Blood Cell Identification – Graded

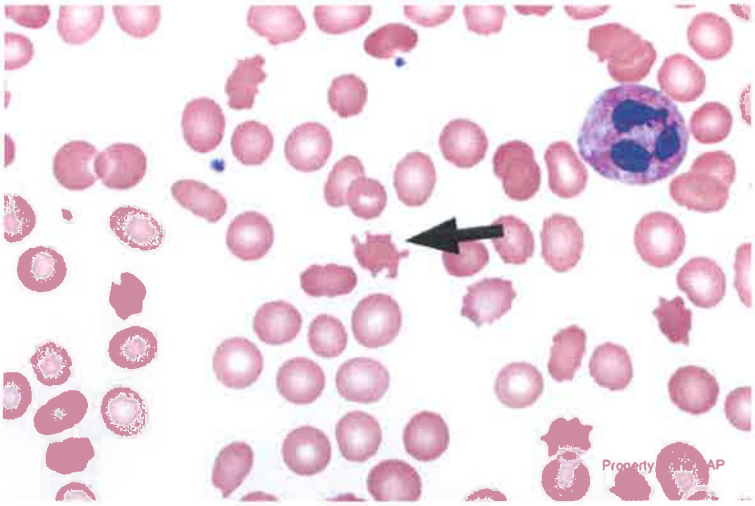
Case History

This peripheral blood smear is from a 61-year-old man with past medical history of alcohol abuse presenting with liver cirrhosis, cardiomyopathy, and anemia. Laboratory data includes: WBC = 12.5 × 10E9/L; RBC = 3.74 × 10E12/L; HGB = 11.5 g/dL; HCT = 32.9%; MCV = 99.0 fL; MCHC = 35.0 g/dL; PLT = 160 × 10E9/L; MPV = 11.5 fL; and RDW = 16.2%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

To access the online Hematology Glossary, please click the hyperlink below:
https://cap.objects.frb.io/documents/2019-hematology-clinical-microscopy-glossary.pdf

BCP-01



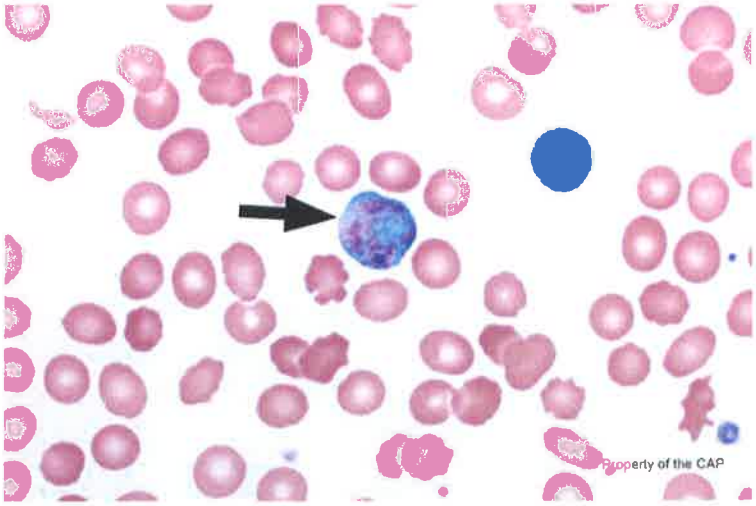
Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Acanthocyte (spur cell)	138	95.2	5139	94.2	Good
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	7	4.8	283	5.2	Unacceptable

The arrowed cell is an acanthocyte (spur cell), as correctly identified by 95.2% of referees and 94.2% of participants. Acanthocytes are densely staining red blood cells that lack central pallor and have multiple irregularly distributed, thorn-like spicules of variable size, often with drumstick ends. While classically associated with hereditary abetalipoproteinemia, acanthocytes may be seen in a variety of other conditions, including cirrhosis. Acanthocytes are rarely encountered in otherwise normal blood smears. Acanthocytes need to be distinguished from echinocyte (burr cells). Echinocytes are red blood cells with 10 - 30 uniform, short, blunt projections distributed evenly that impart a serrated appearance to the red blood cell surface. Echinocytes retain central pallor and are the same size or slightly smaller than normal red blood cells. Echinocytes may be seen as an artifact of smear preparation and in cases of severe renal failure. BCP-04 contains further description and is an example of an echinocyte.

4.8% of referees and 5.2% of participants incorrectly identified the arrowed cell as a fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell). While a subset of fragmented red blood cells (keratocytes) feature spicules, these spicules are typically paired and separated by a concave region, in contrast to the more numerous, irregularly distributed spicules typical of acanthocytes and exhibited by the arrowed cell.

Blood Cell Identification – Graded

BCP-02



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Platelet, giant (macrothrombocyte)	85	58.6	3333	61.2	Non-consensus
Neutrophil necrobiosis (degenerated neutrophil)	16	11.0	450	8.3	Non-consensus
Immature or abnormal cell, would refer	14	9.7	425	7.8	Non-consensus
Megakaryocyte (normal, abnormal, or nuclear fragment)	7	4.8	261	4.8	Non-consensus
Polychromatophilic (non-nucleated) red blood cell	6	4.1	161	3.0	Non-consensus
Basophilic stippling (coarse)	3	2.1	138	2.5	Non-consensus
Lymphocyte, large granular	2	1.4	15	0.3	Non-consensus
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	2	1.4	66	1.2	Non-consensus
Plasmodium sp. (malaria)	2	1.4	70	1.3	Non-consensus
Blast cell	1	0.7	29	0.5	Non-consensus
Howell-Jolly body	1	0.7	33	0.6	Non-consensus
Mitotic figure	1	0.7	64	1.2	Non-consensus
Nucleated red blood cell, normal or abnormal morphology	1	0.7	70	1.3	Non-consensus
Neutrophil, promyelocyte	1	0.7	11	0.2	Non-consensus
Neutrophil, toxic (to include toxic granulation and or Döhle bodies, and/or toxic vacuolization)	1	0.7	38	0.7	Non-consensus
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	1	0.7	37	0.7	Non-consensus
Platelet, hypogranular	1	0.7	16	0.3	Non-consensus

Blood Cell Identification – Graded

BCP-02 (cont)

The arrowed object is a giant platelet, as correctly identified by 58.6% of referees and 61.2% of participants. Giant platelets have diameters exceeding 7 µm, and they typically measure 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 provided MCV of 99 fL in this case supports classifying the arrowed platelet as giant. Giant platelets are a rare finding in normal peripheral blood, but they may be encountered in a variety of reactive, neoplastic, and inherited conditions. Reactive causes include conditions in which platelet turnover is markedly increased, such as immune thrombocytopenia or severe leukemoid reactions. Giant platelets are most often seen in myeloproliferative neoplasms and myelodysplastic syndromes.

11.0% of referees and 8.3% of participants identified the arrowed object as representing neutrophil necrobiosis (degenerated neutrophil). Degenerated neutrophils generally resemble normal segmented neutrophils in terms of size, cytoplasmic characteristics (pale pink with fine granules), and nuclear segmentation. The major distinguishing feature is that the nucleus shows karyorrhexis and/or pyknosis; that is, the chromatin is dense and homogeneous.

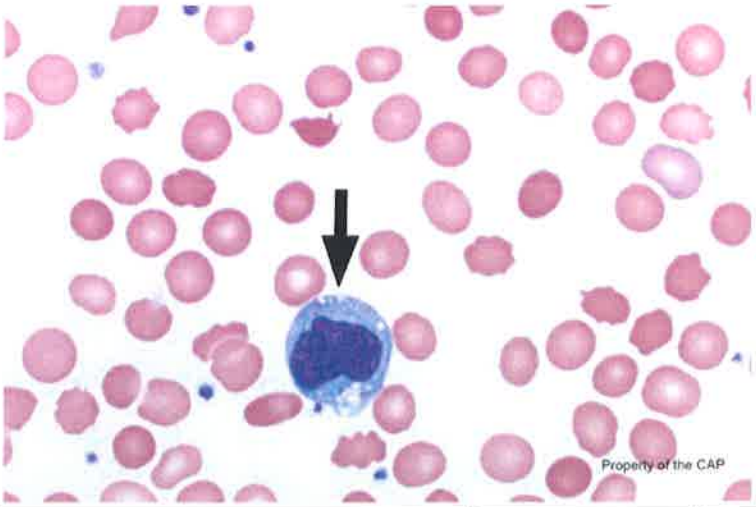
4.8% of referees and 4.8% of participants identified the arrowed object as representing a megakaryocyte (normal, abnormal, or nuclear fragment). While normal mature megakaryocytes are not found in the peripheral blood, megakaryocyte nuclei and micromegakaryocytes may be seen infrequently. Megakaryocyte nuclei feature either a very scant amount of basophilic cytoplasm or no cytoplasm at all. Micromegakaryocytes are abnormally small megakaryocytes that usually measure 20 µm or less in diameter with an N:C ratio of 1:1 or 1:2. The nucleus may be hypolobated or may have multiple small lobes. The cytoplasm is pale blue and may contain pink granules. The presence of micromegakaryocytes in the peripheral blood is usually associated with a myeloproliferative neoplasm or myelodysplastic syndrome.

4.1% of referees and 3.0% of participants identified the arrowed object as a polychromatophilic (non-nucleated) red blood cell. The polychromatophilic red blood cell stains homogeneously pink-gray or pale purple with Romanowsky or Wright-Giemsa stain. Deep blue granular and/or filamentous structures may be seen when these cells are stained using supravital stains such as new methylene blue.

9.7% of referees and 7.8% of participants identified the arrowed object as an immature or abnormal cell, would refer for identification. Immature cells such as blasts have nuclei with finely reticulated chromatin and typically have a high N:C ratio.

Blood Cell Identification – Graded

BCP-03

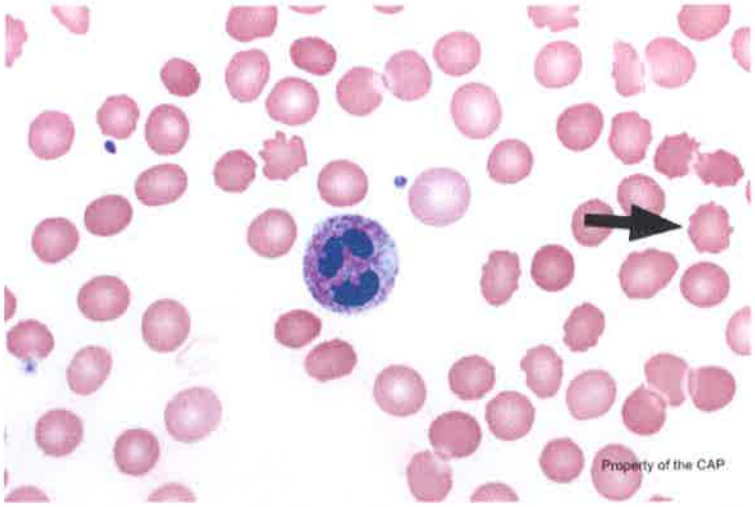


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Monocyte	143	98.6	5345	98.0	Good
Monocyte, immature (promonocyte, monoblast)	2	1.4	65	1.2	Unacceptable

The arrowed cell is a monocyte, as correctly identified by 98.6% of referees and 98.0% of participants. Monocytes are large cells (12 to 20 µm in diameter) with abundant gray or gray-blue cytoplasm that may contain vacuoles and/or fine, evenly distributed azurophilic granules as seen here. 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.

Blood Cell Identification – Graded

BCP-04



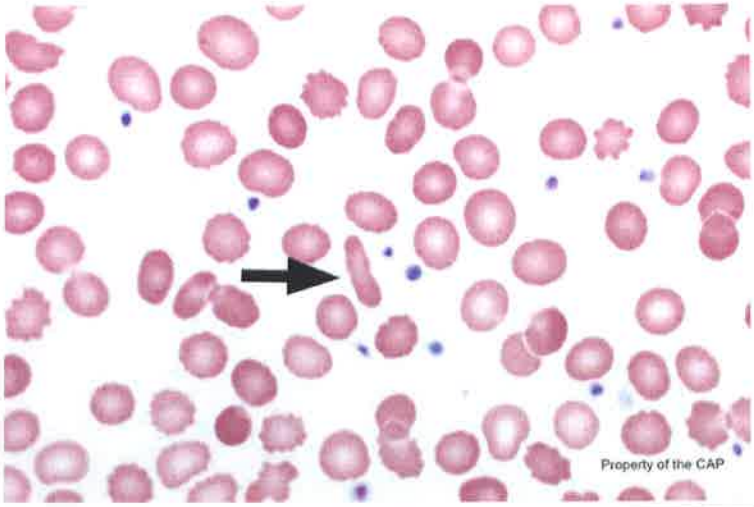
Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Echinocyte (burr cell, crenated cell)	145	100.0	5396	99.0	Good

The arrowed cell is an echinocyte (burr cell), as correctly identified by 100.0% of referees and 99.0% of participants. Echinocytes are red blood cells with 10 - 30 uniform, short, blunt projections distributed evenly that impart a serrated appearance to the red blood cell surface. Echinocytes retain central pallor and are the same size or slightly smaller than normal red blood cells. Their appearance often results from improper smear preparation (slow drying, excessive thickness of smear, use of aged blood). Echinocytes that are not artifacts may be indicative of disease, such as uremia or pyruvate kinase deficiency, and are seen post-splenectomy, in hepatitis of the newborn, and in phosphoglycerate kinase deficiency.

Echinocytes need to be distinguished from acanthocytes (spur cells). Acanthocytes are densely staining red blood cells that lack central pallor and have multiple irregularly distributed, thorn-like spicules of variable size, often with drumstick ends. BCP-01 contains further description and is an example of an acanthocyte.

Blood Cell Identification – Graded

BCP-05



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Ovalocyte (elliptocyte)	145	100.0	5422	99.4	Good

The arrowed cell is an ovalocyte (elliptocyte), as correctly identified by 100.0% of referees and 99.4% of participants. The ovalocyte is characterized by the shape of a pencil or thin cigar, with blunt ends and parallel sides. As seen in the arrowed example, hemoglobin is often concentrated at the ends of the ovalocyte. Small numbers of ovalocytes may be present on the smears of normal individuals, while markedly elevated numbers are observed in patients with hereditary elliptocytosis, an abnormality of erythrocyte skeletal membrane proteins. Ovalocytes are also commonly increased in number in iron deficiency and may be seen in other types of anemia.

Case Presentation:

This peripheral blood smear is from a 61-year-old man with past medical history of alcohol abuse presenting with liver cirrhosis, cardiomyopathy, and anemia. Laboratory data includes: WBC = 12.5 × 10E9/L; RBC = 3.74 × 10E12/L; HGB = 11.5 g/dL; HCT = 32.9%; MCV = 99.0 fL; MCHC = 35.0 g/dL; PLT = 160 × 10E9/L; MPV = 11.5 fL; and RDW = 16.2%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Cirrhosis and anemia

Cirrhosis results from injury to the liver and is characterized by fibrosis and the formation of abnormal nodules that disrupt normal hepatic architecture. Cirrhosis represents the final common pathologic pathway of many chronic liver diseases. Chronic hepatitis C virus infection, alcoholism, and non-alcoholic fatty liver disease are common causes of cirrhosis in Western industrialized nations, while chronic hepatitis B virus infection is a leading cause in the Asia-Pacific region.

While thrombocytopenia is the cytopenia most frequently associated with cirrhosis, anemia is also common in this setting, and its etiology may be multifactorial. Causes of anemia may include hemorrhage, hypersplenism, nutritional deficiencies, and hemolysis. Decreased synthesis of coagulation factors and of thrombopoietin, with the latter contributing to thrombocytopenia, can result in hemorrhage, especially in the gastrointestinal tract. Splenic enlargement resulting from portal hypertension is associated with sequestration and destruction of red blood cells (along with platelets and leukocytes). Deficiencies of folate and/or vitamin B12 may result from poor nutrition or malabsorption in the gastrointestinal tract and contribute to a macrocytic anemia associated with megaloblastic erythropoiesis. Some of the toxins and viruses that cause hepatic injury, including alcohol and hepatitis C virus, have direct or indirect deleterious effects on erythrocyte production in the bone marrow.

Abnormalities of lipid metabolism in patients with cirrhosis can increase the cholesterol content of red blood cell membranes, a factor associated with the formation of acanthocytes. The acanthocyte (derived from the Greek *akantha*, meaning "thorn") has irregularly distributed, thorn-like spicules that vary in size. Spur cells are thought to represent acanthocytes that have undergone remodeling in the enlarged spleen, resulting in blunting of the spicules and a more rounded, spherocytic appearance. Such changes render the red blood cells more inflexible and susceptible to hemolysis, and "spur cell anemia" is a type of hemolytic anemia encountered in patients with advanced cirrhosis. Spur cell anemia has been associated with increased mortality among patients with cirrhosis; resolution of spur cell anemia has been observed following liver transplantation.

Michael R. Lewis, MD
Hematology and Clinical Microscopy Committee

References:

1. Gonzalez-Casas R, Jones EA, Moreno-Otero R. Spectrum of anemia associated with chronic liver disease. *World J Gastroenterol.* 2009;15:4653-4658.
2. Alexopoulou A, Vasilieva L, Kanellopoulou T, et al. Presence of spur cells as a highly predictive factor of mortality in patients with cirrhosis. *J Gastroenterol Hepatol.* 2014;29:830-834.

Blood Cell Identification – Ungraded

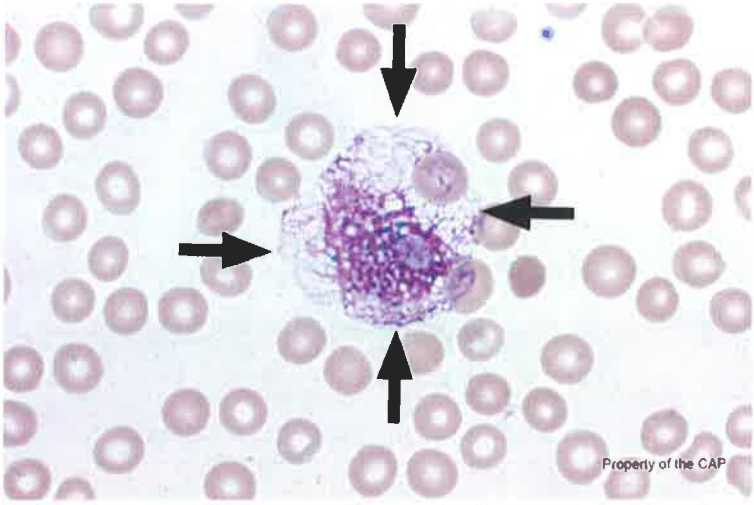
Case History

This peripheral blood smear is from a 49-year-old woman with a past medical history of hypertension presenting with fatigue. Laboratory data includes: WBC = 159.2 × 10E9/L; RBC = 2.98 × 10E12/L; HGB = 10.1 g/dL; HCT = 29.7%; MCV = 90.6 fL; MCHC = 34.0 g/dL; PLT = 90 × 10E9/L; MPV = 10.0 fL; and RDW = 14.5%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

To access the online Hematology Glossary, please click the hyperlink below:
https://cap.objects.frb.io/documents/2019-hematology-clinical-microscopy-glossary.pdf

BCP-06

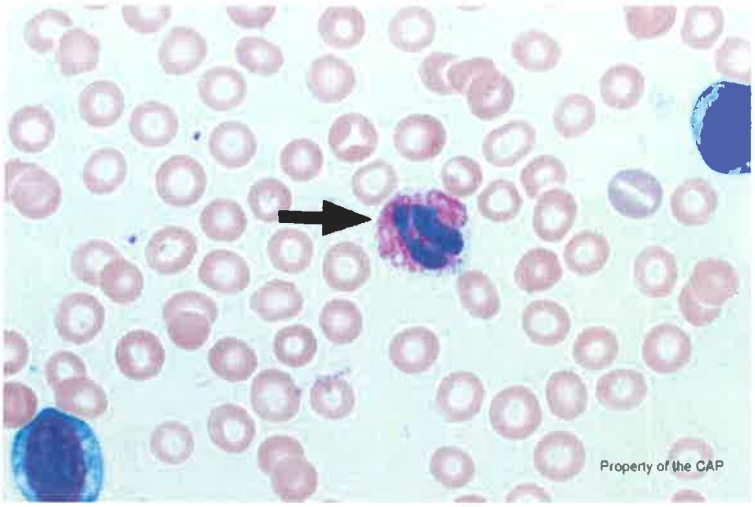


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Basket cell/smudge cell	139	97.2	5247	97.3	Educational
Stain precipitate	2	1.4	110	2.0	Educational
Neutrophil necrobiosis (degenerated neutrophil)	1	0.7	7	0.1	Educational
Squamous epithelial cell/endothelial cell	1	0.7	2	0.0	Educational

The arrowed cell is a basket/smudge cell which has fragmented due to fragility, as correctly identified by 97.2% of referees and 97.3% of participants. The cytoplasm of basket/smudge cells is typically absent or may be indistinct. An albumin preparation is typically used when many basket cells are present for cellular preservation in order to identify them. Basket cells should not be counted in differential counts. Basket cells can be increased in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), other lymphomas within the blood, infectious mononucleosis, and some lymphoblastic leukemias.

Blood Cell Identification – Ungraded

BCP-07

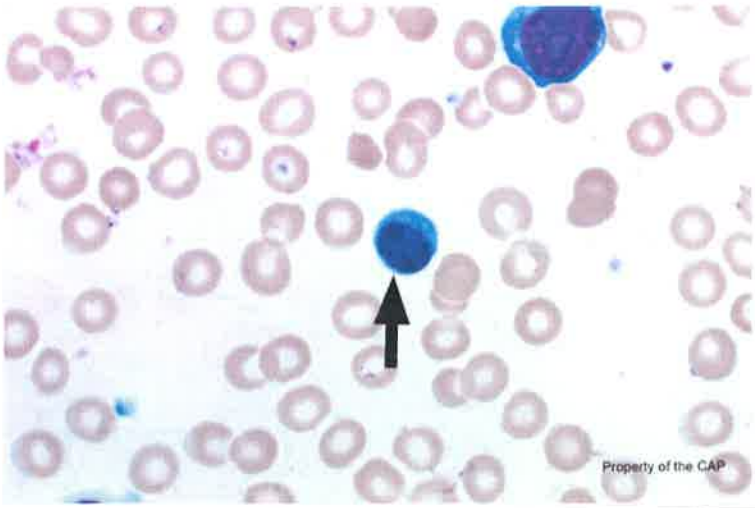


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Eosinophil	142	99.3	5306	99.7	Educational
Neutrophil, toxic (to include toxic granulation and or Döhle bodies, and/or toxic vacuolization)	1	0.7	7	0.1	Educational

The arrowed cell is an eosinophil, as correctly identified by 99.3% of referees and 99.7% of participants. Eosinophils are round to oval with uniform, coarse orange-red granules. About 80% have bilobed nuclei while the remainder may have greater than two lobes. They are the same size as neutrophils, but can be readily differentiated from neutrophils, which have finer granules and usually at least three nuclear lobes. Eosinophils may be increased in certain myeloid neoplasms, in hypereosinophilic syndrome, and in reactive conditions, such as infection or medication use.

Blood Cell Identification – Ungraded

BCP-08

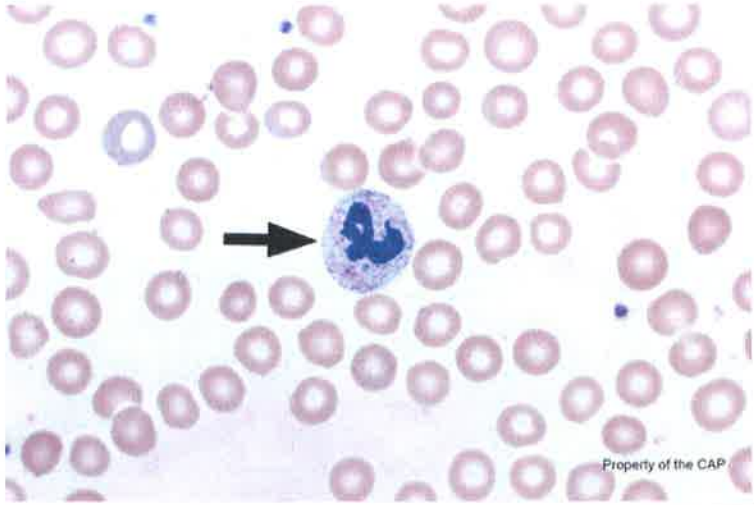


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Lymphocyte	138	96.5	5178	97.3	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	2	1.4	67	1.3	Educational
Nucleated red blood cell, normal or abnormal morphology	2	1.4	48	0.9	Educational
Malignant lymphoid cell (other than blast)	1	0.7	6	0.1	Educational

The arrowed cell is a resting lymphocyte, as correctly identified by 96.5% of referees and 97.3% of participants. It has condensed chromatin, a thin rim of basophilic, agranular cytoplasm, and the nucleus is the size of a normal red blood cell. They range in size from 7 - 15 µm. Lymphocytes can be increased in a variety of conditions, including viral infections and younger ages.

Blood Cell Identification – Ungraded

BCP-09

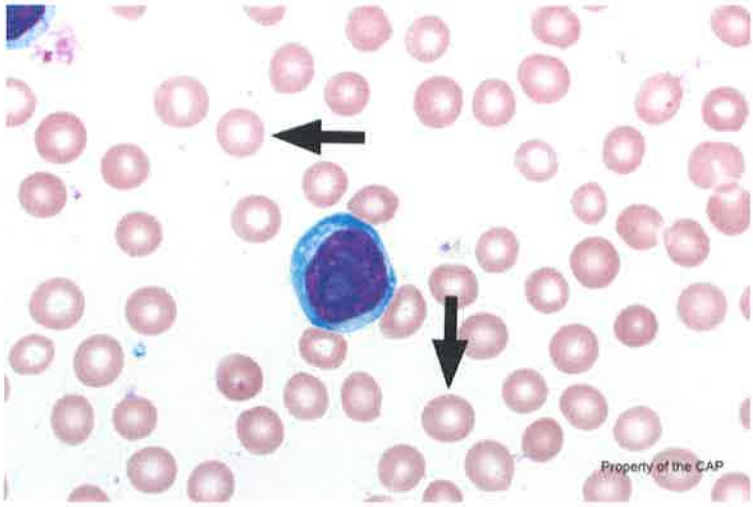


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Neutrophil, segmented or band	112	78.3	4124	77.5	Educational
Neutrophil, toxic (to include toxic granulation and or Döhle bodies, and/or toxic vacuolization)	31	21.7	1155	21.7	Educational

The arrowed cell is a neutrophil, as correctly identified by 78.3% of referees and 77.5% of participants. The nucleus is segmented and contains clumped chromatin. The cytoplasm is abundant and contains specific granules. The neutrophil also has some borderline toxic features including dark granules (although not enlarged) and a cytoplasmic inclusion that could be interpreted as a Döhle body (gray-blue inclusions of variable size), therefore, neutrophil, toxic as identified by 21.7% of referees and 21.7% of participants, is an acceptable answer. Both mature and toxic neutrophils range in size from 10 - 18 µm. The nucleus can be shaped in different ways as is evident in this case. The number of neutrophils may increase in infection or inflammatory conditions.

Blood Cell Identification – Ungraded

BCP-10



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Erythrocyte, normal	140	97.9	5154	96.8	Educational
Microcyte (with increased central pallor)	2	1.4	68	1.3	Educational
Hypochromasia	1	0.7	58	1.1	Educational

The arrowed cells are normal erythrocytes, as correctly identified by 97.9% of referees and 96.8% of participants. Erythrocytes are mature red blood cells (6 - 8 µm or approximately the size of a resting lymphocyte nucleus). Central pallor is present (1/3 of the cell diameter). These cells circulate for approximately 120 days in the blood.

Case Presentation:

This peripheral blood smear is from a 49-year-old woman with a past medical history of hypertension presenting with fatigue. Laboratory data includes: WBC = 159.2 × 10E9/L; RBC = 2.98 × 10E12/L; HGB = 10.1 g/dL; HCT = 29.7%; MCV = 90.6 fL; MCHC = 34.0 g/dL; PLT = 90 × 10E9/L; MPV = 10.0 fL; and RDW = 14.5%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: B-cell prolymphocytic leukemia (B-PLL)

B-cell prolymphocytic leukemia is a rare lymphoproliferative disorder (1% of cases). Patients are typically elderly with a median age of 65 - 70 years. Patients often have splenomegaly with blood and bone marrow involvement. Lymphadenopathy is not typical but may rarely be seen. As in this case, the absolute lymphocyte count is high, often over 100 K/µL.

B-PLL is composed of neoplastic lymphocytes with prominent central nucleoli (i.e. prolymphocytes). Greater than 55% of lymphoid cells should have a central nucleolus. Prolymphocytes typically have deep blue cytoplasm and are typically intermediate in size but may be large. B-PLL differs from chronic lymphocytic leukemia (CLL) which may have some prolymphocytes (usually fewer than 10%) but these may increase over time. B-PLL should only be diagnosed *de novo* and not in the context of CLL/SLL with increasing prolymphocytes. On histologic sections of the bone marrow, prolymphocytes have prominent central eosinophilic nucleoli similar to immunoblasts.

B-PLL has a non-specific immunophenotype by flow cytometry other than displaying a monoclonal mature B-cell phenotype (CD20 and surface immunoglobulin positive). Unlike CLL/SLL, most cases are negative for CD5 and CD23, although some cases may be positive (10 - 30%). The cells are also negative for CD10. The differential diagnosis includes other B-cell lymphoproliferative disorders that cause splenomegaly, including splenic marginal zone lymphoma (which is also CD5 negative) and mantle cell lymphoma (which is often CD5 positive but may be CD5 negative). These cases can be differentiated from leukemic mantle cell lymphoma based on the presence of t(11;14), which is seen in mantle cell lymphoma but not in B-PLL. It is often very difficult to differentiate B-PLL from splenic marginal zone lymphoma, which is much more common, and may have increased numbers of larger cells in the blood. Peripheral blood involvement by diffuse large B-cell lymphoma may also be in the differential, but that is usually not associated with significant splenomegaly.

Many cases of B-PLL have *TP53* mutations. Some cases have recently been recognized that have *MYC* translocations, gains or amplifications. B-PLL is an aggressive disease and has a poor prognosis with a median survival of 30 - 50 months.

Lauren Barrett-Smith, MD
Hematology and Clinical Microscopy Committee

Reference:

1. Swerdlow SH et al, eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Revised 4th ed. IARC: Lyon, France. 2017:222-223.

COLLEGE of AMERICAN PATHOLOGISTS

Attestation of Participation of Self-Reported Training*

We the participants below have completed the review of the CAP FH9-A 2019 (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.*