Blood Cell Identification – Graded

Case History

This peripheral blood smear is from a 34-year-old woman presenting with systemic sclerosis. Laboratory data include: WBC = 3.8 × 10E9/L; RBC = 4.42 × 10E12/L; HGB = 13.3 g/dL; HCT = 39.8%; MCV = 90 fL; and PLT = 215 × 10E9/L. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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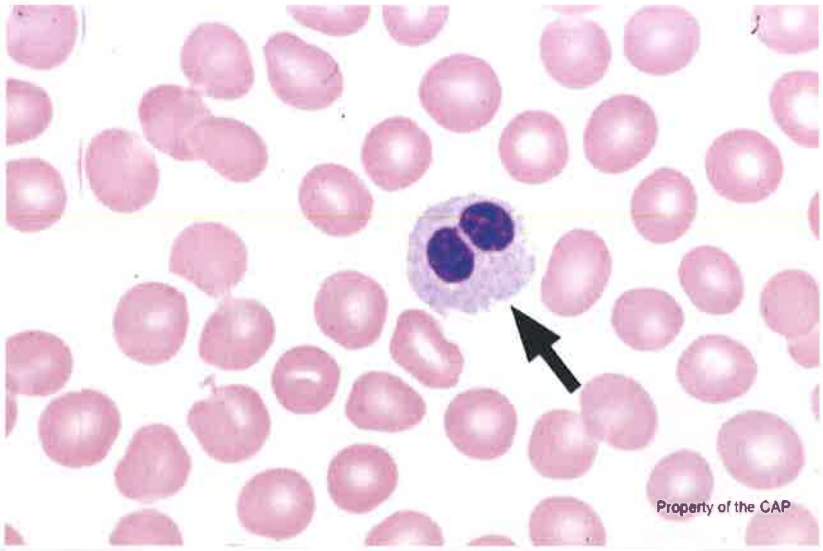
BCP-01

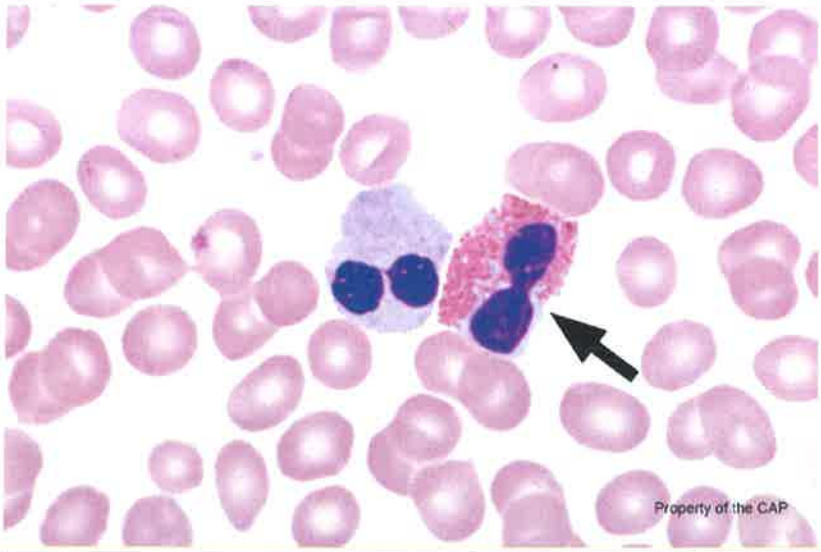


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Neutrophil containing Pelger-Huët nucleus (acquired or congenital)	86	90.5	5092	90.1	Good
Neutrophil, segmented or band	9	9.5	492	8.7	Unacceptable

The arrowed cell is a neutrophil with Pelger-Huët nucleus, as correctly identified by 90.5% of referees and 90.1% of participants. Neutrophils with abnormally unilobed or bilobed nuclei in the pince-nez conformation (two round nuclear lobes connected by a distinct thin filament) are designated as neutrophils with Pelger-Huët nuclei or as Pelger-Huët cells. They occur as an inherited autosomal dominant abnormality of nuclear segmentation referred to as Pelger-Huët anomaly. The nuclear chromatin in Pelger-Huët cells is generally denser than in normal cells. This feature helps to differentiate Pelger-Huët cells from band neutrophils and immature granulocytes such as myelocytes or metamyelocytes which may be seen in the context of a granulocytic left-shift and show more open or lightly staining chromatin. Neutrophils with identical nuclear features are occasionally observed in association with other clinical conditions, including myelodysplastic syndrome (MDS), infection and drug effect. The proportion of nuclei affected in these situations is variable but typically only a small subset of cells are affected, which is a clue since individuals with true Pelger-Huët anomaly usually demonstrate the morphologic abnormality in the majority of their neutrophils. When these cells are seen outside of the context of the congenital abnormality, they are usually referred to as neutrophils with dysplastic nuclei or pseudo-Pelger-Huët cells. However, for proficiency testing purposes, cells with pseudo-Pelger-Huët nuclei are best defined as Pelger-Huët cells.

Blood Cell Identification – Graded

BCP-02

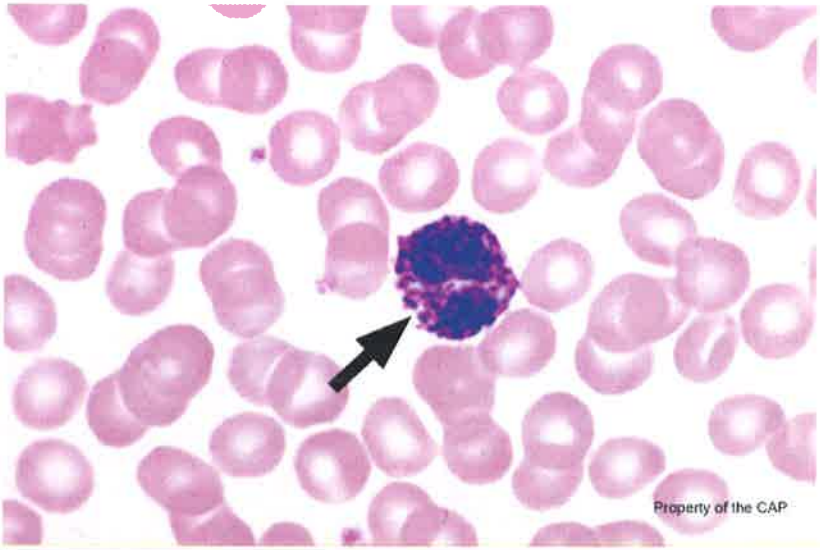


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Eosinophils, any stage	95	100.0	5651	100.0	Good

The arrowed cell is an eosinophil, as correctly identified by 100.0% of referees and 100.0% of participants. The eosinophil is characterized by coarse, orange-red granules of uniform size and is similar to a neutrophil in diameter (10 to 15 µm). Normally, the nucleus demonstrates condensed chromatin and nuclear segmentation with two or three nuclear lobes. This arrowed eosinophil is bilobed, but unilobate forms may also be seen due to the aforementioned Pelger-Huët anomaly.

Blood Cell Identification – Graded

BCP-03

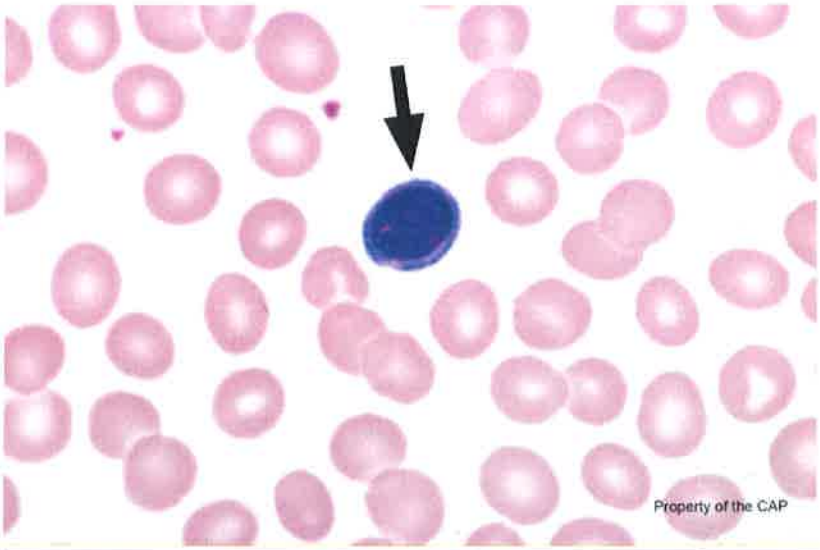


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Basophils, any stage	95	100.0	5635	99.7	Good

The arrowed cell is a basophil, as correctly identified by 100.0% of referees and 99.7% of participants. Basophils are the least common circulating granulocytes. Unlike neutrophils with 3-5 lobed nuclei and fine pink or eosinophilic granules, basophils typically have only two prominent nuclear lobes and cytoplasm with numerous dense purple or basophilic granules, often obscuring the nuclear detail. Basophils are an important part of the allergic immune response, and infrequently circulate in appreciable number (typically representing <0.3% of peripheral leukocytes).

Blood Cell Identification – Graded

BCP-04

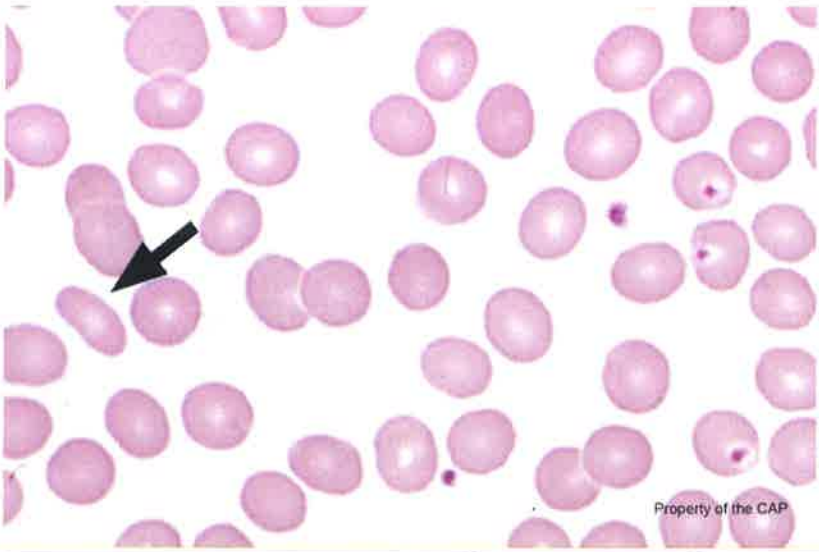


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Lymphocyte	90	94.7	5452	96.4	Good
Lymphocyte, reactive	4	4.2	62	1.1	Unacceptable
Nucleated red cell, normal or abnormal morphology	1	1.1	127	2.3	Unacceptable

The arrowed cell is a lymphocyte, as correctly identified by 94.7% of referees and 96.4% of participants. This cell shows features of mature, non-reactive lymphocytes and is a normal constituent of peripheral blood. The typical lymphocyte is slightly larger than a normal red blood cell with scant to moderate pale blue cytoplasm, round nuclear contours, mature chromatin and inconspicuous nucleoli.

Blood Cell Identification – Graded

BCP-05



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Ovalcyte (elliptocyte)	92	96.8	5619	99.4	Good
Erythrocyte, normal	2	2.1	16	0.3	Unacceptable
Stomatocyte	1	1.1	2	0.0	Unacceptable

The arrowed cell is an ovalocyte (elliptocyte), as correctly identified by 96.8% of referees and 99.4% of participants. The term ovalocyte is used interchangeably with the term elliptocyte, since these red blood cell types have similar disease associations and overlapping morphologic characteristics that make their distinction difficult. Classically, elliptocytes are described as elongated red blood cells with parallel or nearly parallel sides and a concentration of hemoglobin at the ends. Ovalocytes also have an elongated appearance as well but may be differentiated from elliptocytes by having slightly to moderately round rather than straight sides. Central pallor is preserved. Ovalocytes are encountered in a variety of conditions including thalassemia, megaloblastic and iron deficiency anemia, and sickle cell disease. Rare ovalocytes may also be observed in blood smears from normal individuals or as an artifact of smear preparation.

Case Presentation:

This peripheral blood smear is from a 34-year-old woman presenting with systemic sclerosis. Laboratory data include: WBC = 3.8 × 10E9/L; RBC = 4.42 × 10E12/L; HGB = 13.3 g/dL; HCT = 39.8%; MCV = 90 fL; and PLT = 215 × 10E9/L.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Pelger-Huët Anomaly

The Pelger-Huët anomaly refers to a genetic defect which results in characteristically bilobed or unilobed mature granulocyte nuclei. Dr. Karl Pelger, a Dutch hematologist, first described the morphologic features in 1928. Pediatrician G.J Huët established the inherited nature of the abnormality in 1932 when he recognized it in a young girl along with several of the child's relatives. The general incidence of the Pelger-Huët anomaly varies from 0.1-0.01% but may be slightly higher in certain European populations. Neutrophils are most prominently affected and classically show a bilobed nucleus with the lobes separated by a delicate thin filament (so-called spectacle-like or pince-nez formation). The chromatin of affected cells is typically clumped and appears denser than that of normal granulocytes. This feature helps to differentiate Pelger-Huët cells from neutrophil bands which are commonly seen in a granulocytic left-shift and have more open or lightly staining chromatin. Cytoplasmic granulation is usually normal. Other cell lineages, such as monocytes and lymphocytes, are unaffected.

The morphologic phenotype is causally related to mutations in *LBR*, the gene that encodes the lamin B receptor. Lamin B receptor is a constituent of the neutrophil nuclear membrane and is required for normal morphologic development. The Pelger-Huët anomaly is inherited in an autosomal dominant fashion. In the heterozygous state, most of the neutrophils have bilobed nuclei. Individuals with homozygous Pelger-Huët associated gene mutation are very rare and typically demonstrate unilobed nuclei in mature neutrophils. Heterozygous individuals with concurrent infection or systemic inflammation due to granulocytic left-shift may mimic the homozygous state. Detection of Döhle bodies or toxic granulation provides clues to the presence of a left-shift. Notably, individuals with Pelger-Huët anomaly do not appear to be at increased risk for infection, as their neutrophils retain normal functional capability.

Neutrophils with identical nuclear features are occasionally observed as an acquired abnormality in association with various other clinical conditions and in such settings they are referred to as pseudo-Pelger-Huët cells. These include myeloid malignancies such as myelodysplastic syndrome, acute myeloid leukemia, and chronic myelogeneous leukemia. In addition, pseudo-Pelger-Huët cells may be detected in patients with infection and have been linked to HIV, influenza, and mycoplasma. Lastly, a variety of drugs including sulfonamides, colchicine, valproic acid, mycophenolate mofetil, and tacrolimus have been associated with pseudo-Pelger-Huët cells. The proportion of nuclei affected in these situations is variable, but normally segmented neutrophils are usually identifiable.

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1. Kjeldsberg CR, Perkins SL, eds. *Practical Diagnosis of Hematologic Disorders*. 5th ed. Singapore: American Society for Clinical Pathology; 2010.
2. Pereira I, George TI, Arber DA, eds. *Atlas of Peripheral Blood: The Primary Diagnostic Tool*. Philadelphia: Wolters Kluwer; 2012.
3. Colella R, Hollensead SC. Understanding and recognizing the Pelger-Huët anomaly. *American Journal of Clinical Pathology*. 2012;137:358-366.

Blood Cell Identification – Ungraded

Case History

This peripheral blood smear is from a 65-year-old woman with past medical history of breast carcinoma presenting with fatigue. Laboratory data includes: WBC = 14.7 × 10E9/L; RBC = 2.52 × 10E12/L; HGB = 7.6 g/dL; HCT = 22.7%; MCV = 93 fL; PLT = 52 × 10E9/L; and MPV = 7.2 fL. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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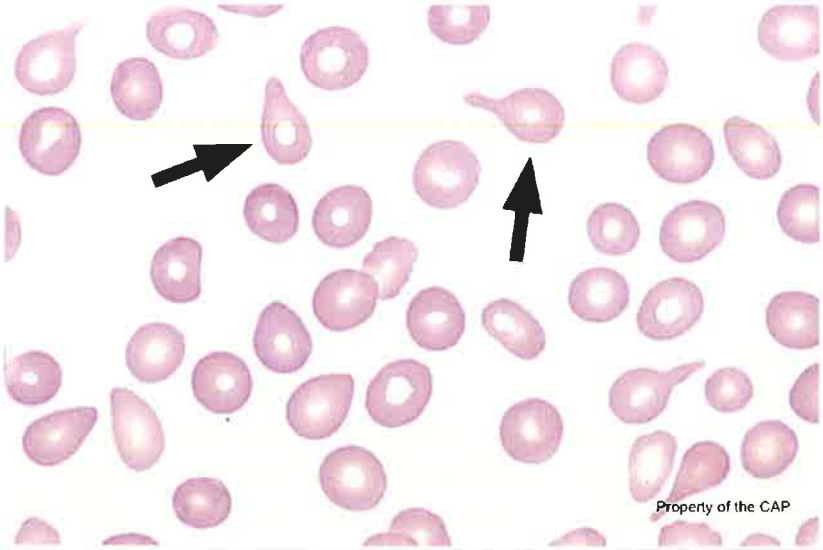
BCP-06

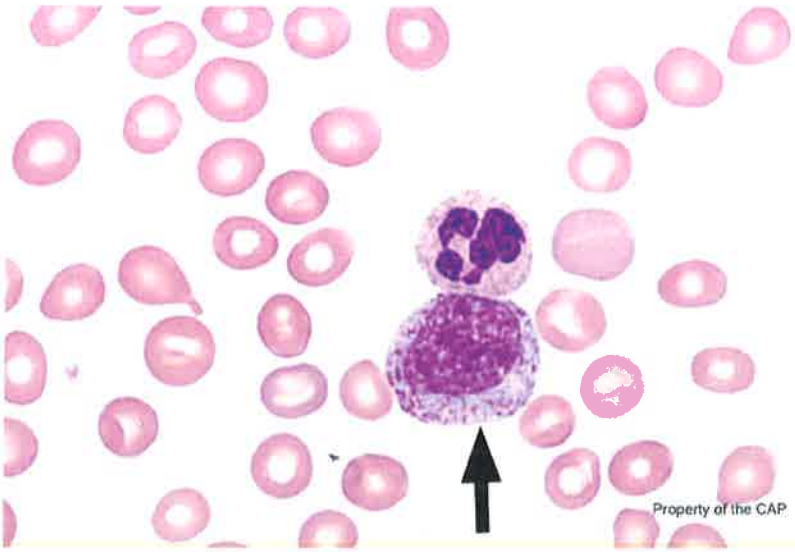


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Teardrop cell (dacrocyte)	95	100.0	5575	99.7	Educational

The arrowed cells are tear drop cells (dacrocytes), as correctly identified by 100.0% of referees and 99.7% of participants. Red cells appearing in the shape of a teardrop or a pear with a single, short or long, often blunted or rounded end are called teardrop cells. These are commonly seen in primary myelofibrosis but may also be seen in pernicious anemia, anemia of renal disease, hemolytic anemias, and other forms of severe anemia. These cells are often associated with an abnormal spleen or bone marrow. Bone marrow infiltration with hematologic and non-hematologic malignancies may also be accompanied by dacrocytosis. Teardrop cells may be seen as an artifact of slide preparation; such dacrocytes are usually easily recognized from the fact that their "tails" all point in the same direction.

Blood Cell Identification – Ungraded

BCP-07

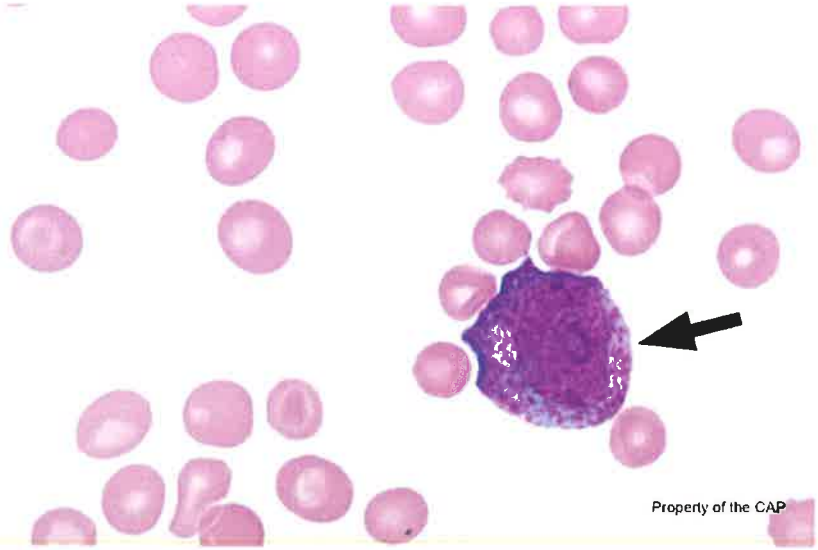


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Neutrophil, myelocyte	83	87.4	4780	87.0	Educational
Neutrophil, promyelocyte	7	7.4	290	5.3	Educational
Neutrophil, toxic	1	1.1	31	0.6	Educational

The arrowed cell is a neutrophil, myelocyte, as correctly identified by 87.4% of referees and 87.0% of participants. The transition from promyelocyte to myelocyte occurs with the end of production of azurophilic (primary) granules and the beginning of production of lilac or pale orange/pink (specific) granules. Myelocytes are usually confined to the marrow where they constitute approximately 10% of the nucleated cells. In pathologic states, myelocytes are seen in blood. The myelocyte is smaller than the earlier precursors, usually 10 to 18 µm. The cells are round to oval in shape and have a nuclear-to-cytoplasmic ratio of 2:1 to 1:1. The nucleus is slightly eccentric, lacks a nucleolus, and begins to demonstrate chromatin clumping; one side often shows slight flattening. Sometimes a clear space or hof is seen adjacent to the nucleus, indicating the location of the Golgi apparatus. The cytoplasm is relatively more abundant than in earlier precursors and is amphophilic. Both azurophilic and specific granules are present in the cytoplasm with specific granules coming to predominate as maturation progresses. See BCP-08 for discussion of a promyelocyte.

Blood Cell Identification – Ungraded

BCP-08



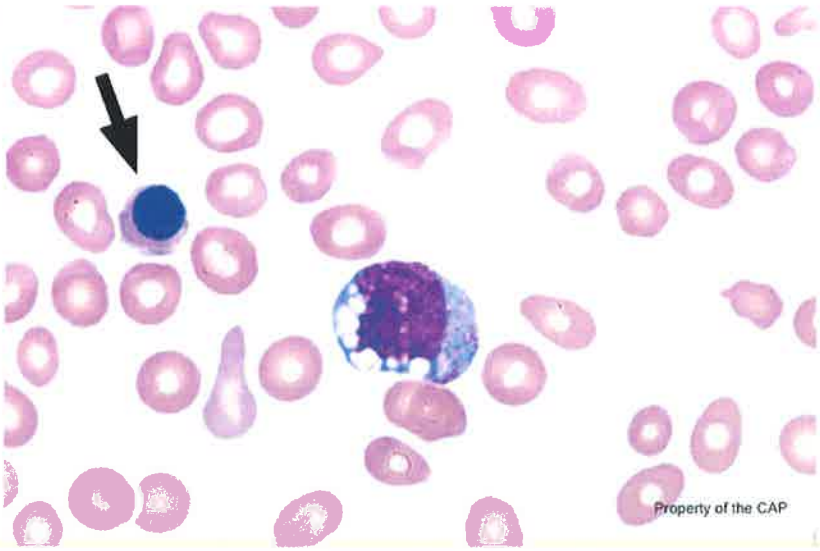
Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Neutrophil, promyelocyte	75	79.0	4503	81.9	Educational
Neutrophil, promyelocyte abnormal containing/lacking Auer rod(s)	12	12.6	399	7.3	Educational
Neutrophil containing dysplastic nucleus and/or hypogranular cytoplasm	1	1.1	2	0.0	Educational
Lymphocyte, large granular	1	1.1	5	0.1	Educational
Malignant lymphoid cell (other than blast)	1	1.1	17	0.3	Educational

The arrowed cell is a neutrophil, promyelocyte, as correctly identified by 79.0% of referees and 81.9% of participants. Promyelocytes are round to oval cells that are generally slightly larger than myeloblasts; the diameter is 12 to 24 µm. They are normally confined to bone marrow, where they constitute less than 2% of nucleated cells; but like the myeloblast, they can be seen in the blood in pathologic states. The nuclear -to-cytoplasmic ratio is high – 5:1 to 3:1. The nucleus is round to oval, has fine chromatin, and contains distinct nucleoli. The cytoplasm is basophilic, more plentiful than in a myeloblast, and contains multiple distinct azurophilic (primary) granules. A paranuclear hof or cleared space may be present.

The arrowed cell is a "neutrophil, promyelocyte" and distinct from a "neutrophil, promyelocyte abnormal", which is the neoplastic cell in acute promyelocytic leukemia (APL). An abnormal promyelocyte differs from a promyelocyte in several respects. The abnormal promyelocyte nucleus is usually folded, bilobed, or reniform, often with overlapping nuclear lobes; a distinct Golgi zone is typically absent. Cytoplasmic granules, while abundant in the classic hypergranular form of APL, may differ in appearance, often being coarser or finer than those seen in normal promyelocytes and slightly darker or more reddish in color. In the microgranular variant of APL, very few granules may be visible and those granules present may be very fine. Finally, the abnormal promyelocyte of APL frequently contains numerous overlapping Auer rods. The arrowed cell in this question has normal nuclear contours and a distinct Golgi zone. Moreover, the granules have a typical appearance in regards to color, number, and texture. Lastly no Auer rod is seen.

Blood Cell Identification – Ungraded

BCP-09

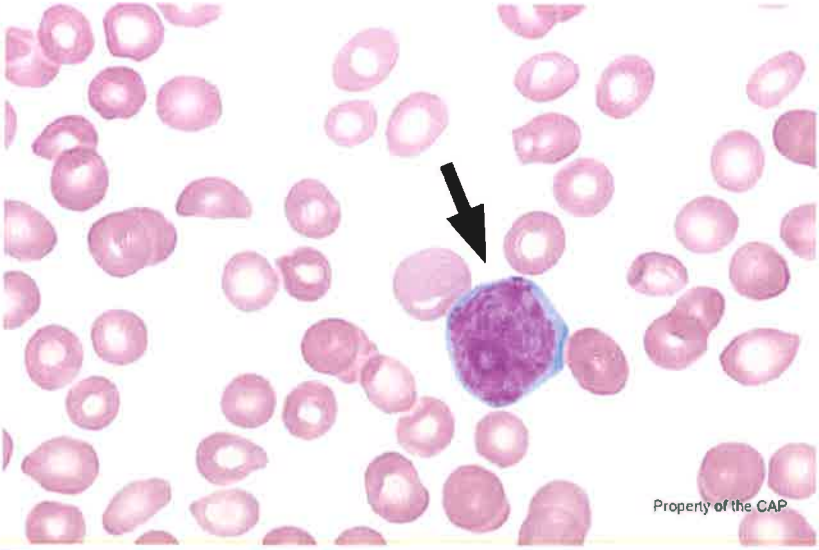


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Nucleated red cell, normal or abnormal morphology	95	100.0	5449	99.1	Educational

The arrowed cell is a nucleated red blood cell (nRBC), as correctly identified by 100.0% of referees and 99.1% of participants. The term *nucleated red blood cell* 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 cell is at the orthochromic stage of differentiation. Both megaloblastic and dysplastic changes can be seen in these circulating red cells, reflecting simultaneous erythroid maturation abnormalities present in the bone marrow. Caution should be used in classifying a circulating nucleated red cell 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 erythroids precursors present in the marrow. For the purposes of proficiency testing, it is adequate to identify a cell as a nucleated red cell when it is present in the peripheral blood, be it normal or abnormal (ie. exhibits megaloblastic or dysplastic changes).

Blood Cell Identification – Ungraded

BCP-10



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Blast cell	81	85.3	4806	87.5	Educational
Lymphocyte, reactive	3	3.2	124	2.3	Educational
Monocyte, immature (promonocyte, monoblast)	3	3.2	85	1.6	Educational
Malignant lymphoid cell (other than blast)	3	3.2	106	1.9	Educational
Lymphocyte, large granular	1	1.1	10	0.2	Educational

The arrowed cell is a blast cell, as correctly identified by 85.3% of referees and 87.5% of participants. 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 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; and it 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). Other cells may have the appearance of a blast, including some lymphoma cells. In the absence of Auer rods, immunophenotyping by flow cytometry, immunohistochemistry on tissue sections or, less commonly, cytochemical staining (eg. peroxidase or Sudan black B reactivity) is required to determine the lineage of a given blast cell. As blasts are quite variable in appearance, it is often impossible to correctly classify an individual cell based on the morphology alone. Blasts can be morphologically indistinguishable from lymphoma cells. For identification purposes, one should classify individual cells exhibiting this type of morphology as blast cells when additional confirmatory information is unavailable.

Case Presentation:

This peripheral blood smear is from a 65-year-old woman with past medical history of breast carcinoma presenting with fatigue. Laboratory data includes: WBC = 14.7 × 10E9/L; RBC = 2.52 × 10E12/L; HGB = 7.6 g/dL; HCT = 22.7%; MCV = 93 fL; PLT = 52 × 10E9/L; and MPV = 7.2 fL.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Myelophthisic Smear

The peripheral blood smear is remarkable for mild leukocytosis with granulocyte left shift including blast cells, normocytic anemia with presence of nucleated red blood cells and many dacrocytes (tear drop cells), and moderate thrombocytopenia. These findings along with the provided clinical history are consistent with myelophthisic anemia. Myelophthisic anemia is defined as anemia secondary to marrow infiltration. This may include overt leukoerythroblastosis with immature granulocytes (often times myelocytes and metamyelocytes but sometimes even myeloblasts) and nucleated red blood cells in the peripheral blood or may present with only a few tear drop cells (dacrocytes) in the smear. Although leukoerythroblastosis may be alarming and raise suspicion of a marrow infiltrative process, several conditions may result in this finding in peripheral blood smear. These include premature infants or newborns, severe infection/trauma, and regeneration after marrow insult/injury including chemotherapy among other etiologies. However, leukoerythroblastosis with associated prominent dacrocytes (tear drop cells) is more ominous and suggestive (although not definitive) of a marrow infiltrative process.

Marrow infiltrative processes include granulomas such as those seen in sarcoidosis or miliary tuberculosis, storage disorders with histiocyte proliferations including Gaucher disease, and metastatic malignancy. Regarding metastasis, most patients present with bicytopenia or pancytopenia with anemia being the most common finding. The anemia may be a result of anemia of chronic disease, nutritional deficiency, microangiopathic process (disseminated intravascular hemolysis is often associated with mucin producing tumors), recent anti-neoplastic therapy, and/or marrow replacement. Interestingly, the mean platelet volume (MPV) can predict likelihood of marrow metastasis in patients with thrombocytopenia and known solid tumor. Specifically, a MPV of < 7.4 fL was found to have significant predictive value and correlates with bone marrow metastasis. Metastatic tumor cells are very rarely seen in the peripheral blood, but when noted are often in the feathered edge and may have the appearance of a lymphoma or blast cell. Ultimately, bone marrow biopsy is needed in patients with myelophthisic anemia to determine exact etiology and multiple and bilateral biopsies may be needed to sample a potentially patchy process.

The likely identification of the metastatic malignancy varies depending on age and sex of the patient. In children, neuroblastoma is the most common metastatic cause by far, but other small round blue cell tumors are reported. In adult females, breast and lung carcinoma (oftentimes small cell carcinoma) predominate. In males, prostate and again lung carcinoma are most frequent. In addition, gastrointestinal adenocarcinomas are reported with some frequency. Rarely sarcomas and melanoma can be seen infiltrating the bone marrow.

Lastly, leukoerythroblastosis and dacrocytes can be seen in patients with hematopoietic neoplasms, with primary myelofibrosis (PMF) being the prototype. However, other hematopoietic neoplasms may also be seen including other myeloproliferative neoplasms, myelodysplastic syndromes with fibrosis, and acute leukemias with fibrosis. Moreover, lymphoma including classical Hodgkin lymphoma can cause marrow replacing lesions with accompanying fibrosis. The finding of large abnormal platelets may suggest PMF among other myeloid neoplasms; the presence of overtly dysplastic granulocytes may also support that a myeloid neoplasm as opposed to a non-hematopoietic neoplasm is inducing a myelophthisic anemia. Finally, overtly malignant myeloblasts such as those containing Auer rods would also confirm a myeloid neoplasm as opposed to a metastasis resulting in myelophthisic anemia.

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Hematology and Clinical Microscopy Committee

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