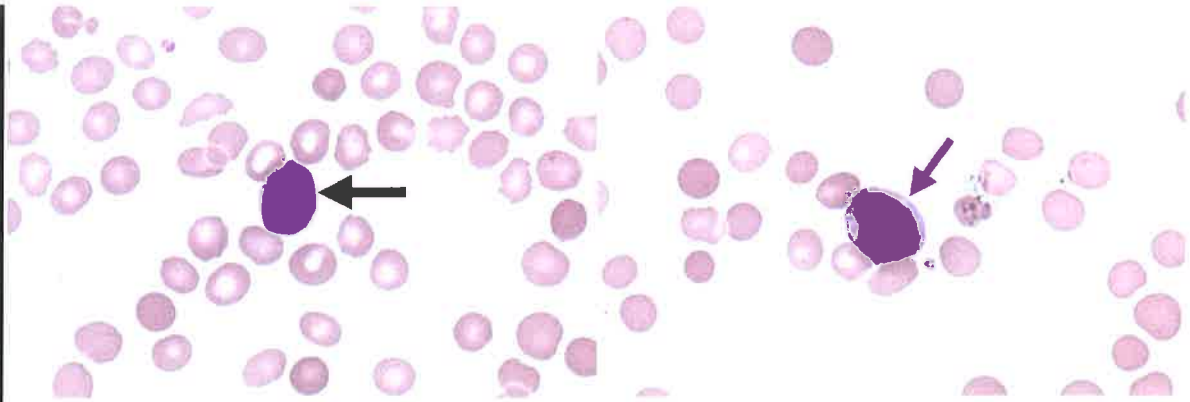
VPBS-03



Identification	Participants No.	%	Evaluation
Blast cell	544	46.0	Educational
Lymphocyte	381	32.2	Educational
Malignant lymphoid cell (other than blast)	148	12.5	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	85	7.2	Educational
Immature or abnormal cell, would refer for identification	16	1.4	Educational
Lymphocyte, large granular	6	0.5	Educational
Neutrophil, myelocyte	2	0.2	Educational

The arrowed cells are blast cells, and more specifically lymphoblasts, as correctly identified by 46.0% of participants. Lymphoblasts are the most immature cells of the lymphoid series. They are most commonly seen in acute lymphoblastic leukemia (ALL) and lymphoid blast crisis of chronic myeloid leukemia (CML). These round to oval cells range in size from 10 to 20 µm. The N:C ratio varies from 7:1 to 4:1. Morphologically, lymphoblasts are variable in appearance, sometimes even within a single case. In this patient, the lymphoblasts are small to medium in size, with high nuclear to cytoplasmic ratio, dispersed (not clumped) chromatin, and scant, agranular cytoplasm. As lymphoblasts may be quite variable in appearance, it is often impossible to correctly classify an individual cell based on the morphology alone. Review of other cells within the sample may help in this regard. Finally, supplemental studies, such as immunophenotyping, are useful to arrive at a definitive diagnosis. Lymphoblasts can sometimes be indistinguishable from other types of blasts and lymphoma cells. For purposes of proficiency testing, one should identify individual cells exhibiting this immature type of morphology as blast cells.

VPBS-03

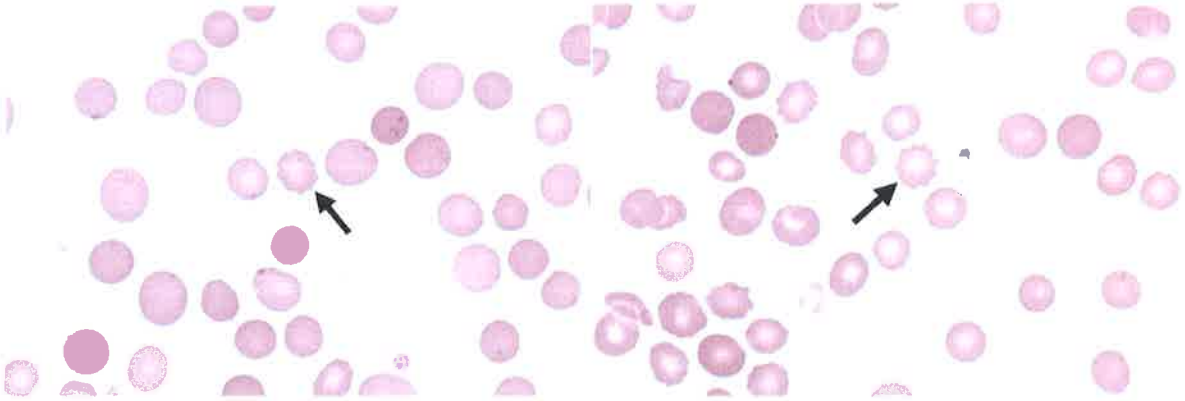
VPBS-03 Discussion, Cont'd:

12.5% of participants have identified the arrowed cells as malignant lymphoid cells (other than blasts). Lymphoma cells can exhibit a variety of appearances depending on the lymphoma subtype, and definitive diagnosis can be difficult. It is critical to obtain an accurate clinical history, since knowledge of a previous diagnosis of lymphoma greatly aids in the identification of these cells. In this patient, the history of B lymphoblastic leukemia/lymphoma is key in guiding the most likely classification of these cells as blast cells. Supplemental studies, such as immunophenotyping, are often necessary to arrive at a definitive diagnosis.

32.2% of participants have identified the arrowed cells as lymphocytes. Most normal lymphocytes are fairly homogeneous, but they do exhibit a range of normal morphology. 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. The arrowed cells are not consistent with lymphocytes as their N:C ratio greatly exceeds the expected 5:1 to 2:1 ratio. Moreover, the chromatin pattern is dispersed and not dense, coarse, or clumped. Lastly, nucleoli are noted in these arrowed cells, thereby excluding a typical lymphocyte.

7.2% of participants have identified the arrowed cells as lymphocytes, reactive. In blood smears, it may be difficult to distinguish reactive lymphocytes from lymphoma cells or lymphoblasts. The most important distinction between these cells is the difference in their N:C ratios. The N:C ratio tends to be low in reactive lymphocytes, while it is typically high in lymphoma cells and lymphoblasts. In addition, reactive lymphocytes are characterized by their wide range of morphologic appearances within the same blood smear. In contrast, while lymphoma cells can exhibit a wide range of morphologic appearances, any individual case of leukemia or lymphoma (such as lymphoblastic leukemia) tends to show a more monotonous population of the cells. In this sample, the arrowed cells have too high of N:C ratios to qualify as reactive lymphocytes.

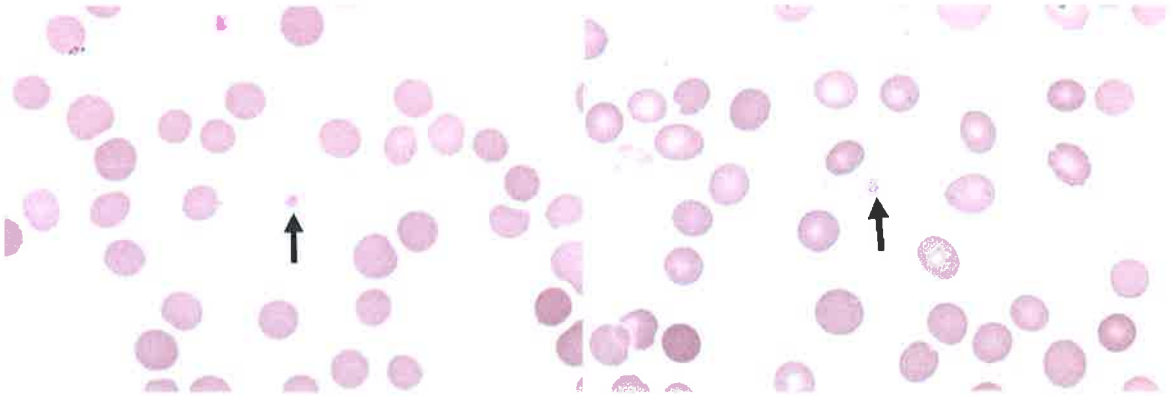
VPBS-04



Identification	Participants No.	%	Evaluation
Echinocyte (burr cell, crenated cell)	1164	98.5	Educational
Acanthocyte (spur cell)	13	1.1	Educational
Erythrocyte, normal	1	0.1	Educational
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	1	0.1	Educational
Howell-Jolly body	1	0.1	Educational
Neutrophil, segmented or band	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational

The arrowed cells are echinocytes, as correctly identified by 98.5% of participants. Echinocytes are red blood cells with 10 - 30 uniform, short, blunt projections distributed evenly that impart a serrated appearance to the red blood cell surface. The red blood cells retain central pallor and are the same size or slightly smaller than normal red blood cells. Echinocytes need to be differentiated from acanthocytes (spur cells). Acanthocytes are densely stained, spheroidal red blood cells that lack or have decreased central pallor and have multiple (usually three to 20), irregularly distributed, thorn-like spicules of variable size, often with drumstick ends. Echinocytes may be present as an artifact or may be an indication of severe renal failure.

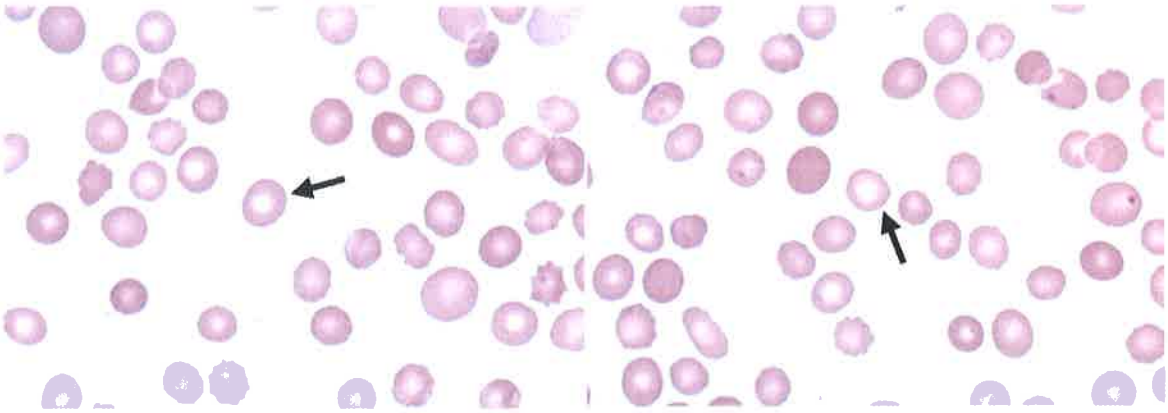
VPBS-05



Identification	Participants No.	%	Evaluation
Platelet, normal	1164	98.5	Educational
Platelet, hypogranular	10	0.9	Educational
Platelet, giant (macrothrombocyte)	4	0.3	Educational
Blast cell	1	0.1	Educational
Erythrocyte, normal	1	0.1	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	1	0.1	Educational
Nucleated red blood cell, normal or abnormal morphology	1	0.1	Educational

The arrowed objects are platelets, as correctly identified by 98.5% of participants. Platelets are blue-gray fragments of megakaryocytic cytoplasm that typically measure 1.5 to 3 µm in diameter and contain fine, purple-red granules. The platelets in this case demonstrate normal size and normal granulation.

VPBS-06



Identification	Participants No.	%	Evaluation
Erythrocyte, normal	1130	95.6	Educational
Hypochromasia	34	2.9	Educational
Microcyte (with increased central pallor)	8	0.7	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	6	0.5	Educational
Nucleated red blood cell, normal or abnormal morphology	2	0.2	Educational
Hemoglobin C crystal	1	0.1	Educational
Platelet, normal	1	0.1	Educational

The arrowed cells are erythrocytes, as correctly identified by 95.6% of participants. An erythrocyte is a mature, non-nucleated, biconcave, disc-shaped cell of fairly uniform diameter (6.7 to 7.8 µm) with a uniform round area of central pallor. It contains hemoglobin and stains uniformly pink-red. The zone of central pallor is due to the biconcavity of the cell and occupies approximately one third (2 to 3 µm) of the cell diameter.

Clinical Presentation:

This peripheral blood smear is from a 70-year-old man with a history of B lymphoblastic leukemia/lymphoma presenting with anemia and thrombocytopenia. WBC = 6.0 x 10E9/L; RBC = 2.90 x 10E12/L; HGB = 8.7 g/dL; HCT = 27.1%; MCV = 88 fL; MCHC = 32.1 g/dL; PLT = 14 x 10E9/L; and RDW = 20%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Recurrent B-Lymphoblastic Leukemia/Lymphoma (B-ALL)

The patient has peripheral blood findings consistent with recurrent B-lymphoblastic leukemia/lymphoma (B-ALL). The CBC demonstrates anemia and thrombocytopenia, and the morphology shows a distinct population of immature lymphoid cells, with very few neutrophils or other leukocytes. In the 2016 WHO classification of hematolymphoid tumors, B-ALL is defined as a neoplasm of precursor lymphoid cells committed to the B-cell lineage, typically composed of small to medium-sized blasts with scant cytoplasm, moderately condensed to dispersed chromatin, and inconspicuous nucleoli, involving bone marrow and blood, and occasionally presenting with primary involvement of nodal or extranodal sites. Unlike with acute myeloid leukemias, there is no agreed-upon lower limit for the proportion of blasts required to establish a diagnosis B-ALL. In general, the diagnosis should be avoided when there are less than 20% blasts in the blood or bone marrow.

ALL is most common in children but may also be seen in adults. Approximately 75% of cases present under the age of 18 and comprise 80 - 85% of leukemias in this age group. The estimated overall number of new cases in the United States is approximately 6,000/year, with approximately 80 - 85% being B-ALL, and the remainder T-ALL. In contrast, acute myeloid leukemia (AML) is diagnosed primarily in adults (median age of 60 years), where it represents 80 - 90% of acute leukemias. Differentiating ALL form AML is important, because the therapeutic regimens differ significantly. The differential diagnosis is based on morphology, cytochemistry, immunophenotyping, and genetics.

While it is sometimes difficult to unequivocally ascribe lineage assignment (myeloid or lymphoid) in acute leukemias based on peripheral blood morphology alone, there are some generic cytologic features that may help in that differential diagnosis (**Table 1**). Of note, these are general descriptors, as lymphoblasts can be variable in appearance, and it may be impossible to correctly classify an individual cell based on the morphology alone. Lymphoblasts can also be indistinguishable from other types of blasts and lymphoma cells. For purposes of proficiency testing, one should identify individual cells exhibiting immature type of morphology as blast cells, unless Auer rods are present which allow for definitive identification as myeloid blasts.

Table 1. Generic cytologic features of blasts in ALL and AML.

	ALL	AML
Blast size	Small to medium; variable	Large and uniform
Chromatin	Coarser than myeloblasts	Finely dispersed
Nucleoli	Absent or 1 - 2; inconspicuous	1 - 4; often prominent
Cytoplasm	Scant to moderate; usually agranular	Moderately abundant; granules may be present
Auer rods	Absent	Often present (60 - 70% cases)
Myelodysplasia	Absent	May be present

Patients with B-ALL may present with an insidious or acute onset. Common, non-specific symptoms include lethargy, fever, joint or bone pain, infection (related to neutropenia), easy bruising (related to thrombocytopenia), and lymphadenopathy or hepatosplenomegaly. From a hematology standpoint, most patients present with anemia and thrombocytopenia. The white blood cell count may be low (or low normal) in 25% of cases, and very high (> 100,000/uL) in only 10% of cases. The presence of increased blasts in the peripheral blood and/or bone marrow biopsy will trigger further workup with ancillary testing, including flow cytometry, FISH, and cytogenetic analysis. In current times, cytochemistry seldom contributes to the diagnosis of ALL, although lymphoblasts would be negative for myeloperoxidase (MPO) granules, if performed. Common B-cell antigens expressed in B-ALL and tested by flow cytometry include CD19, CD20, and CD22. In addition, CD10 is expressed in 90% of cases, and markers of immaturity, such as CD34 and TdT, are also present in most cases. Cytogenetic analysis is very important for risk stratification, with several recognized genetic abnormalities that impart either a good or poor prognosis. The long-term survival in childhood B-ALL is high (approximately 80%) and significantly better than in adults (less than 50%).

Question 1. Which of the following clinical and epidemiologic features are typical for ALL:

A. Most cases are T-ALL
B. Most cases present in the adult age group
C. Most cases present in the pediatric age group
D. Most cases present with a very high WBC count

Question 2. Which of the following best describe cytologic features of lymphoblasts?

A. Auer rods
B. Coarser chromatin than myeloblasts
C. Granular cytoplasm
D. Prominent nucleoli

Question 3. Which of the following ancillary studies would support a diagnosis of B-ALL?

A. Absence of CD10
B. Absence of CD19
C. Expression of MPO
D. Expression of TdT

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References:

1. Borowitz MJ, Chan JKC, Downing JR, LeBeau MM, Arber DA: B-Lymphoblastic leukemia/lymphoma, not otherwise specified (NOS), in Swerdlow SH, Camp E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, eds. *WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues.* Revised 4th ed. Lyon, France: IARC;2017:99-203

ANSWERS TO QUESTIONS:

Question 1: C. Most cases present in the pediatric age group

ALL is most common in children but may also be seen in adults. Approximately 75% of cases present under the age of 18 and comprise 80 - 85% of leukemias in this age group.

Question 2: B. Coarser chromatin than myeloblasts

Lymphoblasts can be variable in appearance, and it may often be impossible to correctly classify an individual cell based on the morphology alone. Lymphoblasts can also be indistinguishable from other types of blasts and lymphoma cells. However, some general cytologic features that may be used to distinguished lymphoblasts from myeloblasts include: variable, small to medium cell size; coarser chromatin; absent or inconspicuous nucleoli; scant to moderate, agranular cytoplasm; and absence of Auer rods.

Question 3: D. Expression of TdT

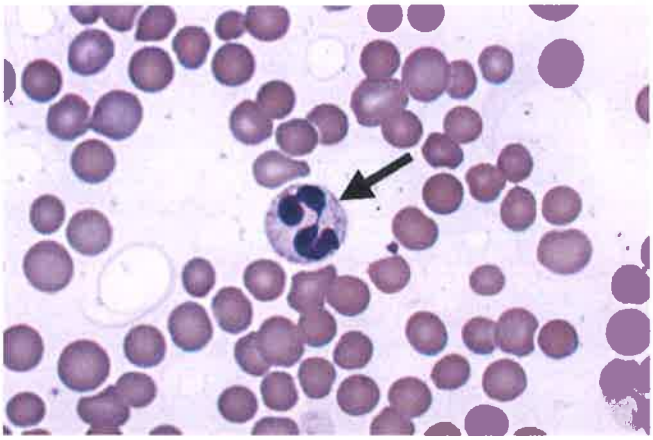
Common B-cell antigens expressed in B-ALL and tested by flow cytometry include CD19, CD20, and CD22. In addition, CD10 is expressed in 90% of cases, and markers of immaturity, such as CD34 and TdT, are present in most cases.

Committee Comments on Peripheral Blood Smear Whole Slide Image

The CBC data are indicative of moderate to marked leukocytosis with mild normocytic anemia and moderate to marked thrombocytopenia. Morphologic examination shows a leukoerythroblastic blood picture without teardrop poikilocytosis observed. The leukocytosis is dominated by neutrophilic cells, many of which are band and segmented forms, while others are immature precursors (ie, promyelocytes, myelocytes, and metamyelocytes). Blasts are rare. Basophils and eosinophils are not significantly increased. The red blood cells are mildly decreased in number. Circulating nucleated red blood cells are readily seen, and polychromasia is increased. Occasional Howell-Jolly bodies are also noted. Platelets are moderately to markedly decreased in number, with a few large and giant forms.

Cell Identification

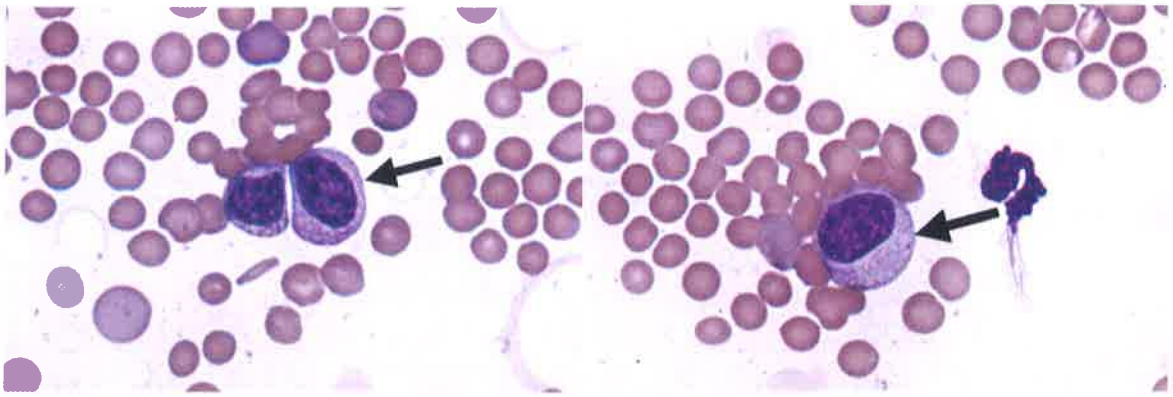
VPBS-08



Identification	Participants No.	%	Evaluation
Neutrophil, segmented or band	1133	95.8	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	35	3.0	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	7	0.6	Educational
Basket cell/smudge cell	1	0.1	Educational
Eosinophil, any stage	1	0.1	Educational
Metastatic tumor cell or tumor cell clump	1	0.1	Educational
Neutrophil, giant band or giant metamyelocyte	1	0.1	Educational
Neutrophil necrobiosis (degenerated nenutrophil)	1	0.1	Educational
Neutrophil with hypersegmented nucleus	1	0.1	Educational
Platelet, normal	1	0.1	Educational

The arrowed object is a segmented neutrophil, as correctly identified by 95.8% of the participants. Segmented neutrophils are typically the predominant leukocyte in the peripheral blood. They are usually about 10 to 15 µm in diameter, round to oval, with segmented to lobated nuclei (typically, three to five lobes), with pale pink cytoplasm with specific granules. The chromatin is highly condensed, and the lobes are connected by solid, dark, thread-like filaments with no internal chromatin. For the purposes of proficiency testing, band and segmented neutrophils are identified together.

VPBS-09



Identification	Participants No.	%	Evaluation
Neutrophil, myelocyte	1024	86.6	Educational
Neutrophil, metamyelocyte	63	5.3	Educational
Lymphocyte	24	2.0	Educational
Neutrophil, promyelocyte	21	1.8	Educational
Blast cell	9	0.8	Educational
Lymphocyte, large granular	8	0.7	Educational
Immature or abnormal cell, would refer for identification	7	0.6	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	7	0.6	Educational
Monocyte	5	0.4	Educational
Monocyte, immature (promonocyte, monoblast)	3	0.3	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	3	0.3	Educational
Myeloblast with Auer rod	2	0.2	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	2	0.2	Educational
Eosinophil, any stage	1	0.1	Educational
Malignant lymphoid cell (other than blast)	1	0.1	Educational
Neutrophil, promyelocyte, abnormal with/without Auer rods	1	0.1	Educational
Neutrophil with Pelger-Huët nucleus (acquired or congenital)	1	0.1	Educational

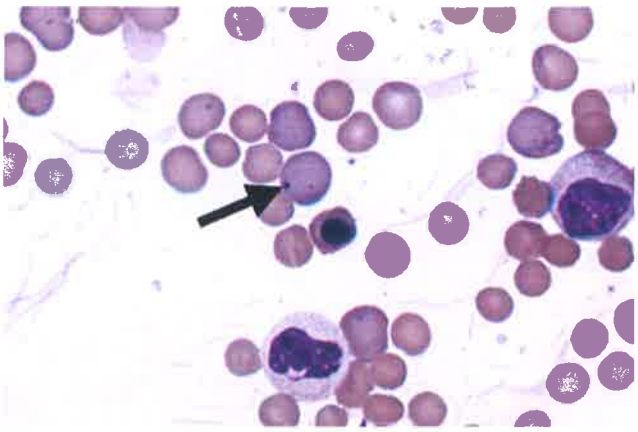
The arrowed object is a neutrophil, myelocyte, as correctly identified by 86.6% of the participants. Myelocytes are usually 10 to 18 µm in diameter, smaller than their earlier precursor, the promyelocyte. Myelocytes are round to oval in shape, with an N:C ratio of 2:1 to 1:1. The nucleus of myelocytes is slightly eccentrically-placed, with chromatin that is more clumped than its earlier precursor. The nucleus may appear slightly flat on one side, sometimes with a perinuclear Golgi hof. Unlike promyelocytes, myelocytes lack a nucleolus. The cytoplasm of myelocytes is relatively abundant and amphophilic, containing both azurophilic and specific granules. Though they comprise about 10% of nucleated cells in the normal bone marrow, myelocytes are not typically seen in the peripheral blood. In this case, myelocytes are readily observed, signifying left-shifted granulocytosis in this patient with a history of myeloproliferative neoplasm.

VPBS-09

VPBS-09 Discussion, Cont'd:

Approximately 5.3% of participants incorrectly identified the arrowed object as a neutrophil, metamyelocyte. In contrast to myelocytes, metamyelocyte nuclear contours not only appear slightly flattened on one side, the contours truly show indentation extending to less than half of the potential round nucleus (that is to say that the indentation is smaller than half of the distance to the farthest nuclear margin), a feature which is not present in the arrowed cells. The metamyelocyte chromatin may be more condensed and coarser when compared to the chromatin of the earlier neutrophilic myelocyte precursor. Although myelocytes may have much more prominent primary (azurophilic) granules and fewer specific (lilac) granules than metamyelocytes, there is a maturational spectrum and the cytoplasmic granularity of myelocytes may closely resemble those of metamyelocytes. The most reliable feature in distinguishing the two neutrophilic precursors is the presence of a bona fide nuclear indentation.

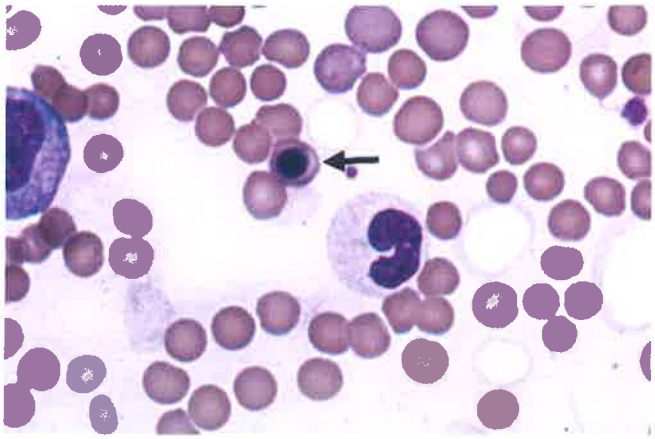
VPBS-10



Identification	Participants No.	%	Evaluation
Howell-Jolly body	1154	97.6	Educational
Polychromatophilic (non-nucleated) red blood cell	14	1.2	Educational
Pappenheimer bodies (iron or Wright stain)	8	0.7	Educational
Basophilic stippling (coarse)	1	0.1	Educational
Echinocyte (burr cell, crenated cell)	1	0.1	Educational
Eosinophil, any stage	1	0.1	Educational
Lymphocyte	1	0.1	Educational
Spherocyte	1	0.1	Educational
Stain precipitate	1	0.1	Educational

The arrowed objects are erythrocytes containing Howell-Jolly bodies, as correctly identified by 97.6% of the participants. Howell-Jolly bodies are DNA remnants manifesting in non-nucleated red blood cells as small, round, dark purple homogeneous masses. At 1 µm in diameter, they are larger, more rounded, and darker staining than Pappenheimer bodies. They are formed in the process of red blood cell nuclear karyorrhexis or when an aberrant chromosome becomes separated from the mitotic spindle and remains behind after the rest of the nucleus is extruded. In normal states, the spleen is usually very efficient in removing Howell-Jolly bodies from red blood cells. However, Howell-Jolly bodies are seen when the spleen is surgically absent or hypofunctional. Many patients with myeloproliferative neoplasms will have extramedullary hematopoiesis in the spleen, which can lead to splenomegaly over a long period of time, and eventually, a hypofunctional spleen. This mechanism explains the presence of Howell-Jolly bodies in this patient's case.

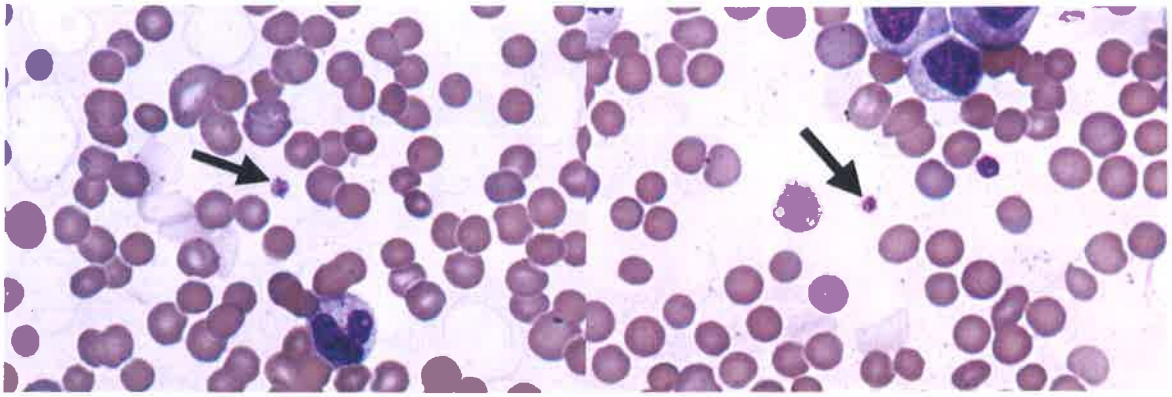
VPBS-11



Identification	Participants No.	%	Evaluation
Nucleated red blood cell, normal or abnormal morphology	1174	99.3	Educational
Erythrocyte, normal	3	0.3	Educational
Lymphocyte	2	0.2	Educational
Basophil, any stage	1	0.1	Educational
Hemoglobin C crystal	1	0.1	Educational
Platelet, normal	1	0.1	Educational

The arrowed object is a nucleated red blood cell (nRBC), as correctly identified by 99.3% of the participants. When seen, circulating nRBCs are usually orthochromic normoblasts (as in this case), which are at the latest stage of normoblast differentiation. Orthochromic normoblasts are round or ovoid (8 to 12 µm in diameter), with a small, often pyknotic, nucleus that sometimes appears as a homogeneous mass of dense chromatin. The cytoplasm usually stains uniformly pinkish orange with little or no basophilia. For purposes of peripheral blood cell identification on CAP proficiency testing, all normal- or abnormal (ie, megaloblastic or dysplastic)-appearing nucleated red blood cells found in the periphery, regardless of maturational stage, are classified as nRBCs.

VPBS-12



Identification	Participants No.	%	Evaluation
Platelet, normal	1167	98.7	Educational
Platelet, giant (macrothrombocyte)	8	0.7	Educational
Erythrocyte, normal	3	0.3	Educational
Lymphocyte	1	0.1	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	1	0.1	Educational
Monocyte	1	0.1	Educational
Platelet, hypogranular	1	0.1	Educational

The arrowed objects are platelets, as correctly identified by 98.7% of the participants. Platelets (also called thrombocytes) are blue-gray fragments of megakaryocytic cytoplasm, which play an essential role in primary hemostasis. In the peripheral blood of healthy individuals, platelets are small and fairly uniform in size, most measuring 1.5 to 3 µm in diameter, with a few smaller forms (less than 1.5 µm) and a few larger forms (4 to 7 µm). Fine, purple-red, alpha granules are dispersed throughout the cytoplasm or are sometimes aggregated at the center. In this case, the platelet sizes vary, and large and giant forms are frequent.

Clinical Presentation:

This peripheral blood smear is from a 55-year old man with a history of myeloproliferative neoplasm now presenting with leukocytosis. Laboratory data include: WBC = 79.4 x 10E9/L; RBC = 3.97 x 10E12/L; HGB = 11.7 g/dL; HCT = 37.2%; MCV = 94 fL; and PLT = 37 x 10E9/L.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA

CASE DISCUSSION: Myeloproliferative neoplasm now with leukocytosis

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic malignancies that are characterized by the overproduction of myeloid progenitor cells, leading to elevated levels of peripheral blood erythrocytes, granulocytes, and/or platelets - whether as isolated elevations of each cell lineage or combinations thereof. MPNs are rare, with all subtypes together having an annual incidence of 6 cases per 100,000 individuals.

EXAMPES OF MYELOPROLIFERATIVE NEOPLASMS

Examples of the more common MPNs include chronic myeloid leukemia (CML), essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF). CML is slightly more commonly seen than the other MPNs, with an annual incidence of 1 - 2 cases per 100,000 persons. The discovery of the *BCR-ABL1*, t(9;22)(q34.1;q11.2) as the causative mutation in CML was a major scientific breakthrough that has advanced the study of hematopoietic malignancies.

The other more common MPNs (PV, ET, and PMF) lack the *BCR-ABL1* fusion (hence the term, *BCR-ABL1*-negative MPNs), but were found to have mutually exclusive driver mutations, including *JAK2* V617F, *JAK2* exon 12, *CALR,* and *MPL*.

Question 1. Which MPN is characterized by the *BCR-ABL1* fusion?

A. Chronic myeloid leukemia
B. Essential thrombocythemia
C. Polycythemia vera
D. Primary myelofibrosis

TYPICAL FINDINGS AND DIAGNOSTIC APPROACH

Typically, patients will present with persistent elevations in blood elements with no clear underlying reactive cause. Typically, in PV, the hemoglobin and/or red blood cell mass is elevated. ET classically presents with an isolated thrombocytosis. In PMF, the CBC findings may be much more variable and can include anemia, leukocytosis, and/or thrombocytosis. On the other hand, CML has a distinctive morphology, with a characteristic left-shifted granulocytosis ("myelocyte bulge"), basophilia, and/or eosinophilia.

The diagnosis of CML is usually straightforward and can be performed on review of the peripheral blood smear, requiring genetic/molecular studies to confirm one's morphologic impression. However, accurate diagnosis of *BCR-ABL1*-negative MPNs requires thorough clinical history, laboratory data, and careful review of well-prepared peripheral blood smears, bone marrow aspirate smears, touch preparation slides, and bone marrow trephine biopsies.

Question 2. True or False Statement: The presence of certain driver mutations (such as *JAK2* V617F) alone is sufficient for the definitive diagnosis of primary myelofibrosis.

A. True
B. False

NATURAL HISTORY OF DISEASE

MPNs may be clinically insidious and patients may be asymptomatic at presentation. Splenomegaly and/or hepatomegaly may be observed because of long-standing extramedullary hematopoiesis in the spleen and/or liver. Depending on the MPN entity, there are varying degrees of risk of progression to any or a combination of the following clinical outcomes: bone marrow failure secondary to myelofibrosis (ie, marrow fibrosis), ineffective hematopoiesis, evolution to accelerated phase with excess blasts, and/or transformation to acute leukemia.

In a patient with a history of MPN, who is presenting with new-onset cytopenias and leukoerythroblastosis (ie, circulating immature granulocytes and nucleated red blood cells (nRBCs)), these findings are a harbinger of disease progression, as they suggest impending bone marrow failure and significant increase in bone marrow fibrosis, respectively.

Question 3. The following peripheral blood findings are uncommon features in patients with long-standing history and disease progression of MPN:

A. Anemia with circulating nucleated red blood cells
B. Circulating immature granulocytes
C. Excess blasts
D. Plasma cells and immunoblasts

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REFERENCES:

1. Vardiman JW. Myeloproliferative neoplasms. In Jaffe ES, Arber DA, Campo E, et al., eds. *Hematopathology.* 2nd edition. Philadelphia, PA: Elsevier 2017.
2. Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues.* Revised 4th ed. Lyon, France: IARC; 2017: 199-203

Answers to Questions:

Question 1: A. Chronic myeloid leukemia
The chromosomal abnormality resulting in the reciprocal translocation of genetic material from chromosomes 9 and 22, leads to the *BCR-ABL1* fusion seen in CML. The other MPNs lack the *BCR-ABL1* fusion, and instead have other driver mutations, including *JAK2* V617F, *JAK2* exon 12, *CALR,* and *MPL*.

Question 2: B. False
This is a false statement. The accurate diagnosis of *BCR-ABL1*-negative MPNs, such as primary myelofibrosis, requires the integration of good clinical history, laboratory data, and careful review of well-prepared peripheral blood smears, bone marrow aspirate smears, touch preparation slides, and bone marrow trephine biopsies.

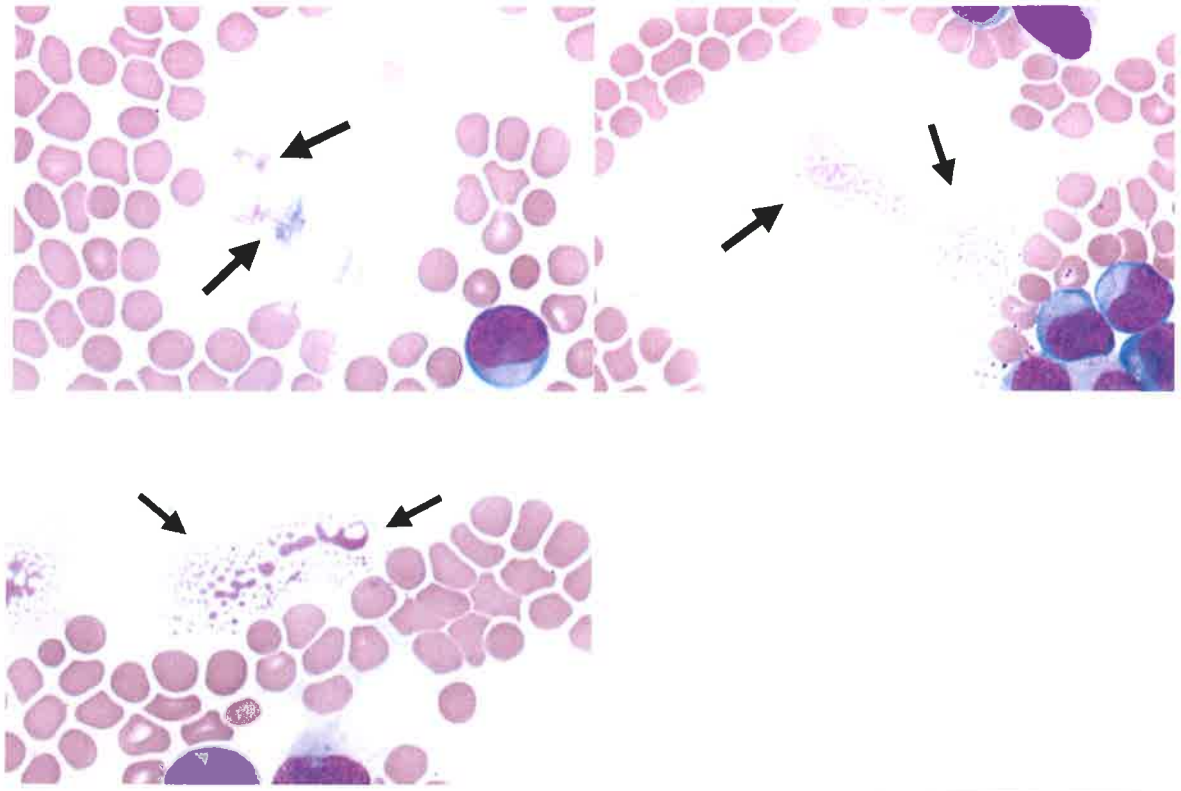
Question 3: D. Plasma cells and immunoblasts
Choices A - C can be seen in a patient with history of MPN, with progression of disease. In particular, the leukoerythroblastic blood picture with circulating immature granulocytes and nRBCs as well as myelophthisic blood picture with the aforementioned leukoerythroblastosis and teardrop poikilocytosis may signal the presence of increasing bone marrow fibrosis. Some patients with long-standing MPN are also at increased risk of evolution to accelerated phase and/or transformation to acute myeloid leukemia, and thus excess blasts may also be seen. On the other hand, the finding of plasma cells and immunoblasts in the peripheral blood would not be associated with MPN disease progression.

Committee Comments on the CBC and Blood Film

The CBC data are indicative of a significant leukocytosis with moderate anemia and severe thrombocytopenia. Morphologic examination shows that the leukocytosis consists of numerous blasts including forms with single to multiple Auer rods. These blasts are large in size with open chromatin, scant to moderate blue cytoplasm, and variably prominent nucleoli. A subset have cytoplasmic vacuoles. Rare mature granulocytes, lymphocytes, and monocytes are seen.

Cell Identification

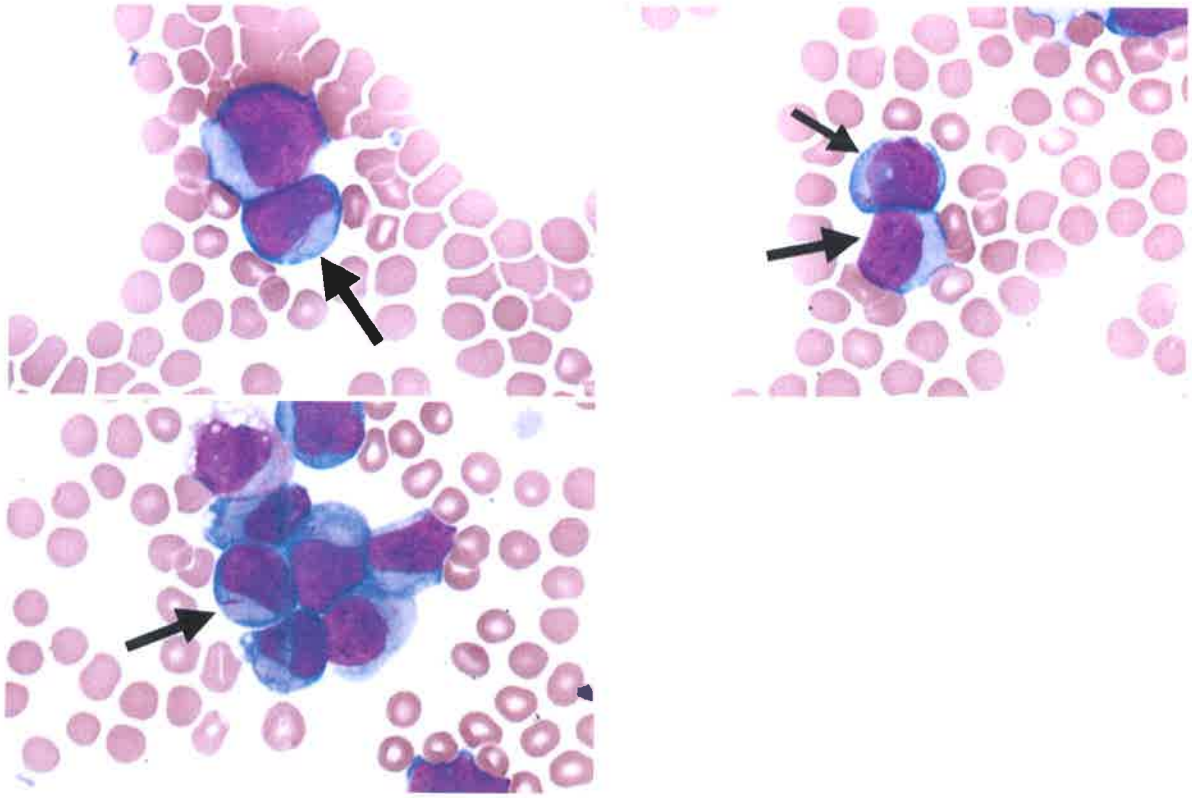
VPBS-14



Identification	Participants No.	%	Evaluation
Stain precipitate	1166	98.7	Educational
Basket cell/smudge cell	10	0.9	Educational
Cryoglobulin	3	0.3	Educational
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	1	0.1	Educational
Immature or abnormal cell, would refer for identification	1	0.1	Educational
Squamous epithelial cell/endothelial cell	1	0.1	Educational

The arrowed feature is stain precipitate, as correctly identified by 98.7% of participants. Stain precipitate is acellular, variably sized, amorphous aggregates or red or purple material that can overlay cells in the smear. While the stain precipitate is clearly acellular in the images shown, it can be mistaken for cellular inclusions, parasites, or microorganisms in some cases.

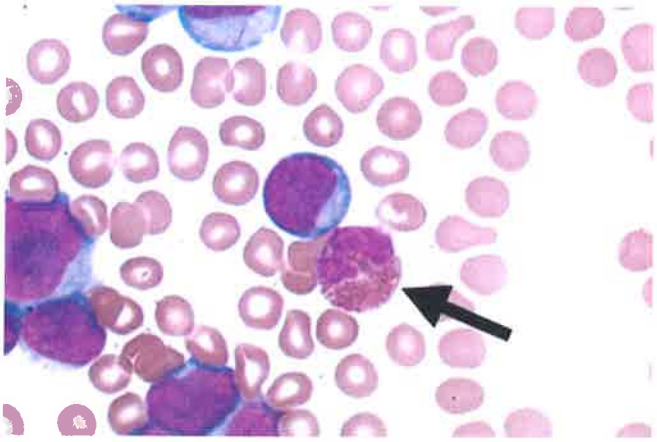
VPBS-15



Identification	Participants No.	%	Evaluation
Myeloblast with Auer rod	989	83.7	Educational
Blast cell	131	11.1	Educational
Neutrophil, promyelocyte, abnormal with/without Auer rod(s)	20	1.7	Educational
Immature or abnormal cell, would refer for identification	17	1.4	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	13	1.1	Educational
Malignant lymphoid cell (other than blast)	7	0.6	Educational
Basket cell/smudge cell	1	0.1	Educational
Erythocyte, normal	1	0.1	Educational
Microcyte (with increased central pallor)	1	0.1	Educational
Neutrophil, myelocyte	1	0.1	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	1	0.1	Educational

The arrowed cells are myeloblasts with Auer rods, as correctly identified by 83.7% of participants. Blast cell, as identified by 11.1 of participants, is an acceptable answer, although not as specific as myeloblast with Auer rods. Myeloblasts are typically large cells (10 - 20 µm) with round or slightly irregular nucleus and characteristic dispersed, finely reticulated chromatin. One or more conspicuous nucleoli can often be seen. The cytoplasm is typically scant and basophilic. Auer rods, as noted in many of the arrowed cells, are identified as pink to red, rod-shaped, crystalline inclusions and are a specific finding associated with some types of myeloblasts, although they are not seen in all myeloblasts. When an Auer rod is present, the cell is definitely able to be identified as a myeloblast.

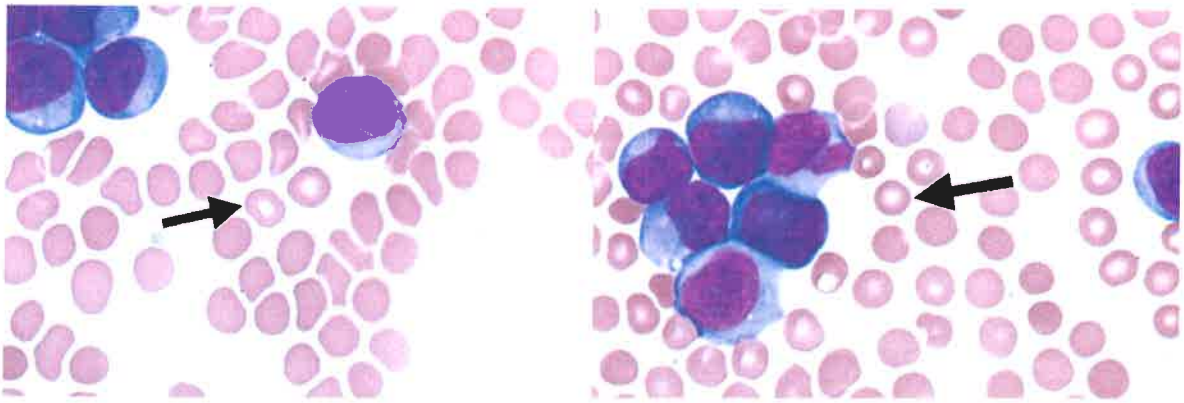
VPBS-16



Identification	Participants No.	%	Evaluation
Eosinophil, any stage	1180	99.8	Educational
Basophil, any stage	1	0.1	Educational
Myeloblast with Auer rod	1	0.1	Educational

The arrowed cell is an eosinophil, as correctly identified by 99.8% of participants. Eosinophils are characterized by coarse, orange-red granules of uniform size and are similar to neutrophils in diameter (10 - 15 µm). Normally, the nucleus demonstrates condensed chromatin and nuclear segmentation typically with two or three lobes.

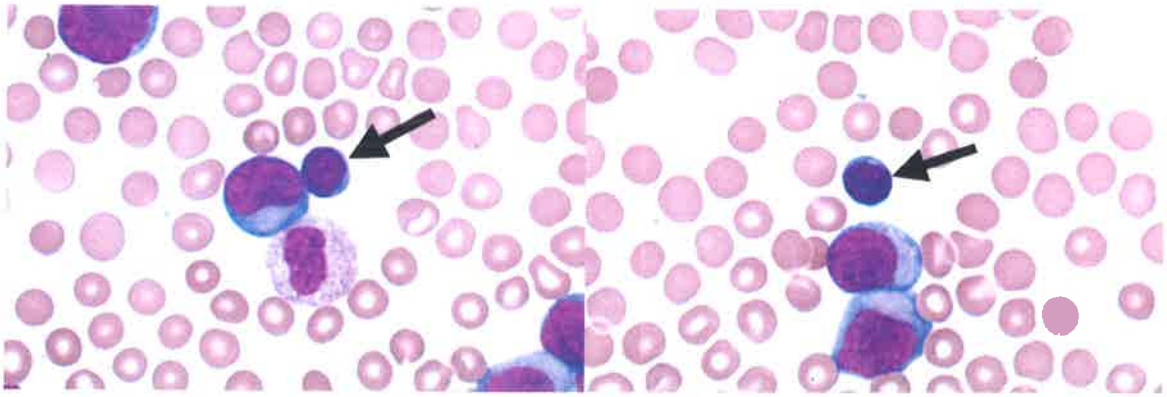
VPBS-17



Identification	Participants No.	%	Evaluation
Erythrocyte, normal	883	74.7	Educational
Microcyte (with increased central pallor)	176	14.9	Educational
Hypochromasia	111	9.4	Educational
Nucleated red blood cell, normal or abnormal morphology	4	0.3	Educational
Spherocyte	3	0.3	Educational
Polychromatophilic (non-nucleated) red blood cell	2	0.2	Educational
Myeloblast with Auer rod	1	0.1	Educational
Platelet, normal	1	0.1	Educational
Stomatocyte	1	0.1	Educational

The arrowed cells are normal erythrocytes, as correctly identified by 74.7% of participants. Erythrocytes are anucleate, biconcave discs containing predominantly hemoglobin. The cells measure approximately 6.7 - 7.8 µm in size, with the area of central pallor measuring one-third of the diameter in normocytic, normochromic red blood cells, as seen in the arrowed cells above. In contrast, microcytes, as identified by 14.9% of participants, are smaller than normal erythrocytes, typically reflected by a low MCV (= (HCT/RBC) x 10), and those with "increased central pallor" have a clear central zone that exceeds 50% of the cell diameter. While erythrocyte size is comparable to a lymphocyte nucleus, this form of relative assessment is only possible when background leukocytes are also morphologically normal.

VPBS-18



Identification	Participants No.	%	Evaluation
Lymphocyte	1170	99.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	4	0.3	Educational
Malignant lymphoid cell (other than blast)	2	0.2	Educational
Bacteria (spirochete), extracellular	1	0.1	Educational
Blast cell	1	0.1	Educational
Monocyte	1	0.1	Educational
Myeloblast with Auer rod	1	0.1	Educational
Nucleated red blood cell, normal or abnormal morphology	1	0.1	Educational

The arrowed cells are lymphocytes, as correctly identified by 99.1% of the participants. These cells have a round to slightly oval nucleus with dense and clumped chromatin, helping to differentiate them from blasts. The lymphocyte is slightly larger than a normal red blood cell with scant to moderate pale blue and typically agranular cytoplasm.

Clinical Presentation:

This peripheral blood smear is from a 51-year-old woman with new onset of leukocytosis presenting with fever and fatigue. Laboratory data include: WBC = 186.9 x 10E9/L; RBC = 3.20 x 10E12/L; HGB = 9.6 g/dL; HCT = 30.0%; MCHC = 32.0 g/dL; and PLT = 29 x 10E9/L.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Acute Myeloid Leukemia (AML)

Acute myeloid leukemia (AML) is a malignancy of early myeloid precursors that have lost the ability to differentiate. These neoplasms were originally defined by their morphology, surface marker expression, and cytochemical staining patterns, and grouped based on which of the myeloid series they most closely resembled. For instance, those with monocytic features would be classified as acute monocytic or monoblastic leukemia, those with promyelocytic characteristics as acute promyelocytic leukemia (APL), and so on. With the application of conventional karyotyping, genetic classification of AML was adopted, and further refined by subsequent molecular studies. In some instances, the genomic changes are prognostic, and in others, predictive of response to specific therapeutic agents.

CLINICAL PRESENTATION:

Clinical manifestations of AML are not typically specific, and usually represent consequences of bone marrow failure and associated cytopenias. Patients can present with severe fatigue and shortness of breath related to a hypoproliferative anemia, opportunistic infections as a result of neutropenia, and/or bruising and petechiae as a result of thrombocytopenia. In many cases, the leukocyte count is elevated due to numerous early myeloid precursors (blasts), that can cause slowing of the blood flow (stasis) through the brain (as well as other organs), and patients can present with mental status changes and somnolence. In some forms of AML, patients can present with disseminated intravascular coagulation (DIC) due to dysregulation of the clotting pathway. Symptoms manifest in weeks to months, and while there is a broad age range, AML is typically considered a disease of older adulthood.

DIAGNOSIS:

The correct diagnosis and classification of acute leukemia rests on a comprehensive morphologic, flow cytometric, and genomic characterization of the neoplastic cells. As prognosis and therapy are distinctly different, differentiation between AML and acute lymphoblastic leukemia (ALL) is of paramount importance. While blasts can be generally identified and enumerated by morphology alone, classification as "myeloid" or "lymphoid" in the current era requires immunophenotypic investigation, typically by flow cytometry. In some subsets of AML, polymerized primary azurophilic granules can form eosinophilic, needle-shaped cytoplasmic inclusions (Auer rods), that are sufficient to assign a "myeloid" lineage. A diagnosis requires a morphologic blast count of at least 20% in the blood or bone marrow aspirate (excluding AML with specific cytogenetic abnormalities such as t(8;21), APL with *PML-RARA*, and AML with inv(16) or t(16;16)). In some cases such as those with a dry tap due to a fibrotic marrow, etc, enumeration of blasts may be difficult and may be aided by performing a differential on a biopsy touch preparation or via immunohistochemical analysis.

Question 1. Auer rods identified in some cases of acute myeloid leukemia are:

A. Also present in many cases of acute lymphoblastic leukemia
B. Basophilic needle-shaped cytoplasmic inclusions
C. Polymerized primary granules
D. Uniformly present in all populations of blasts

Question 2. The diagnosis of Acute Myeloid Leukemia typically requires at least:

A. 20% blasts by flow cytometric analysis of blood or bone marrow
B. 20% blasts by manual differential count in the blood or bone marrow
C. 20% blasts by immunohistochemical staining of the bone marrow biopsy
D. 20% blasts in both the blood and bone marrow

CYTOGENETICS AND MOLECULAR TESTING:

Routine karyotypes are typically performed on cases of AML at diagnosis, and the cytogenetic data provide important diagnostic, prognostic, and predictive information. Some karyotypic changes are so characteristic that they define the specific category of AML. Fluorescent in situ hybridization (FISH) can be used to confirm the karyotypic findings or identify key aberrations when a fresh aspirate specimen is not available for karyotyping. Moreover, FISH analysis may be useful when the cytogenetic aberration is cryptic by karyotype.

More recently, mutations in specific genes have been identified, which similarly provide important clinical information. Next generation sequencing platforms as well as more targeted assays are used to characterize these changes in AML.

PROGNOSIS AND TREATMENT:

Patients with AML have a variable outcome depending on clinical and biologic features. Patients who are younger and otherwise healthy can be treated with conventional cytotoxic chemotherapeutic regimens with the intention of cure. Some categories of AML, particularly highly aggressive forms arising in patients who can tolerate aggressive therapy, can be cured with allogeneic bone marrow transplantation. However, significant morbidity and mortality can be associated with this therapy, and accurate diagnosis and classification is necessary to effectively guide therapy.

Question 3. Which of the following ancillary studies is the most appropriate tool for immunophenotypically characterizing blasts and differentiating AML from ALL?

A. Flow cytometry
B. Fluorescence in situ hybridization
C. Karyotype
D. Next generation sequencing panels

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REFERENCES:

1. Swerdlow SH, Campo E, Harris NL, Jaffee ES, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Revised. 4th Edition, Volume 2. Lyon, France: IARC; 2017.
2. Glassy EF. *Color Atlas of Hematology: An Illustrated Field Guide Based on Proficiency Testing*. Northfield IL: College of American Pathologists; 2018.
3. Arber DA, Borowitz MJ, Cessna M, et al. Initial Diagnostic Workup of Acute Leukemia: a Guideline from the College of American Pathologists and the American Society of Hematology. *Archives of Pathology and Laboratory Medicine*. 2018;141:1342.

ANSWERS TO QUESTIONS:

Question 1: C. Polymerized primary granules.

Auer rods are not seen in all cases of AML, but when present tend to be identified in a subset of the myeloid blasts. These eosinophilic, needle-shaped inclusions represent polymerized primary granules and are diagnostic of the myeloid series. As such, they are not identified in ALL.

Question 2: B. 20% blasts by manual differential count of the blood or bone marrow.

The diagnosis of AML requires no bf 20% blasts enumerated by a morphologic manual count in the blood or bone marrow. In some cases, AML patients are pancytopenic and do not show increased numbers of circulating peripheral blood blasts. The enumeration can be clarified and supported by immunophenotypic studies including flow cytometry and immunohistochemistry. Some cytogenetic abnormalities are so characteristic, that they are independently sufficient to warrant a diagnosis of AML even without 20% blasts in the blood or bone marrow.

Question 3: A. Flow cytometry

Flow cytometry allows for detailed immunophenotypic characterization of blasts in the blood or bone marrow and is typically used to analyze expression of myeloid and lymphoid lineage markers. This process allows for the important differentiation of AML from ALL. The other listed assays are helpful in the subclassification of acute leukemia, and often provide prognostic information.