Blood Cell Identification – Graded

Case History

This peripheral blood smear is from a 35-year-old woman with past medical history of kidney disease presenting with headache and lethargy. Laboratory data includes: WBC = 11.0 x 10E9/L; RBC = 3.53 x 10E12/L; HGB = 10.7 g/dL; HCT = 30.5%; MCV = 120.0 fL; MCHC = 35.1 g/dL; PLT = 204 x 10E9/L; and RDW = 16.1%. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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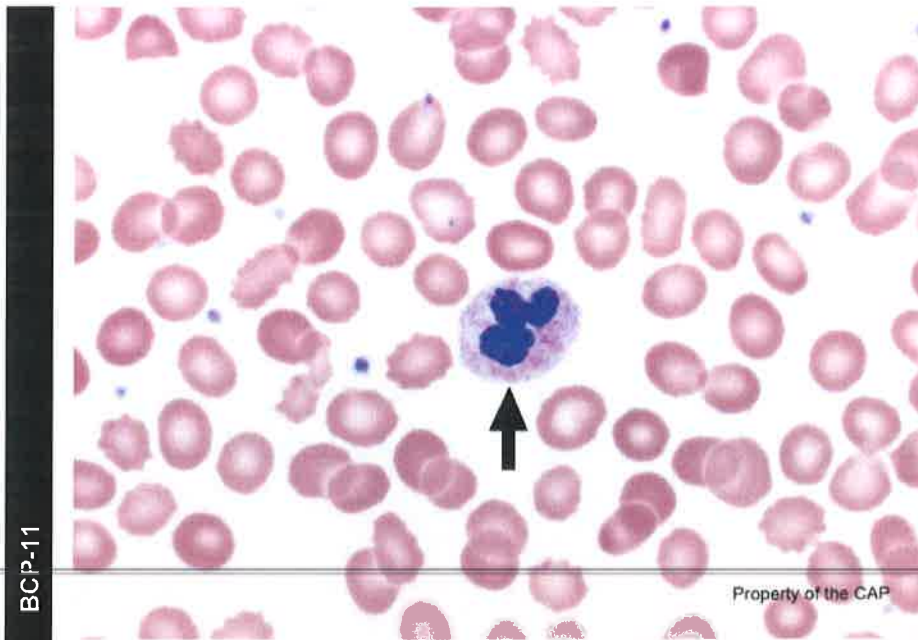
BCP-11



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Neutrophil, segmented/band	119	85.6	4946	87.6	Good
Neutrophil, toxic (to include toxic granulation and or Döhle bodies, and/or toxic vacuolization)	20	14.4	667	11.8	Acceptable

The arrowed cell is a neutrophil, segmented/band, as correctly identified by 85.6 % of referees and 87.6 % of participants. Mature neutrophils are typically the predominant leukocyte in the peripheral blood. This polymorphonuclear cell is 10 to 18 um in diameter, with N:C ratio varying from 1:1.5 to 1:3, and displaying highly condensed nuclear chromatin. An absolute and/or relative neutrophilia can be seen in a number of physiologic and pathologic states, including infectious/inflammatory processes, tissue damage or necrosis, neoplasia, poisoning or intoxication, drug effect, and metabolic abnormalities.

Blood Cell Identification – Graded

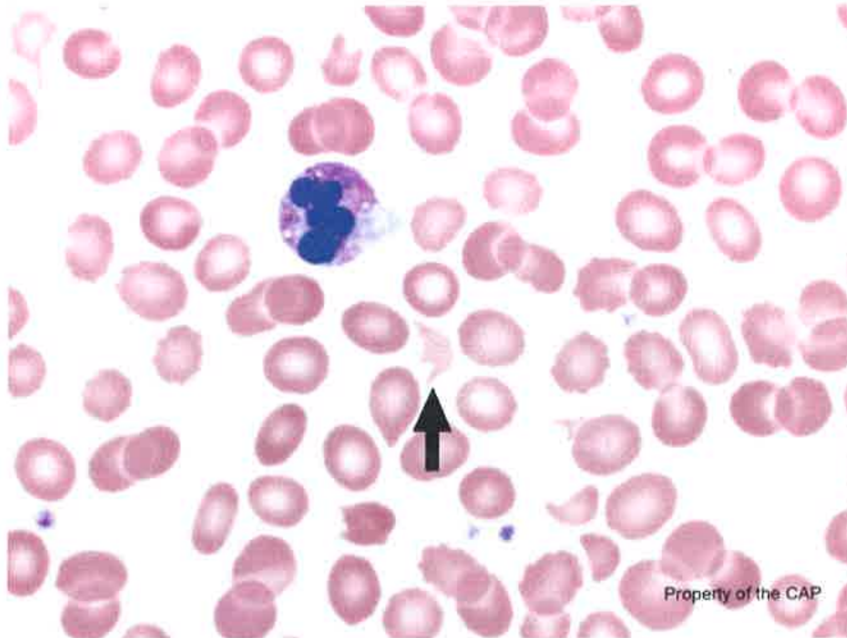
BCP-11 (cont)

The presence of thin, thread-like filaments (which lack internal chromatin) is thought to be the main distinguishing feature of segmented forms as compared to band forms. However, for the purposes of proficiency testing, differentiation of band and segmented neutrophils is not required, given the difficulty in achieving consistent and reproducible differentiation, and the questionable independent contribution of band count results to clinical management in many settings. [For a detailed guideline for the differentiation of segmented and band neutrophils, see Glassy, 2018; for other reading related to the clinical utility of band counts, see Cornbleet, 2002.]

Toxic neutrophil is also considered an acceptable answer, as identified by 14.4% of referees and 11.8% of participants. Toxic changes include toxic granulation, toxic vacuolization, and Döhle bodies (blue or gray-blue cytoplasmic inclusions, representing parallel strands of rough endoplasmic reticulum). In neutrophils displayed in these photopages, the granules may appear to some participants as slightly more prominent than is typically observed in normal neutrophils, with large, purple or dark blue cytoplasmic granules; thus toxic neutrophil is considered an acceptable cell identification. Either toxic granulation or Döhle bodies may be present in an individual neutrophil in isolation and would be sufficient to designate a neutrophil as toxic. On the other hand, vacuolization may be due to either toxic change or degeneration, therefore, the presence of neutrophil vacuolization as a sole finding should not be labeled as toxic vacuoles, unless accompanied by toxic granules and/or Döhle bodies.

Blood Cell Identification – Graded

BCP-12

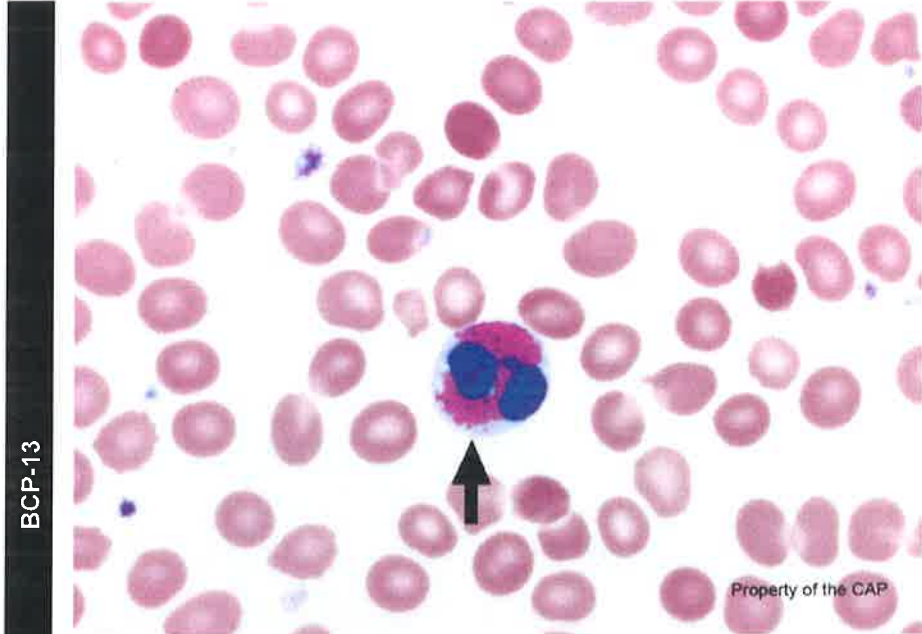


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	137	98.6	5478	97.0	Good
Bite cell (degmacyte)	1	0.7	47	0.8	Unacceptable
Neutrophil, segmented/band	1	0.7	1	0.0	Unacceptable

The arrowed cell is a fragmented red blood cell, as correctly identified by 98.6% of referees and 97.0% of participants. Fragmented red blood cells include helmet cells, keratocytes (horn cells), triangulocytes, and schistocytes. Red blood cells can experience fragmentation from being dragged across fibrin strands in the microcirculation, resulting in rips and tears through their membranes and producing irregularly-shaped red cell fragments with sharp angles, straight borders, small crescents, or helmet cells. Another mechanism of red cell fragmentation is erythrocyte buffeting against unyielding structures in the macrocirculation (as seen in prosthetic cardiac valves or severe valvular stenosis).

Blood Cell Identification – Graded

BCP-13

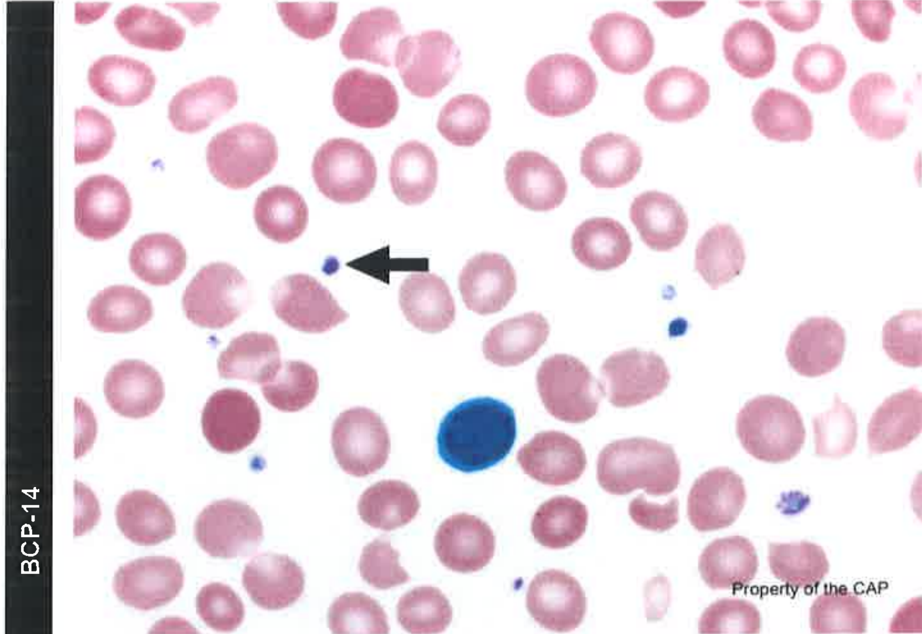


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Eosinophil, any stage	139	100.0	5638	99.8	Good

The arrowed cell is an eosinophil, as correctly identified by 100.0 % of referees and 99.8 % of participants. Mature eosinophils are white blood cells that are readily identified by their characteristically uniform orange-red granules and, oftentimes, bilobed nuclear segmentation. While comparable in size to segmented and band neutrophils (10 to 15 µm in diameter), mature eosinophils have coarse/larger, evenly-sized granules, while neutrophils have smaller and finer granules.

Blood Cell Identification – Graded

BCP-14

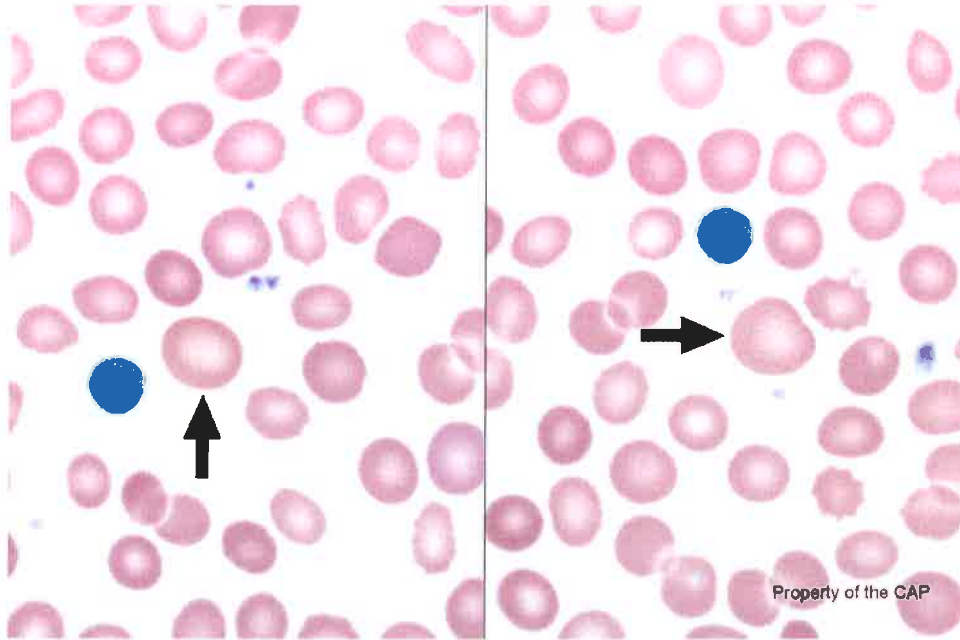


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Platelet, normal	138	99.3	5632	99.7	Good
Megakaryocyte (normal, abnormal, or nuclear fragment)	1	0.7	4	0.1	Unacceptable

The arrowed cell is a normal platelet, as correctly identified by 99.3% of referees and 99.7% of participants. Platelets are fragments of megakaryocytic cytoplasm. They appear as small, acellular blue-gray fragments with fine, purple-red granules, which are dispersed throughout or aggregated centrally. Most of them are small (1.5 to 3 µm in diameter), while a few larger platelets (4 to 7 µm in diameter) can also be seen in normal blood smears. Platelets are involved in primary hemostasis and coagulation.

Blood Cell Identification – Graded

BCP-15



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	139	100.0	5563	98.5	Good

The arrowed cell is a macrocyte, as correctly identified by 100.0% of referees and 98.5% of participants. To qualify as a macrocyte, a mature red cell must be abnormally large, measuring greater than 8.5 µm in diameter on a blood smear and greater than 100 fL on an instrument analyzer.

Macrocytes must be differentiated from polychromatophilic red cells. Like macrocytes, polychromatophilic erythrocytes can be much larger than normal red blood cells. However, polychromatophils continue to have increased RNA content, imparting a gray-blue hue to the cytoplasm; in contrast, macrocytes are mature red blood cells, and as such, lack significant polychromasia.

For morphologic size comparison, one can see that the much larger red blood cell (ie, macrocyte) appears beside other smaller red blood cells as well as a small lymphocyte (which typically measures from 7 - 15 µm in diameter) in the photopage image provided. As an added indicator of mean red cell volume, one should review the patient's MCV to verify one's visual microscopic examination (in this case, MCV is 120 fL).

As a final note, spurious elevations of MCV can occur in the setting of hyperglycemia and/or intravenous line contamination with glucose into the specimen drawn for analysis. Along with confirmation by visual microscopic examination, one can verify that the MCV is abnormally high by cross-checking the value of the MCHC. The MCHC will be normal or elevated in the face of a bona fide significant increase in MCV. On the other hand, abnormally low MCHCs would be seen with spuriously elevated MCVs. In this case, visual examination confirms the presence of true macrocytes, with a concomitant elevation in MCV and normal MCHC (at 35.1 g/dL); the combination of findings validates the existence of true macrocytosis.

Case Presentation:

This peripheral blood smear is from a 35-year-old woman with past medical history of kidney disease presenting with headache and lethargy. Laboratory data includes: WBC = 11.0 × 10E9/L; RBC = 3.53 × 10E12/L; HGB = 10.7 g/dL; HCT = 30.5%; MCV = 120.0 fL; MCHC = 35.1 g/dL; PLT = 204 × 10E9/L; and RDW = 16.1%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Macrocytic anemia

Macrocytic anemia is defined as anemia associated with MCV elevations above the normal population reference range (in adults, generally greater than or equal to 100 fL) and is age-dependent in infants and children. Abnormally large erythrocytes that are greater than or equal to 8.5 µm in diameter (ie. macrocytes) would be seen. To confirm the presence of macrocytes in smears, it is important to compare the size of the red blood cell of interest (ie, candidate macrocyte) against the other red blood cells in the smear and to compare to the size of other white blood cells, which typically are larger than 8 µm in diameter. Round and oval macrocytes are distinguished from large polychromatophilic cells (which represent reticulocytes or less mature anucleate red cells and can mimic macrocytes) by the blue or blue-gray cytoplasmic hue imparted by the increased RNA content in polychromatophilic cells. The increased fragility of macrocytes as they transit through the spleen is thought to lead to increased numbers of fragmented red blood cells and spherocytes often associated with macrocytic anemias. Anisocytosis and poikilocytosis would also be evident. Oval macrocytes and hypersegmented neutrophils could suggest a megaloblastic anemia.

Subclassifying macrocytic anemias can be done in a number of ways. One method would be to use the reticulocyte count as a discriminator. If the absolute reticulocyte count is high, the macrocytosis would likely be attributable to reticulocytosis in response to hemolysis, acute/recent blood loss, or active treatment of folate or vitamin B12 deficiencies. If the absolute reticulocyte count is low, one would more likely consider alcoholism, folate or vitamin B12 deficiency, hypothyroidism, liver disease, drugs that act primarily on DNA synthesis (eg, methotrexate, hydroxyurea, purine and pyrimidine analogues), congenital/inherited disorders, and myelodysplastic syndromes (particularly in elderly patients) in the differential diagnosis.

Another way to subclassify macrocytic anemias would be to first determine whether an individual case falls into one of two forms: megaloblastic versus non-megaloblastic. Clues in the CBC and peripheral blood smear review that could favor a megaloblastic anemia include MCVs exceeding 120 fL, and the presence of oval macrocytes and hypersegmented neutrophils (six or more nuclear segments). Megaloblastic anemia is considered a distinctive type of anemia, most commonly arising from folate or vitamin B12 (cobalamin) deficiency. The coenzyme forms of folate and cobalamin play essential roles in DNA synthesis, and as such, their deficiencies result in ineffective hematopoiesis. The lack of sufficient DNA to undergo mitosis causes a delay in cell division, while RNA synthesis – which is not dependent on folate or cobalamin – proceeds unimpeded. As a result, megaloblastic change or enlargement or "gigantism" of hematopoietic precursors is seen, as is nuclear-to-cytoplasmic dyssynchrony (ie. cytoplasmic differentiation appears more mature as compared to nuclear maturation). Giant C-shaped neutrophilic bands and megaloblastic erythroid precursors can be seen in bone marrow aspirate smears of patients with megaloblastic anemia.

From a treating provider's perspective, common causes of an isolated macrocytic anemia depend on region and setting: alcohol, hemolysis, medication-induced, and vitamin B12/folate deficiency. Careful integration of findings from clinical history (eg, age of the patient, strict vegan diet without vitamin B12 supplementation, medication history, alcohol use, small bowel disease), physical examination (eg. neurologic findings), CBC/peripheral blood smear findings, and laboratory results (eg. reticulocyte count, TSH, serum B12 and folate levels, liver function tests) can lead to focused downstream testing and, ultimately, the identification of the correct etiology for macrocytic anemia to assure appropriate approaches to therapy.

Maria (Ria) Vergara-Lluri, MD
Hematology and Clinical Microscopy Committee

References:

1. Cornbleet PJ. Clinical Utility of the Band Count, In: *Interpretation of the Peripheral Blood Film.* Clin Lab Med. 2002; 22(1): 101-136.
2. Glassy EF, ed. *Color Atlas of Hematology: An Illustrated Field Guide Based on Proficiency Testing,* 2nd ed. Peripheral Blood. Northfield, IL: College of American Pathologists; 2018.
3. Hariz A, Bhattacharya PT. Megaloblastic anemia. [Updated 2019 Jan 23]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019 Jan -. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537254/
4. Nagao T, Hirokawa M. Diagnosis and treatment of macrocytic anemia in adults. *J Gen Fam Med* 2017;18:200-204.
5. Wilson CS, Vergara-Lluri ME, Brynes RK. Evaluation of anemia, leukopenia, and thrombocytopenia. In: Jaffe ES et al, eds. *Hematopathology*, 2nd ed. Philadelphia, PA: Elsevier; 2017.

Blood Cell Identification – Ungraded

Case History

This peripheral blood smear is from a 32-year-old man with no significant past medical history presenting with pallor and fatigue. Laboratory data includes: WBC = 15.2 x 10E9/L; RBC = 3.79 x 10E12/L; HGB = 9.9 g/dL; HCT = 28.5%; MCV = 87 fL; PLT = 70 x 10E9/L; and RDW = 16%. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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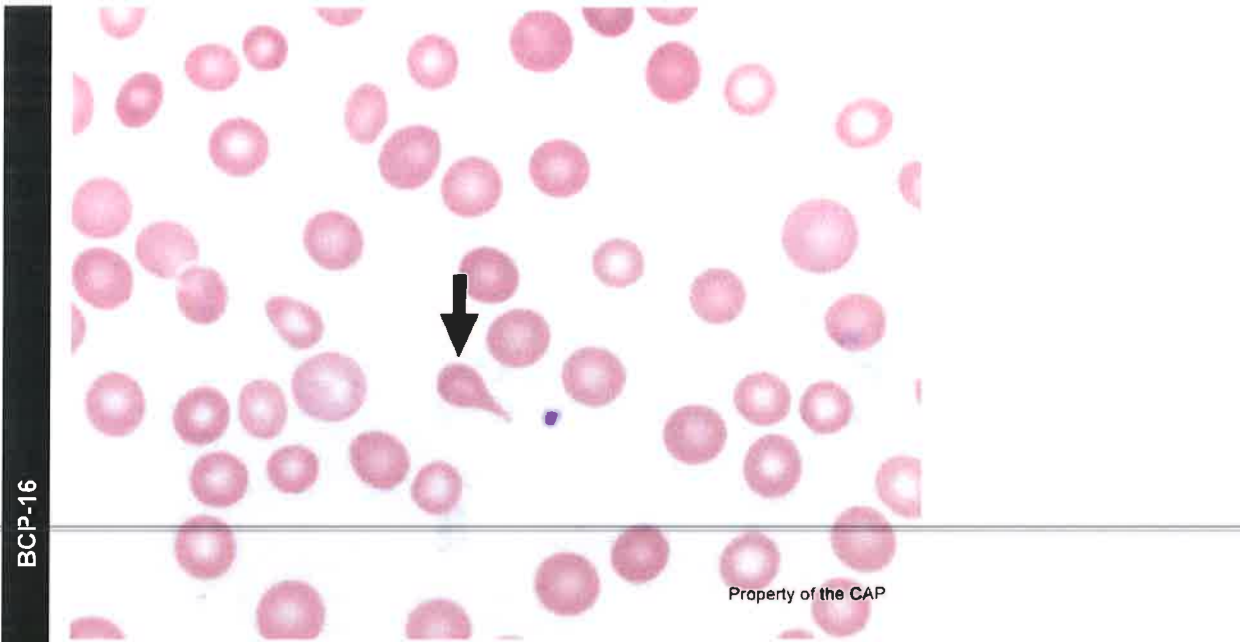
BCP-16

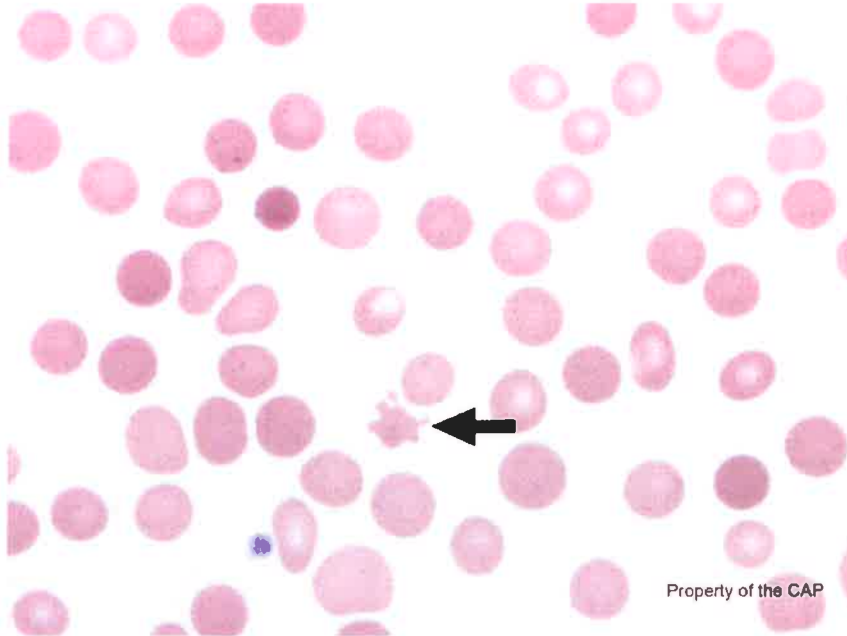


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Teardrop cell (dacrocyte)	139	100.0	5575	99.7	Educational

The arrowed cell is a teardrop cell, as correctly identified by 100.0% of the referees and 99.7% of the participants. The teardrop cell (dacrocyte) is an abnormally shaped red blood cell with a single elongated, tapered tail, resulting in the shape of a teardrop or pear. The teardrop morphology results from stretching of the RBC, most often as it passes through an abnormal/fibrotic bone marrow or the spleen. True teardrop cells should be differentiated from artifactual mimics that may be seen on poorly prepared peripheral blood smears; in this situation, the artificially stretched tails are shorter, sharper, and all point in the same direction whereas true teardrop cells will have tails that point in random directions.

Blood Cell Identification – Ungraded

BCP-17



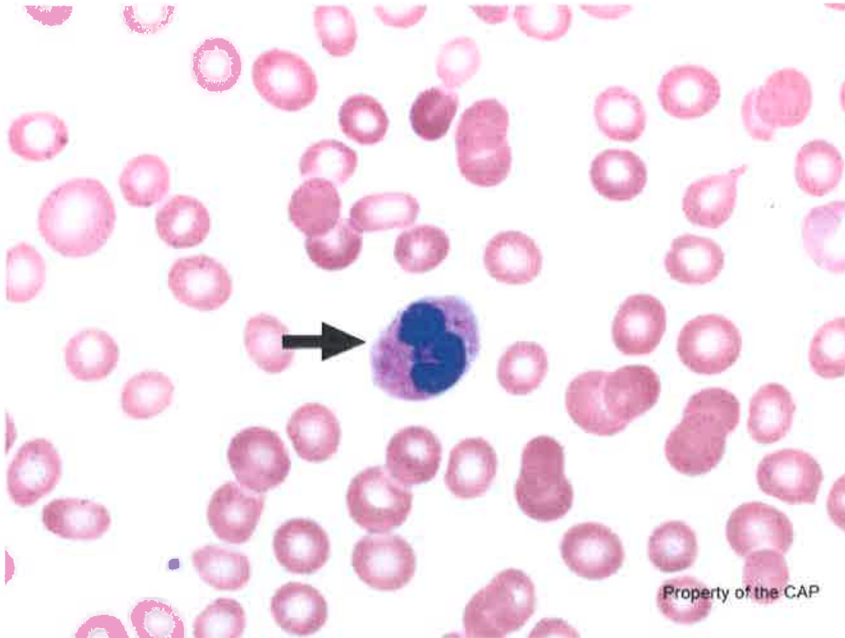
Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Acanthocyte (spur cell)	115	82.7	4796	86.8	Educational
Echinocyte (burr cell, crenated cell)	15	10.8	373	6.8	Educational
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	9	6.5	337	6.1	Educational

The indicated cell is an acanthocyte, as correctly identified by 82.7% of the referees and 86.8% of the participants. The acanthocyte is a densely-stained red blood cell lacking central pallor with characteristic thorn-like or knobby spicules of variable size. The acanthocyte is sometimes confused with the echinocyte (burr cell, crenated cell). However, the echinocyte retains central pallor and shows projections that are uniform and evenly distributed around the periphery of the cell, in contrast to the variability seen in the acanthocyte.

6.5% of referees and 6.1% of participants identified the cell as a fragmented red blood cell. See BCP-12 for the description and image of a fragmented red blood cell.

Blood Cell Identification – Ungraded

BCP-18



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Neutrophil, segmented or band	126	90.7	4831	87.5	Educational
Neutrophil with Pelger-Huët nucleus (acquired or congenital)	7	5.0	296	5.4	Educational
Neutrophil, toxic (to include toxic granulation and or Döhle bodies, and/or toxic vacuolization)	3	2.2	188	3.4	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	3	2.2	162	2.9	Educational

The arrowed cell is a neutrophil, as correctly identified by 90.7% of the referees and 87.5% of the participants. The neutrophil, the most mature stage of myeloid neutrophilic maturation, is 10 to 15 µm in size with an N:C ratio of 1:3. The cytoplasm is characterized by fine, lilac/pink granulation. The nucleus shows condensed chromatin with distinct nuclear lobes, each separated by a thin filament. This lobation pattern distinguishes the segmented neutrophil from its immediate precursor form, the band neutrophil; although for the purposes of proficiency testing, distinction between band and segmented neutrophils is not required.

5.0% of referees and 5.4% of participants identified this cell as a neutrophil with Pelger-Huët nucleus (acquired or congenital). Neutrophils with bilobed nuclei in the "pince-nez" conformation (ie, two round or nearly round lobes connected by a distinct thin filament) are designated as neutrophils with Pelger-Huët nuclei or as Pelger-Huët cells. They may be seen in the context of an inherited autosomal dominant abnormality of nuclear segmentation, referred to as Pelger-Huët anomaly, caused by mutations in the *Lamin B Receptor* (*LBR*) gene. In patients heterozygous for an *LBR* mutation, virtually all of the neutrophils have bilobed nuclei. Individuals homozygous for an *LBR* mutation, however, typically have single lobe neutrophils.

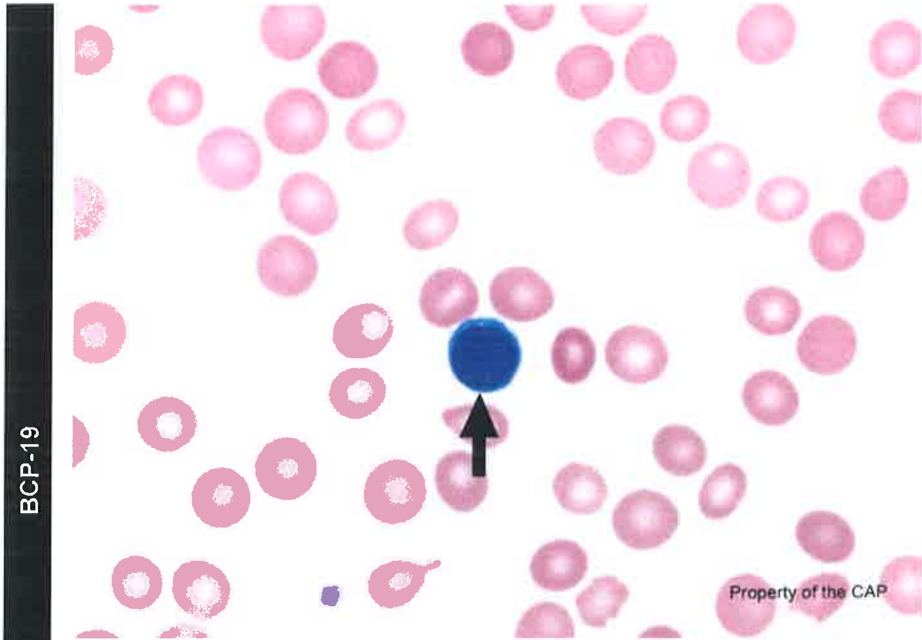
The nuclear chromatin in Pelger-Huët cells is generally denser than in normal neutrophils. Comparable neutrophil morphology, typically involving only a subset of neutrophils, may be seen in association with a number of conditions including myelodysplastic syndromes and other myeloid neoplasms, as a side-effect of certain drugs (eg, sulfonamides, colchicine, and mycophenolate mofetil), and in association with certain infections (eg, HIV and Mycoplasma pneumonia). To distinguish these cells from neutrophils seen in inherited conditions, these cells are designated as pseudo-Pelger-Huët cells. The particular cell in question does not have denser chromatin than typical for a neutrophil. In addition, although one nuclear lobe is round, the other nuclear lobe has more of a kidney bean shape. Therefore, the designation of neutrophil, segmented or band is more appropriate.

2.2% referees and 3.4% of participants identified this cell as a neutrophil, toxic (to include toxic granulation and or Döhle bodies, and/or toxic vacuolization). Toxic changes in neutrophils include toxic granulation, toxic vacuolization, and Döhle bodies. Toxic granulation and Döhle bodies each may be present in an individual cell without the other finding and either change alone is sufficient to designate a neutrophil as toxic. Toxic granulation is defined by the presence of large, purple or dark blue cytoplasmic granules in neutrophils, bands, and metamyelocytes. Vacuoles within the cytoplasm of these same cells define toxic vacuolization. The vacuoles are variable in size and may coalesce, sometimes distorting the neutrophil cytoplasm to form pseudopodia. Döhle bodies appear as single or multiple blue or gray-blue inclusions of variable size (0.1 to 5.0 µm) and shape (round, elongated, or crescent shaped) in the cytoplasm of neutrophils, bands, or metamyelocytes. They are often found at the periphery of the cytoplasm, near the cell membrane. These inclusions represent parallel strands of rough endoplasmic reticulum. Toxic changes result from the action of cytokines released in response to infection, burns, trauma, and granulocyte colony stimulating factor (G-CSF), and they indicate a shortened maturation time and activation of post-mitotic neutrophil precursors. The particular cell in question does not show cytoplasmic vacuolization or Döhle bodies. In addition, the granules appear fine and pale pink in color; they are not large or purple/dark blue in color. Therefore, the designation of neutrophil, segmented or band is more appropriate.

2.2% of referees and 2.9% identified this cell as a neutrophil with dysplastic nucleus and/or hypogranular cytoplasm. Dysplastic neutrophils are characteristic of myelodysplastic syndromes. Morphologically, the normal synchronous maturation of nucleus and cytoplasm is lost. As a result, in the cytoplasm, the primary and secondary granules are often decreased or absent, causing the cytoplasm to appear pale and bluish. Dysplastic neutrophils often have cytoplasm so pale that cytoplasmic borders cannot be easily distinguished from the slide background. The nucleus may show abnormal lobation accompanied by a mature chromatin pattern. In some cases, the nucleus has a "pince-nez" appearance; these cells are known as pseudo Pelger-Huët neutrophils. For proficiency testing purposes, cells with pseudo-Pelger-Huët nuclei with normal cytoplasmic granulation are best labelled as Pelger-Huët cells. Dysplastic neutrophils often have abnormal cytochemical reactivity; levels of myeloperoxidase and neutrophil alkaline phosphatase may be low or absent. The dysplastic neutrophils may also exhibit functional defects. The particular cell in question does not have hypogranular cytoplasm, and although one nuclear lobe is round, the characteristic "pince-nez" appearance is not seen. Therefore, the designation of neutrophil, segmented or band is more appropriate.

Blood Cell Identification – Ungraded

BCP-19

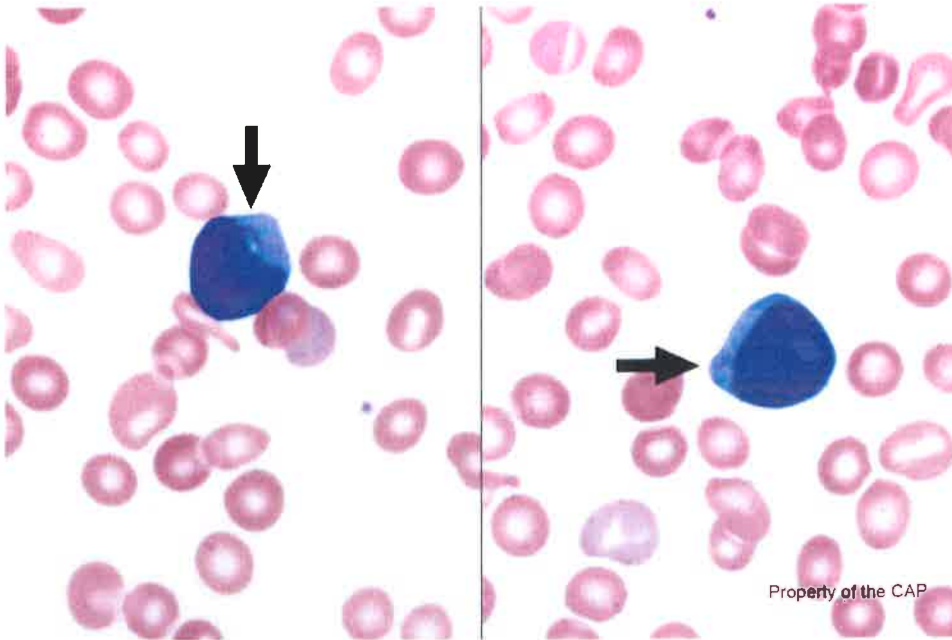


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Lymphocyte	138	99.3	5480	99.2	Educational
Malignant lymphoid cell (other than blast)	1	0.7	3	0.1	Educational

The arrowed cell represents a lymphocyte, as chosen by 99.3% of the referees and 99.2% of the participants. The lymphocyte is a small cell (7 to 15 µm) with rounded nuclear contours, coarse or clumped chromatin, and a scant to modest amount of pale blue cytoplasm.

Blood Cell Identification – Ungraded

BCP-20



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Myeloblast with Auer rod	100	71.9	4102	74.3	Educational
Blast cell	17	12.2	737	13.3	Educational
Lymphocyte, reactive	6	4.3	205	3.7	Educational
Neutrophil, promyelocyte, abnormal with/without Auer rod(s)	4	2.9	88	1.6	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	2	1.4	33	0.6	Educational
Malignant lymphoid cell (other than blast)	1	0.7	62	1.1	Educational

The arrowed cells are myeloblasts with Auer rods, as correctly identified by 71.9% of referees and 74.3% of participants. Myeloblasts are the most immature cells in the myeloid series. They are normally confined to the bone marrow, where they constitute less than 3% of the nucleated cells. They may be present in the blood in leukemic states, in myelodysplastic syndromes, in myeloproliferative neoplasms, and, very rarely, in leukemoid reactions. The myeloblast is usually a fairly large cell, 15 to 20 µm in diameter, with a high N:C ratio, usually 7:1 to 5:1, and typically basophilic cytoplasm. Myeloblasts may occasionally be smaller, similar to the size of a mature myeloid cell. The cell and nucleus are usually round, although irregularly shaped or folded nuclei may be present. The myeloblast nucleus has a characteristically finely reticulated chromatin pattern with distinct nucleoli present. Leukemic myeloblasts may also exhibit a few delicate granules and/or Auer rods. Distinguishing one type of abnormal blast cell from another is not always possible using Wright-Giemsa stains alone. Additional testing such as cytochemical staining (eg, using myeloperoxidase or Sudan black), or immunophenotyping may be required to further define the lineage of a given blast population.

Blood Cell Identification – Ungraded

BCP-20 (cont)

Auer rods are pink or red, rod-shaped cytoplasmic inclusions seen in early myeloid forms and occasionally in early monocytic forms in patients with myeloid lineage neoplasms including leukemia. These inclusions represent a crystallization of azurophilic (primary) granules and are pathognomonic for a high grade myeloid neoplasm.

12.2% of referees and 13.3% of participants identified these cells as blast cells which is an acceptable answer. 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"). Given the presence of distinct Auer rods in the arrowed cells, the more appropriate designation of the cells is myeloblasts with Auer rods as this is a more specific sub-classification. Nonetheless, blast cell is acceptable.

4.3% of referees and 3.7% of participants identified these cells as lymphocytes, reactive. This designation is inappropriate because although reactive lymphocytes may sometimes have a more open chromatin pattern (as seen in these arrowed cells), they will never have Auer rods.

2.9% of referees and 1.6 % of participants identified these cells neutrophils, promyelocytes, abnormal with/without Auer rod(s). Although this may appear as a potential choice given the presence of Auer rods, these arrowed cells do not have folded, bilobed, or reniform nuclear appearance, which is typical for abnormal promyelocytes.

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FH(1-4, 6, 9-10, 13)-B 2019: Acute Myeloid Leukemia

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David Czuchlewski, MD, MEd, FCAP; Stephanie A. Salansky, MEd, MS, MT(ASCP)

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Learning Objectives

Upon completing the reading and answering the learning assessment questions, you should be able to:

1. Describe the diagnostic criteria and common clinical presentation of acute myeloid leukemia (AML)
2. Identify the clinical, morphologic, and common laboratory features of acute promyelocytic leukemia (APL).
3. Utilize the myeloperoxidase (MPO) cytochemical stain in the workup of AML.
4. Describe the utility of common laboratory tests used to confirm and further investigate cases of AML, including flow cytometry, cytogenetics, and molecular sequencing.

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FH(1-4, 6, 9-10, 13)-B 2019: Acute Myeloid Leukemia

Case Presentation

This peripheral blood smear is from a 32-year-old man with no significant past medical history who presents with pallor and fatigue. Laboratory data includes: WBC = 15.2 x 10E9/L; RBC = 3.79 x 10E12/L; HGB = 9.9 g/dL; HCT = 28.5%; MCV = 87 fL; PLT = 70 x 10E9/L; and RDW = 16%. Identify the arrowed object(s) on each image.*

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

**Note:* Image of the slide is located within the FH (1-4, 6, 9-10, 13)-B 2019 Participant Summary.

INTRODUCTION

Acute myeloid leukemia (AML) is a myeloid neoplasm that meets 1 of 2 diagnostic requirements: either there are ≥ 20% myeloblasts (or equivalents) in the peripheral blood or bone marrow, or there are certain specific cytogenetic abnormalities resulting in particular gene fusions.

AML is one of the most consequential diagnoses that can be made based on peripheral blood examination. Indeed, morphologic detection of blasts on the peripheral smear is often the initiating step in a series of investigations and confirmatory steps leading to the diagnosis of AML. In addition, morphology plays a crucial role in the recognition of acute promyelocytic leukemia (APL), a subtype of AML that must be diagnosed as rapidly as possible due to its association with coagulation abnormalities resulting in high mortality at initial presentation. Even the delay of a few hours in recognizing a case of APL can have fatal consequences.

While peripheral blood morphology is the typical starting point for a diagnosis of AML, complete characterization in any given case will most likely include cytochemistry, flow cytometric analysis, cytogenetic studies (including fluorescence in situ hybridization [FISH] and karyotyping), and molecular sequencing for gene mutations. Some of these investigations are not directly within the purview of the hematology laboratory. Yet, because the final diagnosis of AML must integrate these data with the peripheral blood findings, it is helpful for the morphologist to understand how these additional studies can impact the diagnosis and management of AML. For example, the World Health Organization (WHO) 2016 Classification of Hematopoietic Neoplasms includes many subtypes of AML based on genetic and clinical factors (Table 1). While the specific WHO subtype of a given case of AML may not be fully established based on the initial peripheral blood examination, every case of AML must eventually be categorized according to this scheme for proper treatment and management.

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Table 1. WHO 2016 Classification of Acute Myeloid Leukemia (AML)

AML with recurrent genetic abnormalities

- AML t(8;21)(q22;q22.1); *RUNX1- RUNX1T1*
- AML inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
- APL with *PML-RARA*
- AML t(9;11)(p21.3;q23.3); *KMT2A–MLLT3*
- AML t(6;9)(p23;q34.1); *DEK-NUP214*
- AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*
- AML with t(1;22)(p13.3;q13.1); *RBM15-MKL1*
- AML with *BCR-ABL1*
- AML with mutated *NPM1*
- AML with biallelic mutation of *CEBPA*
- AML with mutated *RUNX1*

AML with myelodysplasia-related changes
Therapy-related myeloid neoplasm
AML, not otherwise specified
Myeloid sarcoma
Myeloid proliferations associated with Down syndrome

EPIDEMIOLOGY

AML can occur at any age, though the overall incidence increases with age (median age at diagnosis 65 years). In adults, 70% - 80% of acute leukemia cases represent AML, while, in contrast, acute lymphoblastic leukemia (ALL) is more common in children. The incidence of AML overall in the United States is 5.4 cases per 100,000 persons.

PATHOGENESIS

Multiple acquired genetic changes in a bone marrow stem/progenitor cell are required to give rise to AML. Many cases are characterized by mutations or gene rearrangements that lead to a blockade in cellular differentiation and an abnormal increase in cellular proliferation. The combined effect of these changes is the overproduction of cells at the earliest stage of granulocytic differentiation: the myeloblast. While some recurrent genetic changes — such as those affecting transcription factors and signaling molecules — directly impact these cellular processes, others exert similar effects by altering DNA methylation, chromatin modification, RNA splicing, or other cellular mechanisms. In some cases of AML, mutations and rearrangements occur as random events in the bone marrow. However, there are specific conditions that increase the likelihood of these genetic changes occurring. These include prior treatment with DNA-damaging chemotherapy or radiation therapy, a pre-existing myelodysplastic syndrome, and the presence of certain specific (usually inherited) germline mutations associated with higher risk of myeloid neoplasms. All these clinical settings are associated with increased incidence of AML.

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DIAGNOSIS

The Complete Blood Count (CBC)

The predominance of blasts in the bone marrow results in decreased production of mature peripheral blood elements. Thus, the CBC usually shows anemia, thrombocytopenia, and/or neutropenia. Circulating blasts are often very numerous, resulting in a leukocytosis, though some cases show fewer circulating blasts.

Peripheral Blood Evaluation

The diagnosis of AML depends upon an accurate blast count. Peripheral blood cells that are counted as blasts for this diagnosis include myeloblasts and monoblasts. Promonocytes are considered blast equivalents and are also counted as blasts for purposes of AML diagnosis. Promyelocytes are counted as blast equivalents for the diagnosis of APL specifically. The morphologic features of these various cell types are given in Table 2.

Table 2. Features of Blasts and Commonly Encountered Equivalents

Cell type	Morphologic features
Blast	High nuclear-to-cytoplasmic ratio; fine, dispersed chromatin; variably prominent nucleoli; range of cell size, most often intermediate to large
Monoblast	Large cell, more cytoplasm than a myeloblast; round or oval nucleus with lacy chromatin and nucleoli
Promonocyte	Large cell, relatively abundant gray-blue cytoplasm with a few red granules; irregular, indented, or creased nuclear contour with chromatin condensation between that seen in blast and mature monocyte; considered a blast equivalent for diagnosis of AML
Abnormal promyelocyte (in acute promyelocytic leukemia)	Nucleus is usually folded, bilobed, or reniform (often with overlapping nuclear lobes); distinct Golgi zone is typically absent; abundant cytoplasmic granules, often either coarser or finer than those seen in normal promyelocytes and slightly darker in color; microgranular variant shows very few, fine granules; Auer rods present, in some cells numerous and overlapping

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For purposes of identifying generic myeloblasts, key features include open or powdery chromatin, variably prominent nucleoli, high nucleus to cytoplasmic ratio, and in some cases, Auer rods. If an Auer rod is seen in a blast cell, myeloid lineage is established. It is especially critical to recognize the features of the abnormal promyelocytes seen in APL, including prominent and abnormal cytoplasmic granulation, bilobed nuclei, frequent Auer rods, and multiple Auer rods in single cells. Importantly, some microgranular or hypogranular cases of APL show cytoplasm that lacks the typical granulation. Nevertheless, the other morphologic features including bilobed nuclei remain present. It should be noted that in some cases of APL (especially those with typical hypergranular appearance), these circulating blast equivalents are often relatively scant.

While attention in cases of AML is typically devoted to the blasts, the remaining morphologic aspects of the peripheral smear should not be neglected. Morphologic dysplasia may be present; if confirmed in greater than 50% of 2 lineages (ie, after bone marrow assessment), this would be consistent with AML with myelodysplasia-related changes. Cases of AML, most importantly APL, may also show evidence of disseminated intravascular coagulation, and therefore, the red blood cell morphology should be carefully evaluated for the presence of schistocytes.

A useful adjunct in the initial work-up of AML is the cytochemical stain for myeloperoxidase (MPO). This stain may be performed rapidly at the hematology bench as part of the initial review of the case. If the blasts convincingly contain MPO positive granules, the presence of myeloid lineage differentiation is established. However, some cases of AML are negative for MPO (eg, AML with minimal differentiation or monoblastic leukemia), and therefore, the absence of MPO positive granules does not exclude the diagnosis of AML. The leukemic cells in APL show very strong MPO activity, a feature that may be helpful in rapidly identifying these cases as APL. Of note, even microgranular cases of APL show this characteristic pattern of strong MPO expression. (The granules in these cases are abnormally small, and therefore, cannot be seen using the light microscope, but they are nevertheless present and are revealed by the MPO stain.)

It should be noted that in some cases of AML, the blast count in the peripheral blood will not exceed 20%. As mentioned above, in these cases either the bone marrow blast count would be above 20% or certain specific cytogenetic abnormalities would be present. In cases of AML with monocytic differentiation, it is particularly common that the bone marrow shows a greater degree of immaturity than the peripheral blood.

Flow Cytometry

After the initial examination of the peripheral blood smear, flow cytometric analysis is often the next step in the workup of cases of suspected AML. In this technique, monoclonal antibodies to various cellular proteins are used to interrogate the cells for the presence of specific antigens. Myeloblasts typically show a combination of myeloid antigens (eg, CD13, CD33, MPO) with markers of cellular immaturity such as CD34 and CD117. Flow cytometric analysis is also useful in documenting the presence of monocytic differentiation, including with the expression of monocytic antigens such as CD14, CD36, and CD64. Yet another important observation on flow cytometric analysis is the characteristic absence of CD34 and HLA-DR in cases of APL.

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Cytogenetic Analysis

Two types of cytogenetic analysis are commonly performed in cases of AML. FISH may be performed on either peripheral blood or bone marrow specimens. In this technique, fluorescently labeled probes are hybridized to the cells in order to reveal the presence and location of specific genetic regions. Most critical in the diagnosis of AML is the use of probes for the *PML* and *RARA* regions. Using these probes, a *PML-RARA* gene fusion resulting from the t(15;17) translocation can be definitively demonstrated, thus clinching the diagnosis of APL. FISH for the *PML-RARA* fusion should be performed on a rapid basis, given the critical nature of a prompt diagnosis. However, because of the high mortality associated with APL at diagnosis and the excellent response to all-trans retinoic acid (ATRA) therapy, treatment with ATRA should be initiated as soon as the diagnosis of APL is strongly suspected, rather than waiting for genetic confirmation.

Karyotyping is the second type of cytogenetic analysis performed in cases of AML. In this assay, patient cells are grown in culture and the chromosomes are visualized. In AML, karyotyping provides important information for subclassification as well as risk stratification for prognosis and treatment.

Molecular Sequencing

It is now standard of care to perform molecular sequencing in cases of AML. The number of genes assessed may vary, but for proper WHO classification and for complete prognostic determination, it is important to assess for mutations of the *NPM1*, *FLT3*, *CEBPA*, *RUNX1*, and (in some cases) *KIT* genes. Other genes commonly assessed include *ASXL1* and *TP53*, mutations of which are associated with worse prognosis. With the increasing number of genes that must be tested, it is becoming more common to use next generation sequencing technology to study multiple gene regions simultaneously. Genetic data may also be used to guide targeted therapy. FDA-approved therapies are available for cases bearing mutations of the *FLT3*, *IDH1*, and *IDH2* genes.

Differential Diagnosis

The differential diagnosis of AML is broad and includes non-neoplastic and neoplastic mimics. It is essential that reactive circulating cells such as immunoblasts be accurately differentiated from myeloblasts in order to avoid misdiagnosis. Another non-neoplastic consideration is the administration of G-CSF to patients. This therapy is given to increase the neutrophil count, but may also in some cases lead to the presence of some (though usually far fewer than 20%) circulating blasts. If numerous blasts are indeed present, the differential diagnosis includes ALL, which is best distinguished from AML by immunophenotypic studies. Circulating myeloblasts (< 20%) are seen in many myeloid neoplasms other than AML, including the myelodysplastic syndromes and myeloproliferative neoplasms. Additional morphologic and CBC clues to these alternative myeloid neoplasms, such as dysplasia or significant cytoses, may be seen in the peripheral blood. However, because of the possibility of a low blast count AML, as defined by the presence of specific cytogenetic abnormalities, additional correlation is typically necessary. Other blastic neoplasms such as blastic plasmacytoid dendritic cell neoplasm or blast phase of chronic myeloid leukemia must also be considered. Thus, the detection of blasts is only the first step in a multifaceted clinicopathologic investigation to reach a diagnosis of AML.

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THERAPY AND PROGNOSIS

Cases of non-APL AML are initially treated with cytotoxic chemotherapy, most often using the regimen known as "7+3," consisting of cytarabine and an anthracycline such as daunorubicin. The prognostic expectations are established using cytogenetic and molecular data, in addition to the WHO subtype. The 2 risk stratification systems in common usage are the National Comprehensive Cancer Network (NCCN) guidelines and the European LeukemiaNet (ELN) system. Based in part on the prognostic data thus obtained, some patients with more aggressive disease may be offered allogeneic hematopoietic stem cell transplantation. The detection of certain molecular abnormalities, such as *FLT3* mutation, may lead to the addition of targeted therapies. An important exception to this overall approach occurs in the setting of APL, in which treatment with ATRA overcomes the differentiation blockade and leads to an excellent prognosis.

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David R. Czuchlewski, MD, MEd, FCAP
Hematology and Clinical Microscopy Resource Committee

AUTHOR'S BIOGRAPHY

David R. Czuchlewski, MD, MEd, FCAP, is associate professor of pathology at the University of New Mexico and director of the Genetics and Cytometry Laboratories at TriCore Reference Laboratories in Albuquerque, NM. Dr. Czuchlewski has authored several articles, abstracts, and educational activities and serves as a member of the Hematology and Clinical Microscopy Committee for the College of American Pathologists.

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