Blood Cell Identification – Graded

Case History

This peripheral blood smear is from a 19-year-old woman presenting with headaches and dizziness for two months. Laboratory data includes: WBC = 5.8 × 10E9/L; RBC = 3.05 × 10E12/L; HGB = 4.3 g/dL; HCT = 18.1%; PLT = 391 × 10E9/L; MCV = 60 fL; and RDW = 22%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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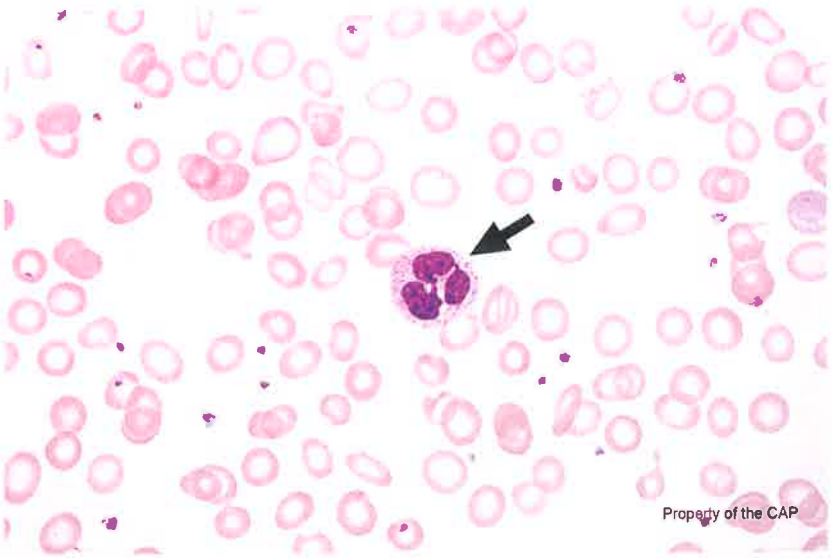
BCP-01



Identification	Referees		Participants		Evaluation
	N	%	N	%	
Neutrophil, segmented or band	180	99.5	5279	98.7	Good
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	-	-	61	1.1	Unacceptable
Neutrophil, polyploid	1	0.5	5	0.1	Unacceptable

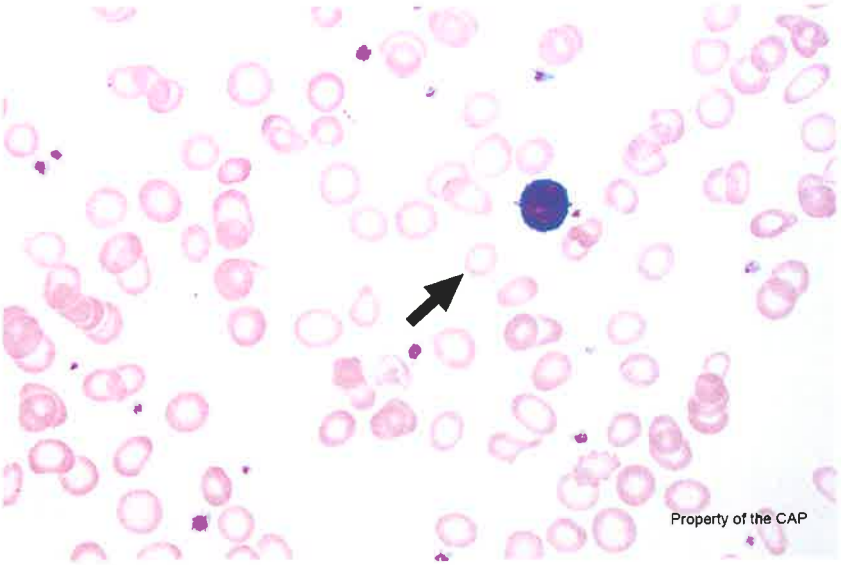
The arrowed cell was correctly identified as a mature neutrophil (segmented or band) by 99.5% of referees and 98.7% of participants. The segmented neutrophil (as in this photomicrograph) is the predominant blood leukocyte. It has a similar size to a band neutrophil (ie, 10 to 15 µm in diameter), as well 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. The presence of these thread-like filaments is the basis for distinguishing the segmented neutrophil from 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.

BCP-01, con't

The arrowed cell was incorrectly identified as neutrophil, toxic (to include toxic granulation, Döhle bodies, and/or toxic vacuolization) by 1.1% of participants. Toxic granulation is defined by the presence of large, purple or dark blue cytoplasmic granules in segmented 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 segmented neutrophils, bands, or metamyelocytes. They are often found at the periphery of the cytoplasm, near the cell membrane. These inclusions represent parallel strands of rough endoplasmic reticulum. Vacuoles in these cells with toxic granulation and/or Döhle bodies define toxic vacuolization. The vacuoles are variable in size and may coalesce, sometimes distorting the neutrophil cytoplasm to form pseudopodia. Neutrophil vacuoles should not be labeled as toxic vacuoles unless accompanied by other toxic changes. The arrowed cell in this case contains no Döhle bodies, no toxic vacuoles, nor large, purple-dark blue toxic cytoplasmic granules.

Blood Cell Identification – Graded

BCP-02



Identification	Referees		Participants		Evaluation
	N	%	N	%	
Microcyte (with increased central pallor)	170	93.9	4971	92.9	Good
Erythrocyte, normal	8	4.4	199	3.7	Unacceptable
Lymphocyte	2	1.1	149	2.8	Unacceptable
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.6	7	0.1	Unacceptable

The arrowed cell was correctly identified as a microcyte (with increased central pallor) by 93.9% of referees and 92.9% of participants. Microcytes are smaller than normal red blood cells, typically 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 (note: see the small lymphocyte in this photomicrograph, as a basis for size comparison). 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 are commonly seen in iron deficiency anemia (as in this case), thalassemia, lead poisoning and some cases of anemia of chronic disease.

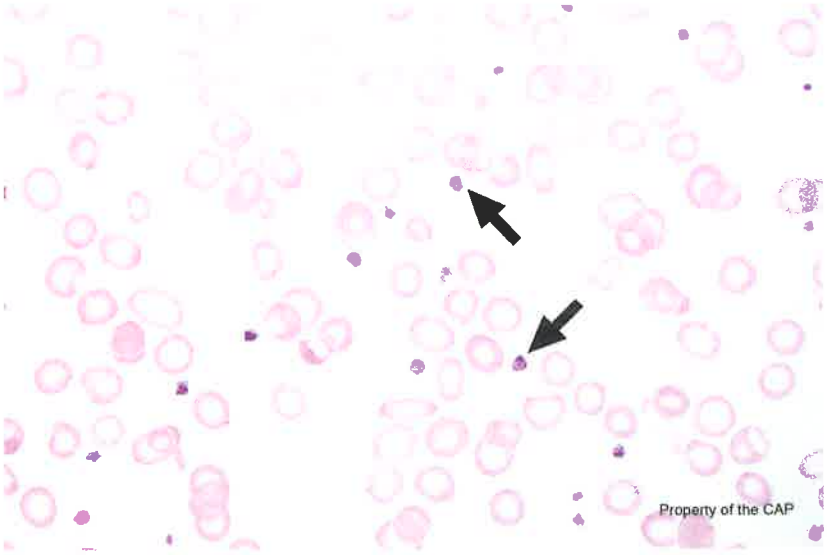
A small number of referees and participants (4.4% and 3.7%, respectively) incorrectly identified this arrowed cell as a normal erythrocyte. The strikingly low MCV of 60 fL (provided in the clinical history of this case) and the small size of this red blood cell (particularly, in comparison to the small lymphocyte in this field) argue strongly against the interpretation of a normal erythrocyte; rather, it is most appropriately identified as a hypochromic, microcytic erythrocyte. Furthermore, the zone of central pallor in normal erythrocytes occupies no more than one-third of the cell diameter. As mentioned above, the central pallor in this cell exceeds 50% of the cell diameter.

BCP-02, con't

The arrowed cell was incorrectly reported as a lymphocyte by 2.8% of participants and 1.1% of referees. Although a small lymphocyte is seen in this photomicrograph, the arrowed cell is clearly pointing to a non-nucleated red blood cell.

Blood Cell Identification – Graded

BCP-03

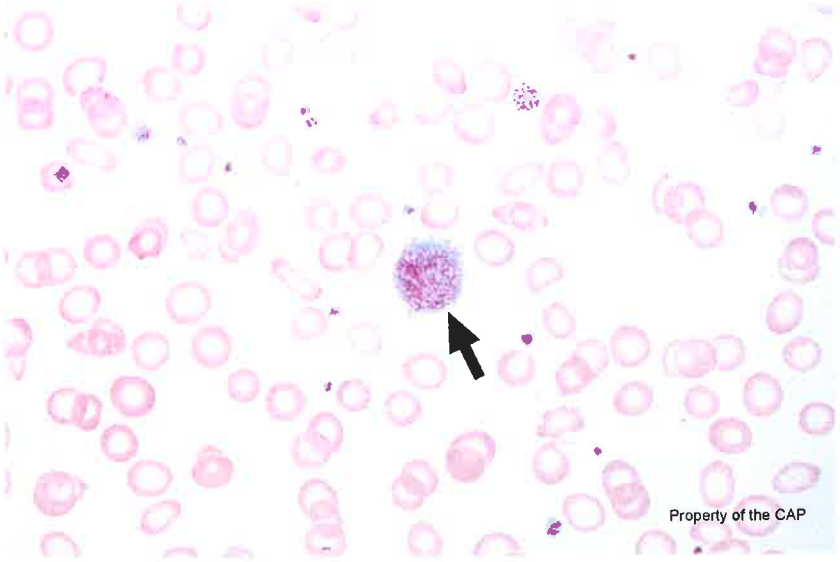


Identification	Referees		Participants		Evaluation
	N	%	N	%	
Platelet, normal	180	99.5	5335	99.7	Good
Blast cell	1	0.5	2	0.0	Unacceptable

The arrowed cells were correctly identified as normal platelets by 99.5% 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-04



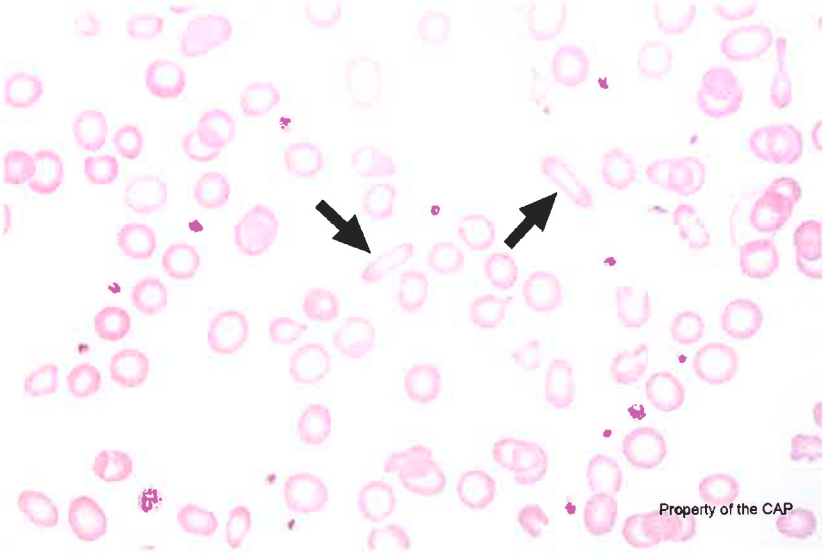
Identification	Referees		Participants		Evaluation
	N	%	N	%	
Platelet, giant (macrothrombocyte)	178	98.3	5278	98.6	Good
Megakaryocyte (normal, abnormal, or nuclear fragment)	3	1.7	56	1.1	Unacceptable

The arrowed cell was correctly identified as a giant platelet (macrothrombocyte) by 98.3% of referees and 98.6% of participants. Giant platelets are larger than 7 µm, usually measuring 10 to 20 µm in diameter. For proficiency testing purposes, the term giant platelet is used when the platelet is larger than the size of the average red blood cell in the field, assuming a normal MCV. The periphery of the giant platelet may be round, scalloped, or stellate. The cytoplasm may contain a normal complement of fine azurophilic granules, or the granules may fuse into giant forms. Giant platelets are a rare finding in normal peripheral blood, but may be seen in many different reactive, neoplastic, and inherited conditions. Reactive causes include conditions in which platelet turnover is markedly increased, such as immune thrombocytopenia or severe leukemoid reactions. Many giant platelets are most often seen in myeloproliferative neoplasms and myelodysplastic syndromes. The inherited conditions associated with giant platelets are rare and have associated thrombocytopenia.

The arrowed cell was incorrectly identified as a megakaryocyte (normal, abnormal, or nuclear fragment) by 1.1% of participants and 1.7% of referees. Megakaryocytes are nucleated cells. In the peripheral blood, they are single-lobed or, less commonly, multilobated. The chromatin is smudged or "puddled" and is surrounded by a scant amount of cytoplasm. At times, the cytoplasm is barely perceptible on light microscopy, giving the impression of a "bare" or "naked" megakaryocyte nucleus. However, upon closer inspection, a small amount of wispy, frilly, or fragmented cytoplasm can still be seen. The arrowed cell contains no nucleus, and instead displays purple-red granules that are finely dispersed throughout the cytoplasm, with ruffled cytoplasmic margins. The texture and quality of this giant platelet is comparable to the nearby small to intermediate-sized platelets seen in this photomicrograph.

Blood Cell Identification – Graded

BCP-05



Identification	Referees		Participants		Evaluation
	N	%	N	%	
Ovalocyte (elliptocyte)	180	99.5	5338	99.8	Good
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	1	0.5	3	0.1	Unacceptable

The arrowed cells were correctly identified as ovalocytes (elliptocytes) by 99.5% of referees and 99.8% of participants. The terms elliptocytes and ovalocytes are used to describe red blood cells appearing in the shape of a pencil or thin cigar, with blunt ends and parallel sides. Hemoglobin is often concentrated at the ends, producing a dumbbell appearance. A small number of elliptocytes/ovalocytes may be present on the smears of normal individuals (< 1%), whereas a moderate to marked elliptocytosis/ovalocytosis (> 25%) is observed in patients with hereditary elliptocytosis, an abnormality of erythrocyte cytoskeletal membrane proteins. Elliptocytes are also commonly increased in number in iron deficiency and in the same states in which teardrop cells are prominent. Some ovalocytes may superficially resemble oval macrocytes but are not as large as macrocytes and tend to be less oval with sides that are nearly parallel. The ends of ovalocytes are always blunt and never sharp, unlike those of sickle cells.

Clinical Presentation:

This peripheral blood smear is from a 19-year-old woman presenting with headaches and dizziness for two months. Laboratory data includes: WBC = 5.8 × 10E9/L; RBC = 3.05 × 10E12/L; HGB = 4.3 g/dL; HCT = 18.1%; PLT = 391 × 10E9/L; MCV = 60 fL; and RDW = 22%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Iron Deficiency Anemia

In this case of severe iron deficiency anemia (IDA), several red blood cell (RBC) indices are markedly decreased, including hemoglobin (HGB), hematocrit (HCT), RBC count, and MCV, while RDW is increased. White blood cells are normal in number and there is slight thrombocytosis. The peripheral blood smear, as exemplified in the photomicrographs, highlights several RBC abnormalities including prominent anisopoikilocytosis with many hypochromic microcytes, elliptocytes/ovalocytes, and occasional prekeratocytes and teardrop poikilocytes.

IDA is a common cause of anemia worldwide and occurs in a wide variety of clinical settings. The most common etiology in developed countries is chronic blood loss, especially due to heavy menses and gastrointestinal bleeding as well as decreased iron absorption. The latter can be due to surgical intervention (eg, gastrectomy, bariatric surgery) or *Helicobacter pylori* infection. In developing countries, low iron bioavailability in the diet is the primary cause of iron deficiency. Meat and fish are rich in iron, but vegetarian diets may predispose individuals to iron deficiency, especially if diets lack other iron sources, such as black beans, soybeans, and cornflower. In times of increased physiologic demand, certain individuals are much more susceptible to IDA, such as infants, adolescents (because of rapid growth), and pregnant women (particularly in the second and third trimesters).

Symptoms of IDA are somewhat nonspecific and can include fatigue, weakness, irritability, headaches, dizziness, and exercise intolerance. Older patients may present with worsening of an underlying illness. One example of this would be an individual with pre-existing chronic obstructive pulmonary disease, who presents with increasing shortness of breath due to worsening anemia. Another case would be a patient with underlying severe coronary artery disease, experiencing increasing angina. In the current clinical vignette, this young woman had a history of heavy menses as well as an iron-poor diet. She now presents with worsening headaches and dizziness with declining hemoglobin levels because of this combination of risk factors.

Review of the peripheral blood smear in a typical case of iron deficiency anemia reveals hypochromic, microcytic red blood cells, as well as ovalocytes/elliptocytes, and prekeratocytes. These may also be seen in thalassemia, anemia of chronic disease, and sideroblastic anemia, although other morphologic findings, red cell indices, and results of iron studies will aid in distinguishing one entity from another.

Maria Vergara-Lluri, MD
Hematology and Clinical Microscopy Committee

References:

1. Camaschella C. Iron-deficiency anemia. *N Engl J Med.* 2015;372(19):1832-43.
2. Camaschella C. Iron deficiency: new insights into diagnosis and treatment. *Hematology Am Soc Hematol Educ Program.* 2015;2015:8-13. doi: 10.1182/asheducation-2015.1.8.
3. Wilson CS, Vergara-Lluri ME, Brynes RK. Evaluation of anemia, leukopenia, and thrombocytopenia. In: *Hematopathology*. 2nd edition. Elsevier; 2017:195-218.

Blood Cell Identification – Ungraded

Case History

This peripheral blood smear is from a 57-year-old man presenting with fatigue and a feeling of abdominal fullness. Laboratory data includes: WBC = 1.9 × 10E9/L; RBC = 4.35 × 10E12/L; HGB = 12.5 g/dL; HCT = 38.0%; PLT = 60 × 10E9/L; MCV = 86 fL; and RDW = 17%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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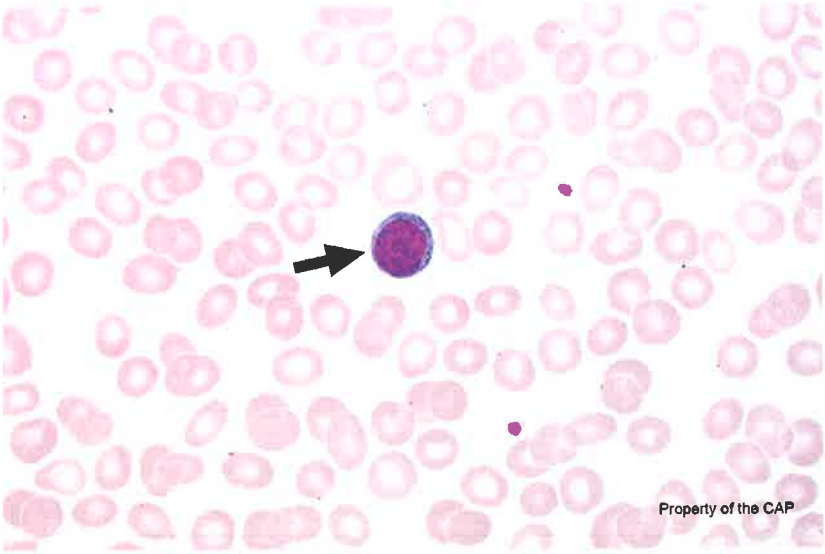
BCP-06

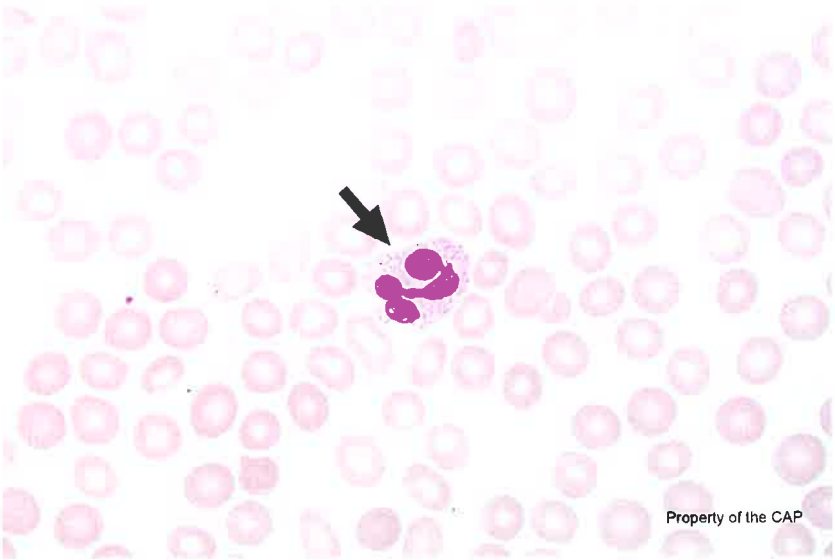


Identification	Referees		Participants		Evaluation
	N	%	N	%	
Lymphocyte	179	99.4	5225	98.5	Educational
Immature/abnormal cell, would refer for identification	1	0.6	7	0.1	Educational

The arrowed cell is a lymphocyte, as correctly identified by 99.4% of the referees and 98.5% of the participants. This cell shows features of a mature, non-reactive lymphocyte and is a normal constituent of peripheral blood. The lymphocyte is slightly larger than a normal red blood cell with scant to moderate pale blue cytoplasm, round nuclear contours, mature chromatin, and no visible nucleolus.

Blood Cell Identification – Ungraded

BCP-07



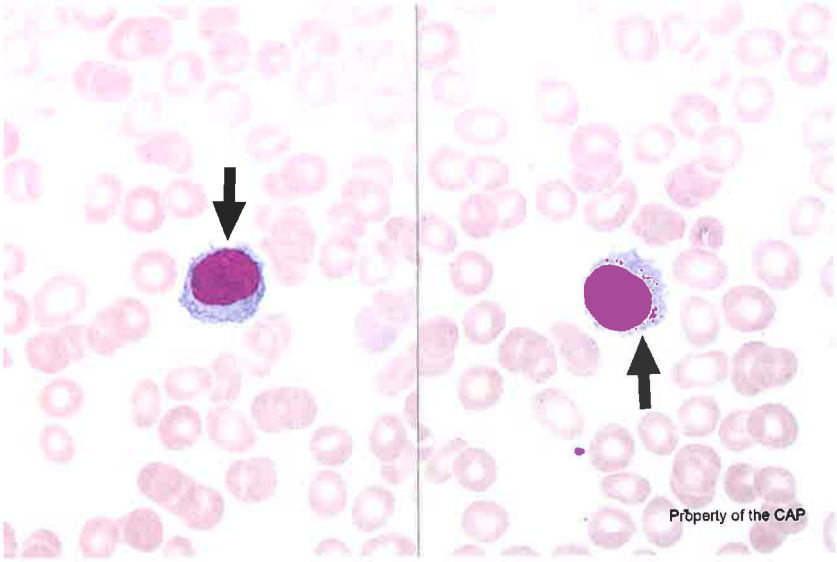
Identification	Referees		Participants		Evaluation
	N	%	N	%	
Neutrophil, segmented or band	176	97.8	5097	97.3	Educational
Neutrophil with hypersegmented nucleus	3	1.7	54	1.0	Educational
Neutrophil, polyploid	1	0.5	16	0.3	Educational

The arrowed cell is a segmented neutrophil, as correctly identified by 97.8% of the referees and 97.3% of the participants. Neutrophils measure 10 to 15 µm in diameter, have round to oval shape, and pale pink cytoplasm with specific granules. 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. The nuclear chromatin is highly condensed.

Neutrophil with hypersegmented nucleus, as reported by 1.7% of referees and 1.0% of participants is an unacceptable response. To be considered a neutrophil with a hypersegmented nucleus, the neutrophil should demonstrate six or more lobes. This image does not contain six or more lobes. Hypersegmented neutrophils are uncommon unless there is megaloblastic hematopoiesis. They may also be seen in sepsis, renal disease, and myeloproliferative neoplasms.

Blood Cell Identification – Ungraded

BCP-08



Identification	Referees		Participants		Evaluation
	N	%	N	%	
Malignant lymphoid cell (other than blast)	133	73.9	3909	74.6	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	23	12.8	688	13.1	Educational
Immature or abnormal cell, would refer for identification	11	6.1	266	5.1	Educational
Lymphocyte	9	5.0	195	3.7	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	2	1.1	88	1.7	Educational
Lymphocyte, large granular	1	0.6	55	1.1	Educational
Basket cell/smudge cell	1	0.6	2	0.0	Educational

The arrowed cells are malignant lymphoid cells (hairy cells), as correctly identified by 73.9% of the referees and 74.6% of the participants. Hairy cells, typical of hairy cell leukemia, are round to ovoid lymphoid cells that measure 12 to 20 µm in diameter (larger than normal, mature lymphocytes). Their N:C ratio ranges from 4:1 to 2:1, and they contain moderate to abundant pale blue to gray-blue cytoplasm. The cell borders are often indistinct, secondary to the presence of characteristic elongated, fine (hairy), cytoplasmic projections. These projections are frequently irregular and may be thick, blunted, smudged, serrated, or short. The cytoplasm typically is agranular, although occasional fine azurophilic granules may be seen. The nuclei of hairy cells are usually oval to indented, but may be folded, bean-shaped, angulated, or dumbbell-shaped and are either centrally or eccentrically located. The chromatin is usually homogeneous, finer than in normal lymphocytes, and evenly distributed with scant intervening parachromatin. Nucleoli, if present, are generally small and single. Occasional cells may have multiple small nucleoli or a single large nucleolus.

BCP-08, con't

Immature cell, would refer for identification, as reported by 6.1% of referees and 5.1% of participants is acceptable only if your laboratory routinely sends the cell in question to an outside laboratory with another CLIA number.

Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms), as reported by 12.8% of referees and 13.1% of participants is an unacceptable response. The hairy projections seen in this image would generally be inconsistent with reactive lymphocytes. The most common type of reactive lymphocytes (Downey type II cells) present as larger cells with round to oval nuclei, moderately condensed chromatin, and absent or indistinct nucleoli. They contain abundant pale gray-blue cytoplasm. Other types of reactive lymphocytes present with a wide range of cellular sizes and shapes. These include Downey type I cells, Downey type III cells (immunoblasts and immunoblastic-like cells), and plasmacytoid lymphocytes.

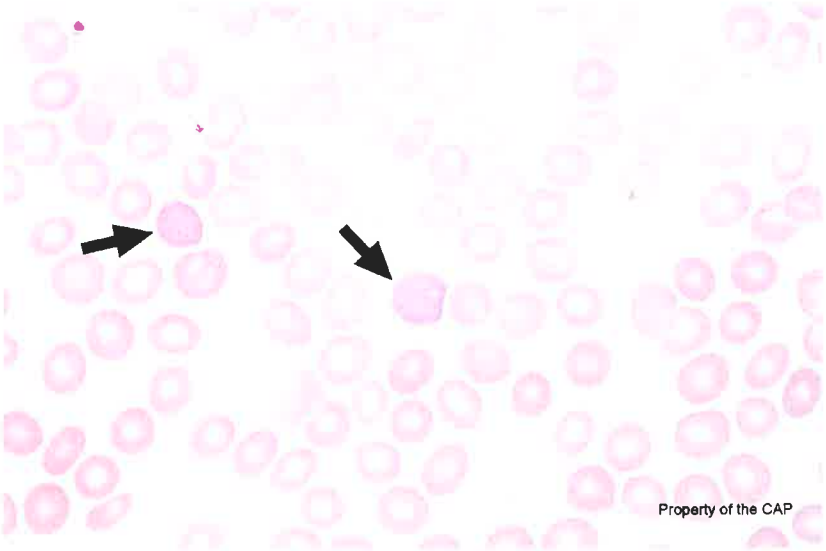
Lymphocyte as reported by 5.0% of referees and 3.7% of participants is an unacceptable response. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 µm with an N:C ratio ranging from 5:1 to 2:1. Most lymphocytes have round to oval nuclei, diffusely dense or coarse and clumped chromatin and a scant amount of pale blue to moderately basophilic, agranular cytoplasm.

Plasma cell, morphologically mature/abnormal containing inclusion (eg. Dutcher body, Russell body) as reported by 1.1% of referees and 1.7% of participants is an unacceptable response. Plasma cells are only rarely seen in normal peripheral blood. They range in size from 10 to 20 µm, and mature plasma cells are often oval shaped with relatively abundant blue cytoplasm, eccentrically located nuclei and prominent perinuclear hof.

Lymphocyte, large granular as reported by 0.6% of referees and 1.1% of participants is an unacceptable response. 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.

Blood Cell Identification – Ungraded

BCP-09

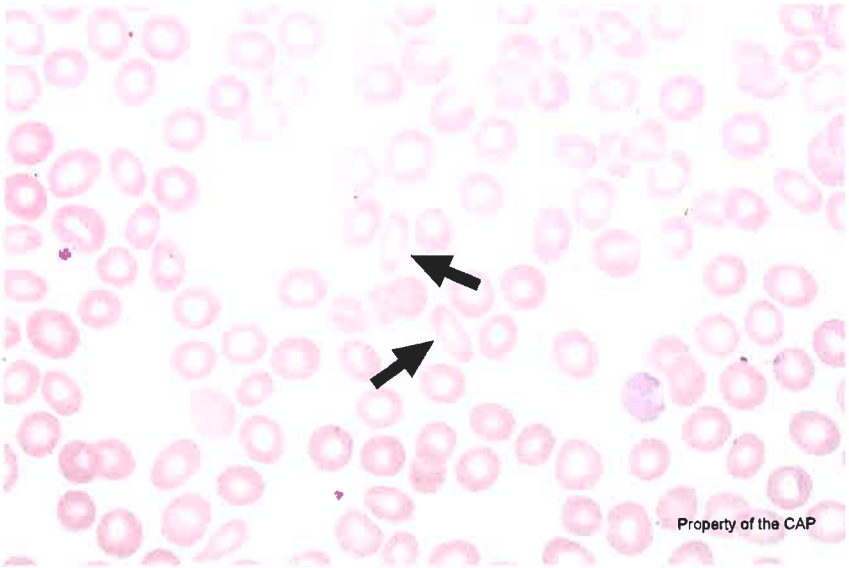


Identification	Referees		Participants		Evaluation
	N	%	N	%	
Polychromatophilic (non-nucleated) red blood cell	179	99.4	5138	98.1	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	1	0.6	25	0.5	Educational

The arrowed cells are polychromatophilic (non-nucleated) red blood cells, as correctly identified by 99.4% of the referees and 98.1% of the 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 homogeneously pink-gray or pale purple with Romanowsky or Wright-Giemsa stain. These cells can be stained using supravital stains, such as new methylene blue and enumerated as reticulocytes. Automated technologies for assessing reticulocytes improve the accuracy and precision of determining reticulocyte numbers.

Blood Cell Identification – Ungraded

BCP-10



Identification	Referees		Participants		Evaluation
	N	%	N	%	
Ovalocyte (elliptocyte)	179	99.4	5223	99.7	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	1	0.6	4	0.1	Educational

The arrowed cells are ovalocytes (elliptocytes), as correctly identified by 99.4% of the referees and 99.7% of the participants. Ovalocytes/elliptocytes are red blood cells appearing in the shape of a pencil or thin cigar, with blunt ends and parallel sides. Hemoglobin is often concentrated at the ends, producing a dumbbell appearance. A small number of elliptocytes/ovalocytes may be present on the smears of normal individuals (< 1%), whereas a moderate to marked elliptocytosis/ovalocytosis (> 25%) is observed in patients with hereditary elliptocytosis, an abnormality of erythrocyte skeletal membrane proteins. Elliptocytes are also commonly increased in number in iron deficiency and in the same states in which teardrop cells are prominent (eg, myelophthisis, myelofibrosis).

Clinical Presentation:

This peripheral blood smear is from a 57-year-old man presenting with fatigue and a feeling of abdominal fullness. Laboratory data includes: WBC = 1.9 × 10E9/L; RBC = 4.35 × 10E12/L; HGB = 12.5 g/dL; HCT = 38.0%; PLT = 60 × 10E9/L; MCV = 86 fL; and RDW = 17%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case discussion: Hairy Cell Leukemia

Hairy cell leukemia (HCL) is a cytologically and immunophenotypically distinct, indolent B-cell lymphoproliferative disorder. It is a rare disease accounting for 2% of lymphoid neoplasms. It occurs in older adults (median age of 58 years) and demonstrates a male predominance (male: female ratio 4:1). Patients typically present clinically with weakness and fatigue, pancytopenia, splenomegaly, and recurrent opportunistic infections. Tumor cells are found predominantly in the bone marrow and spleen. Typically, a small number of circulating cells are also noted in the peripheral blood. Tumor infiltrates may also occur in the liver and lymph nodes.

The diagnosis of HCL can usually be made by careful morphologic examination of a well-prepared peripheral blood smear and/or bone marrow biopsy sample in conjunction with flow cytometry immunophenotyping. Hairy cells are round to ovoid lymphoid cells that are slightly larger than normal, mature lymphocytes. They contain moderate to abundant pale blue to gray-blue cytoplasm. The cell borders are often indistinct, secondary to the presence of characteristic elongated, fine (hairy), cytoplasmic projections. The nuclei of hairy cells are usually oval to indented, but may be folded, bean-shaped, and are either centrally or eccentrically located. Monocytopenia characteristically accompanies HCL and is a helpful diagnostic feature. Because of significant bone marrow reticulin fibrosis associated with HCL, nucleated red blood cells and left-shifted myeloid cells may be seen in the peripheral blood.

Immunophenotypic analysis, preferably by flow cytometry, is crucial for making a diagnosis of HCL. The classic immunophenotypic profile of hairy cells consists of bright monotypic surface immunoglobulins, bright expression of B-cell markers CD19, CD20, CD22, and co-expression of CD11c, CD103, CD25, CD123, annexin A1 (most specific), FMC7, CD200, and cyclin D1 (weak and variable). CD10 may be expressed in a minor subset of cases (10 - 20%) and CD5 may be expressed rarely (0 - 2%). Often, due to the abundant cytoplasm and larger cell size, the neoplastic cells will be found outside of the typical lymphocyte gate. Cytochemical staining for tartrate resistant acid phosphatase (TRAP) is also typical of HCL and may show a strong, diffuse or finely granular staining pattern. *BRAF V600E* mutation is present in the vast majority of cases and can be tested for by molecular genetic methods or immunohistochemistry using a mutation-specific antibody.

The first line treatment for HCL includes cladribine +/- rituximab or pentostatin, with which patients often achieve complete and durable remission. In refractory or relapsed cases, salvage therapeutic options include chemotherapy combined with rituximab, anti-CD22 immunotoxin therapy, and *BRAF* inhibitors (vemurafenib).

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Hematology and Clinical Microscopy Committee

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