Blood Cell Identification – Graded

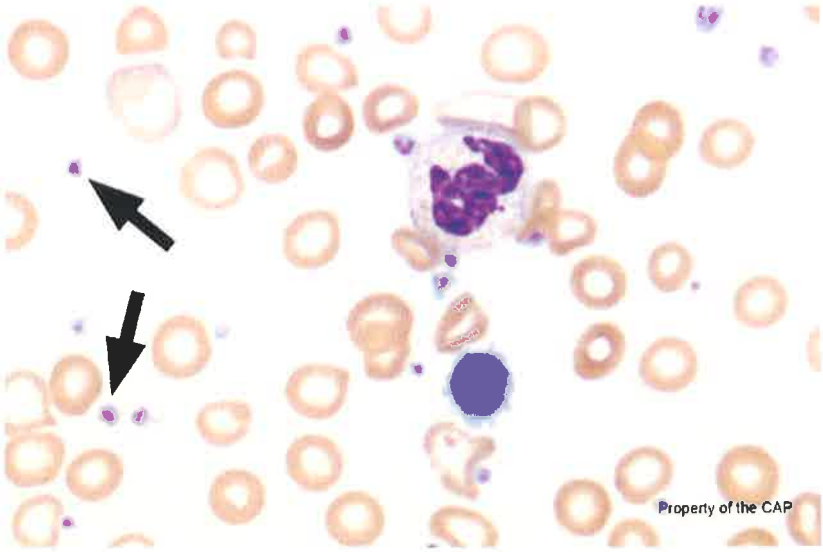
Case History

This blood film is from a 20-year-old woman with a month long history of appearing pale and feeling fatigued. Laboratory data include: WBC = 18.2 x 10E9/L; RBC = 2.12 x 10E12/L; HGB = 3.2 g/dL; HCT = 11.8%; MCV = 56 fL; MCHC = 27.1 g/dL; RDW = 22%; and PLT = 839 x 10E9/L. Identify the arrowed image(s).

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

To access the online Hematology Glossary, please click the hyperlink below:
http://www.cap.org/ShowProperty?nodePath=/UCMCon/Contribution%20Folders/WebContent/pdf/hematology-glossary.pdf

BCP-01

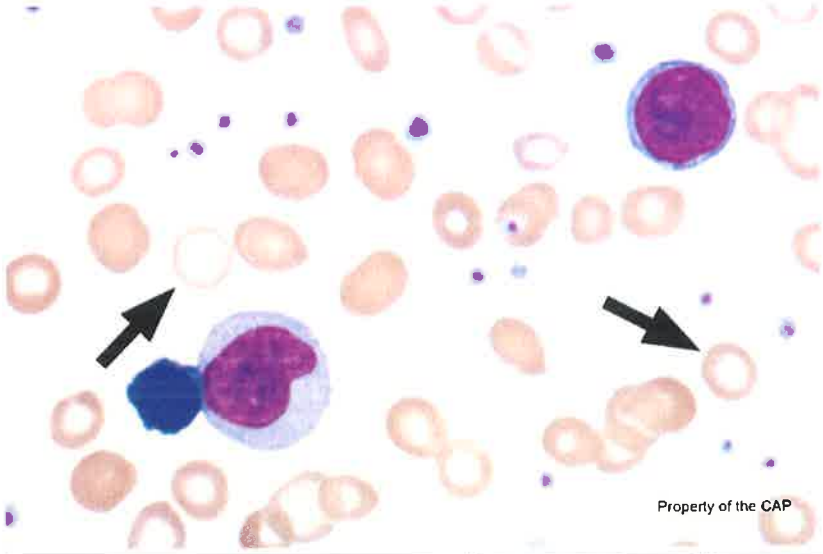


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Platelet, normal	117	100.0	4955	99.5	Good

The arrowed cells are normal platelets, as correctly identified by 100.0% of referees and 99.5% of participants. Platelets are also known as thrombocytes, measure 1.5 - 3 µm in diameter, and contain fine purple-red granules. Platelets are essential for normal hemostasis.

Blood Cell Identification – Graded

BCP-02



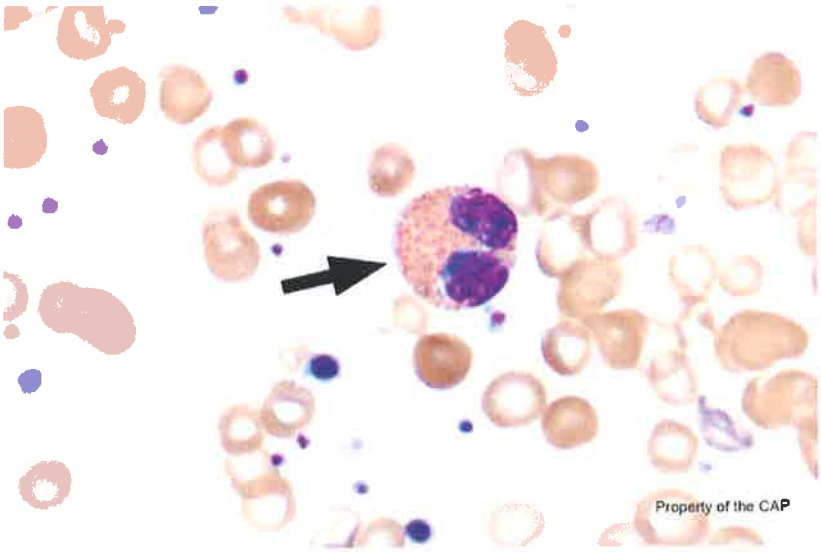
Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Microcyte (with increased central pallor)	34	29.1	1316	26.4	Good
Hypochromasia	83	70.9	3653	73.4	Good

The arrowed cells are microcytes (with increased central pallor), as correctly identified by 29.1% of referees and 26.4% of participants. These erythrocytes demonstrate greater than 50% central pallor and are smaller than the nucleus of a resting lymphocyte (less than 6 µm in diameter). These cells are often seen in iron deficiency anemia but can also be seen in other types of anemias as well.

Another appropriate response is hypochromasia as identified by 70.9% of referees and 73.4% of participants. In iron deficiency anemia, cells are often smaller and have less hemoglobin which makes them appear paler than normal red cells. In the laboratory, hypochromasia can be confirmed using the MCHC, calculated as 27 g/dL, which is low in this case.

Blood Cell Identification – Graded

BCP-03

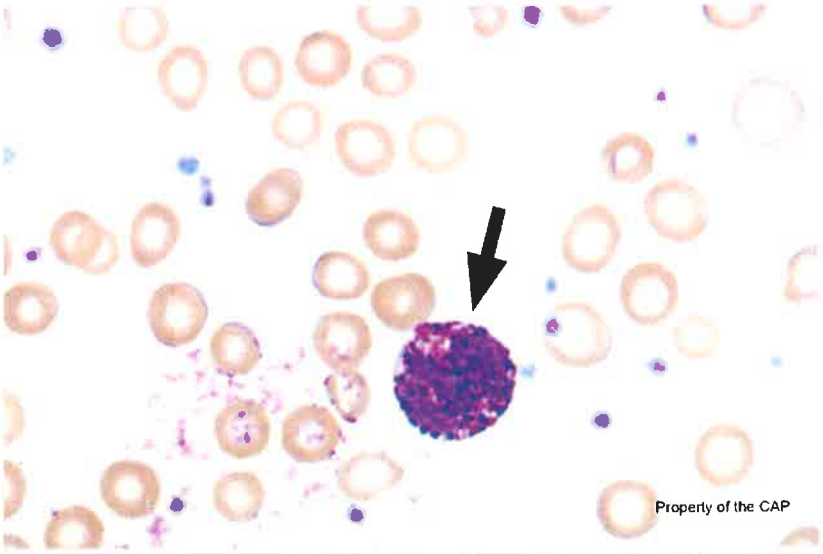


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Eosinophil, any stage	117	100.0	4974	99.9	Good

The arrowed cell is an eosinophil, as correctly identified by 100.0% of referees and 99.9% of participants. The eosinophil is characterized by coarse, orange-red granules of uniform size and is similar to a neutrophil in diameter (10 - 15 µm). Normally, the nucleus demonstrates condensed chromatin and nuclear segmentation with two or three nuclear lobes.

Blood Cell Identification – Graded

BCP-04

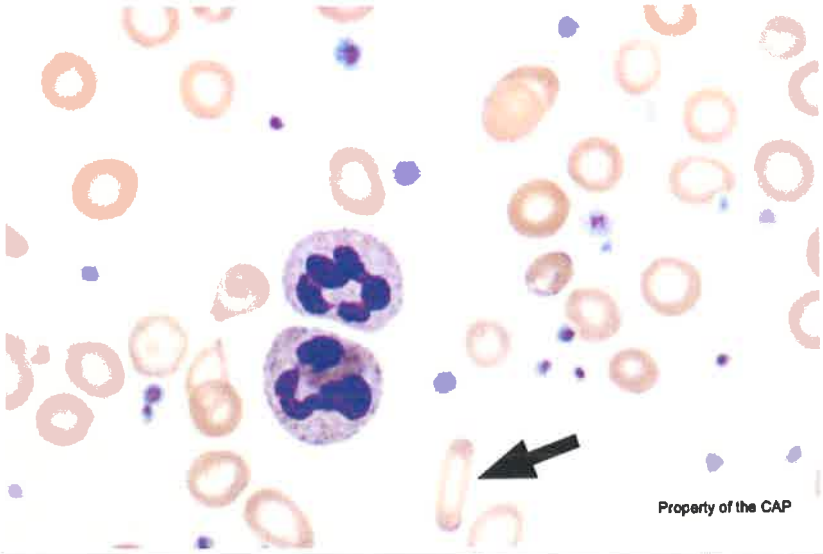


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Basophil, any stage	116	99.2	4968	99.8	Good
Basophilic stippling (coarse)	1	0.8	4	0.1	Unacceptable

The arrowed cell is a basophil, as correctly identified by 99.2% of referees and 99.8% of participants. Basophils are the least common circulating granulocytes. Unlike neutrophils with 3 - 5 lobed nuclei and fine pink or eosinophilic granules, basophils typically have only two prominent nuclear lobes and cytoplasm with numerous dense purple or basophilic granules, often obscuring the nuclear detail. Basophils are an important part of the allergic immune response, and infrequently circulate in appreciable number (typically representing < 0.3% of peripheral leukocytes).

Blood Cell Identification – Graded

BCP-05



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Ovalocyte (elliptocyte)	117	100.0	4958	99.6	Good

The arrowed cell is an ovalocyte (elliptocyte), as correctly identified by 100.0% of referees and 99.6% of participants. These cells are often seen in patients with iron deficiency anemia. They have blunt ends and parallel sides which help differentiate ovalocytes from sickle cells. They are also seen in patients with hereditary elliptocytosis (greater than 25% of erythrocytes).

Case Presentation:

This blood film is from a 20-year-old woman with a month long history of appearing pale and feeling fatigued. Laboratory data include: WBC = 18.2 x 10E9/L; RBC = 2.12 x 10E12/L; HGB = 3.2 g/dL; HCT = 11.8%; MCV = 56 fL; MCHC = 27.1 g/dL; RDW = 22%; and PLT = 839 x 10E9/L.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Iron deficiency anemia

Iron deficiency anemia is a very common cause of microcytic, hypochromic anemia. The etiology in adults is often blood loss, either from menstruation or loss from the gastrointestinal/genitourinary tract. If a source of blood loss is not readily apparent, the patient should be evaluated for an occult malignancy. In children and infants, deficiency may arise due to insufficient iron intake or absorption to meet growth requirements. In infants, iron deficiency anemia can occur with breast feeding around six months of age. The clinical symptoms of iron deficiency vary. If the anemia is severe, the patient will have symptoms related to diminished oxygen-carrying capacity including pallor, dizziness, fatigue, and palpitations. Rarely, patients may have nail abnormalities or suffer from cheilitis or pica.

Morphologic features of iron deficiency anemia are usually readily apparent when it is severe, as in this case. Increased central pallor in erythrocytes is often present. The normal amount of central pallor should be 1/3 or less of the diameter of the cell. If the central pallor is more than 1/2 of the cell diameter, this is considered hypochromic. The cells will also be smaller than normal erythrocytes. Normal erythrocytes should be the size of a resting (small) lymphocyte nucleus. If many of the cells are smaller than normal, this is consistent with microcytosis (reflected by the low MCV). Anisocytosis (variation in cell size as reflected by the RDW) is typically increased in iron deficiency anemia and elliptocytes (pencil cells or ovalocytes) may be seen. In addition to the red cell abnormalities, the patient may have a reactive thrombocytosis as is present in this case. Basophilic stippling should be absent. As in any cause of anemia, the cells may be widely-spaced at the feathered edge of the smear, indicating a low red blood cell count. In mild cases of iron deficiency anemia, the morphology may be nearly normal and difficult to discern on routine peripheral blood smear review. Correlation of peripheral blood smear morphology with CBC indices is necessary.

Laboratory testing can be used to confirm iron deficiency anemia. The serum ferritin will be low unless the patient has an elevated ferritin from a concomitant inflammatory disorder. The total iron-binding capacity is usually normal or high. The serum iron level is low. The percent transferrin saturation is typically less than 15% in iron deficiency anemia. An algorithmic approach to laboratory testing can be used to differentiate between iron deficiency anemia and other causes of microcytic anemia including thalassemia trait and anemia of chronic disease.

Lauren B. Smith, MD
Hematology and Clinical Microscopy Resource Committee

References:

1. Kjeldsberg CR, Perkins SL, eds. *Practical Diagnosis of Hematologic Disorders*. 5th ed. Singapore, China: American Society for Clinical Pathology; 2010.

Blood Cell Identification – Ungraded

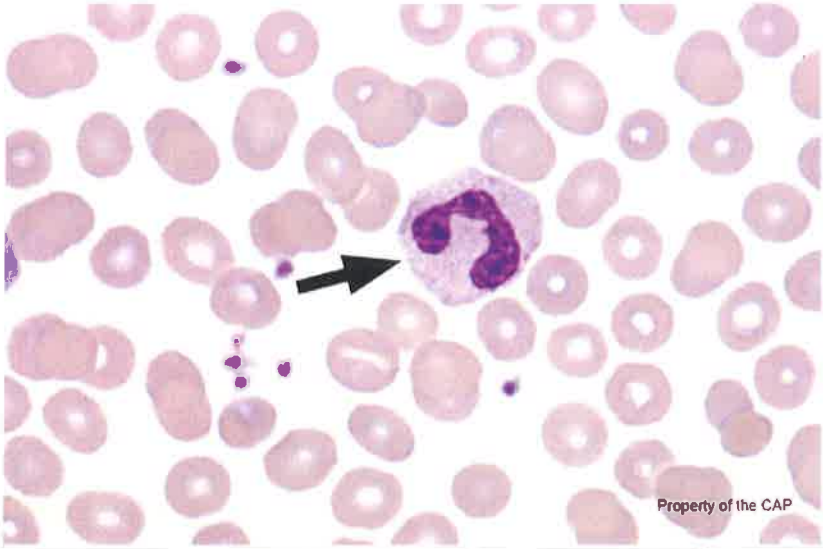
Case History

This peripheral blood smear is from a 72-year-old man presenting with painless cervical lymphadenopathy, weight loss, and fatigue. Laboratory data include: WBC = 45.2 x 10E9/L; RBC = 3.67 x 10E12/L; HGB = 10.7 g/dL; HCT = 33.0%; MCV = 90 fL; MCHC = 32.4 g/dL; RDW = 20%; and PLT = 200 x 10E9/L. Identify the arrowed image(s).

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

To access the online Hematology Glossary, please click the hyperlink below:
http://www.cap.org/ShowProperty?nodePath=/UCMCon/Contribution%20Folders/WebContent/pdf/hematology-glossary.pdf

BCP-06



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Neutrophil, segmented or band	113	100.0	4909	99.6	Educational

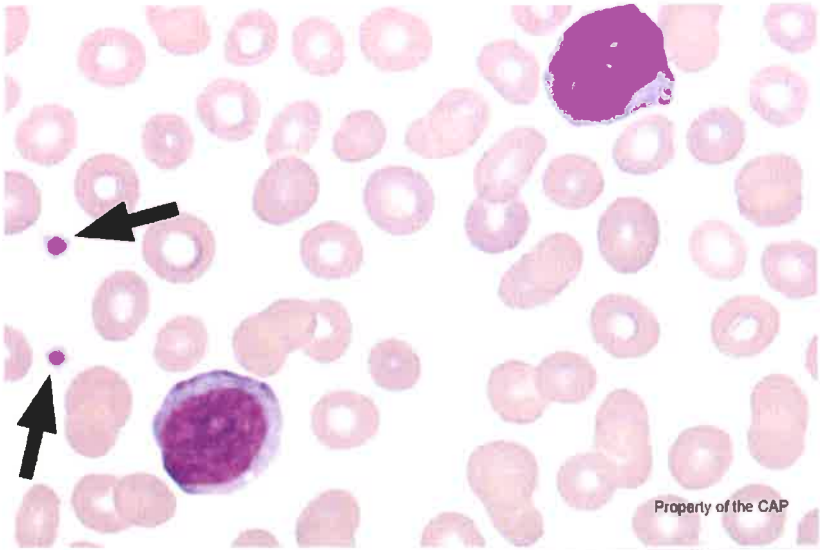
The arrowed cell is a neutrophil, segmented/band, as correctly identified by 100.0% of referees and 99.6% of participants. Segmented neutrophils, the mature cells of the myeloid series, constitute 40% to 70% of the white blood cells in the peripheral blood. Band neutrophils, also known as stabs, are the immediate precursors of segmented neutrophils and constitute 5% - 10% of the white blood cells in the peripheral blood during normal conditions. Increased numbers of bands appear in the blood in a number of physiologic and pathologic states. The band is round to oval and 10 - 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 in no area is the chromatin condensed to a single filament. The nucleus can assume many shapes: it can be band-like; sausage-like; S-, C-, or U-shaped; and twisted and 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 mimics the band in size (10 - 15 µm), shape (round to oval), and cytoplasmic appearance (pale pink cytoplasm with specific granules).

BCP-06 (cont)

The N:C ratio is 1:3, the most mature of any cell in the neutrophilic series, and the nuclear chromatin is condensed. The nucleus is segmented or lobated (two to five lobes normally). The lobes are connected by a thin filament that contains no internal chromatin, giving it the appearance of a solid, thread-like dark 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 these maturational stages be differentiated.

Blood Cell Identification – Ungraded

BCP-07

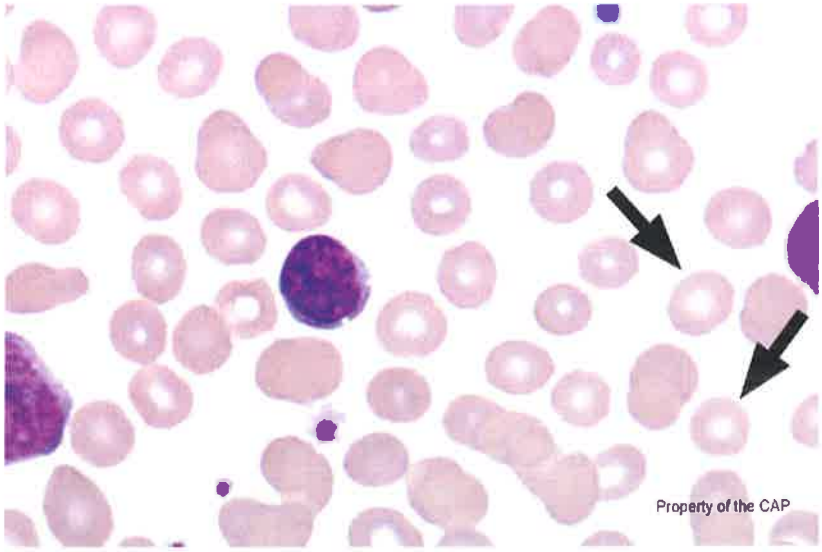


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Platelet, normal	112	99.1	4814	99.4	Educational
Platelet, hypogranular	1	0.9	10	0.2	Educational

The arrows point to platelets, normal, as correctly identified by 99.1% of referees and 99.4% of participants. Platelets, also known as thrombocytes, are small, blue-gray fragments of megakaryocytic cytoplasm. Most are 1.5 to 3 µm in diameter. A few small platelets, less than 1.5 µm in diameter, and a few large platelets, 4 - 7 µm in diameter, can 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 – Ungraded

BCP-08

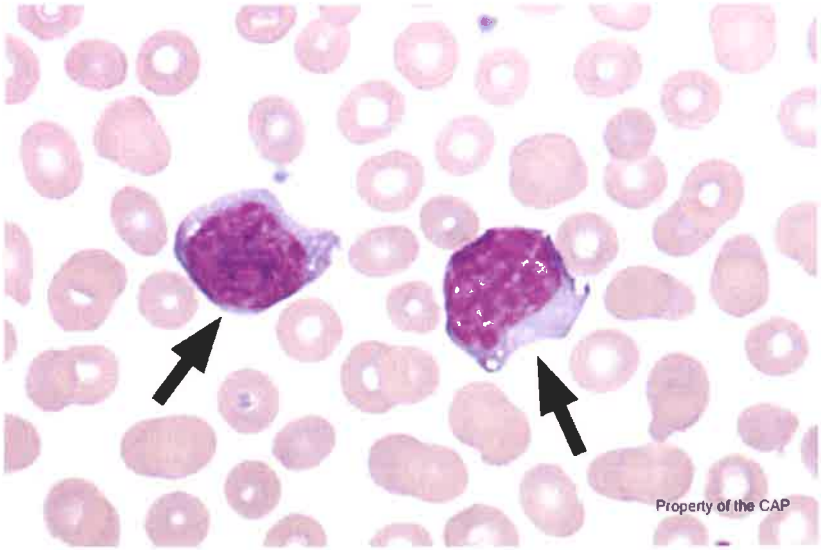


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Erythrocyte, normal	112	99.1	4615	95.3	Educational
Microcyte (with increased central pallor)	1	0.9	119	2.5	Educational

The arrowed cells are erythrocytes, normal, as correctly identified by 99.1% of referees and 95.3% of participants. An erythrocyte is a mature, non-nucleated biconcave cell of fairly uniform diameter (6.7 - 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 - 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.

Blood Cell Identification – Ungraded

BCP-09



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Malignant lymphoid cell (other than blast)	48	42.5	1630	33.7	Educational
Monocyte	20	17.7	725	15.0	Educational
Lymphocyte, reactive (to include plasmacytoid and immunoblastic forms)	19	16.8	1001	20.7	Educational
Blast cell	11	9.7	657	13.6	Educational
Monocyte, immature (promonocyte, monoblast)	4	3.5	384	7.9	Educational
Neutrophil, myelocyte	2	1.8	94	1.9	Educational
Lymphocyte	1	0.9	74	1.5	Educational
Neutrophil, promyelocyte	1	0.9	22	0.5	Educational

The arrowed cells are malignant lymphoid cells (other than blasts), as correctly identified by 42.5% of referees and 33.7% of participants. Lymphoma cells can exhibit a variety of appearances depending on the lymphoma subtype and definitive diagnosis can be difficult. These cells can exhibit a variety of sizes, shapes, and nuclear and cytoplasmic characteristics. Cell size ranges from 8 - 30 µm, and the nuclear to cytoplasmic 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. Supplemental studies, such as immunophenotyping, are often necessary to arrive at a definitive diagnosis. In blood smears, it may be difficult to distinguish reactive lymphocytes from lymphoma cells. However, careful examination can aid in distinguishing these two. The nuclear to cytoplasmic ratio tends to be low in reactive lymphocytes, while it is high in lymphoma cells. In addition, reactive lymphocytes are characterized by a spectrum of morphologic appearances within the same blood smear. In contrast, while lymphoma cells can exhibit a wide range of morphologic appearances, any individual case tends to show a more monotonous population of the abnormal cells.

BCP-09 (cont)

17.7% of referees and 15.0% of participants chose monocyte. Monocytes are slightly larger than neutrophils, ranging from 12 - 20 µm in diameter. The majority of monocytes are round with smooth edges, but some may have pseudopod-like cytoplasmic extensions. The cytoplasm is abundant, with a gray or gray-blue ground-glass appearance, and may contain vacuoles or fine, evenly distributed azurophilic granules. The nuclear to cytoplasmic ratio ranges from 4:1 to 2:1. The nucleus is usually indented, often resembling a three-pointed hat, but it can also be folded or band-like. The chromatin is condensed, but is usually less dense than that of a neutrophil or lymphocyte. Nucleoli are generally absent, but occasional monocytes may contain a small, inconspicuous nucleolus. Monocyte is an incorrect choice in this example as the cells within the photomicrograph show prominent nucleoli. Moreover, the nuclear to cytoplasmic ratios are more increased than typically seen in monocytes.

16.8% of referees and 20.7% of participants chose lymphocyte, reactive. The key distinguishing feature of reactive lymphocytes is their wide range of cellular sizes and shapes, as well as nuclear sizes, shapes, and chromatin patterns. These lymphocytes are reacting to an immune stimulus and are frequently increased in viral illnesses. 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 - 25 µm in size with a nuclear to cytoplasmic ratio that varies from 3:1 to 1:2. Lymphocyte, reactive is an incorrect choice in this example as the cells within the photomicrograph are monotonous in appearance, consistent with a neoplastic/clonal process (ie, lymphoma).

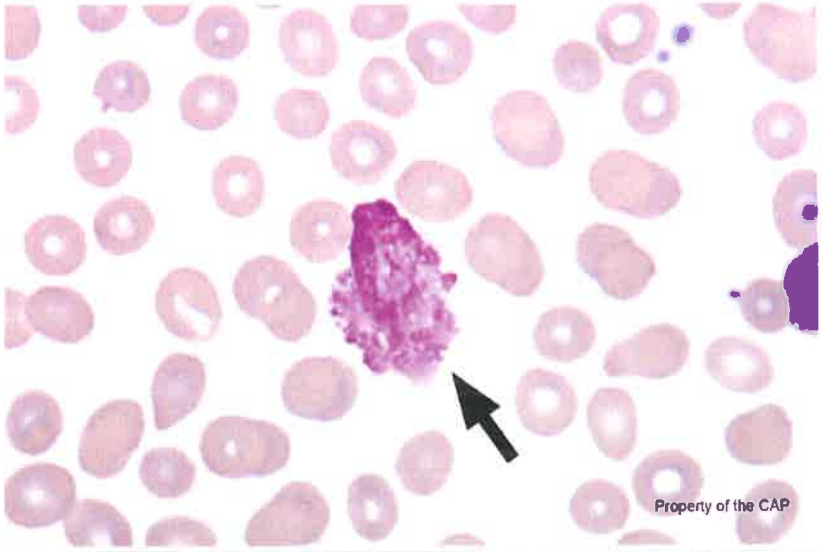
9.7% of referees and 13.6% of participants chose blast. A blast is a large, round-to-oval cell, 10 - 20 µm in diameter. The nuclear to cytoplasmic ratio is high, varying from 7:1 to 5:1. The blast often has a round to oval nucleus, but sometimes it is indented or folded. The blast cell has fine, lacy, 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 frequently 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. In the absence of Auer rods, immunophenotyping is required to determine the lineage of a given blast cell. 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. Given that confirmatory testing was not provided in this example, blast is an acceptable choice. However, the chromatin pattern within the cells in the photomicrograph is more condensed than typically seen within a blast. Therefore, malignant lymphoid cell is the more appropriate choice.

BCP-09 (cont)

3.5% of referees and 7.9% of participants chose monocyte, immature. For the purposes of proficiency testing, selection of the response "monocyte, immature" should be reserved for malignant cells in the context of acute monocytic/monoblastic leukemia, acute myelomonocytic leukemia, chronic myelomonocytic leukemia, or myelodysplastic syndromes (ie, promonocytes and monoblasts). The malignant monoblast is a large cell, usually 15 - 25 µm in diameter. It has relatively more cytoplasm than a myeloblast with the nuclear to cytoplasmic ratio ranging from 7:1 to 3:1. The monoblast nucleus is round or oval and has finely dispersed chromatin and distinct nucleoli. The cytoplasm is blue to gray-blue and may contain small, scattered azurophilic granules. Some monoblasts cannot be distinguished morphologically from other blast forms; in these instances, additional tests are required to accurately assign blast lineage. Promonocytes have nuclear and cytoplasmic characteristics that are between those of monoblasts and mature monocytes. They are generally larger than mature monocytes, but they have similar-appearing gray-blue cytoplasm that often contains uniformly distributed, fine azurophilic granules. Cytoplasmic vacuolization is not a typical feature. The nuclei show varying degrees of lobulation, usually characterized by delicate folding or creasing of the nuclear membrane, in contrast to the rounder nuclear profile of monoblasts. Nucleoli are present but may not be as distinct as in monoblasts. Monocyte, immature is an incorrect choice in this example as the cells within the photomicrograph show more condensed chromatin than would be expected for a monoblast/promonocyte (ie, monocyte, immature).

Blood Cell Identification – Ungraded

BCP-10



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Basket cell/smudge cell	113	100.0	4773	98.6	Educational

The arrowed cell is a basket cell/smudge cell, as correctly identified by 100.0% of referees and 98.6% of participants. Basket cells 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 the chromatin strands may spread 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.

Case Presentation:

This peripheral blood smear is from a 72-year-old man presenting with painless cervical lymphadenopathy, weight loss, and fatigue. Laboratory data include: WBC = 45.2 x 10E9/L; RBC = 3.67 x 10E12/L; HGB = 10.7 g/dL; HCT = 33.0%; MCV = 90 fL; MCHC = 32.4 g/dL; RDW = 20%; and PLT = 200 x 10E9/L.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Mantle cell lymphoma

The CBC indices are indicative of leukocytosis with accompanying anemia. Platelets are normal in number. Review of the images reveals malignant lymphoid cells. The malignant lymphoid cells are quite large in size, using background red blood cells for reference. They have partially condensed chromatin with prominent nucleoli. Basophilic cytoplasm is seen with few cytoplasmic vacuoles. Although it is not possible to sub-classify this lymphoma on morphology alone, the cells are clearly neoplastic given the aforementioned features and their monotonous appearance.

Further workup of this patient, including immunophenotyping by flow cytometry, reveals findings diagnostic of mantle cell lymphoma. Mantle cell lymphoma is a mature B-cell neoplasm, which comprises approximately 5-10% of all non-Hodgkin lymphomas in the United States. It typically occurs in middle aged or older individuals with a male predominance. Lymph nodes are the most common involved site. The spleen, peripheral blood, and bone marrow are frequently involved as well. Moreover, gastrointestinal involvement, sometimes in the form of lymphomatous polyposis, is not uncommon. Most patients present with high stage disease (stage III or IV), which correlates with poor clinical outcome. Unlike the other "small B-cell lymphomas", mantle cell lymphoma is not an indolent disease with a median survival of only approximately 3 - 5 years. However, a subset of mantle cell lymphoma patients will have a more indolent disease course. Today, there is no definitive marker which identifies indolent cases. However, a subset of indolent cases are characterized by leukemic, non-nodal presentation, splenomegaly, mutated immunoglobulin genes, low CD38 expression, interstitial involvement of the bone marrow (ie, non-nodular), and a low number of genomic aberrations. These cases are frequently SOX11 negative by immunohistochemistry.

Although mantle cell lymphoma is typically comprised of monotonous small to medium sized lymphoid cells with irregular nuclear contours, a spectrum of morphologic variants are recognized including the blastoid and pleomorphic variants. These two variants are significant with poorer prognosis noted. The blastoid variant may resemble lymphoblasts with more dispersed chromatin. The pleomorphic variant shows many large cells with oval to irregular nuclear contours and prominent nucleoli, as in our case.

Immunophenotyping studies, via flow cytometry and/or immunohistochemistry, are routinely performed in the workup of patients with possible lymphoma. Mantle cell lymphoma frequently expresses intense surface IgM/IgD with lambda light chain restriction. The lymphoma cells usually express CD5, FMC-7, and CD43. They are typically negative for CD10 and BCL6, markers of germinal center derivation. Unlike chronic lymphocytic leukemia/small lymphocytic lymphoma (another CD5 positive non-Hodgkin B-cell lymphoma), CD23 is usually negative. Almost all cases express cyclin D1. Cyclin D1 negative cases can be identified via SOX11 staining. Cytogenetic analysis will usually show t(11;14)(q13;q32), which results in an abnormal *IGH-CCND1* (cyclin D1) fusion gene that drives lymphomagenesis in these patients.

Natasha M. Savage, MD, FCAP
Hematology and Clinical Microscopy Resource Committee

References:

1. Sander B, Quintanilla-Martinez L, Ott G, et al. Mantle cell lymphoma-a spectrum from indolent to aggressive disease. *Virchows Arch*. 2016;468(3):245-257.

2. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*. 2016;127(20):2375-2390.

COLLEGE of AMERICAN PATHOLOGISTS

Attestation of Participation of Self-Reported Training*

We the participants below have completed the review of the CAP FH9-A 2018 (Product Mailing, Year) Participant Summary/Final Critique report and can self-report the recommended 0.5 (Education Hours) hours towards fulfilling education and certification of maintenance requirements.

Participant	Date	Participant	Date

Director (or Designee) Signature - I have verified that the individuals listed above have successfully participated in this activity. Date

Retain this page for record-keeping and auditing purposes.

1. Go to www.cap.org
2. Click **LOG IN / LOG IN** and enter your User ID and Password.
   - If you are unsure whether you have an *individual* web account with the CAP, or do not remember your user ID and password, click on **PASSWORD HINT**.
   - If you do not have an *individual* web account, click **CREATE AN ACCOUNT**. Complete and submit the account request form. You will be notified within one business day that your individual account has been activated.
3. Click **Learning** from the top menu bar
4. Click **Learning Transcript** from the menu bar
5. Click **Add My Own Activity**
6. Follow prompts to enter 'Self-Reported Training Activities'.

For assistance, call our Customer Contact Center at 800-323-4040 or 847-832-7000 option 1.

**CAP Self-Reported Training activities do not offer CE credit, but can be used towards fulfilling requirements for certification of maintenance by agencies such as the American Society of Clinical Pathology (ASCP). Please verify with your certifying agency to determine your education requirements.*