer. This is considered to be a correct response. These arrowed cells are small to intermediate in size and have nuclear clefts, moderately coarse chromatin and scant cytoplasm. This is a case of follicular lymphoma and cells with nuclear clefts are consistent with centrocytes.

28.9% of referees and 31.1% of participants incorrectly identified the arrowed cells as reactive lymphocytes. 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. A variety of reactive lymphocyte forms have been described and they are

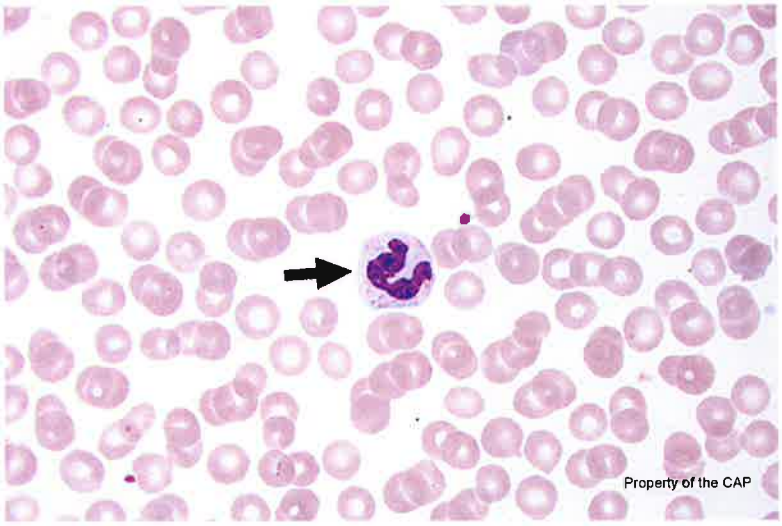
often seen concurrently in the same blood film. These round to ovoid to irregular cells range from 10 to 25 µm in size with an N:C ratio that varies from 3:1 to 1:2. The most common type of reactive lymphocyte resembles a larger lymphocyte and corresponds to a Downey type II cell. These cells have round 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. 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.

6.4% of referees and 6.8% of participants incorrectly identified the arrowed cells as lymphocytes. While most normal lymphocytes are fairly homogeneous, they do exhibit 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, adjacent to one side of the nucleus.

3.5% of referees and 4.0% of participants incorrectly identified the arrowed cells as monocytes. 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 nuclear-to-cytoplasmic ratio is 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 less dense than that of a neutrophil or lymphocyte. Nucleoli are generally absent, but occasional monocytes may contain a small, inconspicuous nucleolus.

Blood Cell Identification – Ungraded

BCP-29



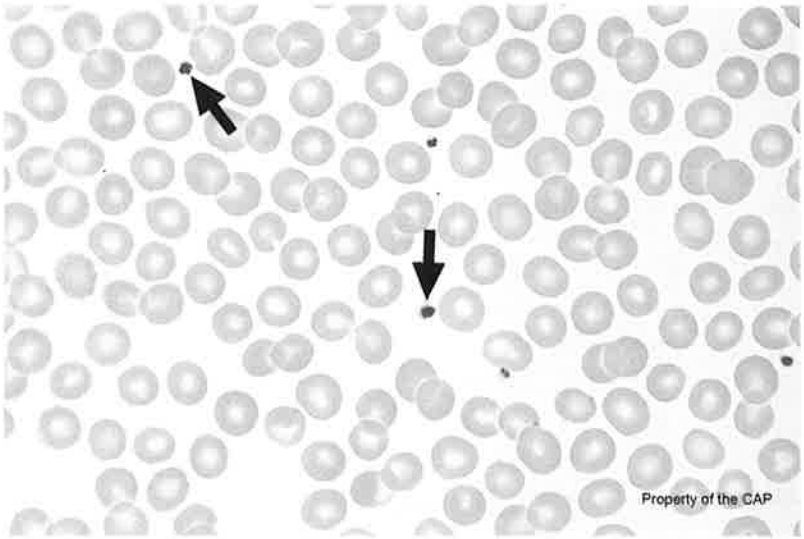
Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Neutrophil, segmented or band	169	97.7	5149	98.4	Educational
Neutrophil with hypersegmented nucleus	2	1.2	12	0.2	Educational
Nucleated red blood cell, normal or abnormal morphology	1	0.6	-	-	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	1	0.6	36	0.7	Educational

The arrowed cell is a segmented neutrophil, as correctly identified by 97.7% of referees and 98.4% of participants. Segmented neutrophils and their immediate precursors, bands, constitute 12% to 25% of the nucleated cells in the bone marrow. The segmented neutrophil is the predominant blood leukocyte. It has a similar size to a band neutrophil (ie, 10 to 15 µm in diameter), as well as comparable shape (round to oval), and cytoplasmic appearance (pale pink cytoplasm with specific granules). The N:C ratio is 1:3 and the nuclear chromatin is highly condensed. The nucleus is segmented or lobated (with a normal range of three to five lobes). The lobes are connected by a thin filament that contains no internal chromatin, giving it the appearance of a solid, dark, thread-like line.

1.2% of referees and 0.2% of participants incorrectly identified the arrowed cell as hypersegmented neutrophil. To be considered a neutrophil with hypersegmented nucleus, the neutrophil should demonstrate six or more lobes separated by thin filaments. Hypersegmented neutrophils are uncommon unless there is megaloblastic hematopoiesis. Hypersegmented neutrophils may also be seen in sepsis, renal disease, and myeloproliferative neoplasms. Megaloblastic hematopoiesis occurs when DNA synthesis is impaired. Such conditions include deficiency of cofactors for nucleotide synthesis, such as vitamin B12 and folate, and cases when patients are receiving a nucleotide analog (such as 6-mercaptopurine) or nuclear cofactor blocking agents (such as methotrexate) for neoplastic or rheumatologic conditions.

Blood Cell Identification – Ungraded

BCP-30



Identification	Referees		Participants		Evaluation
	Freq	%	Freq	%	
Platelet, normal	171	98.8	5212	99.6	Educational
Platelet satellitism	1	0.6	1	0.0	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.6	2	0.0	Educational

The arrowed objects are platelets, as correctly identified by 98.8% of referees and 99.6% 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.

Clinical Presentation:

This peripheral blood smear is from a 48-year-old man presenting with a lump in his left groin. Laboratory data includes: WBC = 6.6 × 10E9/L; RBC = 4.52 × 10E12/L; HGB = 13.5 g/dL; HCT = 40.2%; PLT = 151 × 10E9/L; MCV = 89 fL; and RDW = 14%. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Follicular lymphoma

Follicular lymphoma is a common low-grade B-cell non-Hodgkin lymphoma that accounts for approximately 20% of the lymphomas diagnosed in the United States. It tends to affect middle-aged to older patients with a median onset in the 6th decade and a male-to-female ratio of approximately 1:1.7. Although the most common clinical presentation is peripheral lymphadenopathy, it infrequently (up to 10% of cases) presents with peripheral blood lymphocytosis, usually in cases with widespread involvement. The majority of patients have bone marrow involvement with paratrabecular lymphoid aggregates/infiltrates.

Lymph nodes demonstrate a nodular appearance due to the presence of numerous follicles composed of neoplastic lymphocytes. The neoplastic lymphocytes characteristically have nuclear clefts or irregular nuclear contours and are called centrocytes. Occasional larger cells with prominent nucleoli, consistent with centroblasts may be admixed. The grade depends on the number of large cells (centroblasts) and can be low grade (grade 1 - 2) or high grade (grades 3A and 3B), with the more centroblasts the higher the grade. Grading is required for prognostic purposes and is performed in tissue biopsies. Even though morphologic features are suggestive, definitive diagnosis of follicular lymphoma usually requires additional studies, such as immunophenotyping and genetic studies. The peripheral blood, when involved by follicular lymphoma, may show occasional atypical lymphocytes or, less commonly, frank lymphocytosis.

Flow cytometric immunophenotyping is needed to distinguish follicular lymphoma from other B-cell lymphoproliferative disorders involving peripheral blood. Follicular lymphoma is characterized by expression of CD10, CD19, CD20, CD22, CD38, and surface light and heavy chain restriction. This immunophenotype may be seen with lymphomas of follicle center cell origin including follicular lymphoma, Burkitt lymphoma, and some diffuse large B-cell lymphomas (DLBCL). Morphologic correlation is required to exclude other lymphomas with similar immunophenotype. In rare cases of follicular lymphoma, CD10 may be negative, and thus the differential diagnosis should include other B-cell lymphomas such as marginal zone lymphoma or lymphoplasmacytic lymphoma.

Follicular lymphomas demonstrate a characteristic genetic translocation t(14;18)(q32q21), *BCL2::IGH,* which is seen in up to 90% of cases; this translocation is more common in lower grade follicular lymphomas. A minority of cases (10 - 15%), have *BCL6* gene rearrangements. Both of these cytogenetic abnormalities can also be seen in DLBCL, so correlation with histologic findings is essential for the diagnosis. Patients with follicular lymphoma may have concomitant DLBCL at the time of diagnosis or may transform to DLBCL during their disease course, with a transformation risk of 2 - 3% per year. Prognosis in follicular lymphoma is correlated with several factors, the most important being the extent of disease. Although an incurable lymphoma, some patients can be managed conservatively by "watch and wait approach", while others have a more aggressive clinical course and require therapy.

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References

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2. Kahl BS, Yang DT. Follicular lymphoma: Evolving therapeutic strategies. *Blood*. 2016;127(1):2055-63.
3. Maeshima AM, Taniguchi H, Tanioka K, et al. Clinicopathologic characteristics of follicular lymphoma with peripheral blood involvement. *Leuk Lymphoma*. 2015;56(7):2000-4.

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