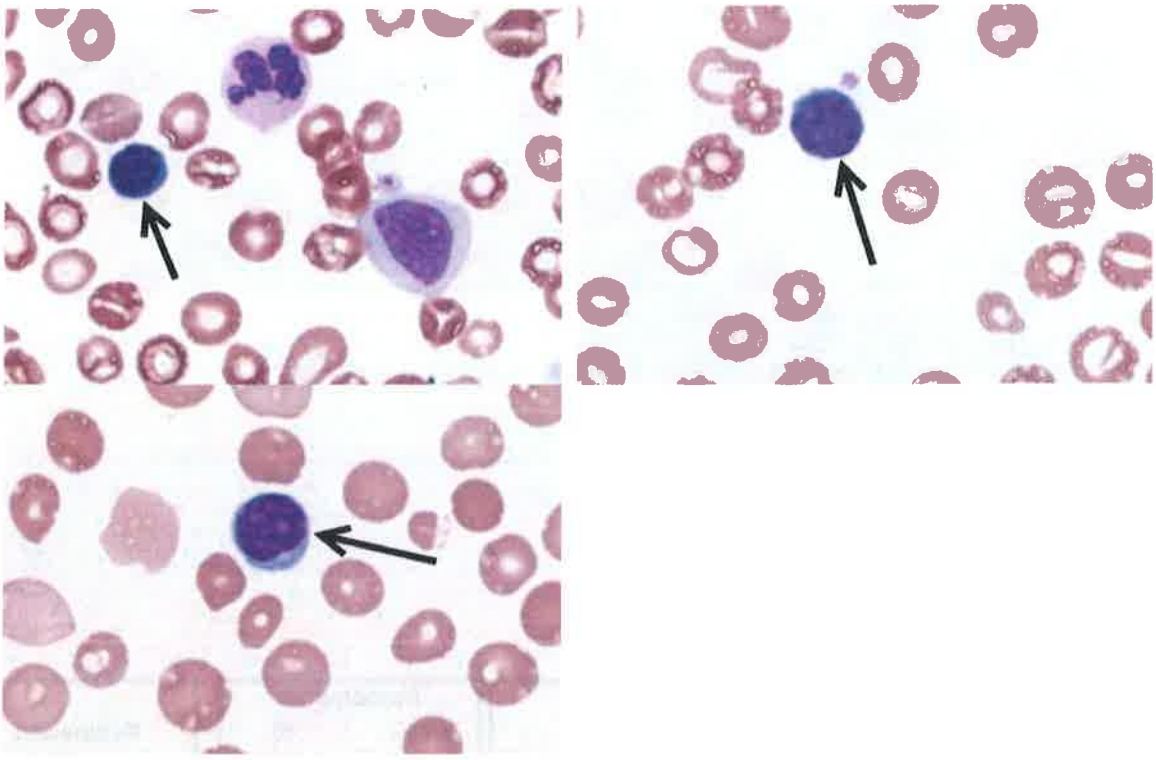
## Cell Identification

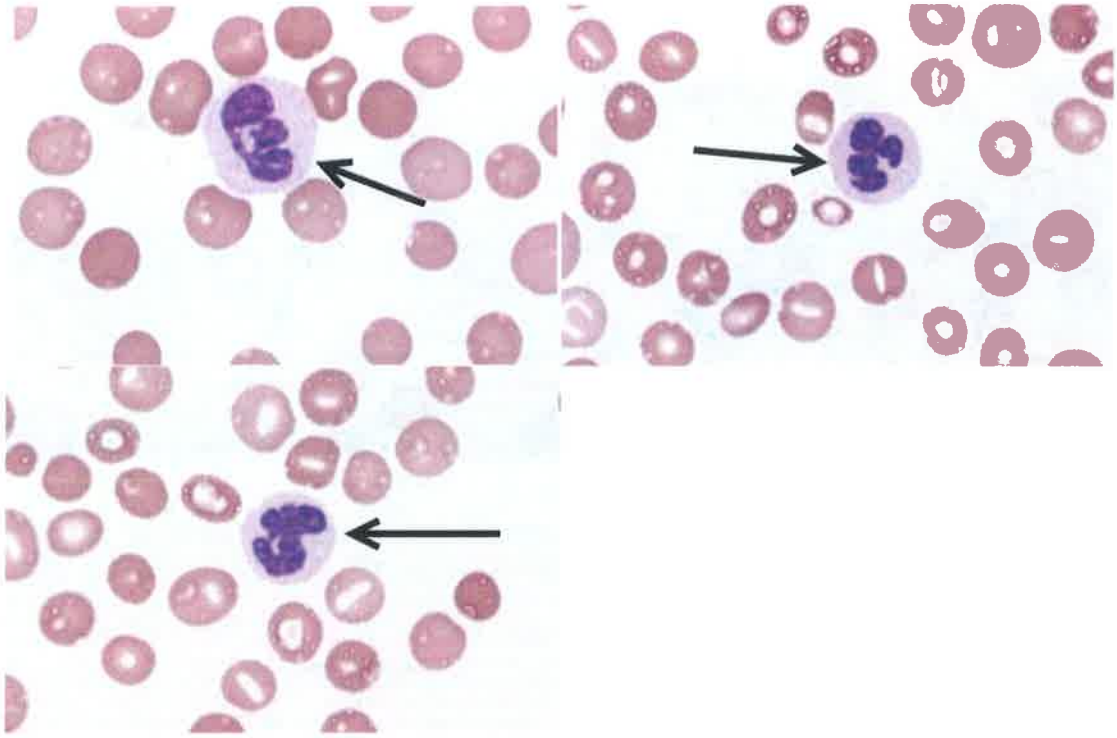
VPBS-20



| Identification | Participants No. | Participants % | Evaluation |
|---|---|---|---|
| Lymphocyte | 1140 | 97.8 | Educational |
| Lymphocyte, reactive | 15 | 1.3 | Educational |
| Malignant lymphoid cell (other than blast) | 6 | 0.5 | Educational |
| Blast cell | 2 | 0.2 | Educational |
| Basophil, any stage | 1 | 0.1 | Educational |
| Lymphocyte, large granular | 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 97.8% of participants. While most normal lymphocytes are fairly homogeneous, 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 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.

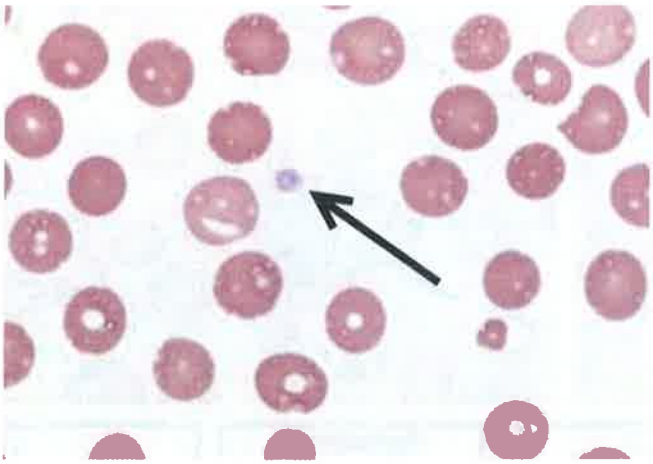
VPBS-21



| Identification | Participants No. | % | Evaluation |
|---|---|---|---|
| Neutrophil, segmented or band | 1123 | 96.3 | Educational |
| Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm | 29 | 2.5 | Educational |
| Neutrophil with hypersegmented nucleus | 7 | 0.6 | Educational |
| Neutrophil, polyploid | 2 | 0.2 | Educational |
| Neutrophil, toxic | 2 | 0.2 | Educational |
| Neutrophil, giant band or giant metamyelocyte | 1 | 0.1 | Educational |
| Platelet, hypogranular | 1 | 0.1 | Educational |
| Platelet, normal | 1 | 0.1 | Educational |

The arrowed cells are segmented neutrophils, as correctly identified by 96.3% of participants. 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.

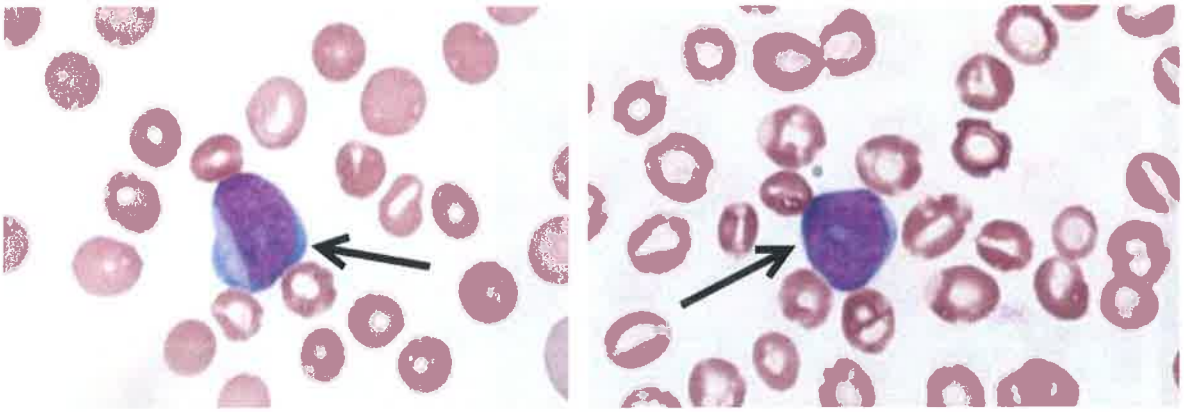
VPBS-22



| Identification | Participants No. | % | Evaluation |
|---|---|---|---|
| Platelet, normal | 1128 | 96.7 | Educational |
| Platelet, hypogranular | 29 | 2.5 | Educational |
| Platelet, giant | 5 | 0.4 | Educational |
| Megakaryocyte | 2 | 0.2 | Educational |
| Leukocyte containing bacteria | 1 | 0.1 | Educational |
| Monocyte | 1 | 0.1 | Educational |

The arrowed object is a normal platelet, as correctly identified by 96.7% of participants. Platelets, also known as thrombocytes, are small, blue-gray fragments of megakaryocytic cytoplasm. Most are 1.5 to 3 µm in diameter. A few small platelets, less than 1.5 µm in diameter, and a few large platelets, 4 to 7 µm in diameter, can also be seen in normal blood films. Fine, purple-red granules are dispersed throughout the cytoplasm or are sometimes aggregated at the center. These granules are platelet alpha granules. Platelet delta granules (or dense granules) are not visible on light microscopy. Platelets may be variable in shape, but most normal platelets are round or very slightly elliptical. Some have short cytoplasmic projections or ruffled margins.

VPBS-23



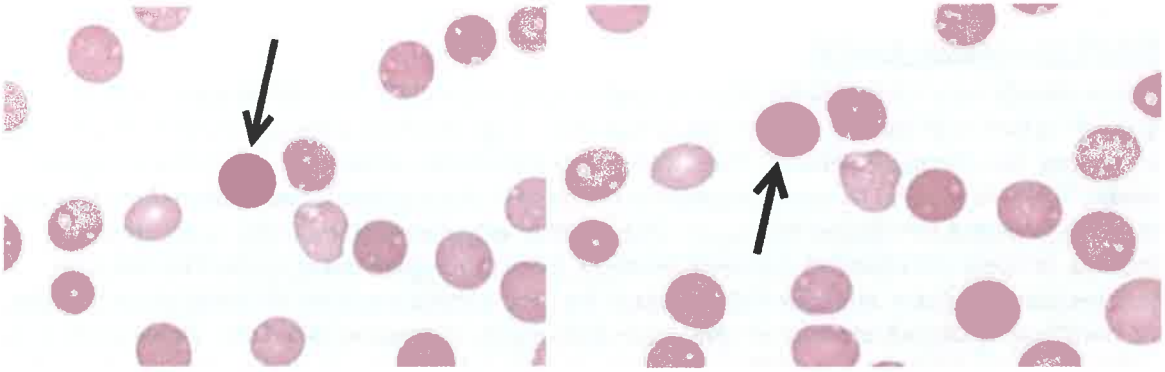
| Identification | Participants No. | Participants % | Evaluation |
|---|---|---|---|
| Blast cell | 988 | 84.7 | Educational |
| Lymphocyte, reactive | 87 | 7.5 | Educational |
| Immature/abnormal cell, would refer | 20 | 1.7 | Educational |
| Monocyte | 12 | 1.0 | Educational |
| Lymphocyte | 11 | 0.9 | Educational |
| Malignant lymphoid cell (other than blast) | 11 | 0.9 | Eduational |
| Monocyte, immature (promonocyte, monoblast) | 11 | 0.9 | Educational |
| Neutrophil, myelocyte | 8 | 0.7 | Educational |
| Neutrophil, promyelocyte | 8 | 0.7 | Educational |
| Myeloblast with Auer rod | 5 | 0.4 | Educational |
| Neutrophil, metamyelocyte | 2 | 0.2 | Educational |
| Lymphocyte, large granular | 2 | 0.2 | Educational |
| Plasma cell, mature/abnormal | 1 | 0.1 | Educational |

The arrowed cells are blasts, as correctly identified by 84.7% of participants. Myeloblasts are the most immature cells in the myeloid series. They are normally confined to the bone marrow, where they constitute less than 3% of the nucleated cells. They may be present in the blood in leukemic states, myelodysplastic syndromes, myeloproliferative neoplasms, and, very rarely, leukemoid reactions. The myeloblast is usually a fairly large cell, 15 to 20 µm in diameter, with a high nuclear-to-cytoplasmic (N:C) ratio, usually 7:1 to 5:1, with cytoplasm that is basophilic. Myeloblasts may occasionally be smaller, similar to the size of a mature myeloid cell. The cell and nucleus are usually rounded, although irregularly shaped or folded nuclei may be present. The myeloblast nucleus has finely reticulated chromatin pattern with distinct nucleoli present.

**VPBS-23 Discussion, Cont'd:**

The arrowed cells were incorrectly identified as reactive lymphocytes by 7.5% of participants. 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. These cells are reacting to an immune stimulus and are frequently increased in viral illnesses. The classic example is infectious mononucleosis (acute Epstein-Barr virus infection). Reactive lymphocytes can also be found in a variety of other viral infections (including cytomegalovirus, adenovirus, or acute HIV infection), protozoal infections (such as toxoplasmosis), some drug reactions, connective tissue diseases, and after major stress to the body's immune system. A variety of reactive lymphocyte forms have been described and they are often seen concurrently in the same blood film. These round to ovoid to irregular cells range from 10 to 25 µm in size with a nuclear-to-cytoplasmic ratio that varies from 3:1 to 1:2. The most common type of reactive lymphocyte resembles a large lymphocyte and corresponds to a Downey type II cell. These cells have round to oval nuclei, moderately condensed chromatin (giving it a smeared appearance), and absent or indistinct nucleoli. They contain abundant pale gray-blue cytoplasm. Granules, if present, are usually small and few in number. Frequently, these reactive lymphocytes have an amoeboid cytoplasm that partially surrounds adjacent red cells (ballerina skirt appearance) and has a darker-staining, furled margin. Basophilia radiating out from the nucleus may also be present.

VPBS-24



| Identification | Participants No. | % | Evaluation |
|---|---|---|---|
| Spherocyte | 1008 | 86.5 | Educational |
| Erythrocyte, normal | 151 | 12.9 | Educational |
| Macrocyte oval round | 3 | 0.3 | Educational |
| Microcyte (with increased central pallor) | 3 | 0.3 | Educational |
| Polychromatophilic (non-nucleated) red blood cell | 1 | 0.1 | Educational |

The arrowed cells are spherocytes, as correctly identified by 86.5% of participants. Spherocytes are identified as densely staining, spherical, or globular red blood cells with normal or slightly reduced volume (MCV) and increased thickness (more than 3 µm), but with decreased diameter (usually less than 6.5 µm) and without central pallor. These cells appear denser than normal RBCs and are commonly found in hereditary spherocytosis and immune hemolytic anemias. Micro-spherocytes (spherocytes measuring 4 µm or less in diameter), frequently seen in severe burns or microangiopathies, probably represent rounded-up fragments of red blood cells.

The arrowed cells were incorrectly identified as normal erythrocytes by 12.9% of participants. An erythrocyte is a mature, non-nucleated biconcave disc-shaped cell that typically ranges from 6.7 to 7.8 µm in diameter. The cytoplasm contains hemoglobin and stains uniformly pink-red. Normal erythrocytes have a round zone of central pallor that occupies approximately one-third of the cell diameter.

**Clinical Presentation:**
This peripheral blood smear is from a 60-year-old woman who presents with fatigue and bruising. Laboratory data include: WBC = 4.8 x 10E9/L; RBC = 2.62 x 10E12/L; HGB = 7.8 g/dL; HCT = 24.3%; MCV = 97 fL; and PLT = 32 x 10E9/L.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

**CASE DISCUSSION: Acute myeloid leukemia**

Acute myeloid leukemia (AML) represents a heterogeneous set of disorders, with a generally poor outcome; the overall long-term survival of patients with AML is only ~ 25%. AML is a systemic neoplasm of myeloid progenitor cells that primarily involves the peripheral blood and bone marrow, although it may also involve extramedullary sites. By definition, a diagnosis of AML requires the presence of ≥ 20% myeloblasts in the peripheral blood or bone marrow biopsy; AMLs with certain recurrent cytogenetic abnormalities, such as t(15;17) (seen in acute promyelocytic leukemia, APL), t(8;21) or inv(16) / t(16;16) are an exception to this rule. AML can be diagnosed in patients with these cytogenetic abnormalities even when the blast percentage is < 20%.

From an epidemiologic standpoint, AML is a relatively uncommon condition, with an incidence of ~3 cases/100,000 population per year, and affect primarily adults, with a median age of 60 years. The incidence increases with age, with AML representing 80 - 90% of acute leukemias in adults. This is in contrast with lymphoblastic leukemia/lymphoma (ALL), which is more common in children and represents 80 - 85% of leukemias in pediatric patients under the age of 18. AML has an equal gender distribution (male:female ratio = 1:1).

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**Question 1. What is the minimum blast percentage required for a diagnosis of AML, in the absence of certain cytogenetic abnormalities [such as t(15;17), t(8;21) or inv(16)/t(16;16)]?**

A. 5%
B. 10%
C. 20%
D. 30%

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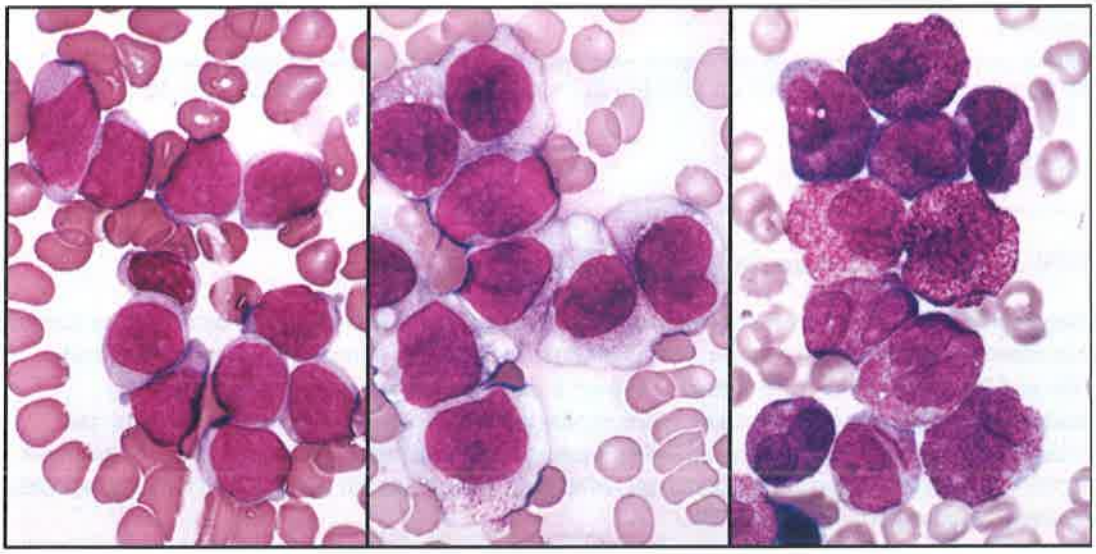
The diagnosis of AML is based on clinical findings, morphology, cytochemistry, immunophenotyping (by flow cytometry or immunohistochemistry) and genetic/molecular analysis. The clinical features are generally related to cytopenias, and may include weakness, fatigue (due to anemia), petechiae (secondary to thrombocytopenia), or infection (due to neutropenia). Less common findings are hepatosplenomegaly, lymphadenopathy, and infiltration of other extramedullary tissues, such as gingivae. Coagulopathy may be also present, particularly in specific variants (APL). From a laboratory standpoint, patients may present with a variable WBC count, ranging from severe leukopenia to marked leukocytosis, although anemia and thrombocytopenia are the norm. The peripheral blood smear shows increased blasts, and the bone marrow biopsy typically demonstrates increased cellularity, with an expanded population of blasts and variable (but by definition, limited) ability of the leukemic clone to mature beyond the blast stage.

The blast morphology may be variable, depending on the subtype of AML, as illustrated in the image below, which shows three different cases of AML:



Distinguishing one type of abnormal blast cell from another is not always possible using Wright-Giemsa stains alone. Additional testing such as enzyme cytochemistries (eg, myeloperoxidase, non-specific esterase or Sudan black reactivity), or immunophenotyping by flow cytometry may be required to further define the lineage of a given blast population. The morphologic features of a blast cell may also not allow definite determination of the cell lineage, ie., myeloblasts versus lymphoblast. The one exception is the presence of Auer rods, which are diagnostic of myeloid lineage. Auer rods are pink or red, rod-shaped cytoplasmic inclusions seen in early myeloid forms and occasionally in early monocytic forms in patients with myeloid lineage leukemia. These inclusions represent a crystallization of azurophilic (primary) granules. A cell containing multiple Auer bodies clumped together is referred to as a faggot cell (from the Latin term meaning cord of wood). Faggot cells are most commonly seen in APL.

Immunophenotyping, either by flow cytometry or immunohistochemistry, serves many purposes. It is helpful in differentiating AML from ALL; it distinguishes subtypes of AML; it may predict certain cytogenetic abnormalities with prognostic significance and therapeutic implications; and it can be used as a fingerprint for minimal residual disease analysis.

Cytogenetic and molecular analysis is important for defining subgroups of AML, with different biologic and prognostic characteristics. The current 2016 WHO classification of hematolymphoid neoplasms lists four main categories of AML:

1. AML with recurrent cytogenetic abnormalities
2. AML with myelodysplasia-related changes
3. AML and MDS, therapy-related
4. AML, not otherwise categorized

This classification highlights the biological differences between different types of AML. As such, AML with recurrent cytogenetic abnormalities generally has reciprocal translocations; a flat incidence rate over different age groups; distinctive morphologic features; no antecedent myelodysplastic syndrome or prominent dysplasia of maturing lineages; and often times, a favorable prognosis. Examples of recurrent cytogenetic abnormalities associated with a good prognosis in AML include t(8;21), inv16 / t(16;16), and t(15;17). In contrast, AML with myelodysplasia-related changes may be preceded by an antecedent MDS; shows prominent multilineage dysplasia and MDS-type cytogenetic abnormalities (such as complex karyotypes and losses of chromosomes or parts of chromosomes); has an increasing incidence with age; and overall, a poor prognosis.

Specific genes that have been shown to affect prognosis in AML include *FLT3*, *NPM1*, *CEBPA*, *RUNX1*, and *KIT*. A mutated *NPM1* (in isolation) or a biallelic mutation in *CEBPA* are associated with a good prognosis, while mutated *FLT3*, *RUNX1*, or *KIT* confer a poor prognosis.

**Question 2. Which of the following genetic or molecular abnormalities is associated with an unfavorable prognosis in AML?**

A. Translocation t(8;21)
B. Translocation t(16;16)
C. Mutated NPM1 gene
D. Mutated FLT3 gene

**Horatiu Olteanu, MD, PhD**
**Hematology and Clinical Microscopy Resource Committee**

**References:**

Arber DA, Brunning RD, LeBeau MM, Falini B, Vardiman JW, Porwit A, Thiele J, Bloomfield CD (2008). Acute myeloid leukemia with recurrent genetic abnormalities. In SH Swerdlow, E Campo, NL Harris, ES Jaffe, SA Pileri, H, Stein, J, Thiele, JW Vardiman (Eds.), *WHO Classification of Tumours of Haematopoietic and Lymphoid Tlsssues*, 4th Edition, pp. 109-148. Lyon: IARC Press.

Arber DA, Orazi A, Hasseriian R, Thiele J, Borowitz MJ, LeBeau MM, Bloomfield CD, Cazzola M, Vardiman JW. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-2405.

**Answers to questions:**

**Question 1: C. 20%**
By definition, a diagnosis of AML requires the presence of ≥ 20% myeloblasts in the peripheral blood or bone marrow biopsy; an important exception to this rule can occur in cases with certain recurrent cytogenetic abnormalities, such as t(15;17) (seen in acute promyelocytic leukemia), t(8;21) or inv(16) / t(16;16), that allow rendering an AML diagnosis even with a blast percentage < 20%.

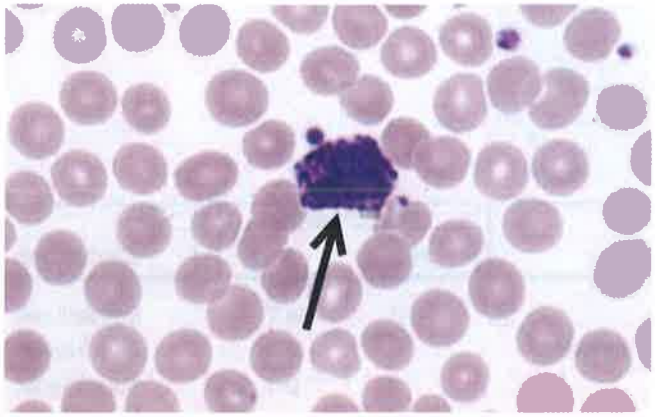
**Question 2: D. Mutated *FLT3* gene**
Genetic and molecular findings are useful in identifying prognostic features in patients with AML. Examples of recurrent cytogenetic abnormalities associated with a good prognosis in AML include t(8;21), inv16 / t(16;16), and t(15;17). Specific genes that have been shown to affect prognosis in AML include *FLT3*, *NPM1*, *CEBPA*, *RUNX1*, and *KIT*. A mutated NMP1 (in isolation) or a biallelic mutation in CEBPA are associated with a good prognosis, while mutated *FLT3*, *RUNX1*, or *KIT* confer a poor prognosis.

## Committee Comments on Peripheral Blood Smear Whole Slide Image

CBC data indicate mild leukocytosis without other abnormalities. The blood smear shows a mild increase in mature segmented neutrophils (mild neutrophilia). There is no significant left shift, although a very rare metamyelocyte or myelocyte may be found. The remaining leukocytes show no abnormality. Platelets and red blood cells are morphologically normal.

### Cell Identification

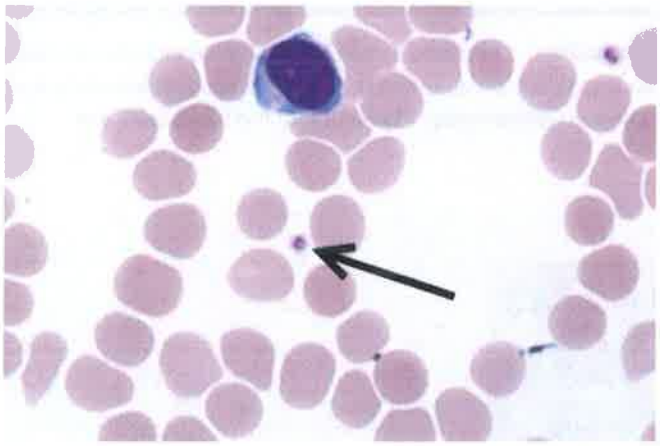
VPBS-26



| Identification | Participants No. | Participants % | Evaluation |
|---|---|---|---|
| Basophil, any stage | 1153 | 99.0 | Educational |
| Basophilic stippling | 8 | 0.7 | Educational |
| Eosinophil, any stage | 1 | 0.1 | Educational |
| Leukocyte containing Alder (Alder-Reilly) anomaly inclusion(s) | 1 | 0.1 | Educational |
| Lymphocyte | 1 | 0.1 | Educational |
| Mast cell | 1 | 0.1 | Educational |

The arrowed cell is a basophil, as correctly identified by 99.0% of participants. The cell shows the characteristic coarse and densely-stained granules that are larger than the granules seen in neutrophils. Basophil granules are usually blue-black and unevenly distributed within the cell. They frequently overlay and obscure the nucleus, as seen in this example.

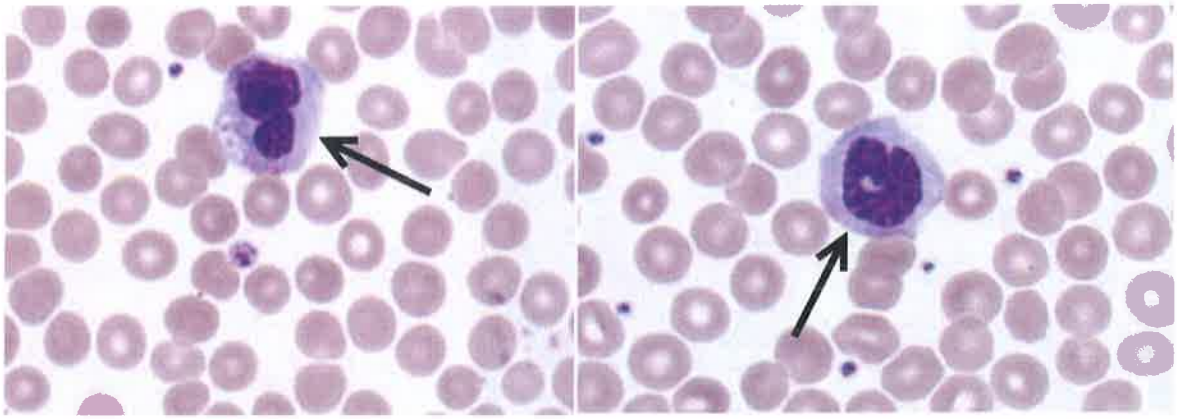
VPBS-27



| Identification | Participants No. | % | Evaluation |
|---|---|---|---|
| Platelet, normal | 1161 | 99.7 | Educational |
| Megakaryocyte (normal, abnormal or nuclear fragment) | 2 | 0.2 | Educational |
| Neutrophil, segmented or band | 1 | 0.1 | Educational |
| Platelet, hypogranular | 1 | 0.1 | Educational |

The arrowed object is a normal platelet, as correctly identified by 99.7% of participants. Platelets are small, round or slightly elliptical, blue-gray fragments of megakaryocyte cytoplasm. Most are 1.5 to 3 µm in diameter, although a few small platelets (< 1.5 µm in diameter) or large platelets (4 to 7 µm in diameter) can also be seen in normal blood films. Fine, purple-red granules are dispersed throughout the cytoplasm or are sometimes aggregated at the center.

VPBS-28

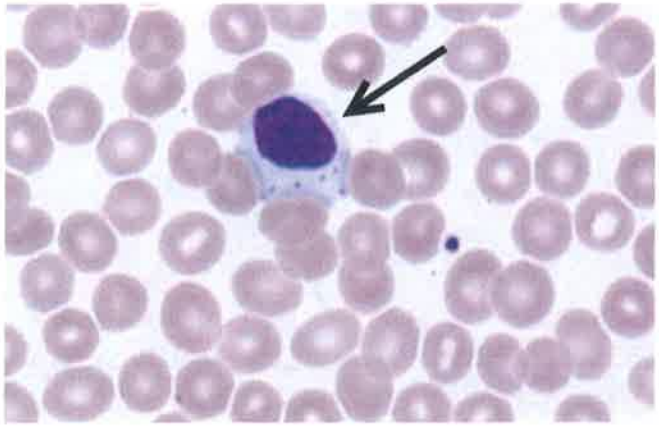


| Identification | Participants No. | % | Evaluation |
|---|---|---|---|
| Monocyte | 982 | 84.3 | Educational |
| Neutrophil, segmented or band | 132 | 11.3 | Educational |
| Neutrophil, toxic | 27 | 2.3 | Educational |
| Monocyte, immature (promonocyte, monoblast) | 10 | 0.9 | Educational |
| Neutrophil with dyplastic nucleus and/or hypogranular cytoplasm | 6 | 0.5 | Educational |
| Neutrophil, giant band or giant metamyelocyte | 2 | 0.2 | Educational |
| Neutrophil with Pelger-Huët nucleus | 2 | 0.2 | Educational |
| Leukocyte containing bacteria | 1 | 0.1 | Educational |
| Neutrophil, metamyelocyte | 1 | 0.1 | Educational |
| Neutrophil, promyelocyte | 1 | 0.1 | Educational |
| Platelet, normal | 1 | 0.1 | Educational |

The arrowed cells are monocytes, as correctly identified by 84.3% of participants. Monocytes are generally the largest white blood cell with a normal size that ranges from 12 to 20 µm in diameter. The cytoplasm is abundant, with a gray or gray-blue ground-glass appearance, and often contains several vacuoles. Some normal monocytes have visible fine, evenly distributed azurophilic granules. The nucleus is usually indented, folded, or band-like.

These cells were incorrectly identified as segmented or band neutrophils by 11.3% of participants. Compared to monocytes, segmented neutrophils are slightly smaller, have visible azurophilic cytoplasmic granules (rather than the slate gray or gray-blue cytoplasm of monocytes), and have more clearly segmented nuclei that are connected by thin chromatin filaments. The presence of cytoplasmic vacuoles, as seen in one of the arrowed cells, is typical of monocytes. The nucleus of one of the arrowed monocytes is somewhat band-shaped (as may occur in normal monocytes). In a neutrophilic band the width of the nucleus is usually fairly constant, in contrast to the variable width of the nucleus seen in this monocyte.

VPBS-29



| Identification | Participants No. | % | Evaluation |
|---|---|---|---|
| Lymphocyte, large granular | 570 | 48.9 | Educational |
| Lymphocyte, reactive | 419 | 36.0 | Educational |
| Lymphocyte | 166 | 14.3 | Educational |
| Leukocyte containing bacteria | 3 | 0.3 | Educational |
| Leukocyte containing Chediak-Higashi anomaly inclusion(s) | 2 | 0.2 | Educational |
| Bacteria (spirochete), extracellular | 1 | 0.1 | Educational |
| Basophilic stippling | 1 | 0.1 | Educational |
| Blast cell | 1 | 0.1 | Educational |
| Monocyte | 1 | 0.1 | Educational |
| Platelet satellitism | 1 | 0.1 | Educational |

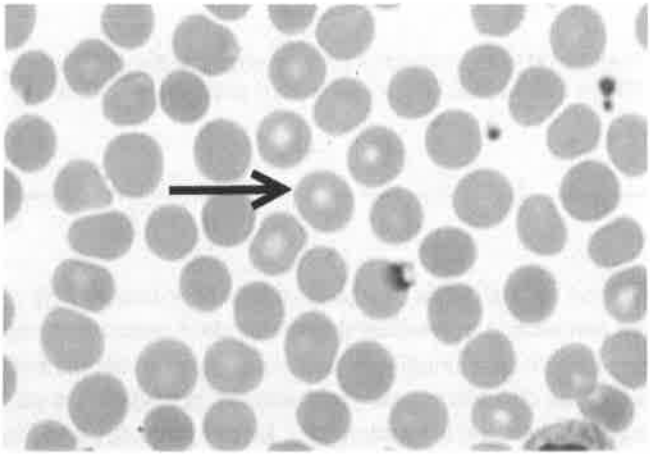
The arrowed cell is a large granular lymphocyte (LGL), as correctly identified by 48.9% of participants. LGLs characteristically have abundant clear to lightly basophilic cytoplasm that contains several coarse, unevenly distributed, azurophilic granules. The nucleus is generally round with condensed chromatin and absent nucleoli, similar to typical lymphocytes.

LGLs typically represent < 5% of leukocytes in normal blood smears and are either natural killer cells or suppressor/cytotoxic T cells. However, these two cell types cannot be distinguished by morphology alone and thus require immunophenotyping (eg. flow cytometry) for lineage assignment. Increased numbers of LGLs may be seen in patients with LGL leukemia or in certain benign conditions such as autoimmune diseases, viral infections, or following transplant.

The arrowed cell was incorrectly identified as a reactive lymphocyte by 36.0% of participants.Reactive lymphocytes often have abundant cytoplasm but usually lack the scattered coarse cytoplasmic granules seen in LGLs. The cytoplasm of reactive lymphocytes often shows accentuated basophilia radiating in bands from the nucleus toward the cell membrane ("radiating basophilia"), and the cytoplasm often partially surrounds adjacent red blood cells. In addition, reactive lymphocytes characteristically exhibit a wide variety of morphologic appearances within a blood film whereas LGLs are relatively uniform from cell to cell.

The arrowed cell was incorrectly identified as a lymphocyte by 14.3% of participants. Proficiency testing requires distinction between large granular lymphocytes and typical lymphocytes. Typical lymphocytes are small cells that lack the abundant cytoplasm and cytoplasmic granules characteristic of LGLs.

VPBS-30



| Identification | Participants No. | % | Evaluation |
|---|---|---|---|
| Erythrocyte, normal | 1120 | 96.1 | Educational |
| Hypochromasia | 36 | 3.1 | Educational |
| Macrocyte oval/round | 3 | 0.3 | Educational |
| Nucleated red blood cell, normal/abnormal | 2 | 0.2 | Educational |
| Erythrocyte with overlying platelet | 1 | 0.1 | Educational |
| Microcyte (with increased central pallor) | 1 | 0.1 | Educational |
| Spherocyte | 1 | 0.1 | Educational |
| Stomatocyte | 1 | 0.1 | Educational |

The arrowed cells are normal red blood cells, as correctly identified by 96.1% of participants. An erythrocyte is a mature, non-nucleated biconcave disc-shaped cell that typically ranges from 6.7 to 7.8 µm in diameter. The cytoplasm contains hemoglobin and stains uniformly pink-red. Normal erythrocytes have a round zone of central pallor that occupies approximately one-third of the cell diameter.

**Clinical presentation**:

This peripheral blood smear is from a 27-year-old man who was seen for a routine physical examination. Laboratory data include: WBC = 13.3 x 10E9/L; RBC = 5.26 x 10E12/L; HGB = 15.8 g/dL; HCT = 47.4%; MCV = 92 fL; and PLT = 216 x 10E9/L.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

**Case Discussion: Reactive Neutrophilia**

Neutrophils are the most abundant leukocyte in the blood, with a normal range for adults of approximately 1.5 - 8.0 x 10E9/L. The absolute number and relative percentage of neutrophils are standard parameters reported by automated hematology analyzers but can also be determined manually. In manual differential counts bands and segmented neutrophils should be grouped together, as distinction between these two cell types has poor reproducibility among observers, resulting in significant imprecision in band counts. Metamyelocytes and more immature neutrophil precursors should be counted separately from segmented neutrophils/bands. Some hematology analyzers provide an aggregate percentage for metamyelocytes, myelocytes, and promyelocytes, which are collectively reported as immature granulocytes (IG%) in the automated differential.

Neutrophils have a lifespan of approximately 5 days in the circulation. They play an essential role in immunity, primarily defense from bacterial infections, via their production of various cytokines and chemokines as well as their interaction with other immune cells such as B cells, T cells, NK cells, and macrophages. In response to infection, inflammation, or other stimuli, neutrophils migrate within the vasculature to the site of irritation, roll along the endothelial surface, adhere to the endothelium, and then cross through the vessel wall into the adjacent tissue. After exiting the blood vessel, neutrophils migrate toward the site of injury by a process known as chemotaxis, which occurs in response to peptides, lipids, or other chemoattractants released by bacteria or other pathogenic stimuli. The offending agents are then eliminated via phagocytosis and release of various enzymes or other microbicidal products by the neutrophils.

Neutrophilia is defined as an absolute neutrophil count (ANC) that exceeds the upper limit of the reference range. In reactive conditions the ANC rarely exceeds 40 x 10E9/L. A notable exception is neutrophilia associated with endogenous overproduction of granulocyte-colony stimulating factor (G-CSF), such as by a tumor (eg. some lung carcinomas), or exogenous administration of recombinant G-CSF, either of which can produce marked neutrophilia that may even exceed 60 x 10E9/L. Marked reactive neutrophilia is often referred to as a leukemoid reaction because it mimics a leukemic leukocytosis. Reactive neutrophilia of any etiology may be accompanied by monocytosis or eosinophilia but rarely is associated with basophilia. This is in contrast to chronic myeloid leukemia, which characteristically exhibits significant basophilia in addition to marked neutrophilia with a left shift.

Reactive neutrophils may exhibit morphologic features of activation within the cytoplasm such as toxic granulation, vacuolization, and Döhle bodies, particularly when associated with sepsis. Toxic granulation is characterized by large, distinct, purple or dark-blue cytoplasmic granules and is often accompanied by variably-sized cytoplasmic vacuoles. Döhle bodies represent parallel strands of rough endoplasmic reticulum and appear as single or multiple blue or blue-gray inclusions that are typically located adjacent to the cell membrane.

**Question 1. Which of the following morphologic findings in neutrophils effectively rules out a septic neutrophilia?**

A. Auer rods
B. Cytoplasmic vacuolization
C. Döhle bodies
D. Toxic granulation

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There are numerous causes of reactive neutrophilia, the most common being bacterial infections, drug effects, trauma, and inflammatory disorders (Table 1). The etiology of neutrophilia can be broadly divided into two categories: shift neutrophilia and true neutrophilia. Shift neutrophilia is sometimes referred to as pseudoneutrophilia and occurs in response to an acute stress such as strenuous exercise, electric shock, seizure, or other cause of epinephrine release. Shift neutrophilia occurs rapidly (less than 30 minutes) in response to a stress and is the result of detachment of marginated neutrophils from the luminal side of the endothelium where they are normally attached. This process is referred to as demargination. Shift neutrophilia does not result in a true increase in the total blood granulocyte pool (TBGP) because the marginated neutrophils are already present in the blood. In contrast, true neutrophilia is due to an influx of neutrophils from the bone marrow and thus represents a true increase in the TBGP. This influx can be rapid (several hours) due to mobilization of already-mature bone marrow neutrophils or can occur over a period of days and be sustained due to increased marrow granulopoiesis. The capacity for the marrow to increase the TBGP significantly exceeds that attainable by shift neutrophilia alone.

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**Question 2. Which of the following is most consistent with shift neutrophilia rather than true neutrophilia? (ANC = absolute neutrophil count)**

A. Hospital inpatient with left-shifted leukocytosis and basophilia, ANC 87.0 x 10E9/L
B. Patient with an autoimmune disease who has received corticosteroids for 3 weeks, ANC 15.6 x 10E9/L
C. Previously healthy patient who is status post seizure 10 minutes prior to CBC collection, ANC 12.5 x 10E9/L
D. Septic patient with neutrophilia and toxic granulation, ANC 19.3 x 10E9/L

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| **Table 1: Causes of Reactive Neutrophilia** |
|---|
| Infection, primarily bacterial but sometimes associated with viral, fungal, parasitic, or other infectious organisms |
| Drug effects, particularly corticosteroids, epinephrine, lithium, and some poisons/toxins |
| Burns or other trauma |
| Inflammatory disorders such as autoimmune disorders or collagen vascular disorders |
| Endogenous or exogenous granulocyte-colony stimulating factor (G-CSF) |
| Acute stress |
| Seizure |
| Exercise |
| Pregnancy |
| Smoking |

**Kyle Bradley, MD**
**Hematology and Clinical Microscopy Resource Committee**

**References:**

Chabot-Richards DS, George TI. Leukocytosis. *Int J Lab Hematol.* 2014 Jun;36(3):279-288.

Cornbleet PJ. Clinical utility of the band count. *Clin Lab Med.* 2002;22(1):101-136.

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Yang F, Feng C, Zhang X, Lu J, Zhao Y. The diverse biological functions of neutrophils, beyond the defense against infections. *Inflammation.* 2017 Feb;40(1):311-323.

**Answers to Questions:**

**Question 1: A. Auer rods**
Toxic neutrophilia is commonly associated with sepsis. Toxic neutrophils exhibit varying combinations of toxic granulation, cytoplasmic vacuolization, and Döhle bodies. Auer rods represent an abnormal fusion of primary granules and are associated with myeloid malignancies. In addition, Auer rods, when present, are usually seen in blasts and are extremely rare in more mature granulocytes.

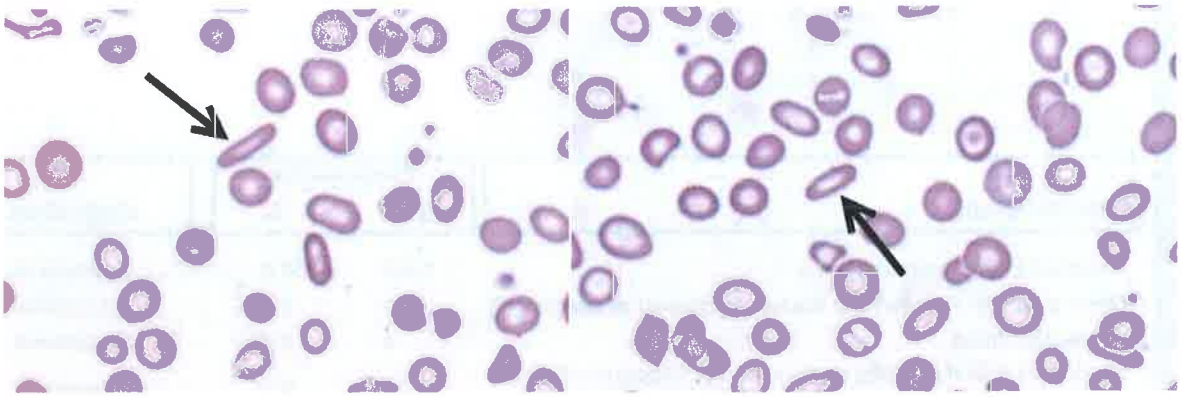
**Question 2: C. Previously healthy patient who is status post seizure 10 minutes prior to CBC collection, ANC 12.5 x 10E9/L**
Shift neutrophilia occurs as an immediate response to an acute stress such as a seizure and is due to intravascular demargination of neutrophils. This contrasts with true neutrophilia, which is due to migration of neutrophils from the bone marrow into the circulation. Neutrophilia secondary to corticosteroid therapy (Answer B) or sepsis (Answer D) are typical examples of true neutrophilia. The finding of marked left-shifted leukocytosis with basophilia (Answer A) should raise concern for a myeloproliferative neoplasm such as chronic myeloid leukemia.

## Committee Comments on the CBC and Blood Film

The CBC data reveal a prominent, isolated microcytic anemia while the peripheral smear shows generally unremarkable leukocyte and platelet morphology. Red blood cell anisopoikilocytosis is significantly increased by many elliptocytes/ovalocytes, occasional target cells, hypochromic microcytes, and scattered spherocytes. Polychromasia is sparse to absent. Additional laboratory studies provided disclose markedly decreased levels of serum iron, iron saturation, and ferritin. The combined morphologic and laboratory findings support an iron deficiency anemia.

### Cell Identification

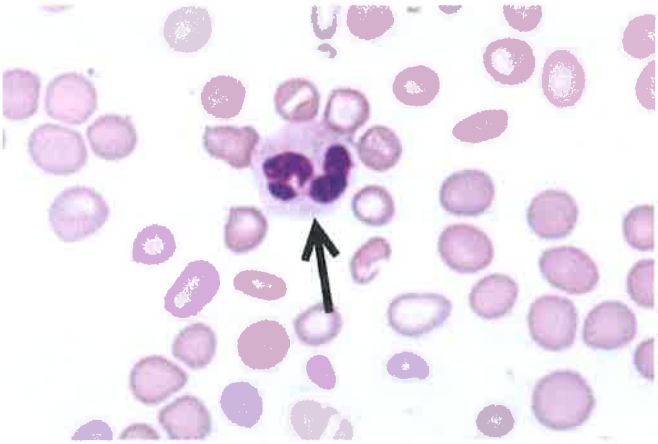
VPBS-32



| Identification | Participants No. | % | Evaluation |
|---|---|---|---|
| Ovalocyte (elliptocyte) | 1164 | 99.7 | Educational |
| Monocyte | 1 | 0.1 | Educational |
| Sickle cell (drepanocyte) | 1 | 0.1 | Educational |
| Stomatocyte | 1 | 0.1 | Educational |

The arrowed cells are ovalocytes/elliptocytes, as correctly identified by 99.7% of participants. These red blood cells are pencil- or cigar-shaped, with parallel sides and blunt ends. Hemoglobin is often concentrated at the ends, producing a dumbbell appearance. An increased number of elliptocytes may be seen in individuals with hereditary elliptocytosis, an abnormality of skeletal membrane proteins. They are also commonly increased in patients with iron deficiency anemia.

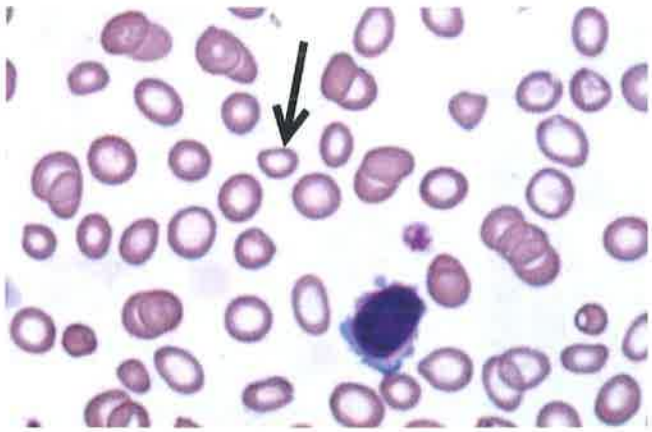
VPBS-33



| Identification | Participants No. | % | Evaluation |
|---|---|---|---|
| Neutrophil, segmented or band | 1144 | 98.0 | Educational |
| Neutrophil with Pelger-Huët nucleus (acquired or congenital) | 9 | 0.8 | Educational |
| Neutrophil, toxic | 5 | 0.4 | Educational |
| Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm | 4 | 0.3 | Educational |
| Microcyte (with increased central pallor) | 1 | 0.1 | Educational |
| Neutrophil with hypersegmented nucleus | 1 | 0.1 | Educational |
| Neutrophil, giant band | 1 | 0.1 | Educational |
| Neutrophil, polyploid | 1 | 0.1 | Educational |
| Platelet, hypogranular | 1 | 0.1 | Educational |

The arrowed cell is a neutrophil, segmented or band, as correctly identified by 98.0% of participants. Segmented neutrophils are the predominant leukocyte in the peripheral blood. They are similar in many respects to their immediate precursor, the band neutrophil, including their size (10 to 15 µm in diameter), shape (round to oval), and cytoplasmic appearance (pale pink cytoplasm with specific granules). The nuclear chromatin is highly condensed. The nucleus is segmented or lobated, with variably three to five lobes that are connected by a thin, thread-like filament without internal chromatin.

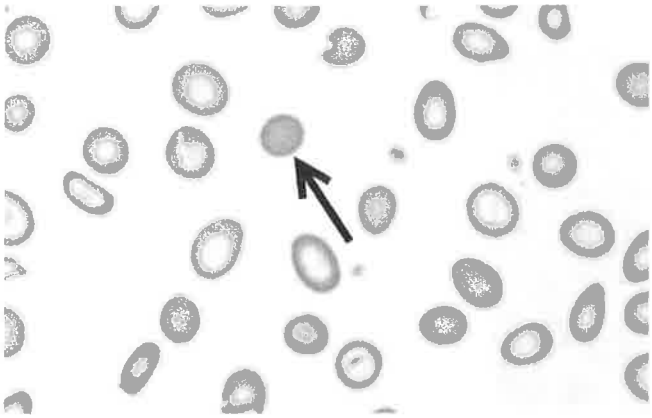
VPBS-34



| Identification | Participants No. | % | Evaluation |
|---|---|---|---|
| Microcyte (with increased central pallor) | 1075 | 92.1 | Educational |
| Hypochromasia | 65 | 5.6 | Educational |
| Erythrocyte, normal | 8 | 0.7 | Educational |
| Lymphocyte | 7 | 0.6 | Educational |
| Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell) | 5 | 0.4 | Educational |
| Lymphocyte, reactive | 3 | 0.3 | Educational |
| Echinocyte (burr cell, crenated cell) | 1 | 0.1 | Educational |
| Macrocyte oval/round | 1 | 0.1 | Educational |
| Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm | 1 | 0.1 | Educational |
| Stomatocyte | 1 | 0.1 | Educational |

The arrowed cell is a microcytic/hypochromic red blood cell, as correctly identified by 97.7% of participants. As the name implies, microcytes are smaller than normal red blood cells, measuring less than 6 µm in diameter. They also appear smaller than the nucleus of a small lymphocyte. Microcytes retain their central pallor and may be either normochromic or hypochromic. When the central pallor exceeds 50% of the cell diameter, then the red blood cell is considered to be hypochromic. Microcytes can be seen in several settings, including iron deficiency anemia, thalassemias, and anemia of chronic disease.

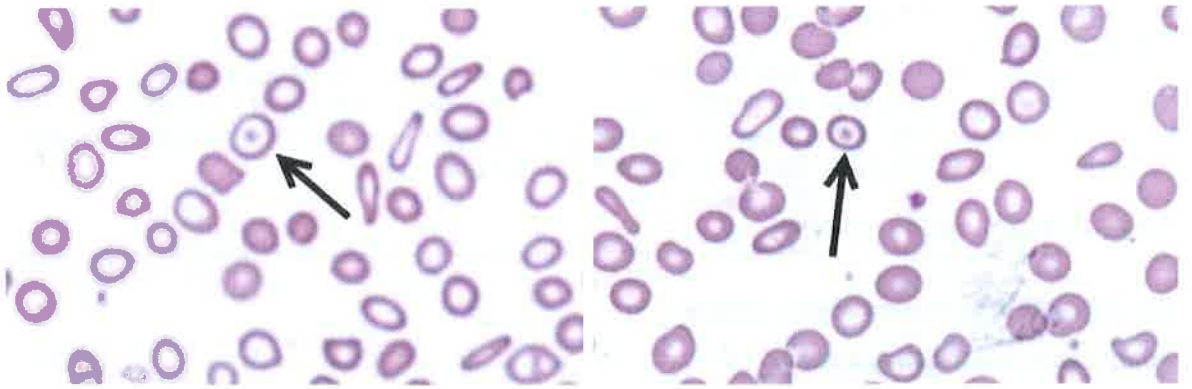
VPBS-35



| Identification | Participants No. | % | Evaluation |
|---|---|---|---|
| Spherocyte | 1130 | 96.8 | Educational |
| Erythrocyte, normal | 23 | 2.0 | Educational |
| Microcyte (with increased central pallor) | 10 | 0.9 | Educational |
| Stomatocyte | 2 | 0.2 | Educational |
| Ovalocyte (elliptocyte) | 1 | 0.1 | Educational |
| Polychromatophilic (non-nucleated) red blood cell | 1 | 0.1 | Educational |

The arrowed cell is a spherocyte, as correctly identified by 96.8% of participants. Spherocytes are "rounded up," densely staining red blood cells that lack central pallor. The loss of the biconcave architecture of normal red blood cells imparts a spherical shape to these cells and the increased thickness results in their densely-staining appearance. The presence of spherocytes should raise the concern for hereditary spherocytosis and immune hemolytic anemias, although they may also be seen in cases of iron deficiency anemia.

VPBS-36



| Identification | Participants No. | % | Evaluation |
|---|---|---|---|
| Target cell (codocyte) | 1163 | 99.7 | Educational |
| *Plasmodium* spp. (malaria) | 2 | 0.2 | Educational |
| Teardrop cell (dacrocyte) | 2 | 0.2 | Educational |

The arrowed cells are target cells (codocytes), as correctly identified by 99.7% of participants. Target cells are red blood cells with an increased surface membrane-to-volume ratio. They are thought to arise from disturbances in red blood cell membrane cholesterol and lecithin content or decreased cytoplasmic hemoglobin content. This imparts a "targetoid" appearance, with a central hemoglobinized area surrounded by an area of pallor, which is in turn bounded by a peripherally hemoglobinized zone. Target cells are typically seen in peripheral smears of individuals with thalassemias, iron deficiency anemia, after splenectomy, or in patients with chronic liver disease. They may also appear as an artifact from slow drying slides in a humid environment or in specimens anticoagulated with excessive amounts of EDTA. A clue to this being an artifactual finding is the presence of numerous target cells in some fields, while being sparse to absent in other fields.

**Clinical Presentation:**
This peripheral blood smear is from a 48-year-old woman who presents with fatigue. Laboratory data include: WBC = 4.7 x 10E9/L; RBC = 2.68 x 10E12/L; HGB = 7.3 g/dL; HCT = 24.2%; MCV = 53 fL; MCHC = 30 g/dL; RDW = 22%; PLT = 234 x 10E9/L; iron = 24 µg/dL (normal range 50 - 160 µg/dL); total iron binding capacity = 468 µg/dL (normal range = 250 – 400 µg/dL); iron saturation = 5%; and ferritin = 8 ng/dL.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

## CASE DISCUSSION: Iron deficiency anemia

### *Diagnosis of iron deficiency anemia*

Iron deficiency anemia (IDA) is a common cause of anemia worldwide. The peripheral blood smear findings, red blood cell indices, and results of the iron studies in this case support the diagnosis of IDA. Red blood cell abnormalities in IDA include increased anisopoikilocytosis with many hypochromic microcytes, elliptocytes/ovalocytes, target cells, and occasional spherocytes and prekeratocytes. Several RBC indices are decreased, including RBC count, MCH, MCHC, and MCV. Iron studies show low levels of serum iron, ferritin, and iron saturation (ie. serum iron/iron binding capacity), while iron binding capacity is elevated. Serum ferritin level (an indicator of iron stores) is the most sensitive and specific test to identify iron deficiency, with levels below 10 µg/L considered supportive of the diagnosis of IDA. Iron saturation (also referred to as transferrin saturation) levels below 16% signify IDA, as iron saturation is typically around 20 - 45% in normal individuals.

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**Question 1.The following red blood cell morphologic abnormality essentially rules out the diagnosis of iron deficiency anemia:**

A. Bite cells
B. Elliptocytes
C. Hypochromic microcytes
D. Target cells

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### *Causes of iron deficiency anemia*

Iron is essential for the production of red cells, as it is the rate-limiting step in heme synthesis. IDA occurs when loss and/or utilization of iron exceeds iron absorption, leading to inadequate hemoglobinization of erythroblasts and diminution of iron stores. The peptide hormone, hepcidin, is central to the regulation of iron homeostasis. As iron levels diminish (in chronic blood loss, for example), insufficient hemoglobinization eventually leads to production of microcytic red cells. As a compensatory response in iron deficiency, hepcidin is downregulated to allow increased iron absorption in the gut and liberation of iron stores from macrophages. Conversely, high hepcidin levels will limit iron bioavailability.

IDA occurs in a wide variety of clinical settings. The most common etiology for IDA is chronic blood loss, especially due to heavy menses and gastrointestinal bleeding. Decreased iron absorption due to surgical intervention (eg. gastrectomy, bariatric surgery) or *Helicobacter pylori* infection may also lead to IDA. Inadequate iron intake from malnutrition or from vegetarian, vegan, or iron-poor diets can likewise result in IDA. In times of increased physiologic demand, certain individuals are much more susceptible to IDA, such as infants, adolescents (because of rapid growth), and pregnant women (particularly in the second and third trimesters). Finally, an uncommon, congenital cause of iron refractory iron deficiency anemia (IRIDA) is due to a mutation in *TMPRSS6* gene (encodes transmembrane protease, serine 6; also known as matriptase-2), which inhibits the signaling pathway that activates hepcidin.

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**Question 2. Isolated iron deficiency anemia occurs in which of the following settings?**

A. Elderly patient with sepsis and disseminated intravascular coagulation
B. Middle-aged man with prosthetic cardiac valve
C. Patient with long-standing history of alcohol use and poor dietary intake
D. Young woman with heavy menstrual bleeding

***The differential diagnosis for hypochromic, microcytic red blood cells***

Aside from iron deficiency anemia, hypochromic microcytic red blood cells may also be seen in thalassemia, anemia of chronic disease, and sideroblastic anemia. Each entity may be distinguished from IDA by additional morphologic findings, red cell indices, and/or results of iron studies.

Thalassemias arise from absent to diminished synthesis of alpha globin chains due to gene deletions (in the case of alpha thalassemias) or from absent to diminished synthesis of beta globin chains due to gene mutations (in beta thalassemias). In addition to target cells in these peripheral blood smears, coarse basophilic stippling in red cells may also be characteristically observed. The red cell indices may show a normal to elevated RBC count, low MCV, reduced MCHC, and normal to increased RDW.

Anemia of chronic disease (ACD) more often presents as a normochromic, normocytic anemia, although hypochromic microcytic red cells may also be observed with this entity. These occur in the setting of chronic disease or chronic inflammation, with elevations in hepcidin levels that lead to sequestration of iron stores in macrophages and lack of iron bioavailability for heme synthesis. Red blood cell indices show decreased RBC counts, normal to reduced MCV, MCH, and MCHC, and normal RDW. The distinction from IDA is usually straightforward, as iron studies in ACD show normal to decreased serum iron, with elevated ferritin levels and normal iron saturation; on the other hand, these parameters are all decreased in IDA.

Sideroblastic anemias characteristically display a dimorphic population of red cells: one that is normochromic and normocytic, and another that is hypochromic and microcytic. There are various congenital and acquired causes for sideroblastic anemias. Their unifying features are an abnormal accumulation of mitochondrial iron, impaired heme synthesis, and formation of increased numbers of pathologic siderotic granules in bone marrow erythroid precursors (so-called "ring sideroblasts"). Although hypochromic microcytes are seen in this entity, the presence of the dimorphic population of red cells, coarse basophilic stippling, and Pappenheimer bodies should prompt consideration for sideroblastic anemia rather than IDA.

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**Hematology and Clinical Microscopy Committee**

**References:**

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Wilson CS, Vergara-Lluri ME, Brynes RK. Evaluation of anemia, leukopenia, and thrombocytopenia. In: *Hematopathology*. 2nd edition. Philadelphia, PA: Elsevier; 2017;195-218.

**Answers to questions:**

**Question 1: A. Bite cells**
Morphologic abnormalities typically found in patients with overt IDA include elliptocytes/ovalocytes, target cells, and hypochromic microcytes. Bite cells are characteristically observed in patients with enzyme deficiency, such as glucose-6-phosphate deficiency, who have undergone oxidant injury.

**Question 2: D. Young woman with heavy menstrual bleeding**
IDA can occur in many clinical settings. One of the most common causes is in patients who experience chronic blood loss, such as in a young woman with heavy menstrual bleeding. Alcohol use and poor dietary intake (potentially resulting in vitamin B12 and/or folate deficiency) are typically associated with macrocytic, not microcytic, anemia. In patients with prosthetic cardiac valves, physicomechanical disruption of red cells can result in an increased number of schistocytes (ie., fragmented red cells). Patients with sepsis and disseminated intravascular coagulation (DIC) usually present with additional CBC abnormalities like leukocytosis or neutropenia, and anemia is more likely secondary to microangiopathic hemolytic anemia rather than IDA.

COLLEGE of AMERICAN PATHOLOGISTS

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