Blood Cell Identification – Graded

Case History

This peripheral blood smear is from a 26-year-old woman with past medical history of beta thalassemia trait (minor) presenting with fatigue. Laboratory data includes: WBC = 9.8 x 10E9/L; RBC = 5.45 x 10E12/L; HGB = 8.7 g/dL; HCT = 31.9%; MCV = 62 fL; PLT = 170 x 10E9/L; and RDW = 22.0%.
Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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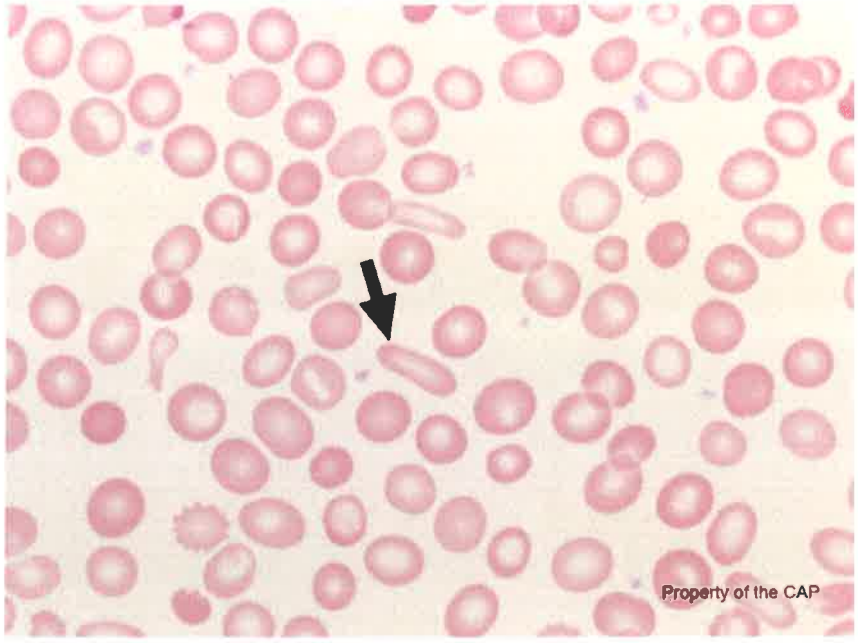
https://cap.objects.frb.io/documents/2019-hematology-clinical-microscopy-glossary.pdf

BCP-21

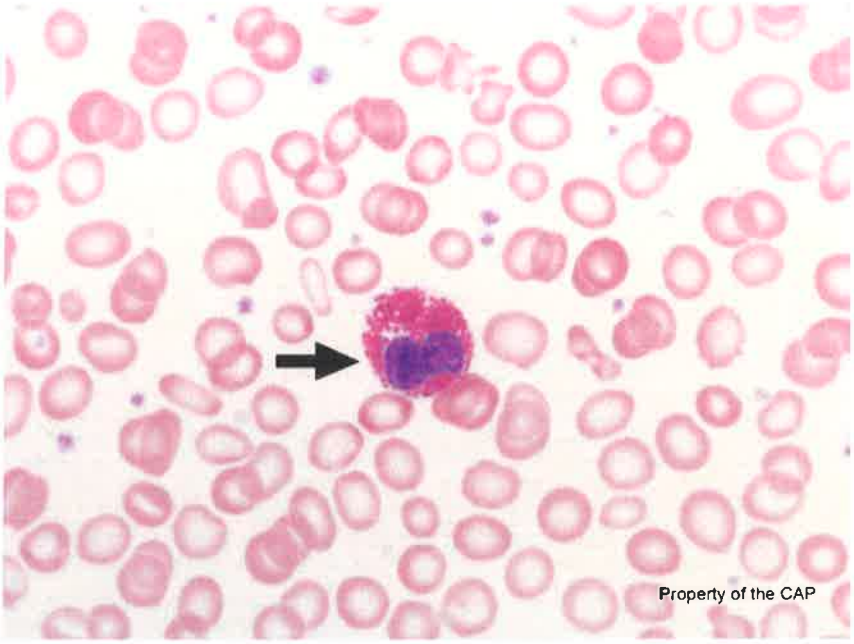


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Ovalocyte (elliptocyte)	134	100.0	5484	99.8	Good

The arrowed cell is an elliptocyte, as correctly identified by 100.0% of referees and 99.8% of participants. The terms elliptocyte and ovalocyte are used to describe red blood cells appearing in the shape of a pencil or thin cigar, with blunt ends and parallel sides. Hemoglobin is often concentrated at the ends, producing a dumbbell appearance. A small number of elliptocytes/ovalocytes may be present in the smears of normal individuals (< 1%), whereas a moderate to marked elliptocytosis/ovalocytosis (> 25%) is observed in patients with hereditary elliptocytosis, an abnormality of erythrocyte skeletal membrane proteins. Elliptocytes are also commonly increased in number in iron deficiency and in the same states in which teardrop cells are prominent.

Blood Cell Identification – Graded

BCP-22

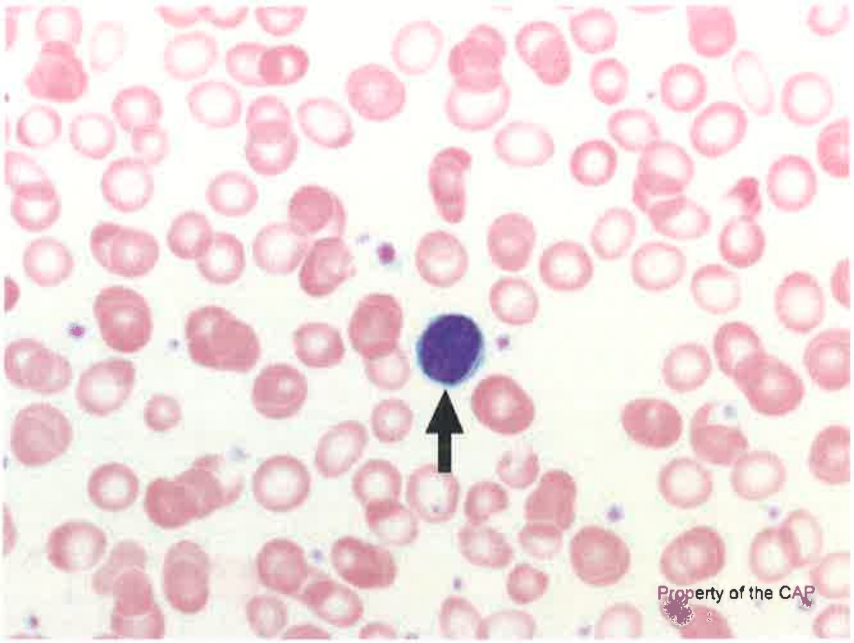


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Eosinophil, any stage	134	100.0	5484	99.8	Good

The arrowed cell is an eosinophil, as correctly identified by 100.0% of referees and 99.8% of participants. Eosinophils are round-to-oval leukocytes that are recognizable by their characteristic coarse, orange-red granulation. They are comparable in size to neutrophils, ie, 10 to 15 µm in diameter, in their mature forms. The eosinophil nuclear-to-cytoplasmic ratio ranges from 1:3 for mature forms to 2:1 for immature forms. The eosinophil cytoplasm is generally evenly filled with numerous coarse, orange-red granules of uniform size. These granules rarely overlie the nucleus and are refractile by light microscopy due to their crystalline structure. This refractile appearance is not apparent in photomicrographs or pictures, however. Due to inherent problems with color rendition on photomicrographs, which is sometimes imperfect, eosinophil granules may appear lighter or darker than on a freshly stained blood film. Discoloration may give the granules a blue, brown, or pink tint. Nonetheless, the uniform, coarse nature of eosinophil granules is characteristic and differs from the smaller, finer granules of neutrophils. In the most mature eosinophil form, the nucleus segments into two or more lobes connected by thin filaments. About 80% of segmented eosinophils will have the classic two-lobed appearance. Typically, these lobes are of equal size and round to ovoid or potato-shaped with dense, compact chromatin. The remainder of segmented eosinophils will have three lobes and an occasional cell will exhibit four to five lobes. Eosinophils exhibit the same nuclear characteristics and the same stages of development as neutrophils. Immature eosinophils are rarely seen in the blood, but they are identifiable in bone marrow smears.

Blood Cell Identification – Graded

BCP-23

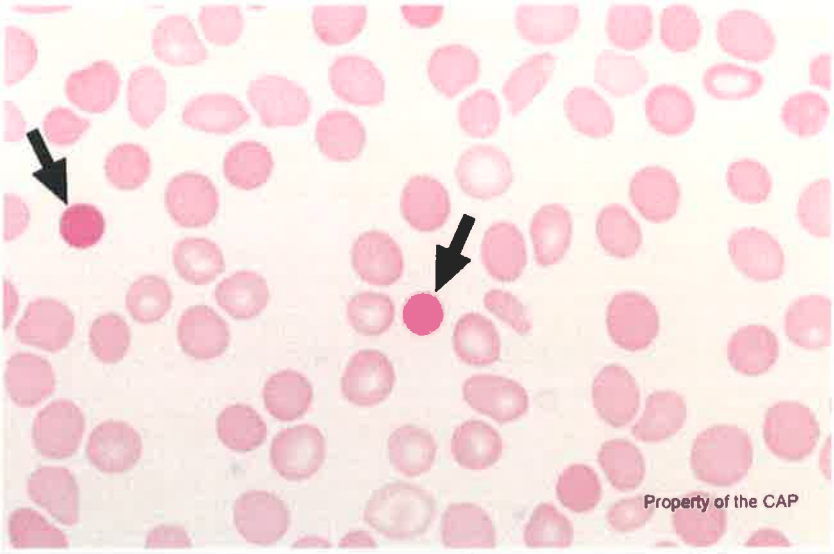


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Lymphocyte	134	100.0	5476	99.7	Good

The arrowed cell is a lymphocyte, as correctly identified by 100.0% of referees and 99.7% of participants. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 µm with a nuclear-to-cytoplasmic ratio ranging from 5:1 to 2:1. Most lymphocytes have round to oval nuclei that may be slightly indented or notched. The chromatin is diffusely dense or coarse and clumped. Nucleoli are not visible, although some cells may exhibit a small, pale chromocenter that may be mistaken for a nucleolus. Most lymphocytes have a scant amount of pale blue to moderately basophilic, agranular cytoplasm. Occasionally, the edges may be slightly frayed or pointed due to artifacts induced during smear preparation. Occasional lymphocytes will have a small clear zone, or hof, adjacent to one side of the nucleus.

Blood Cell Identification – Graded

BCP-24



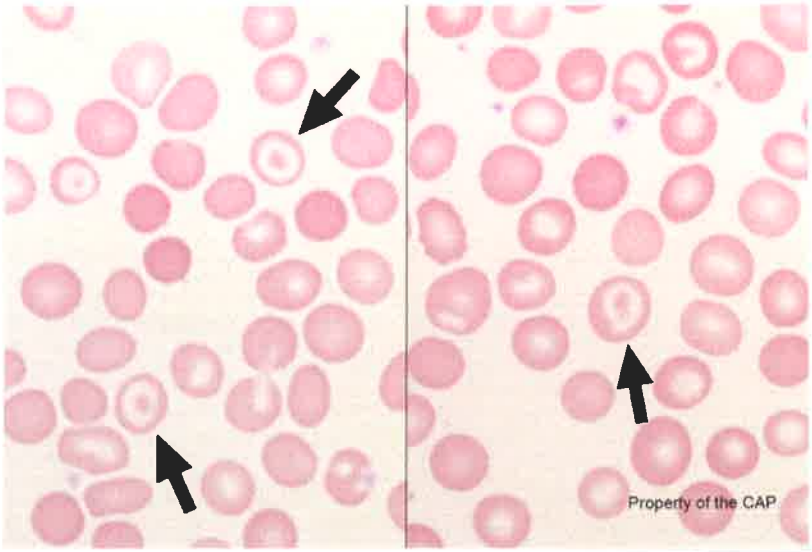
Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Spherocyte	134	100.0	5424	98.8	Good
Microcyte (with increased central pallor)	-	-	33	0.6	Unacceptable

The arrowed cells are spherocytes, as correctly identified by 100.0% of referees and 98.8% of participants. Spherocytes are identified as densely staining, spherical, or globular red blood cells with normal or slightly reduced volume (ie, normal or low MCV) and increased thickness (more than 3 µm), but with decreased diameter (usually less than 6.5 µm) and usually without central pallor. These cells appear denser than normal red blood cells and are commonly found in hereditary spherocytosis and immune hemolytic anemias. Microspherocytes (spherocytes measuring 4 µm or less in diameter) are frequently seen in severe burns or microangiopathies and represent rounded-up fragments of red blood cells.

0.6% of participants identified these cells as microcytes. Microcytes are smaller than normal red blood cells, measuring less than 6 µm in diameter and less than 80 fL in volume. On the blood film, they generally appear smaller than the nucleus of a small lymphocyte. When there is little or no variation in red blood cell size, morphology is less reliable than instrument-generated MCVs in determining if microcytosis is present. On a peripheral blood film, microcytes retain central pallor, appearing either normochromic or hypochromic. Red blood cells are considered hypochromic when central pallor exceeds 50% of cell diameter. Although other poikilocytes, such as spherocytes and fragmented red blood cells, can be very small in size, these red blood cells lack central pallor and should be specifically identified rather than classified as "microcytes." Microcytes are commonly seen in iron deficiency anemia, thalassemia, lead poisoning, and some cases of anemia of chronic disease. In this photomicrograph, the arrowed cells have no central pallor; therefore, microcytes would be an inappropriate designation.

Blood Cell Identification – Graded

BCP-25



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Target cell (codocyte)	134	100.0	5479	99.8	Good

The arrowed cells are target cells, as correctly identified by 100.0% of referees and 99.8% of participants. Target cells are thin red blood cells with an increased surface membrane-to-volume ratio. They are often flattened out on the smears and may appear macrocytic. Target cells are believed to arise from disturbances in red blood cell membrane cholesterol and lecithin content or decreased cytoplasmic hemoglobin content. Target cells are characterized by a central hemoglobinized area within the surrounding area of pallor, which in turn is surrounded by a peripheral hemoglobinized zone giving target cells the appearance of a bull's-eye. Target cells are usually seen in thalassemias, iron deficiency anemia, following splenectomy, or in patients who are jaundiced or who have chronic liver disease; in the latter two conditions, the MCV may be normal or increased. Target cells may also appear as artifacts from slow drying the slides in a humid environment or from specimens anticoagulated with excessive EDTA. The drying artifact results in the presence of numerous target cells in some fields, but none or few in other fields.

Case Presentation:

This peripheral blood smear is from a 26-year-old woman with past medical history of beta thalassemia trait (minor) presenting with fatigue. Laboratory data includes: WBC = 9.8 × 10E9/L; RBC = 5.45 × 10E12/L; HGB = 8.7 g/dL; HCT = 31.9%; MCV = 62 fL; PLT = 170 × 10E9/L; and RDW = 22.0%.

Case Discussion: Beta thalassemia trait and concordant iron deficiency

Beta thalassemia is usually due to point mutations in the DNA coding region for the beta globin chain. It is more common in individuals of Mediterranean, Middle Eastern, Indian, Pakistani, and Southeast Asian ancestry. Numerous mutations have been described, accounting for the heterogeneity in disease severity. These are divided into those mutations that result in complete loss of beta chain production (ie, β^0) and those that only result in decreased production (ie, β^+). If only one gene mutation is inherited, this is diagnosed as beta thalassemia trait (minor) whereas if two gene mutations are inherited, this is termed as beta thalassemia major (Cooley anemia). Clearly, the amount of beta chain produced correlates with disease severity.

Beta thalassemia minor is asymptomatic, with very mild anemia (if any), microcytosis (MCV ranging from 50 - 70 fL), characteristically elevated red blood cell (RBC) counts (ie, thalassemic indices), and absolute reticulocyte counts usually twice the normal value. Blood smears will show mild hypochromia without prominent anisocytosis. However, poikilocytosis with target cells are routinely seen, and can be quite prominent.

Diagnosis of beta thalassemia is suggested by the finding of increased hemoglobin A_2 (> 3.5% of hemoglobin) by high performance liquid chromatography. Although hemoglobin A_2 (Hb A_2) will be increased, it will represent no more than 10% of hemoglobin in typical states. If Hb A_2 is elevated beyond 10%, other diagnoses should be entertained (ie, another abnormal hemoglobin migrating with Hb A_2).

The differential diagnosis includes iron deficiency and other hemoglobinopathies/thalassemias. Iron deficiency will not result in increased Hb A_2, allowing differentiation from hemoglobinopathies/thalassemias. However, iron deficiency can cause a decrease in Hb A_2, particularly in severe cases. Therefore, in a patient with both beta thalassemia trait and severe iron deficiency, the Hb A_2 value may be within reference range (< 3.5%). In patients with suspected concomitant beta thalassemia and iron deficiency, repeat testing of Hb A_2 level is suggested if microcytosis persists despite iron repletion.

Iron deficiency is relatively common and may be due to chronic blood loss (from the gastrointestinal tract or menstrual bleeding, etc.), chronic hemolysis (such as that seen with thalassemia/hemoglobinopathies, among other entities), inadequate intake, and/or decreased absorption. In patients with isolated iron deficiency, anisocytosis is increased, resulting in an RBC distribution width (RDW) higher than is seen in beta thalassemia. Testing would reveal decreased ferritin levels and low serum iron. Total iron binding capacity would be increased. Finally, unlike beta thalassemia, the RBC and absolute reticulocyte counts would usually be reduced, not elevated.

In our patient with known history of beta thalassemia trait (minor), the red cell indices raise the concern of superimposed iron deficiency. The severity of anemia is far worse than is typically observed for beta thalassemia minor alone. The elevated RDW favors iron deficiency; however, the elevated-for-gender RBC count supports underlying beta thalassemia. Target cells can be seen in both entities – though elliptocytes are more typically observed in iron deficiency and spherocytes suggest extravascular hemolysis, such as can be seen in beta

thalassemia. In summary, the overall findings reveal a mixed picture of beta thalassemia trait with coexisting iron deficiency. Hb A_2 levels should be tested after iron is replaced if the diagnosis had not been previously confirmed.

Rebecca Kunak, DO
Hematology and Clinical Microscopy Committee

References:

1. Hoyer JD, Kroft SH. *Color Atlas of Hemoglobin Disorders: A Compendium Based on Proficiency Testing.* Northfield, Illinois: College of American Pathologists; 2003.

Blood Cell Identification – Ungraded

Case History

This peripheral blood smear is from a 35-year-old man with no significant past medical history presenting with headache and easy bruising. Laboratory data includes: WBC = 78.1 x 10E9/L; RBC = 2.38 x 10E12/L; HGB = 7.5 g/dL; HCT = 21.1%; PLT = 20 x 10E9/L; MPV = 11.3 fL; MCV = 90 fL; and RDW = 15%. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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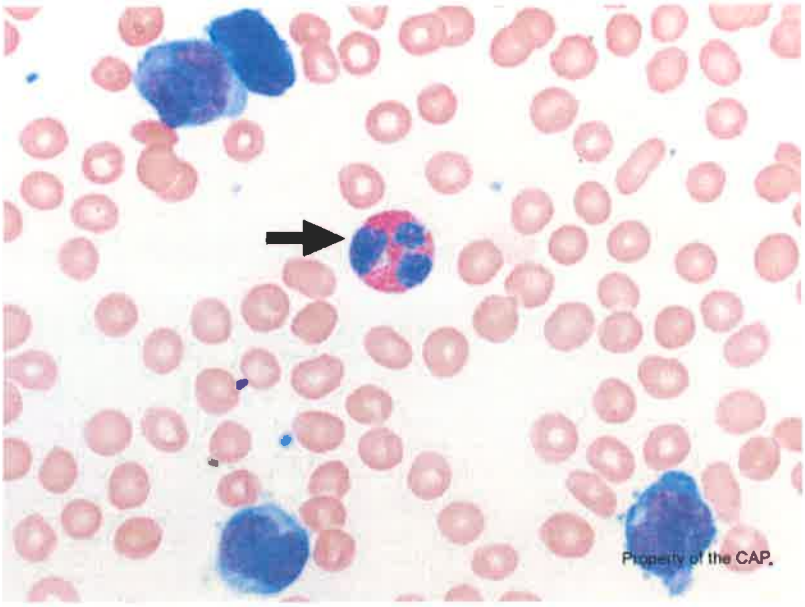
BCP-26

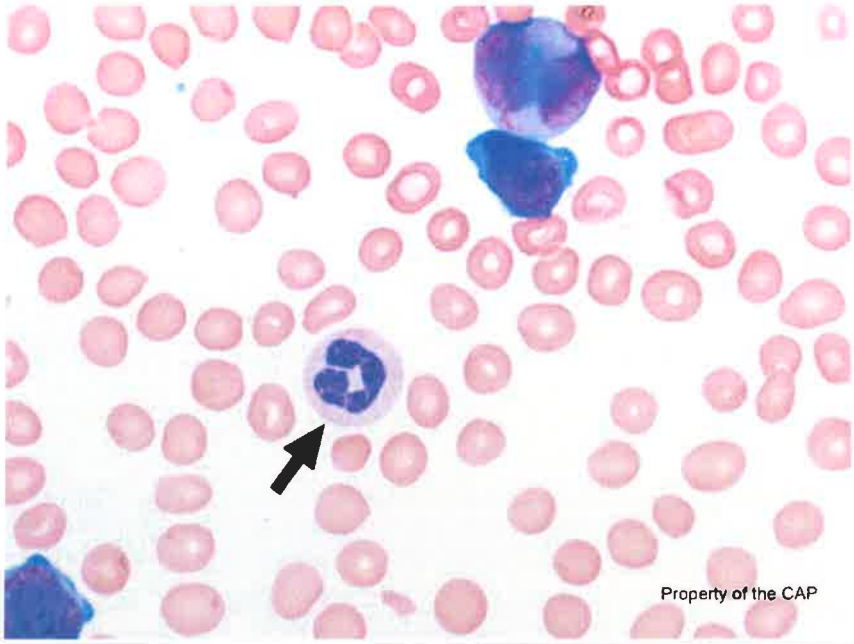


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Eosinophil, any stage	134	100.0	5418	99.6	Educational

The arrowed cell is a mature eosinophil, as correctly identified by 100.0% of referees and 99.6% of participants. Eosinophils are round-to-oval leukocytes that are recognizable by their characteristic coarse, orange-red granulation. They are comparable in size to neutrophils, ie, 10 to 15 µm in diameter in their mature forms, and 10 to 18 µm in diameter in immature forms. The eosinophil N:C ratio ranges from 1:3 for mature forms to 2:1 for immature forms. The eosinophil cytoplasm is evenly filled with numerous coarse, orange-red granules of uniform size. These granules rarely overlie the nucleus and are refractile by light microscopy due to their crystalline structure. In the most mature eosinophil form, the nucleus segments into two or more lobes connected by a thin filament. About 80% of segmented eosinophils will have the classic two-lobed appearance. Typically, these lobes are of equal size and round to ovoid or potato-shaped with dense, compact chromatin. The remainder of segmented eosinophils will have three lobes (as seen in the photomicrograph) and an occasional cell will exhibit four to five lobes.

Blood Cell Identification – Ungraded

BCP-27



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Neutrophil, segmented or band	130	97.0	5309	98.9	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	3	2.2	41	0.8	Educational
Neutrophil with hypersegmented nucleus	1	0.8	7	0.1	Educational

The arrowed cell is a neutrophil, segmented/band, as correctly identified by 97.0% of referees and 98.9% of participants. Segmented neutrophils and their immediate precursors, bands, are typically the predominant leukocyte in peripheral blood. Band neutrophils, also known as stabs, constitute 5% to 10% of the nucleated cells in the blood under normal conditions. An increased number of bands may be noted in the blood in a number of physiologic and pathologic states (eg, infectious/inflammatory processes, tissue damage or necrosis, neoplasia, poisoning or intoxication, drug effect, and metabolic abnormalities). The band is round- to-oval and 10 to 18 µm in diameter. The N:C 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 the chromatin is not condensed to a single filament (as is the defining feature of the fully mature neutrophil). The nucleus can assume many shapes: it can be band- or sausage-like; S-, C-, or U-shaped (as seen in the photomicrograph); and twisted or folded on itself. The cytoplasm is similar to that of other post-mitotic neutrophils, with specific granules predominating in an otherwise pale cytoplasm.

Blood Cell Identification – Ungraded

BCP-27 (cont)

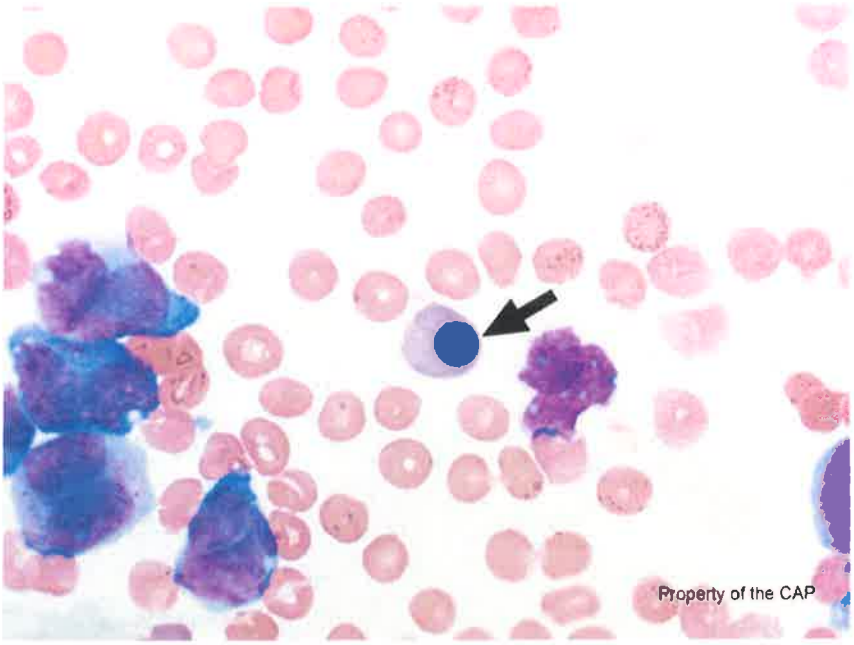
The segmented neutrophil is the predominant blood leukocyte. It has a similar size to a band neutrophil (ie, 10 to 15 µm in diameter), as well as comparable shape (round to oval), and cytoplasmic appearance (pale pink cytoplasm with specific granules). The N:C ratio is 1:3 and the nuclear chromatin is highly condensed. The nucleus is segmented or lobated (with a normal range of three to five lobes). The lobes are connected by a thin filament that contains no internal chromatin, giving it the appearance of a solid, dark, thread-like line. The presence of these thread-like filaments is the basis for distinguishing the segmented neutrophil from the band neutrophil. However, in repeated proficiency testing studies, it has not been possible to achieve consistent differentiation between bands and segmented neutrophils. Therefore, for the purposes of proficiency testing, it is not required that they be differentiated.

2.2% of referees and 0.8% of participants identified this cell as neutrophil with dysplastic nucleus and/or hypogranular cytoplasm. Hypogranular cytoplasm is characterized by absence or decrease in primary and secondary granules, causing the cytoplasm to appear pale and bluish, with cytoplasmic borders that 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.

0.8% of referees and 0.1% of participants identified this cell as neutrophil with hypersegmented nucleus. To be considered a neutrophil with a hypersegmented nucleus, the neutrophil should demonstrate six or more lobes, which are not present in this cell.

Blood Cell Identification – Ungraded

BCP-28

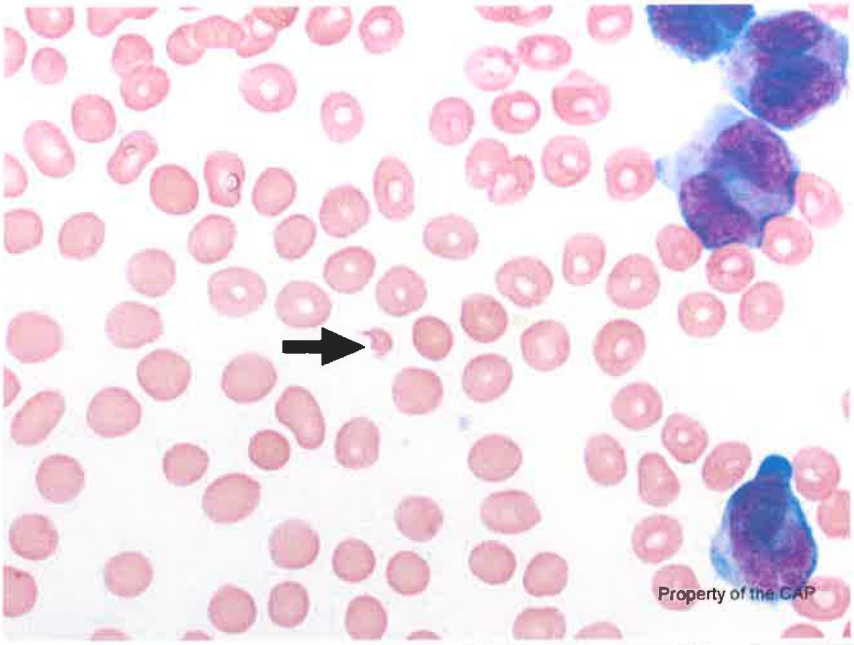


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Nucleated red blood cell, normal or abnormal morphology	134	100.0	5336	99.5	Educational

The arrowed cell is a normal nucleated red blood cell (nRBC), as correctly identified by 100.0% of referees and 99.5% of participants. The term nRBC is used to state the presence of normoblasts in the peripheral blood and includes all normoblasts regardless of the stage of maturation. Typically, the circulating nucleated red blood cell is at the orthochromic stage of differentiation (as seen in the photomicrograph). Both megaloblastic and dysplastic changes can be seen in these circulating red blood cells, reflecting simultaneous erythroid maturation abnormalities present in the bone marrow. Caution should be used in classifying a circulating nRBC as dysplastic on the basis of abnormal nuclear shape (lobated or fragmented), as these changes may occur during their egress from the marrow space and may not be present in the maturing erythroid precursors in the marrow. For the purposes of proficiency testing, it is adequate to identify a cell as a nRBC when it is present in the peripheral blood, be it normal or abnormal (ie, exhibits megaloblastic or dysplastic changes).

Blood Cell Identification – Ungraded

BCP-29



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	129	96.3	5128	95.6	Educational
Acanthocyte (spur cell)	3	2.2	50	0.9	Educational
Bite cell (degmacyte)	2	1.5	176	3.3	Educational

The arrowed cell is a schistocyte, a fragmented red blood cell, as correctly identified by 96.3% of referees and 95.6% of participants. Fragmented red blood cells include helmet cells, keratocytes (horn cells), triangulocytes and a more inclusive term, schistocytes. A zone of central pallor is rarely present in fragmented cells. Occasional spherocytes are almost invariably present in association with fragmented cells (several cells at the base of the arrow). Fragmented cells are seen in severe burns, disseminated intravascular coagulation (DIC) (as in the case of acute promyelocytic leukemia), thrombotic thrombocytopenic purpura (TTP), and other microangiopathic hemolytic anemias, in patients with prosthetic cardiac valves or severe valvular stenosis, malignant hypertension, or other mechanical trauma to the cell (eg, march hemoglobinuria, marathon running). When present in large numbers, they may cause the MCV to fall into the microcytic range or interfere with platelet enumeration.

In addition, on the right-hand side, the photomicrograph shows two abnormal promyelocytes, that are typically seen in acute promyelocytic leukemia (APL). These leukemic cells differ from their normal promyelocyte counterparts in several respects. The nucleus is usually folded, bilobed, or reniform (often with overlapping nuclear lobes). In contrast to normal promyelocytes, a distinct Golgi zone is typically absent in leukemic promyelocytes. Cytoplasmic granules, while abundant in the classic hypergranular form of APL, may be coarser or finer than those seen in normal promyelocytes and slightly darker or more reddish in color. Finally, the abnormal promyelocyte of APL (particularly the hypergranular variant) frequently contains Auer rods, that sometimes can be numerous and overlapping.

Blood Cell Identification – Ungraded

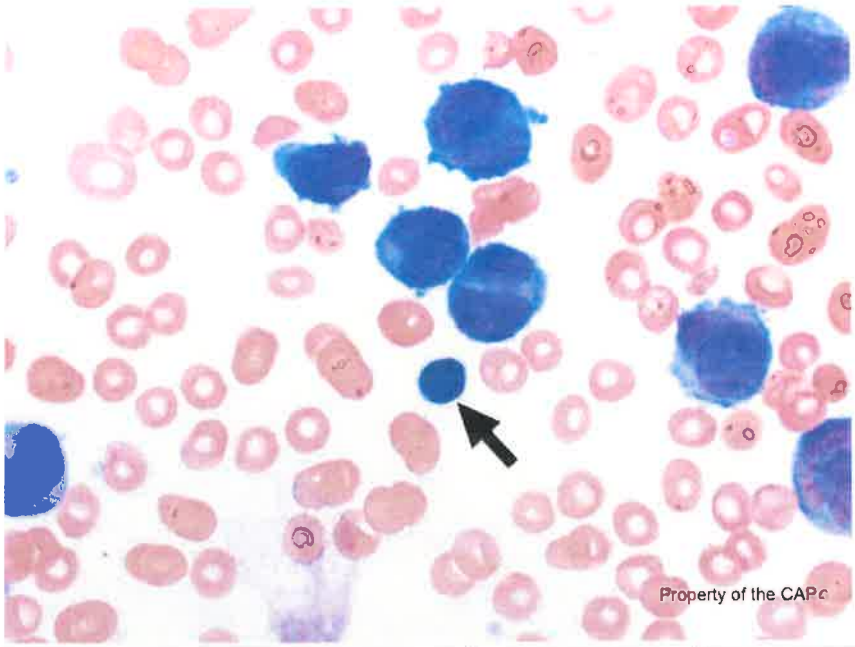
BCP-29 (cont)

2.2% of referees and 0.9% of participants identified this cell as acanthocytes (spur cell). Acanthocytes are densely stained, spheroidal red blood cells that lack central pallor and have multiple (usually three to 20), irregularly distributed, thorn-like spicules of variable size, often with drumstick ends.

1.5% of referees and 3.3% of participants identified this cell bite cell (degmacyte). Bite cells are red blood cells from which precipitated, denatured masses of hemoglobin (Heinz bodies) have been pitted out by the spleen, which results in a variety of peripheral red blood cell defects, ranging from tiny arc-like "nibbles" to large "bites."

Blood Cell Identification – Ungraded

BCP-30



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Lymphocyte	125	93.3	4893	91.2	Educational
Malignant lymphoid cell (other than blast)	3	2.2	67	1.3	Educational
Nucleated red blood cell, normal or abnormal morphology	3	2.2	84	1.6	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	2	1.5	83	1.6	Educational
Lymphocyte, reactive	1	0.8	45	0.8	Educational

The arrowed cell is a normal lymphocyte, as correctly identified by 93.3% of referees and 91.2% of participants. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 µm with an N:C ratio ranging from 5:1 to 2:1. Most lymphocytes have round to oval nuclei that may be slightly indented or notched. The chromatin is diffusely dense or coarse and clumped. Nucleoli are not visible, although some cells may exhibit a small, pale chromocenter that may be mistaken for a nucleolus. Most lymphocytes have a scant amount of pale blue to moderately basophilic, agranular cytoplasm. Occasionally, the edges may be slightly frayed or pointed due to artifacts induced during smear preparation. Occasional lymphocytes will have a small clear zone, or hof, adjacent to one side of the nucleus. While most normal lymphocytes are fairly homogeneous, they do exhibit a range of normal morphology.

2.2% of referees and 1.3% of participants identified this cell as malignant lymphoid cell (other than blast). 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 to 30 µm and the N:C ratio varies from 7:1 to 3:1. However, the small cell size with round nucleus with dense chromatin and scant cytoplasm, as seen on the cell identified by an arrow, are the features of a normal lymphocyte. The other cells on the image are abnormal promyelocytes.

Blood Cell Identification – Ungraded

BCP-30 (cont)

2.2% of referees and 1.6% of participants identified this cell as nucleated red blood cell, normal or abnormal morphology. The term nucleated red blood cell (nRBC) is used to state the presence of normoblasts in the peripheral blood and includes all normoblasts regardless of the stage of maturation. Typically, the nRBC is at the orthochromic stage of differentiation and described as round or ovoid cell (8 to 12 µm in diameter) with very small, often pyknotic, nucleus that sometimes appears as a homogeneous mass of dense chromatin. It is often eccentrically placed and at times may be extruding or fragmented. The cytoplasm usually stains uniformly pinkish orange with little or no basophilia. The N:C ratio is approximately 1:2.

1.5% of referees and 1,6% of participants identified this cell as megakaryocyte (normal, abnormal, or nuclear fragment). Megakaryocyte nuclei and micromegakaryocytes may infrequently be seen in the peripheral blood, however, normal mature megakaryocytes are not found in the peripheral blood. After discharging their cytoplasm to form platelets, megakaryocyte nuclei or nuclear fragments may sometimes enter the bloodstream, particularly in conditions associated with marrow fibrosis, such as primary myelofibrosis. The cell nucleus is single-lobed or, less commonly, multilobated. The chromatin is smudged or "puddled" and is surrounded by a very scant amount of basophilic cytoplasm or no cytoplasm at all. If a small amount of cytoplasm is present, it is often wispy, frilly, or fragmented. Rarely, there may be a few localized areas of cytoplasmic blebs or adherent platelets. If the nuclear characteristics are not appreciated, megakaryocyte nuclei may be mistakenly identified as lymphocytes. 0.8% of referees and 0.8% of participants identified this cell as 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. Reactive lymphocytes are round to ovoid to irregular cells range from 10 to 25 µm in size with an N:C ratio that varies from 3:1 to 1:2. These cells are usually characterized by abundant cytoplasm that can be basophilic, which is not present on the cell identified by an arrow.

Clinical Presentation:

This peripheral blood smear is from a 35-year-old man with no significant past medical history presenting with headache and easy bruising. Laboratory data includes: WBC = 78.1 x 10E9/L; RBC = 2.38 x 10E12/L; HGB = 7.5 g/dL; HCT = 21.1%; PLT = 20 x 10E9/L; MPV = 11.3 fL; MCV = 90 fL; and RDW = 15%.

Case Discussion: Acute promyelocytic leukemia

Acute promyelocytic leukemia (APL) is a biologically and clinically distinct variant of acute myeloid leukemia (AML), characterised by the presence of morphologically abnormal promyelocytes. APL is associated with a specific cytogenetic translocation, t(15;17), resulting in a *PML-RARA* fusion, that is highly sensitive to treatment with all-trans retinoic acid (ATRA). Treatment with ATRA should be initiated as soon as the APL diagnosis is suspected based on abnormal cell morphology. Delays in treatment result in up to early mortality rate of 20% due to cerebrovascular or pulmonary hemorrhage as complications of disseminated intravascular coagulation (DIC) or primary fibrinolysis, which frequently develop in patients with APL.

Patients with APL typically present with leukopenia, accompanied by thrombocytopenia and variable degrees of anemia; however, some cases can present with leukocytosis. In cases with markedly decreased white blood cell (WBC) counts, the abnormal promyelocytes are usually sparse in peripheral blood. Therefore, a careful morphologic evaluation of the peripheral blood smear with identification of abnormal promyelocytes is crucial for a timely presumptive diagnosis of APL. Abnormal promyelocytes are large cells (12 - 24 micron) with morphologic features different from normal promyelocytes. Abnormal promyelocytes have creased, folded, bi-lobed, kidney-shaped, or dumbbell-shaped nuclei with fine chromatin, and prominent nucleoli. In the typical variant of APL (termed "hypergranular" variant), the neoplastic cells contain numerous dense bright pink, reddish-blue, or dark purple granules often obscuring the nucleus. Frequent Auer rods occasionally forming bundles are typically present. This variant accounts for approximately 75% of cases. In contrast, the "hypogranular" or "microgranular" variant, as the name implies, displays sparse to inconspicuous granules on Wright-stained slides by light microscopy. As a result, the hypogranular promyelocytes can be easily confused with immature monocytic cells. Thus, identification of characteristic nuclear shapes is vital to avoid incorrect cell classification. The WBC count is usually higher in the hypogranular variant and a careful review of the peripheral blood smear may reveal occasional typical hypergranular promyelocytes.

In addition to a CBC with differential and review of the peripheral blood smear, all patients with a presumptive APL diagnosis must undergo an urgent laboratory evaluation. This should include coagulation screen to evaluate for the presence of DIC, flow cytometry Immunophenotyping, and cytogenetic and molecular analyses to confirm the presence of an underlying *PML-RARA* translocation. An abnormal coagulation screen with prolonged prothrombin and activated partial thromboplastin times, elevated D-dimers, and low fibrinogen levels is diagnostic of acute DIC. In addition, peripheral blood smear from patients in DIC often contains fragmented red blood cells or schistocytes and occasional spherocytes. Abnormal promyelocytes have a characteristic flow cytometric immunophenotype. The leukemic promyelocytes in both the hypergranular and hypogranular variants demonstrate heterogeneously low to high side scatter; express bright cytoplasmic myeloperoxidase (which can also be assessed by cytochemistry on peripheral blood or bone marrow aspirate smears), CD13 and CD33, and are usually negative for CD34 and HLA-DR. In contrast to normal promyelocytes, APL cells express abnormally low levels of CD15 and weak or variable CD117. The hypogranular variant shows frequent co-expression of CD2 and can sometimes express CD34. The detection of this immunophenotype should immediately prompt a rapid evaluation for APL by detection of t(15;17) which is a disease-defining translocation between the long arms of chromosomes 15 and 17. This creates a fusion gene, *PML-RARA* which links the retinoic acid receptor alpha (*RARA*) gene on chromosome 17 with the promyelocytic leukemia (*PML*) gene on chromosome 15. The

translocation could be rapidly detected by three methods within 24-48 hours of a suspected APL diagnosis: conventional karyotype, fluorescence in situ hybridization (FISH) for the *PML/RARA* fusion, or reverse transcriptase polymerase chain reaction (RT-PCR) for *PML-RARA* RNA. Often all three methods are performed at diagnosis.

To prevent very early deaths occurring prior to treatment, all patients with suspected APL should be hospitalized and managed as a medical emergency. Treatment with ATRA (a myeloid differentiating agent) and measures to counteract coagulopathy should be initiated immediately based on discovery of abnormal promyelocytes on peripheral blood smear review and abnormal coagulation screen, respectively, even before genetic confirmation of the *PML-RARA* translocation. For patients presenting with low WBC counts (≤ 10 x 10E9/L), administration of other antileukemic agents such as arsenic trioxide (ATO) or chemotherapy may be delayed until the genetic diagnosis is confirmed; however, in patients with leukocytosis (ie, WBC count >10 x 10E9/L), chemotherapy should be started without delay even if the diagnostic molecular results are still pending. The induction of tumor cell differentiation with retinoic acid, plus appropriate supportive therapy, leads to rapid improvement in coagulopathy. With timely modern therapy, APL is associated with the highest rate of overall survival out of all subtypes of acute myeloid leukemia.

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