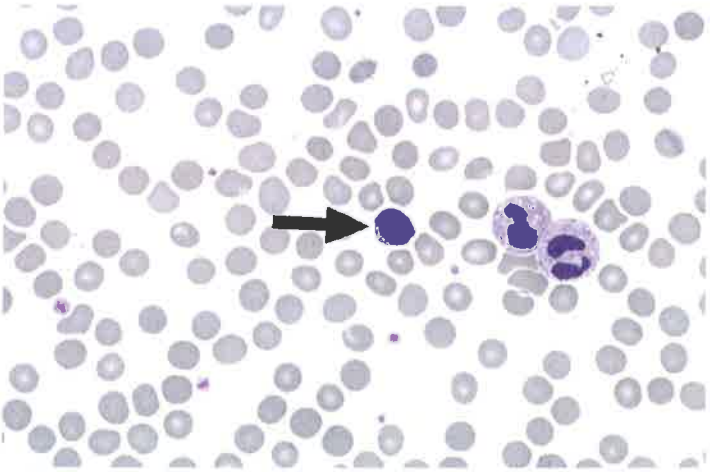
Committee Comments on the CBC and Blood Film

The provided CBC data indicate normocytic anemia and thrombocytopenia, accompanied by a neutrophilic leukocytosis. Low level anisopoikilocytosis is noted, with numerous echinocytes and occasional ovalocytes, acanthocytes and spherocytes. Platelets are generally unremarkable, although occasional hypogranular large/giant forms are also noted. In addition to the neutrophilia, neutrophil toxic granules, vacuoles and occasional Dohle bodies are noted.

Cell Identification

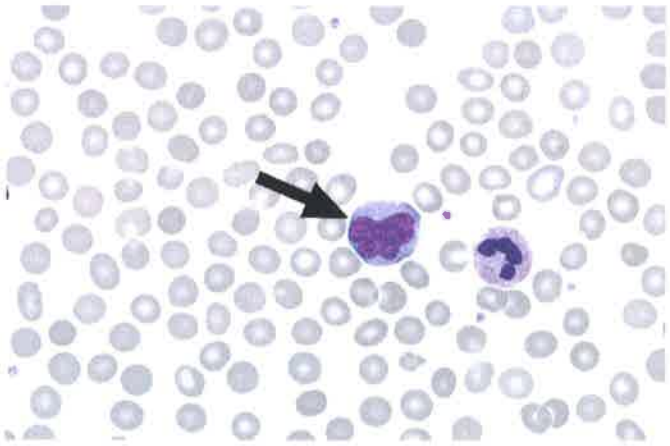
VPBS-20



Identification	Participants No.	Participants %	Evaluation
Lymphocyte	1215	99.7	Educational
Lymphocyte, reactive (to include plasmacytoid and immunoblastic forms)	3	0.3	Educational
Nucleated red blood cell, normal or abnormal morphology	1	0.1	Educational

The arrowed cell is a lymphocyte, as correctly identified by 99.7% of participants. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 µm with an N:C ratio ranging from 5:1 to 2:1. Most lymphocytes have round to oval nuclei that may be slightly indented or notched. The chromatin is 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.

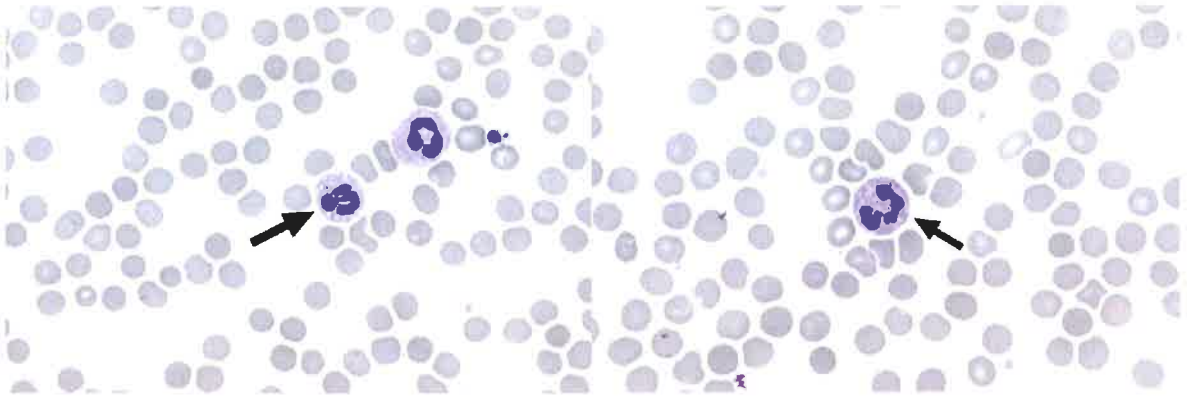
VPBS-21



Identification	Participants No.	%	Evaluation
Monocyte	1169	95.9	Educational
Monocyte, immature (promonocyte, monoblast)	17	1.4	Educational
Neutrophil, metamyelocyte	14	1.1	Educational
Lymphocyte, reactive (to include plasmacytoid and immunoblastic forms)	6	0.5	Educational
Blast cell	4	0.3	Educational
Neutrophil, myelocyte	2	0.2	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	2	0.2	Educational
Immature or abnormal cell, would refer for identification	1	0.1	Educational
Lymphocyte, large granular	1	0.1	Educational
Neutrophil, promyelocyte	1	0.1	Educational
Neutrophil, segmented or band	1	0.1	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	1	0.1	Educational

The arrowed cell is a monocyte, as correctly identified by 95.9% of participants. Monocytes are slightly larger than neutrophils, ranging from 12 to 20 µm in diameter.The majority of monocytes are round with smooth edges, but some may have pseudopod-like cytoplasmic extensions.The cytoplasm is abundant, with a gray or gray-blue ground-glass appearance, and may contain vacuoles or fine, evenly distributed azurophilic granules.The nucleus is usually indented, often resembling a three-pointed hat, but it can also be folded or band-like.The chromatin is condensed, but is usually less dense than that of a neutrophil or lymphocyte. Nucleoli are generally absent, but occasional monocytes may contain a small, inconspicuous nucleolus. For the purposes of proficiency testing, selection of the response "monocyte, immature (promonocyte, monoblast)" should be reserved for malignant cells in the context of acute monocytic/monoblastic leukemia, acute myelomonocytic leukemia, chronic myelomonocytic leukemia, or myelodysplastic syndromes.

VPBS-22

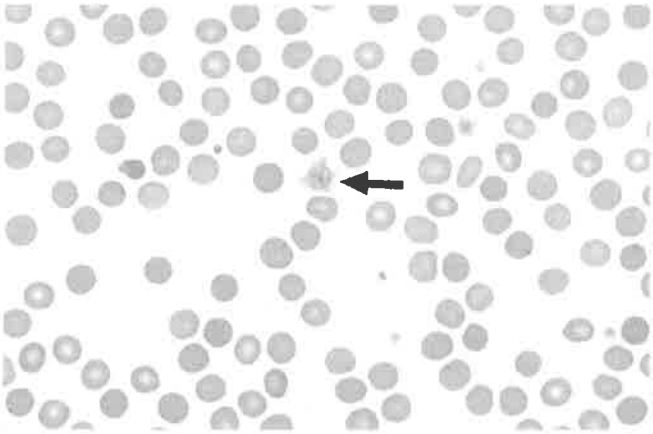


Identification	Participants No.	%	Evaluation
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	697	57.2	Educational
Neutrophil, segmented or band	513	42.1	Educational
Neutrophil with hypersegmented nucleus	4	0.3	Educational
Echinocyte (burr cell, crenated cell)	1	0.1	Educational
Monocyte, immature (promonocyte, monoblast)	1	0.1	Educational
Neutrophil with Pelger-Huët nucleus (acquired or congenital)	1	0.1	Educational
Platelet, normal	1	0.1	Educational
Platelet satellitism	1	0.1	Educational

The arrowed cells are toxic neutrophils, as correctly identified by 57.2% of participants. Toxic changes in neutrophils include toxic granulation, toxic vacuolization, and Döhle bodies. Toxic granulation and Döhle bodies each may be present in an individual cell without the other finding and either change alone is sufficient to designate a neutrophil as toxic. Toxic granulation is defined by the presence of large, purple or dark blue cytoplasmic granules in neutrophils, bands, and metamyelocytes. Vacuoles within the cytoplasm of these same cells define toxic vacuolization. The vacuoles are variable in size and may coalesce, sometimes distorting the neutrophil cytoplasm to form pseudopodia. Ethylenediaminetetraacetic acid (EDTA) blood collection may produce degenerative vacuolization; in this context, only a few, small, punched-out appearing vacuoles may be found. However, as it may be difficult to distinguish toxic from degenerative vacuoles, neutrophil vacuoles should not be labeled as toxic vacuoles unless accompanied by other toxic changes.

42.1% of participants identified the arrowed cells as neutrophils, segmented or band. Neutrophils normally constitute the predominant blood leukocyte, with sizes typically ranging from 10 to 15 µm in diameter. The nucleus of the mature neutrophil is characteristically 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. Resting neutrophils are typified by a pale pink cytoplasm with specific granules.

VPBS-23



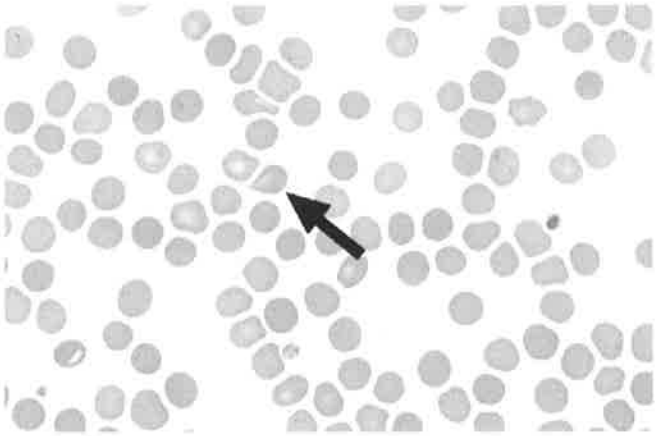
Identification	Participants No.	%	Evaluation
Platelet, giant (macrothrombocyte)	1138	93.4	Educational
Platelet, normal	49	4.0	Educational
Platelet, hypogranular	17	1.4	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	10	0.8	Educational
Basket cell/smudge cell	1	0.1	Educational
Cryoglobulin	1	0.1	Educational
Monocyte, immature (promonocyte, monoblast)	1	0.1	Educational
Platelet satellitism	1	0.1	Educational
Stain precipitate	1	0.1	Educational

Due to the difficulty in determining whether this platelet is large but not giant sized as compared with the surrounding red blood cells, the responses of giant platelet and platelet are both acceptable.

93.4% of participants identified the arrowed entity as a giant platelet. Giant platelets are larger than 7 µm, usually measuring 10 to 20 µm in diameter. For proficiency testing purposes, therefore, the term "giant platelet" is used when the platelet is larger than the size of the average red blood cell in the field, assuming a normal MCV. Giant platelets are a rare finding in normal peripheral blood, but may be seen in many different reactive, neoplastic, and inherited conditions. Reactive causes include conditions in which platelet turnover is markedly increased, such as immune thrombocytopenia or severe leukemoid reactions. Giant platelets are most often seen in myeloproliferative neoplasms and myelodysplastic syndromes.

4.0% of participants identified this entity as a platelet. Platelets, also known as thrombocytes, are small, blue-gray cellular fragments derived from megakaryocyte cytoplasmic blebs. 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 may be centrally aggregated. 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-24



Identification	Participants No.	Participants %	Evaluation
Teardrop cell (dacrocyte)	1179	97.0	Educational
Erythrocyte, normal	26	2.1	Educational
Target cell (codocyte)	5	0.4	Educational
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	3	0.3	Educational
Acanthocyte (spur cell)	1	0.1	Educational
Pappenheimer bodies (iron or Wright stain)	1	0.1	Educational
Platelet, giant (macrothrombocyte)	1	0.1	Educational

The arrowed anucleate cell is a teardrop red blood cell (dacrocyte), as correctly identified by 97.0% of participants. Red blood cells appearing in the shape of a teardrop or a pear with a single, short or long, often blunted or rounded end are called teardrop cells. Teardrop cells may be seen as an artifact of slide preparation; such dacrocytes are usually easily recognized due to the fact that their "tails" all point in the same direction. Teardrop cells are often seen in patients with bone marrow fibrosis, but may also be seen in pernicious anemia, anemia of renal disease, hemolytic anemias, and other forms of severe anemia. These cells are often associated with an abnormal spleen or bone marrow, moreover, and bone marrow infiltration with hematologic and non-hematologic malignancies may be accompanied by peripheral dacrocytosis.

CASE PRESENTATION:

This peripheral blood smear is from a 78-year-old woman presenting with fever and pain in the abdominal and back regions. Laboratory data include: WBC = 28.1 x 10E9/L; RBC = 3.85 x 10E12/L; HGB = 11.8 g/dL; HCT = 35.6%; PLT = 106 x 10E9/L; MCV = 92 fL; and RDW = 16%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Sepsis

Sepsis refers to the general state of hemodynamic dysregulation, with associated organ dysfunction, originating from an underlying infectious etiology (Verdonk, Blet, et al., 2017). Timely diagnosis of sepsis requires a high degree of contextual suspicion, facilitated by a combination of clinical and laboratory data. Diagnosis can be aided by clinical decision tools, which consider a series of biomarkers of organ dysfunction (Vincent, Moreno, et al., 1996). Biomarkers of import derive from a variety of respiratory, cardiovascular, coagulation, liver, central nervous and renal systems, culminating in the so-called SOFA score (Vincent, Moreno, et al., 1996).

Unlike historical definitions of sepsis, which previously included a metric of systemic inflammation inferred from extremes of neutrophil counts, current definitions do not require substantive perturbations in neutrophil parameters (Verdonk, Blet, et al., 2017). Nevertheless, accepted indicators of a hematological response to infection remain in use, including changes in neutrophil counts and so-called "toxic" neutrophil changes. Neutrophil toxic changes include neutrophil hypergranulation, vacuolation, and/or prominence of Döhle bodies, reflective of altered neutrophil biology in response to infection or inflammation (Shen, Cao, et al., 2017).

Question 1. Which statement is correct?

A. Current sepsis definitions require the identification of profound neutropenia.
B. Decision-aids in the diagnosis of sepsis do not exist, or are regarded as clinically ineffective.
C. Identification of neutrophil toxic changes is highly specific for sepsis.
D. Sepsis usually exists in the setting of infection.

Recent data suggest that sepsis indicators may be identifiable in 6% of hospital-admitted patients, with sepsis present in 35% of hospital admissions that end in death (Rhee, Valenzuela-Sánchez, et al., 2017). Older data suggest that emergency department admissions for sepsis in the United States number over 500,000 per annum (Wang, Shapiro, et al., 2007). Although the optimal therapeutic interventions in diagnosed or suspect sepsis remain controversial, there are data supporting early intervention (Seymour et al., 2017). However, data derived from early intervention studies, suggest that overall outcomes may be indifferent to the type of early intervention (The PRISM Investigators, 2017).

Question 2. Which statement is correct?

A. Clinical intervention invariably results in improved sepsis outcomes.
B. Data relating to incidence and outcomes for sepsis are currently unavailable.
C. Early intervention may be helpful in the treatment of sepsis, but may not significantly alter outcomes.
D. Sepsis presents an infrequent burden to both inpatient and outpatient clinical care.

Therapeutic guidance for sepsis relates to our current understanding of the underlying pathophysiology of sepsis (Rello, Valenzuela-Sánchez, et al., 2017). General recommendations include catered but aggressive antibiotic therapy, with deference to identified or presumed foci of infection, local microorganism epidemiology, and patient factors (especially patient factors that may portend increased risk to certain types of infections or colonization by certain microorganisms) (Rello, Valenzuela-Sánchez, et al., 2017). These interventions are generally accompanied by concurrent attempts to improve hemodynamic stability, typically by means of fluid resuscitation, transfusion, and supplementation (Rello, Valenzuela-Sánchez, et al., 2017).

The role of the hematology laboratory in the diagnosis and management of sepsis relate mainly to assisting with initial diagnostic work-up (Fan, Miller, et al., 2016). While not essential, the identification of neutrophilia and toxic changes in the appropriate clinical setting remains an important function of laboratory hematology staff (Fan, Miller, et al., 2016). The hematology laboratory may also need to work in careful and timely coordination with other laboratories (especially clinical chemistry and microbiology) to ensure rapid diagnosis (Fan, Miller, et al., 2016)

Question 3. Which statement is correct?

A. Antibiotic treatment and hemodynamic support are the main focuses for therapeutic intervention for sepsis.
B. Patient factors and local microbiologic factors are irrelevant to the general approach to sepsis management.
C. The hematology lab is not involved in the work-up of putative sepsis patients.
D. Understanding the pathophysiology of sepsis is not considered informative in prioritizing sepsis therapeutic interventions.

Etienne Mahe, MD, MSc
Hematology and Clinical Microscopy Resource Committee

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9. Wang, HE, Shapiro, N, et al. National estimates of severe sepsis in United States emergency departments: *Critical Care Medicine*. 2007;35(8):1928-1936. https://doi.org/10.1097/01.CCM.0000277043.85378.C1. Accessed 2018-APRIL-10

Answers to Questions:

Question 1: D Sepsis usually exists in the setting of infection
Although not all cases of sepsis have an appertaining diagnostic source of infection, an underlying infectious source is presumed in most definitions of sepsis.

Question 2: C. Early intervention may be helpful in the treatment of sepsis, but may not significantly alter outcomes
While a recent large study (Seymour et al, see references) does suggest a benefit to early intervention, other data (specifically originating from the PRISM data cohort) suggest that early goal-directed therapy for sepsis may not significantly improve overall outcomes.

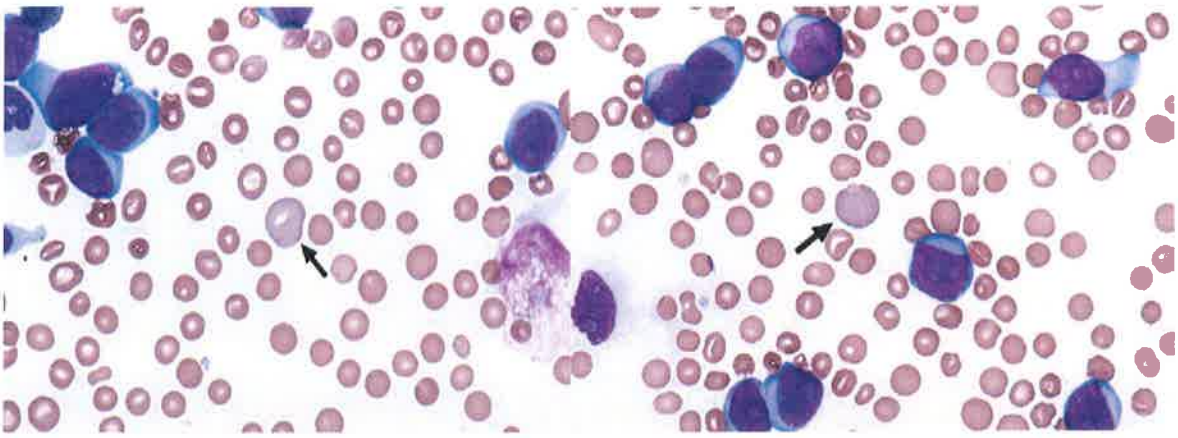
Question 3: A. Antibiotic treatment and hemodynamic support are the main focuses for therapeutic intervention for sepsis.
Despite ongoing questions relating to outcomes from sepsis interventions, most authors hold a need for combination antibiotic therapy and hemodynamic support for patients with diagnosed or suspected sepsis.

Committee Comments on Peripheral Blood Smear Whole Slide Image

CBC data indicate marked leukocytosis accompanied by moderate macrocytic anemia and thrombocytopenia. The peripheral blood smear shows a predominant population of blasts that are generally large with high nuclear to cytoplasmic ratio, variably irregular nuclear contours, dispersed chromatin, prominent nucleoli, and moderate amounts of cytoplasm with scattered examples of vacuolization but no definite granules or Auer rods. Some of the neutrophils appear hypogranular, and basket cells (smudge cells) are readily seen. The red blood cells demonstrate anisopoikilocytosis with polychromasia, and circulating nucleated red blood cells are present. The platelets include a subset of large and/or hypogranular forms.

Cell Identification

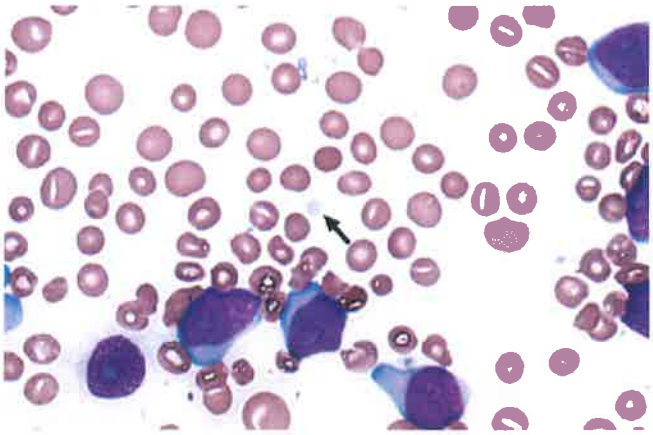
VPBS-26



Identification	Participants No.	Participants %	Evaluation
Polychromatophilic (non-nucleated) red blood cell	1116	91.8	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	86	7.1	Educational
Spherocyte	6	0.5	Educational
Blister cell/Prekeratocyte	3	0.3	Educational
Bite cell (degmacyte)	1	0.1	Educational
Hypochromasia	1	0.1	Educational
Neutrophil, segmented or band	1	0.1	Educational
Red blood cell agglutinates	1	0.1	Educational

The arrowed cells are polychromatophilic (non-nucleated) red blood cells (RBCs), as correctly identified by 91.8% of participants. Polychromatophilic RBCs are round or ovoid red blood cells that are non-nucleated and represent the final stage of RBC maturation after exiting the bone marrow. They are larger than mature RBCs and lack central pallor. Polychromatophilic RBCs primarily contain hemoglobin but also contain a small amount of RNA, which causes them to stain homogeneously pink-gray or pale purple with Romanowsky or Wright-Giemsa stain. These cells can be stained as reticulocytes and enumerated by using supravital stains such as new methylene blue. Although the cell is quite large,"polychromatophilic non-nucleated) red blood cell" is considered the most specific identification (see kit instructions) and therefore "macrocyte", as identified by 7.1% of participants, would not be considered as an acceptable answer.

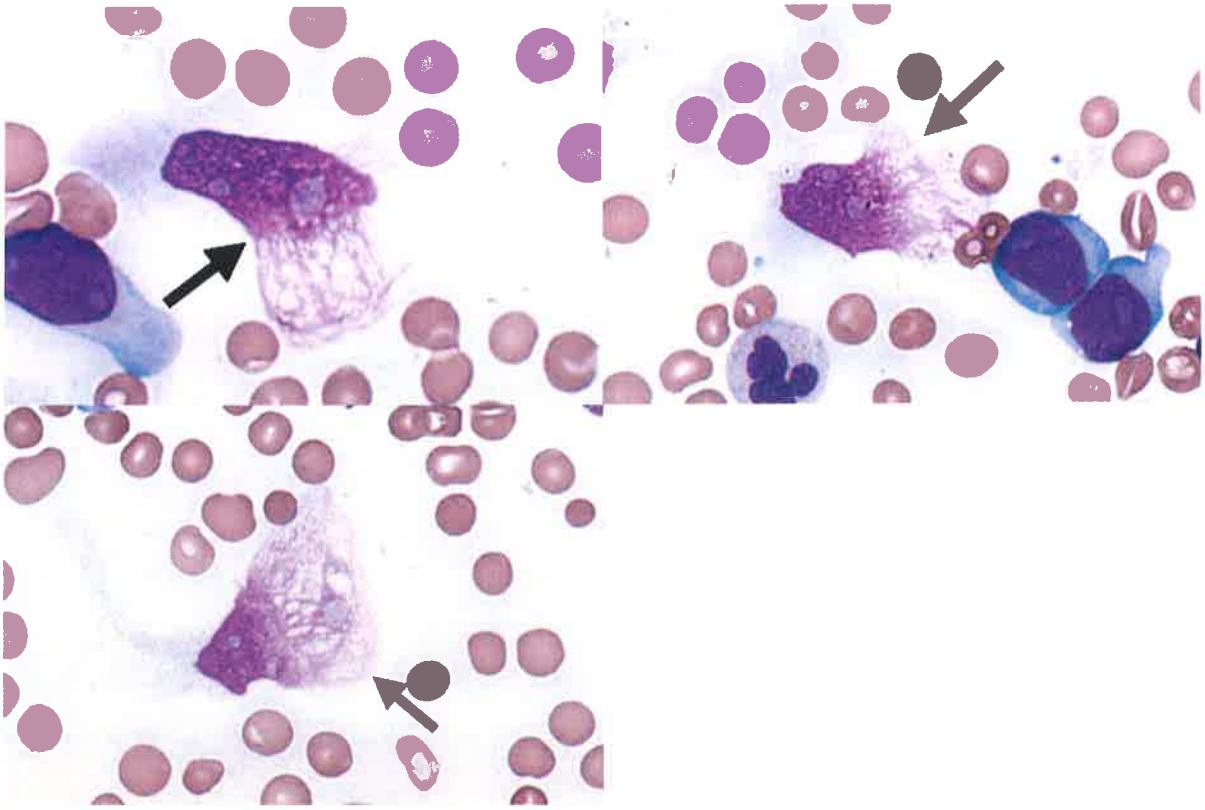
VPBS-27



Identification	Participants No.	%	Evaluation
Platelet, hypogranular	692	57.0	Educational
Platelet, normal	491	40.4	Educational
Platelet, giant (macrothrombocyte)	14	1.1	Educational
Stain precipitate	11	0.9	Educational
Polychromatophilic (non-nucleated) red blood cell	2	0.2	Educational
Blast cell	1	0.1	Educational
Hypochromasia	1	0.1	Educational
Lymphocyte	1	0.1	Educational
Neutrophil with hypersegmented nucleus	1	0.1	Educational

The arrowed object is a hypogranular platelet, as correctly identified by 57.0% of participants. Normal platelets, as identified by 40.4% of participants, are small, blue-gray fragments of megakaryocytic cytoplasm, and normal platelets feature fine, purple-red granules. Hypogranular platelets either have substantially reduced numbers of granules or lack granules entirely. Hypogranular platelets may be normal in size (1.5 - 3 µm in diameter) and shape, or they may be enlarged and misshapen. If granules are absent, alternation of lighter and darker areas within the cytoplasm, known as *zoning*, is needed to confidently identify the structure as a platelet. Hypogranular and other dysplastic platelet types are encountered in myeloproliferative neoplasms and myelodysplastic syndromes. Much less frequently, hypogranular platelets are also seen in the inherited condition known as "gray platelet syndrome" or as a result of artifact associated with difficult venipuncture. When platelets are entirely agranular, they may be easy to miss on peripheral blood smear review without careful scrutiny.

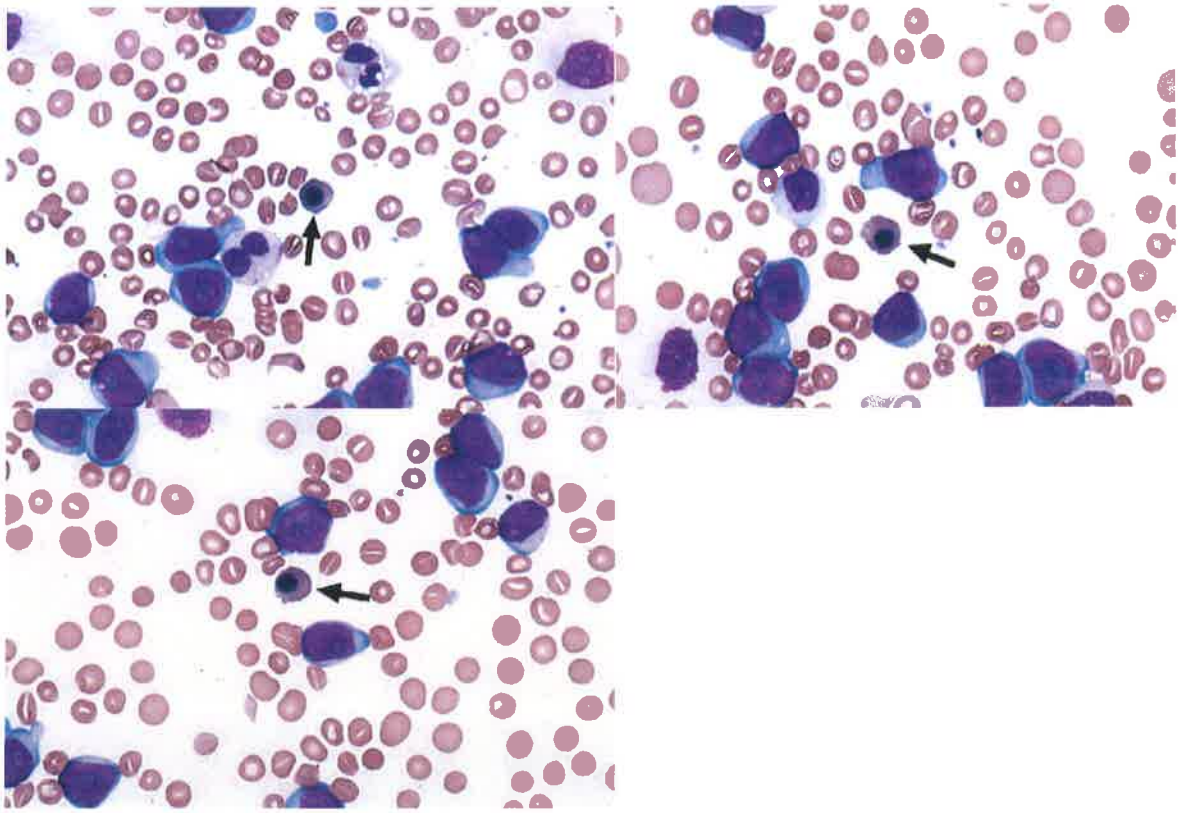
VPBS-28



Identification	Participants No.	%	Evaluation
Basket cell/smudge cell	1206	99.3	Educational
Nucleated red blood cell, normal or abnormal morphology	2	0.2	Educational
Blast cell	1	0.1	Educational
Lymphocyte, reactive (to include plasmacytoid and immunoblastic forms)	1	0.1	Educational
Mast cell	1	0.1	Educational
Microcyte (with increased central pallor)	1	0.1	Educational
Neutrophil necrobiosis (degenerated neutrophil)	1	0.1	Educational
Squamous epithelial cell/endothelial cell	1	0.1	Educational
Stain precipitate	1	0.1	Educational

The arrowed objects are basket cells/smudge cells, as correctly identified by 99.3% of participants. Basket cells or smudge cells result from damage to fragile cells in the process of making a peripheral blood smear. The nucleus may be seen as a non-descript chromatin mass, or strands of chromatin may spread out from a condensed nuclear remnant, resulting in a basket-like appearance. Cytoplasm is either absent or indistinct. Smudge cells are usually lymphocytes, though blasts may also smudge. There is no recognizable cytoplasm to permit definite identification of the origin of the cell, however. Such cells are seen most frequently chronic lymphocytic leukemia and infectious mononucleosis, which are characterized by lymphocyte fragility.

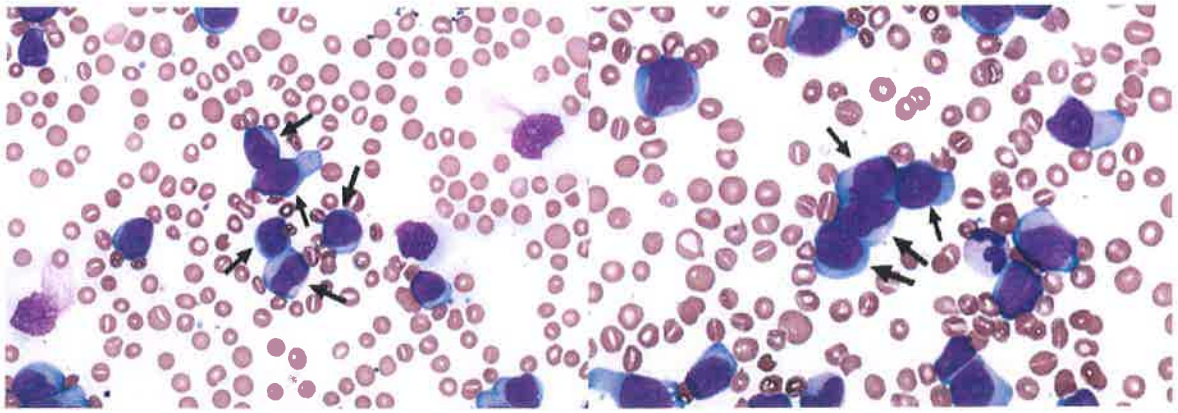
VPBS-29



Identification	Participants No.	%	Evaluation
Nucleated red blood cell, normal or abnormal morphology	1209	99.5	Educational
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	1	0.1	Educational
Lymphocyte	1	0.1	Educational
Neutrophil necrobiosis (degenerated neutrophil)	1	0.1	Educational
Platelet, giant (macrothrombocyte)	1	0.1	Educational
Platelet, normal	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational

The arrowed cells are nucleated red blood cells, normal or abnormal morphology, as correctly identified by 99.5% of participants. The term nucleated red blood cell encompasses any normoblasts in the peripheral blood, regardless of their stage of maturation. Frequently, circulating nucleated red blood cells are at the orthochromic stage of differentiation, though earlier forms may be seen. Both megaloblastic and apparently dysplastic changes can be seen in circulating nucleated red blood cells, though caution should be used in classifying a circulating nucleated red blood cell as dysplastic on the basis of abnormal nuclear shape (lobated or fragmented). Such changes may occur during egress of nucleated red blood cells from the marrow space and may not be present in the maturing erythroid precursors present in the bone marrow. For the purposes of proficiency testing, it is adequate to identify a cell as a nucleated red blood cell when it is present in the peripheral blood, whether it appears normal or abnormal.

VPBS-30



Identification	Participants No.	%	Evaluation
Blast cell	1119	92.1	Educational
Monocyte, immature (promonocyte, monoblast)	34	2.8	Educational
Immature or abnormal cell, would refer for identification	23	1.9	Educational
Lymphocyte, reactive (to include plasmacytoid and immunoblastic forms)	15	1.2	Educational
Malignant lymphoid cell (other than blast)	7	0.6	Educational
Myeloblast with Auer rod	5	0.4	Educational
Metastatic tumor cell or tumor cell clump	3	0.3	Educational
Neutrophil, promyelocyte, abnormal with/without Auer rod(s)	3	0.3	Educational
Basophil, any stage	2	0.2	Educational
Basket cell/smudge cell	1	0.1	Educational
Lymphocyte, large granular	1	0.1	Educational
Neutrophil, polyploid	1	0.1	Educational
Neutrophil, segmented or band	1	0.1	Educational

The arrowed cells are blasts, as correctly identified by 92.1% of participants. Monocyte, immature (promonocyte, monoblast), as identified by 2.8% of participants is an acceptable identification.
Blasts are large, round to oval cells typically measuring 10 - 20 µm in diameter, though blasts with monocytic features (monoblasts) may range up to 25 µm. In the blood film, blasts may appear flattened or compressed by adjacent red blood cells or by other blasts. The nuclear-to-cytoplasmic ratio is typically high, varying from 7:1 - 5:1, though monoblasts may have relatively more cytoplasm than myeloblasts (nuclear-to-cytoplasmic ratio 3:1). The blast often has a round to oval nucleus, but sometimes it is indented or folded. The blast cell has fine, lacy or reticular chromatin, and one or more prominent nucleoli may be seen. The cytoplasm is variably basophilic and often agranular, though small, scattered azurophilic granules may be seen. The morphologic features of a blast cell often do not permit determination of the cell lineage, ie, myeloblast versus lymphoblast. If present, however, Auer rods are diagnostic of myeloid lineage (ie, "myeloblast"). Other cells may have the appearance of a blast, including some lymphoma cells. In the absence of Auer rods, immunophenotyping by flow cytometry, immunohistochemistry on tissue sections, or, less commonly, cytochemical staining (eg, peroxidase or Sudan black) is required to determine the lineage of a given blast cell.

CASE PRESENTATION:

This peripheral blood smear is from a 65-year-old man with a history of myelodysplastic syndrome (MDS). Laboratory data include: WBC = 121.9 × 10E9/L; RBC = 2.49 × 10E12/L; HGB = 8.1 g/dL; HCT = 25.1%; MCV = 105 fL; MCHC = 32.3 g/dL; PLT = 59 × 10E9/L; and RDW = 24%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Acute myeloid leukemia with monocytic features

The peripheral blood smear findings in this case include the presence of numerous large neoplastic cells with morphologic features of blasts and relatively abundant cytoplasm. These cells account for more than 20% of circulating leukocytes, indicating involvement by acute leukemia. Results of flow cytometry (not shown) confirm that the blasts are of myeloid lineage and that they exhibit monocytic immunophenotypic features. Based on these data, a diagnosis of acute myeloid leukemia (AML) can be established; however, correlation with additional clinical and genetic data would be necessary to permit full classification.

AML is a morphologically and genetically heterogeneous disease characterized by a clonal expansion of myeloid progenitor cells. The primary criterion for establishing a diagnosis of AML is the presence of ≥ 20% myeloid blasts (or blast equivalents, eg, promonocytes) in the peripheral blood or bone marrow. Exceptions include the presence of certain recurrent genetic abnormalities, such as t(8;21), inv(16)/t(16;16), or t(15;17), or the presence of myeloid sarcoma (a mass composed of myeloid blasts occurring outside of the bone marrow), as AML can be diagnosed in patients with these abnormalities even when the blood and marrow blast percentages are less than 20%.

While many patients diagnosed with AML have no known history of predisposing conditions, diseases categorized as myelodysplastic syndromes (MDS) or myelodysplastic/myeloproliferative neoplasms (MDS/MPN) are associated with elevated risk for evolution to AML. Subtypes of MDS may categorized by risk based on survival time and on likelihood of evolution to AML; MDS with excess blasts (MDS-EB, as defined in the 2016 WHO Classification) falls within the high risk group, as 25 - 33% of cases progress to AML.

In patients with a history of MDS, a myeloid blast count of at least 20% in peripheral blood or bone marrow establishes a diagnosis of AML. Unless the patient has previously had cytotoxic chemotherapy or radiation therapy (eg, as treatment for an unrelated malignancy), or a recurrent genetic abnormality specified in the 2016 WHO Classification is present, AML in this setting is classified as AML with myelodysplasia-related changes (AML-MRC). AML-MRC, which occurs predominantly in older adults and has a poor prognosis, may also be diagnosed on the basis of a morphologic finding of multilineage dysplasia or the presence of an MDS-related cytogenetic abnormality (of which a complex karyotype is but one example). Most cases of AML-MRC have morphologic evidence of dysplasia affecting multiple lineages, regardless of whether an MDS had been previously diagnosed. AML-MRC accounts for at least one-quarter to one-third of total adult cases of AML.

Question 1. For a patient with a history of myelodysplastic syndrome (MDS), which of the following is required to establish a diagnosis of acute myeloid leukemia with myelodysplasia-related changes (AML-MRC)?

A. A complex karyotype
B. At least 20% blasts in peripheral blood or bone marrow
C. Presence of particular recurrent genetic abnormalities [eg, t(8;21) or inv(16)/t(16;16)]
D. Prior cytotoxic or radiation therapy for unrelated disease

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Diagnosis and classification of AML requires combining clinical findings with results of morphologic, immunophenotypic and genetic evaluation. A particular genetic abnormality (eg, t(8;21) is the defining diagnostic criterion for some subtypes of AML. Morphologic features (eg, Auer rods) and immunophenotypic profiles of leukemic blasts can help to determine the lineage of new acute leukemias, and in some instances characteristic morphologic and/or immunophenotypic features can point to a specific subtype (eg, acute promyelocytic leukemia with *PML-RARA*) and guide clinical management before complete data are available.

Monocytic differentiation of leukemic blasts may be suggested by morphologic findings, immunophenotypic features, and/or clinical data. Monoblasts are generally large, as in the present case. While their nuclear-to-

cytoplasmic ratio is high, cytoplasm is relatively abundant when compared to that seen in other myeloid blasts. Cytoplasmic vacuoles and granules, while not specific, may be seen in monoblasts. Expression of markers such as CD14, CD64, and CD11c is associated with monocytic differentiation, as are clinical findings such as gingival infiltration and formation of masses outside the bone marrow.

Evidence of monocytic differentiation of blasts is not specific for WHO-defined subtypes of AML. Monoblastic morphologic features may be seen in a subset of therapy-related AML cases (especially those following topoisomerase II inhibitor therapy), in some cases of AML with myelodysplasia-related changes (AML-MRC), and in some subtypes of AML with recurrent genetic abnormalities, including inv(16)/t(16;16), t(9;11), and mutated *NPM1*. Some AML cases with monocytic differentiation do not meet criteria for any of these categories, in which case they are classified as AML, not otherwise specified (AML, NOS); in these instances, monocytic differentiation is not of prognostic significance.

Question 2. Which of the following genetic or immunophenotypic findings is associated with monocytic differentiation in AML?

A. A complex karyotype
B. Expression of CD64
C. Mutated *FLT3* gene
D. Translocation t(15;17)

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References:

1. Arber DA, Brunning RD, Orazi A, et al. Acute myeloid leukemia with myelodysplasia-related changes. In SH Swerdlow, E Campo, NL Harris, ES Jaffe, SA Pileri, H Stein, J Thiele, DA Arber, RP Hasserjian, MM Le Beau, A Orazi, R Siebert (Eds.), *WHO Classification of Tumours of Haematopoietic and Lymphoid Tlsssues*, Revised 4th Edition. Lyon, France: IARC Press; 2016:150-152.
2. Hasserjian RP, Orazi A, Brunning RD, et al.Myelodysplastic syndromes. In SH Swerdlow, E Campo, NL Harris, ES Jaffe, SA Pileri, H Stein, J Thiele, DA Arber, RP Hasserjian, MM Le Beau, A Orazi, R Siebert (Eds.), *WHO Classification of Tumours of Haematopoietic and Lymphoid Tlsssues*, Revised 4th Edition. Lyon, France: IARC Press; 2016:98-120.

Answers to questions:

Question 1: B. At least 20% blasts in peripheral blood or bone marrow
By definition, a diagnosis of AML with myelodysplasia-related changes (AML-MRC) requires: 1) the presence of ≥ 20% blasts in the peripheral blood or bone marrow; 2) a history of myelodysplastic syndrome or myelodysplastic/myeloproliferative neoplasm, an MDS-related cytogenetic abnormality, or multilineage dysplasia; and 3) absence of prior cytotoxic or radiation therapy and absence of recurrent cytogenetic abnormalities such as t(15;17) (seen in acute promyelocytic leukemia), t(8;21) or inv(16) / t(16;16), characteristic of AML with recurrent cytogenetic abnormalities. A complex karyotype may be seen in AML-MRC but is not necessary to establish the diagnosis. Detection of any of the recurrent genetic abnormalities specified in the 2016 WHO Classification excludes a patient's AML from the AML-MRC category, as recurrent genetic abnormalities are associated with characteristic clinicopathologic features and are of prognostic significance. A history of cytotoxic or radiation therapy should prompt classification of the patient's disease as therapy-related AML (t-AML)

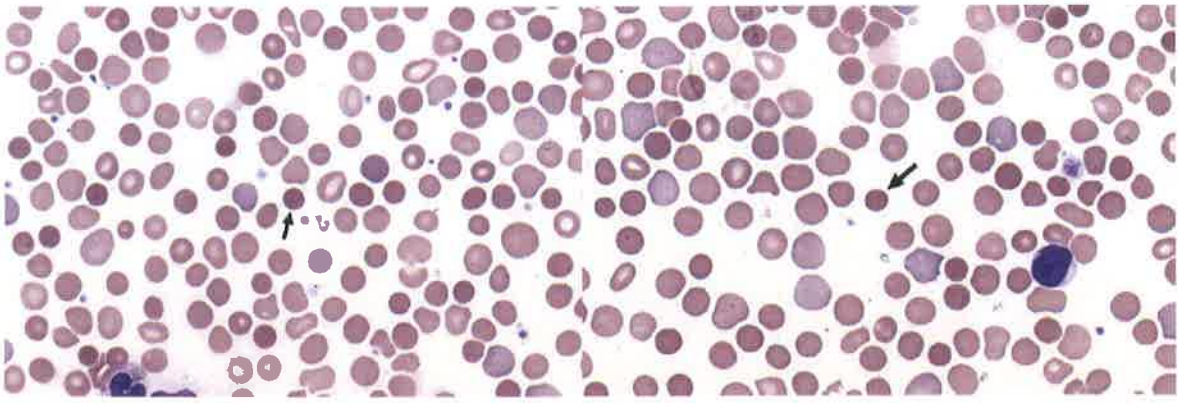
Question 2: B. Expression of CD64
Monocytic differentiation in AML is associated in some cases with characteristic genetic and immunophenotypic findings. Examples of immunophenotypic markers detectable by flow cytometry and associated with monocytic differentiation include CD64 and CD14. Mutated *FLT3* is seen across AML subtypes, while AML with mutated *NPM1* frequently exhibits monocytic or myelomonocytic differentiation. The t(15;17) is characteristic of acute promyelocytic leukemia with *PML-RARA*; karyotypic abnormalities associated with monocytic differentiation include t(9;11) and inv(16)/t(16;16).

Committee Comments on the CBC and Blood Film

The whole slide image of the peripheral blood smear from a newborn girl demonstrates mild macrocytic anemia with increased polychromasia, numerous spherocytes, and circulating nucleated red blood cells (nRBCs). Review of the leukocytes reveals neutrophilia with mild left shift and prominent toxic changes, including prominent purple granules and occasional cytoplasmic vacuoles. The platelets are normal in number and show appropriate size and granulation. The morphologic findings are consistent with a spherocytic hemolytic anemia.

Cell Identification

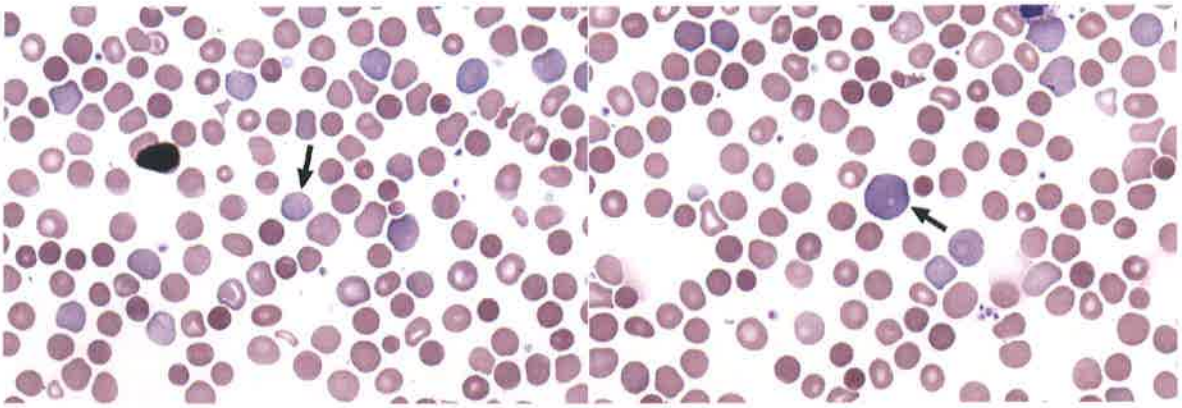
VPBS-32



Identification	Participants No.	%	Evaluation
Spherocyte	1172	96.2	Educational
Microcyte (with increased central pallor)	25	2.0	Educational
Erythrocyte, normal	18	1.5	Educational
Polychromatophilic (non-nucleated) red blood cell	2	0.2	Educational
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	1	0.1	Educational

The arrowed cells are spherocytes, as correctly identified by 96.2% of participants. Spherocytes are erythrocytes that are hyperchromic and lack central pallor due to their spherical shape. This contrasts with normal erythrocytes, which have a biconcave shape and visible central pallor on smear preparations. Spherocytes are often smaller than normal erythrocytes and may be very small (ie, microspherocytes, defined as < 4 μm in diameter). They form as a consequence of membrane loss, resulting in a decreased ratio of cell surface membrane to cytoplasmic volume. Increased spherocytes are most commonly seen in cases of immune hemolytic anemia and hereditary spherocytosis, but also are typically encountered in smaller numbers in patients with Heinz body anemias where they are associated with bite cells.

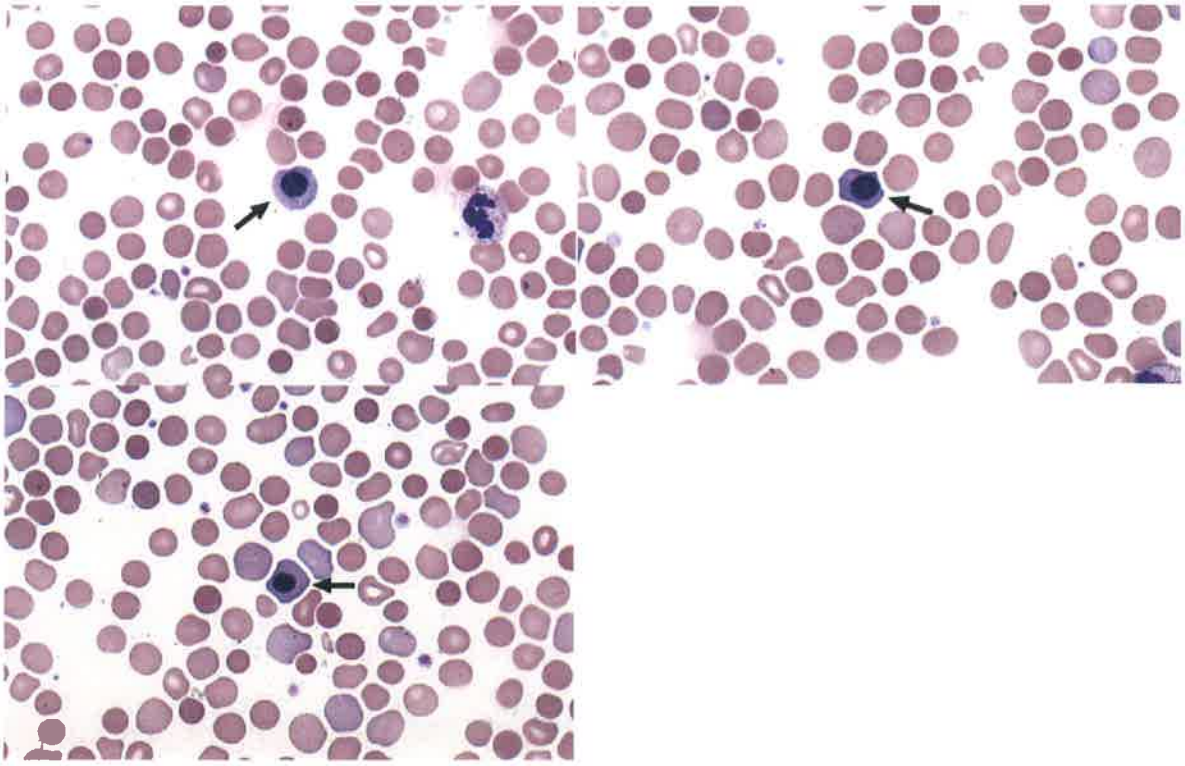
VPBS-33



Identification	Participants No.	%	Evaluation
Polychromatophilic (non-nucleated) red blood cell	1204	98.8	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	8	0.7	Educational
Spherocyte	2	0.2	Educational
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	1	0.1	Educational
Hypochromasia	1	0.1	Educational
Platelet, hypogranular	1	0.1	Educational
Stain precipitate	1	0.1	Educational

The arrowed cells are polychromatophilic (non-nucleated) red blood cells (RBCs), as correctly identified by 98.8% of participants. Mature RBCs (erythrocytes) are round to oval disc shaped cells measuring ~ 7 µm in diameter, and contain central pallor occupying less than one third (2 - 3 µm) of the cell diameter. In contrast, reticulocytes appear polychromatophilic on Wright stains and are slightly larger in size compared to mature RBCs, have a blue-grey cytoplasm and occasionally contain fine basophilic granules.

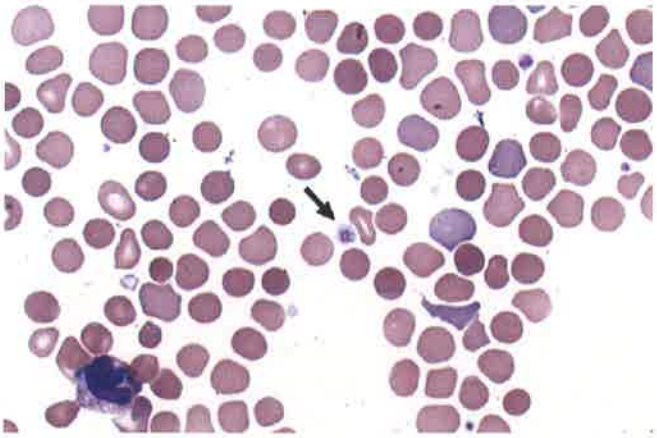
VPBS-34



Identification	Participants No.	Participants %	Evaluation
Nucleated red blood cell, normal or abnormal morphology	1121	99.4	Educational
Basket cell/smudge cell	2	0.2	Educational
Acanthocyte (spur cell)	1	0.1	Educational
Erythrocyte, normal	1	0.1	Educational
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	1	0.1	Educational
Lymphocyte	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational

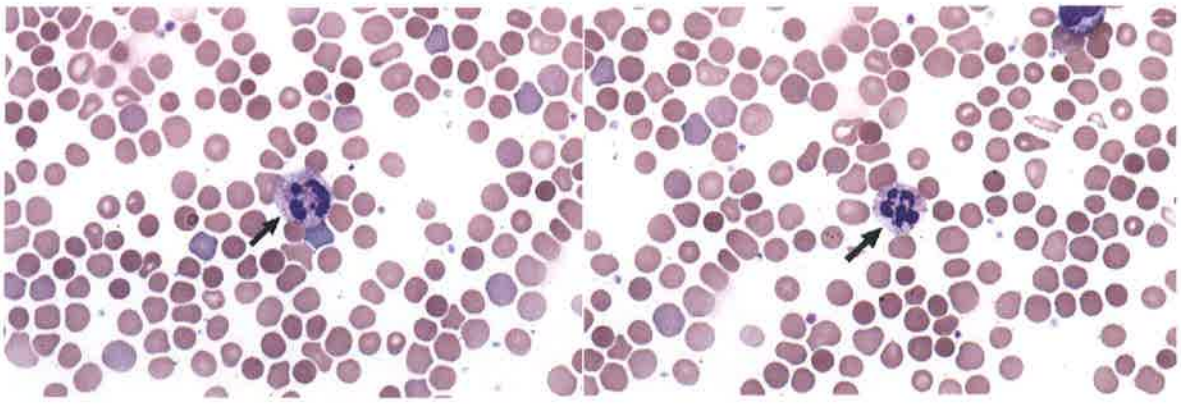
The arrowed cells are nucleated red blood cells (nRBCs), normal or abnormal morphology, as correctly identified by 99.4% of participants. As is generally the case, the circulating nRBCs present in this peripheral blood smear are the orthochromic stage of differentiation with a small pyknotic nucleus and partially hemoglobinized cytoplasm.

VPBS-35



Identification	Participants No.	%	Evaluation
Platelet, normal	1096	90.0	Educational
Platelet, giant (macrothrombocyte)	109	8.9	Educational
Platelet, hypogranular	5	0.4	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	3	0.3	Educational
Microcyte (with increased central pallor)	2	0.2	Educational
Neutrophil, segmented or band	1	0.1	Educational
Nucleated red blood cell, normal or abnormal morphology	1	0.1	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	1	0.1	Educational

The arrowed object is a normal platelet, as correctly identified by 90.0% of participants. Platelets are blue-gray fragments of megakaryocytic cytoplasm that typically measure 1.5 - 3 µm in diameter and contain fine, purple-red granules. Large platelets measure approximately 4 - 7 µm in diameter. 8.9% of participants identified this object as a giant platelet. The term "giant platelet" is used when the platelet is larger than the size of an average red cell, assuming a normal MCV (see VPBS-23 for further description). The platelets in this case demonstrate normal size when compared with the surrounding red blood cells and the monocyte in the lower left hand corner.



Identification	Participants No.	%	Evaluation
Neutrophil, segmented or band	839	68.9	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	312	25.6	Educational
Neutrophil with hypersegmented nucleus	54	4.4	Educational
Blast cell	2	0.2	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	2	0.2	Educational
Neutrophil with Pelger-Huët nucleus (acquired or congenital)	2	0.2	Educational
Erythrocyte, normal	1	0.1	Educational
Metastatic tumor cell or tumor cell clump	1	0.1	Educational
Neutrophil, polyploid	1	0.1	Educational
Platelet, giant (macrothrombocyte)	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational

The arrowed cells are neutrophils, segmented or band, as correctly identified by 68.9% of participants. Segmented neutrophils are the most mature form of the neutrophilic series and the most predominant white cell in human blood of adults. Neutrophil's size ranges from10 to 15 µm; its shape is round to oval; and its cytoplasm is pale pink with specific granules. The N:C ratio is 1:3, and the nuclear chromatin is condensed. The nucleus is segmented or lobated (two to five lobes normally). The lobes are connected by a thin filament that contains no internal chromatin, giving it the appearance of a solid, thread-like dark line.

25.6% of participants identified the arrowed cells as toxic neutrophils (includes toxic granulation and/or Döhle bodies, and/or toxic vacuolization). Toxic neutrophils contain toxic granulation which refers to the presence of large, purple or dark blue cytoplasmic granules in neutrophils, bands, and metamyelocytes. Vacuoles within the cytoplasm of these same cells constitute toxic vacuolization. The vacuoles are variably sized and may coalesce, sometime distorting the neutrophil cytoplasm to form pseudopodia. Toxic changes result from the action of cytokines released in response to infection, burns, trauma, and G-CSF (granulocyte colony stimulating factor), and they indicate a shortened maturation time and activation of postmitotic neutrophil precursors. In the arrowed example, the cytoplasmic granules are relatively prominent, suggestive of toxic granulation. Other neutrophils present this case show similar prominent specific granules, and some have small cytoplasmic vacuoles, supporting an interpretation of toxic neutrophils.

VPBS-36 Discussion, Cont'd:

4.4% of participants called these cells hypersegmented neutrophils. To be considered a neutrophil with a hypersegmented nucleus, the neutrophil should demonstrate six or more lobes. Hypersegmented neutrophils are uncommon unless there is megaloblastic hematopoiesis. Hypersegmented neutrophils may also be seen in sepsis, renal disease, and myeloproliferative neoplasms. Megaloblastic hematopoiesis occurs when DNA synthesis is impaired. Such conditions include deficiency of cofactors for nucleotide synthesis, such as vitamin B12 and folate, and cases in which patients are receiving a nucleotide analog drug (such as 6-mercaptopurine) or nuclear cofactor blocking agents (such as methotrexate) for the treatment of neoplastic or rheumatologic conditions.

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The following authors/planners have no financial relationships to disclose:
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The following authors/planners have financial relationships to disclose:

Author	Commercial Interest	Your Role	What was received
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Learning Objectives

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

1. Identify the clinical features and pathogenesis of hemolytic disease of the fetus and newborn (HDFN).
2. Describe morphologic and laboratory features seen in HDFN.
3. Understand the importance and findings of laboratory studies in the diagnosis, treatment, and prognosis of HDFN.