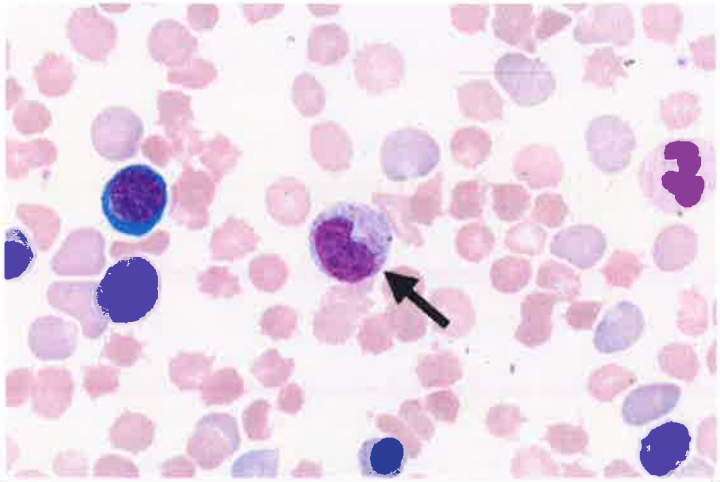
Cell Identification

VPBS-02

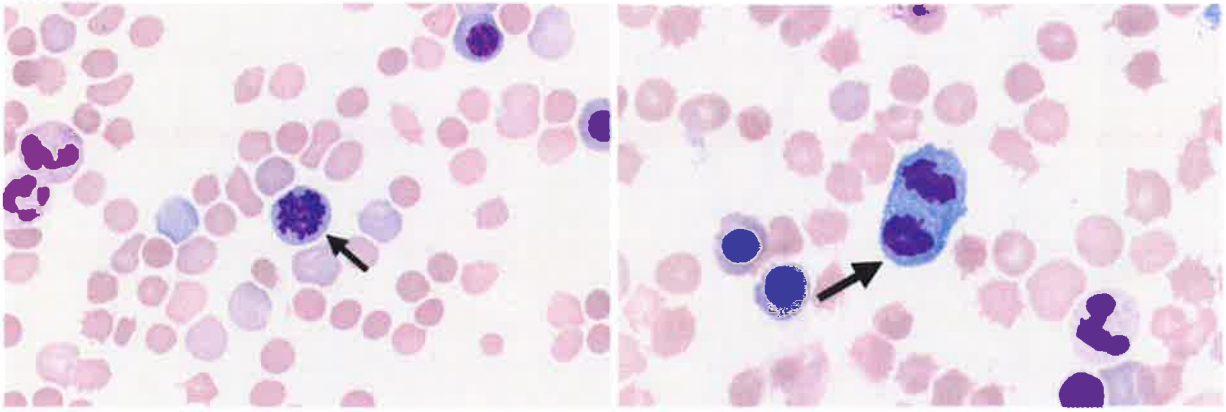


Identification	Participants Labs	Participants %	Evaluation
Neutrophil, metamyelocyte	1126	94.3	Educational
Neutrophil, myelocyte	20	1.7	Educational
Monocyte	11	0.9	Educational
Neutrophil, segmented or band	11	0.9	Educational
Immature or abnormal cell, would refer for identification	10	0.8	Educational
Neutrophil, giant band or giant metamyelocyte	6	0.5	Educational
Neutrophil, promyelocyte	5	0.4	Educational
Lymphocyte, large granular	2	0.2	Educational
Monocyte, immature (promonocyte, monoblast)	2	0.2	Educational
Nucleated red blood cell, normal or abnormal morphology	1	0.1	Educational

The arrowed cell is a metamyelocyte, as correctly identified by 94.3% of participants. Metamyelocytes are the first of the postmitotic myeloid precursors. They constitute 15% to 20% of nucleated cells in the bone marrow and may be seen in the blood in pathologic states and in response to stress. They are approximately 10 to 18 µm in diameter. They are round to oval with a nuclear to cytoplasmic (N:C) ratio of 1.5:1 to 1:1. The nuclear chromatin is condensed, and the nucleus is indented to less than half of the maximal nuclear diameter (ie, the indentation is smaller than half of the distance to the farthest nuclear margin). The cytoplasm is amphophilic containing rare azurophilic or purple (primary) granules and many fine lilac or pale orange/pink specific granules.

1.7% identified the arrowed cell as a myelocyte. The myelocyte immediately precedes the metamyelocyte in development. Like metamyelocytes, myelocytes are approximately 10 to 18 µm in diameter, have an N:C ratio of 2:1 to 1:1, and are usually confined to the bone marrow but may be seen in peripheral blood in pathologic states. However, unlike the metamyelocyte, the myelocyte nucleus lacks indentation and is instead round to oval. In addition, the nuclear chromatin of the myelocyte is less condensed than that of the metamyelocyte: the myelocyte nucleus is just beginning to demonstrate chromatin clumping, often shows slight flattening on one side, and may show a clear space (hof) adjacent to the nucleus, indicating the location of the Golgi apparatus. Lastly, specific granules in the cytoplasm are less abundant in the myelocyte than in the metamyelocyte.

VPBS-03

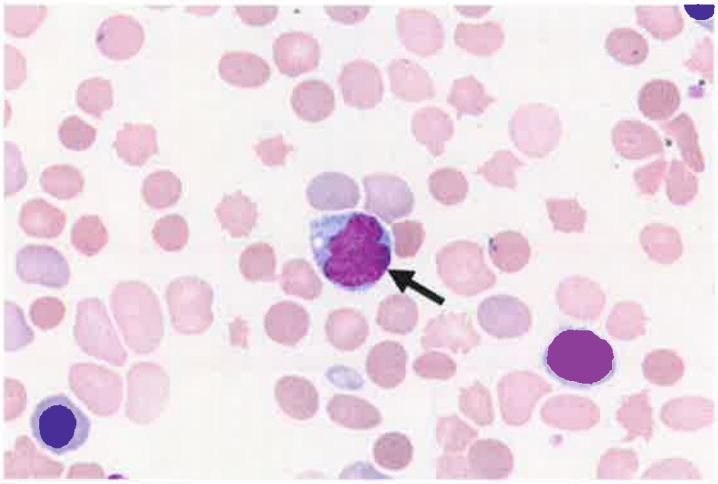


Identification	Participants Labs	Participants %	Evaluation
Mitotic figure	1101	92.2	Educational
Nucleated red blood cell, normal or abnormal morphology	45	3.8	Educational
Immature or abnormal cell, would refer for identification	12	1.0	Educational
Neutrophil necrobiosis (degenerated neutrophil)	12	1.0	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	8	0.7	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	3	0.3	Educational
Neutrophil, polyploid	3	0.3	Educational
Blast cell	2	0.2	Educational
Stain precipitate	2	0.2	Educational
Acanthocyte (spur cell)	1	0.1	Educational
Basophil, any stage	1	0.1	Educational
Lymphocyte	1	0.1	Educational
Mast cell	1	0.1	Educational
Parasite(s) seen, referred for definitive identification	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational

The arrowed cells contain mitotic figures, as correctly identified by 92.2% of participants. A cell containing a mitotic figure is variable in size; it may be larger than the surrounding cells. The cytoplasm has color and granulation characteristic of the resting cell. When a cell undergoes mitosis, typical nuclear features are no longer present. Instead, the nucleus appears as a dark, irregular mass, often with a clear central zone. It may take various shapes, including a daisy-like form or a mass with irregular projections. In metaphase, the individual chromosomes become visible. Arranged equatorially, they begin to separate and to move toward opposite poles. Rarely, the anaphase or telophase of mitosis may be seen, with two separating masses of chromosomes forming two daughter cells, as seen in the example on the right for this VPBS-03 cell identification. A mitotic cell can be distinguished from a degenerating cell by a relatively compact nucleus (or nuclei); a degenerating cell often displays a pyknotic nucleus that has been fragmented into numerous purple, roundish inclusions.

3.8% identified the arrowed cells as normal nucleated red blood cells. The term "nucleated red blood cell" (nRBC) is used to state the presence of normoblasts in the peripheral blood and includes all normoblasts regardless of the stage of maturation. Typically, the circulating nucleated red blood cell is at the orthochromic stage of differentiation. Both megaloblastic and dysplastic changes can be seen in these circulating red blood cells, reflecting simultaneous erythroid maturation abnormalities present in the bone marrow. Megaloblastic and dysplastic changes are distinct from mitotic figures, which specifically show condensed chromosomes.

VPBS-04



Identification	Participants Labs	%	Evaluation
Monocyte	1075	90.0	Educational
Monocyte, immature (promonocyte, monoblast)	61	5.1	Educational
Lymphocyte, large granular	13	1.1	Educational
Neutrophil, myelocyte	13	1.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	11	0.9	Educational
Immature or abnormal cell, would refer for identification	6	0.5	Educational
Neutrophil, metamyelocyte	4	0.3	Educational
Blast cell	3	0.3	Educational
Lymphocyte	3	0.3	Educational
Neutrophil, promyelocyte	3	0.3	Educational
Malignant lymphoid cell (other than blast)	1	0.1	Educational
Mitotic figure	1	0.1	Educational

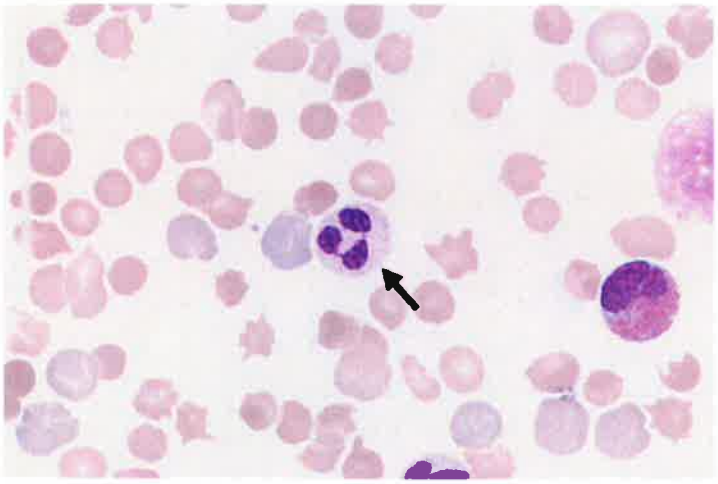
The arrowed cell is a monocyte, as correctly identified by 90.0% of participants. Monocytes are slightly larger than neutrophils, ranging from 12 to 20 µm in diameter. The majority of monocytes are round with smooth edges, but some may have pseudopod-like cytoplasmic extensions. The cytoplasm is abundant, with a gray or gray-blue ground-glass appearance, and may contain vacuoles or fine, evenly distributed azurophilic granules. The N:C ratio ranges from 4:1 to 2:1. 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.

5.1% identified the arrowed cell as monocyte, immature (promonocyte, monoblast). Selection of the response "monocyte, immature (promonocyte, monoblast)" should be reserved for malignant cells in acute monocytic/monoblastic leukemia, acute myelomonocytic leukemia, chronic myelomonocytic leukemia, and myelodysplastic syndromes. The malignant monoblast is a large cell, 15 to 25 µm in diameter. It has relatively more cytoplasm than a myeloblast with the nuclear-to-cytoplasmic ratio ranging from 7:1 to 3:1. The monoblast nucleus is round or oval and has finely dispersed chromatin and distinct nucleoli. The cytoplasm is blue to gray-blue and may contain small, scattered azurophilic granules. Some monoblasts cannot be

VPBS-04, cont'd.

distinguished morphologically from other blast forms, hence the need for using other means (eg, cytochemistry and flow cytometry) are required to accurately assign blast lineage. Promonocytes have nuclear and cytoplasmic characteristics that are between those of monoblasts and mature monocytes. They are generally larger than mature monocytes, but they have similar appearing gray-blue cytoplasm that often contains uniformly distributed, fine azurophilic granules. Cytoplasmic vacuolization is not a typical feature. The nuclei show varying degrees of lobulation, usually characterized by delicate folding or creasing of the nuclear membrane, in contrast to the rounder nuclear profile of monoblasts. Nucleoli are present but not as distinct as in monoblasts.

VPBS-05

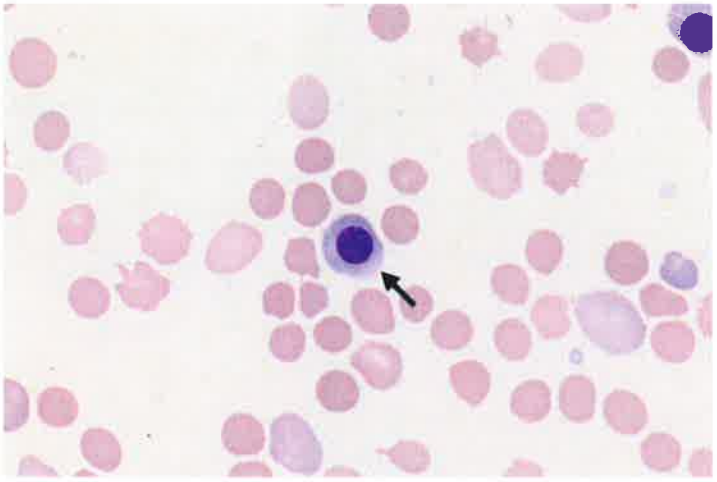


Identification	Participants Labs	%	Evaluation
Neutrophil, segmented or band	1169	97.9	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	15	1.3	Educational
Neutrophil with Pelger-Huët nucleus (acquired or congenital)	4	0.3	Educational
Platelet, hypogranular	2	0.2	Educational
Monocyte	1	0.1	Educational
Neutrophil with hypersegmented nucleus	1	0.1	Educational
Neutrophil, polyploid	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational

The arrowed cell is a neutrophil, as correctly identified by 97.9% of participants. The segmented neutrophil is the predominant blood leukocyte. It has a similar size to its immediate precursor, the 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. An increased number of bands may be noted in the blood in a number of physiologic and pathologic states (eg, infectious/inflammatory processes, tissue damage or necrosis, neoplasia, poisoning or intoxication, drug effect, and metabolic abnormalities). However, in repeated proficiency testing studies, it has not been possible to achieve consistent differentiation between bands and segmented neutrophils. Therefore, for the purposes of proficiency testing, it is not required that they be differentiated.

1.3% identified the arrowed cell as a toxic neutrophil. Toxic changes in neutrophils include toxic granulation, toxic vacuolization, and Döhle bodies. Toxic granulation is the presence of large, purple or dark blue cytoplasmic granules in neutrophils, bands, and metamyelocytes, and based on this definition is not present in the arrowed cell. Of note, toxic granulation and Döhle bodies each may be present in an individual cell without the other finding. Either change alone is sufficient to designate a neutrophil as toxic. Döhle bodies are also not present in the arrowed cell.

VPBS-06



Identification	Participants Labs	%	Evaluation
Nucleated red blood cell, normal or abnormal morphology	1172	98.2	Educational
Lymphocyte	9	0.8	Educational
Blast cell	2	0.2	Educational
Polychromatophilic (non-nucleated) red blood cell	2	0.2	Educational
Erythrocyte, normal	1	0.1	Educational
Immature or abnormal cell, would refer for identification	1	0.1	Educational
Lymphocyte, large granular	1	0.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.1	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	1	0.1	Educational
Microcyte (with increased central pallor)	1	0.1	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	1	0.1	Educational
Neutrophil, metamyelocyte	1	0.1	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	1	0.1	Educational

The arrowed cell is a nucleated red blood cell as correctly identified by 98.2% of participants. The term nucleated red blood cell is used to state the presence of normoblasts in the peripheral blood and includes all normoblasts regardless of the stage of maturation. Typically, the circulating nucleated red blood cell is at the orthochromic stage of differentiation. Caution should be used in classifying a circulating nucleated red blood cell as dysplastic on the basis of abnormal nuclear shape (lobated or fragmented), as these changes may occur during their egress from the marrow space and may not be present in the maturing erythroid precursors present in the 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, be it normal or abnormal (ie, exhibits megaloblastic or dysplastic changes).

Case Presentation:

This peripheral blood smear is from a 2-day-old boy presenting with hemolytic disease of the newborn, who required platelet transfusion and red blood cell exchange transfusion (initial hemoglobin 8.0 g/dL). Laboratory data include: Corrected WBC = 10.4 x 10E9/L; RBC = 5.08 x 10E12/L; HGB = 15.4 g/dL; HCT = 44.3%; MCV = 87 fL; PLT = 37 x 10E9/L; and RDW = 15%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Hemolytic Disease of the Newborn

Pathophysiology

Hemolytic disease of the fetus and newborn (HDFN, or HDN; also known as erythroblastosis fetalis) is caused by differences in antigens expressed on the surface of red blood cells (RBCs) between mother and baby. The fetus or neonate may inherit genes from the father that lead to surface expression of RBC protein antigens that the mother does not have. If the mother is exposed to fetal/neonatal RBCs during pregnancy and labor, she may mount an antibody response to these "non-self" antigens. During a future pregnancy, with repeat exposure to the same type of fetal RBC antigens, these alloantibodies (IgG) may quickly reform in the maternal circulation, cross the placenta into the fetal circulation, and lyse fetal/neonatal RBCs, causing potentially fatal hemolytic disease. Maternal alloimmunization to the RhD antigen (RhD-positive fetus of an RhD-negative mother) is the most common and most severe cause of HDN.

Question 1. Which of the following parental red blood cell antigen combinations would be expected to put a fetus at risk for hemolytic disease of the newborn (HDN)?

A. Father: RhD-negative; Mother: RhD-negative
B. Father: RhD-negative; Mother: RhD-positive
C. Father: RhD-positive; Mother: RhD-negative
D. Father: RhD-positive; Mother: RhD-positive

Clinical and Laboratory Features

Like any form of hemolytic anemia, HDN ranges in severity. Infants show mild jaundice and anemia only, but severely affected patients may show respiratory distress due to marked anemia, hydrops (edema, pleural effusions, and ascites), kernicterus (hyperbilirubinemia affecting the brain), and splenic rupture. Complete blood count (CBC) shows low hemoglobin and hematocrit levels and elevated reticulocyte count. The CBC may show nucleated red blood cells due to bone marrow response to anemia, and low platelets due to splenomegaly and/or consumptive coagulopathy. Peripheral blood smear review from a neonate with severe HDN may show marked red blood cell anisopoikilocytosis.

In the Blood Bank, the direct antiglobulin test (DAT) is positive because the hemolysis is due to alloantibodies from the mother. The most common maternal blood type seen in HDN is Rh negative (see "Prevention" section below).

Question 2. Which of the following complete blood count (CBC) values is most consistent with hemolytic disease of the newborn (HDN)?

A. Leukocytosis
B. Leukopenia
C. Thrombocytopenia
D. Thrombocytosis

Prevention

Severe HDN due to RhD incompatibility is largely preventable today through prenatal maternal administration of Rh immunoglobulin (Rh Ig; trade name: Rhogam). Rh Ig is thought to block maternal anti-Rh antibodies before they can cross the placenta and lyse fetal red blood cells. However, HDN continues to affect fetuses and neonates throughout the world and be a cause of perinatal morbidity and mortality. This is because Rh Ig may not be administered in time or at all due to limited prenatal care. Additionally, not all cases of HDN are due to incompatibility with the RhD antigen. There are many other antigen groups expressed on the surface of red blood cells (eg, Kell, Duffy antigens). With some exceptions, maternal-fetal incompatibilities between these antigens do not tend to cause severe hemolysis.

Question 3. When should Rh immunoglobulin (Rh Ig) be administered to a pregnant woman?

A. To an RhD-negative woman during pregnancy
B. To an RhD-negative woman after delivery
C. To an RhD-positive woman during pregnancy
D. To an RhD-positive woman after delivery

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Hematology and Clinical Microscopy Committee

References:

1. de Haas M, Thurik FF, Koelewijn J.M., van der Schoot C.E. Haemolytic disease of the fetus and newborn. *Vox Sanguinis.* 2015;109:99-113. PMID:25899660.
2. Hendrickson JE, Delaney M. Hemolytic disease of the fetus and newborn: modern practice and future investigations. *Transfus Med Rev*. 2016;30(4):159-164. PMID:27397673.
3. Tormey CA, Hendrickson JE. Transfusion-related red blood cell alloantibodies: induction and consequences. *Blood*. 2019;133(17):1821-1830. PMID:30808636.

Answers to Questions:

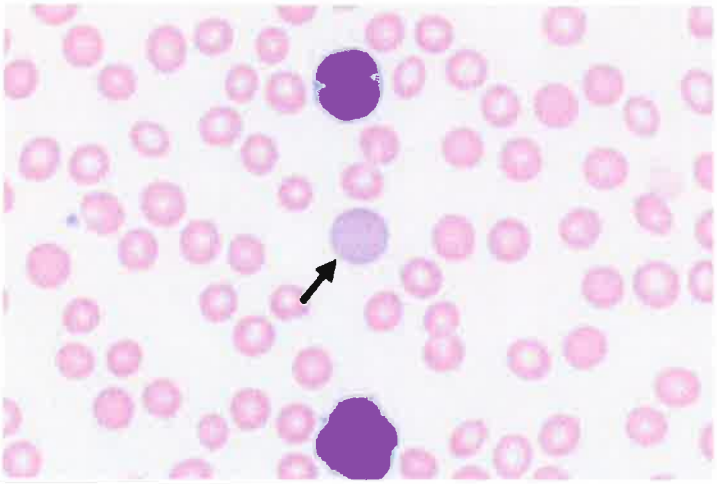
Question 1: C. Father: RhD-positive; Mother: RhD-negative
A father with the *RHD* gene (whose red blood cells express the RhD antigen) may pass this gene, and red blood cell antigen expression, to his fetus. If the mother does not share this antigen, she may produce an antibody response against the fetal red blood cells.

Question 2: C. Thrombocytopenia
HDN may be associated with low platelets (thrombocytopenia) due to platelets being sequestered by an enlarged spleen or consumed in a hemolysis-induced coagulopathy.

Question 3: A. To an RhD-negative woman during pregnancy
Maternal anti-RhD alloantibodies cross the placenta and lyse fetal red blood cells. Rh immunoglobulin (Rh Ig) is administered during pregnancy to RhD-negative women to prevent this.

Cell Identification

VPBS-08

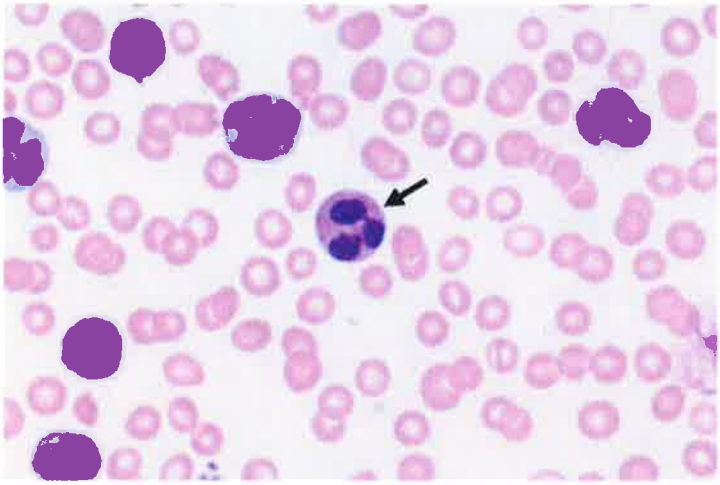


Identification	Participants Labs	Participants %	Evaluation
Polychromatophilic (non-nucleated) red blood cell	1166	97.5	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	15	1.3	Educational
Spherocyte	5	0.4	Educational
Basophilic stippling (coarse)	4	0.3	Educational
Nucleated red blood cell, normal or abnormal morphology	2	0.2	Educational
Acanthocyte (spur cell)	1	0.1	Educational
Blast cell	1	0.1	Educational
Blister cell/Prekeratocyte	1	0.1	Educational
Erythrocyte with overlying platelet	1	0.1	Educational

The arrowed cell is a polychromatophilic (non-nucleated) red blood cell, as correctly identified by 97.5% of participants. Polychromatophilic red blood cells are non-nucleated and correspond to the final stage of red blood cell maturation after exiting the bone marrow. These cells are usually round or oval and are larger than mature erythrocytes. They primarily contain hemoglobin with a small amount of RNA, and thereby stain homogeneously pink-gray or pale purple with Romanowsky or Wright-Giemsa stains. These cells can be stained as reticulocytes and enumerated using supravital stains.

1.3% of participants identified the cell as a macrocyte, oval or round. Macrocytes are abnormally large red blood cells (diameter > 8.5 µm). They are best detected by comparing to other red blood cells in a smear in the context of the MCV. In this case, the MCV was normal. Macrocytes may be oval or round. The hemoglobin concentration is normal; cells lack significant polychromasia (if polychromasia is readily identified, the term polychromatophilic red blood cell is preferred for proficiency testing purposes). As the arrowed cell has overt polychromasia, the choice polychromatophilic (non-nucleated) red blood cell is more appropriate and macrocyte is incorrect.

VPBS-09

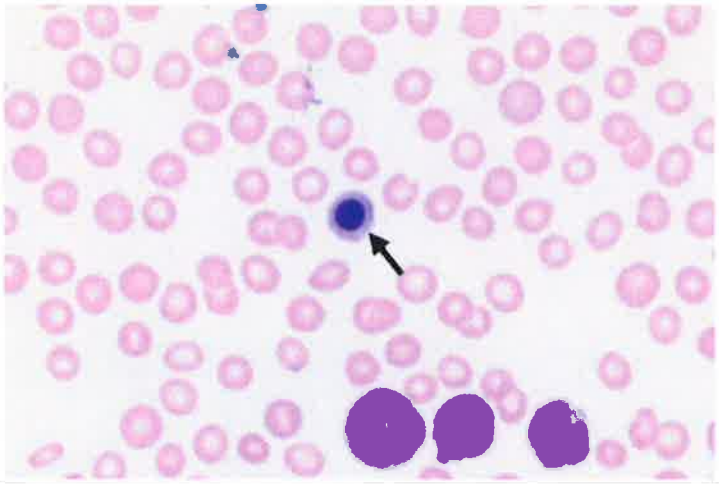


Identification	Participants Labs	Participants %	Evaluation
Neutrophil, segmented or band	1138	95.2	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	19	1.6	Educational
Eosinophil, any stage	14	1.2	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	13	1.1	Educational
Neutrophil with hypersegmented nucleus	5	0.4	Educational
Neutrophil with Pelger-Huët nucleus (acquired or congenital)	4	0.3	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 cell is a segmented neutrophil, as correctly identified by 95.2% of participants. In normal blood smears, segmented neutrophils are the predominant white blood cell. They are round to oval cells, 10 - 15 µm in diameter, with a nuclear-to-cytoplasmic ratio of approximately 1:3. The cytoplasm contains numerous pale pink specific granules. The nucleus is segmented or lobated (two to five lobes normally) and contains condensed chromatin.

1.6% of participants identified the arrowed cell as a neutrophil with dysplastic nucleus. Dysplastic neutrophils are characteristic of myelodysplastic syndromes. Morphologically, the normal synchronous maturation of nucleus and cytoplasm is lost. As a result, in the cytoplasm, the primary and secondary granules are often decreased or absent, causing the cytoplasm to appear pale and bluish. Dysplastic neutrophils often have cytoplasm so pale that cytoplasmic borders cannot be easily distinguished from the slide background. The nucleus may show abnormal lobation accompanied by a mature chromatin pattern. The arrowed neutrophil shows appropriate cytoplasmic granulation and normal nuclear lobation thereby excluding a neutrophil with dysplastic nucleus.

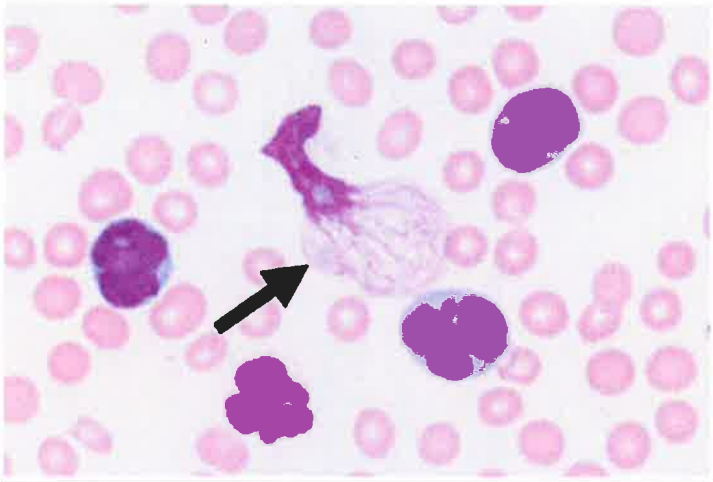
VPBS-10



Identification	Participants Labs	%	Evaluation
Nucleated red blood cell, normal or abnormal morphology	1185	99.1	Educational
Lymphocyte	3	0.3	Educational
Blast cell	2	0.2	Educational
Erythrocyte, normal	2	0.2	Educational
Lymphocyte, large granular	2	0.2	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational

The arrowed cell is a nucleated red blood cell (nRBC), as correctly identified by 99.1% of participants. The term nucleated red blood cell (nRBC) is used to state the presence of normoblasts in the peripheral blood and includes all normoblasts regardless of the stage of maturation. Typically, the circulating nRBC is at the orthochromic stage of differentiation. Orthochromic normoblasts are round or ovoid (8 to 12 um in diameter), with a small, often pyknotic nucleus that sometimes appears as a homogeneous mass of dense chromatin. The cytoplasm usually stains uniformly pinkish orange with little or no basophilia. For purposes of peripheral blood cell identification on CAP proficiency testing, all normal- or abnormal (ie, megaloblastic or dysplastic)-appearing nucleated red blood cells found in the peripheral blood, regardless of maturational stage, are classified as nRBCs.

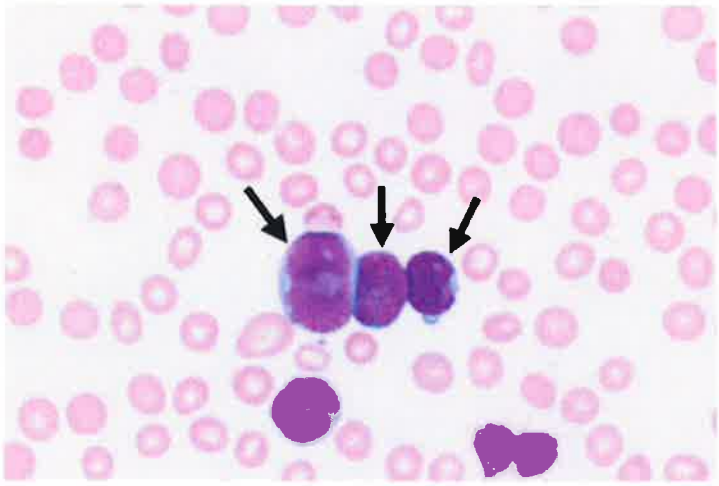
VPBS-11



Identification	Participants Labs	Participants %	Evaluation
Basket cell/smudge cell	1188	99.3	Educational
Stain precipitate	3	0.3	Educational
Malignant lymphoid cell (other than blast)	1	0.1	Educational
Monocyte	1	0.1	Educational
Neutrophil necrobiosis (degenerated neutrophil)	1	0.1	Educational
Squamous epithelial cell/endothelial cell	1	0.1	Educational
Target cell (codocyte)	1	0.1	Educational

The arrowed cell is a basket cell/smudge cell, as correctly identified by 99.3% of participants. Basket/smudge cells are artifacts produced when fragile cells, typically lymphocytes, are subjected to the shearing forces of the peripheral smear production process. The "basket" appearance results when chromatin strands are spread-out from a condensed nuclear remnant. Basket/smudge cells are most commonly encountered in (though not limited to) disorders of increased lymphocyte fragility, such as infectious mononucleosis or chronic lymphocytic leukemia.

VPBS-12



Identification	Participants Labs	Participants %	Evaluation
Blast cell	995	83.2	Educational
Malignant lymphoid cell (other than blast)	139	11.6	Educational
Immature or abnormal cell, would refer for identification	25	2.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	23	1.9	Educational
Monocyte, immature (promonocyte, monoblast)	6	0.5	Educational
Metastatic tumor cell or tumor cell clump	2	0.2	Educational
Neutrophil, myelocyte	2	0.2	Educational
Basket cell/smudge cell	1	0.1	Educational
Monocyte	1	0.1	Educational
Neutrophil, promyelocyte	1	0.1	Educational
Teardrop cell (dacrocyte)	1	0.1	Educational

The arrowed cells are blasts, as correctly identified by 83.2% of participants. Blasts are large, round-to-oval cells with high nuclear-to-cytoplasmic ratio. They often have a round to oval nucleus, sometimes with indentations or folds. Blasts demonstrate characteristically immature chromatin, described as fine, lacy, or reticular. One or more nucleoli may be seen. The cytoplasm may be variably basophilic and typically agranular, although some myeloid blasts may demonstrate cytoplasmic granules or Auer rods. The morphologic features of a blast usually do not permit determination of the cell lineage (ie, myeloblast vs lymphoblast). The one exception is the presence of Auer rods, which is diagnostic of myeloid lineage. The use of other techniques (such as immunophenotypic analysis or cytochemical staining) is required for proper classification.

11.6% of participants identified the arrowed cells as malignant lymphoid cell (other than blast). Lymphoma cells can exhibit a variety of appearances depending on the lymphoma subtype, and definitive diagnosis can be difficult. These cells can exhibit a variety of sizes, shapes, and nuclear and cytoplasmic characteristics. Cell size ranges from 8 to 30 µm and the nuclear to cytoplasmic ratio varies from 7:1 to 3:1. It is critical to obtain an accurate clinical history, since knowledge of a previous diagnosis of lymphoma greatly aids in the identification of these cells. Supplemental studies, such as immunophenotyping, are often necessary to arrive at a diagnosis.

VPBS-12, cont'd.

In this patient with T lymphoblastic leukemia, the more appropriate choice is "blast" for the arrowed cells. While there is variation in size, the history along with the fine chromatin and high N:C ratio make "blast" the best choice. For proficiency testing purposes, malignant lymphoid cell should be chosen if the cell is malignant, of lymphoid origin, and not a blast.

1.9% of participants identified the arrowed cells as lymphocyte, reactive (includes plasmacytoid and immunoblastsic forms). 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 lymphocytes 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) and 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. In this case, the blast cells are too monotonous to be classified as reactive lymphocytes, excluding the choice of lymphocyte, reactive and confirming the choice of blast.

Case Presentation:

This peripheral blood smear is from a 23-year-old man with a bulky mediastinal mass and a history of T-lymphoblastic leukemia. Laboratory data include: WBC = 154.6 x 10E9/L; RBC = 3.14 x 10E12/L; HGB = 9.8 g/dL; HCT = 28.2%; MCV = 92 fL; PLT = 18 x 10E9/L; and RDW = 22%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: T lymphoblastic leukemia/lymphoma

T-ALL/LBL is a neoplasm of lymphoblasts committed to the T-cell lineage. Typically, lymphoblasts are small to medium-sized with scant cytoplasm, moderately condensed to open chromatin, and inconspicuous nucleoli. However, significant variation can be present. At one end of the spectrum are small lymphoblasts with dense but not clumped chromatin, inconspicuous or absent nucleoli, and extremely scant cytoplasm. At the other end are large lymphoblasts with finely dispersed chromatin, variable numbers of distinct nucleoli, and moderate amounts of cytoplasm. Morphologically, the blasts are identical to those seen in B lymphoblastic leukemia/lymphoma (B-ALL/LBL) and are frequently indistinguishable from those of acute myeloid leukemia. The one exception is the presence of Auer rods, which is diagnostic of myeloid lineage. Other techniques (such as immunophenotypic analysis or cytochemical staining) are required for proper classification.

T-ALL/LBL frequently presents with mediastinal involvement (ie, thymic mass) as T-ALL/LBL is derived from thymocytes, the T-cell precursors. Variable degree of involvement of lymph nodes, extranodal sites, and bone marrow/peripheral blood also occurs. By convention, T-LBL is the appropriate term when the neoplasm is confined to a mass lesion with no or minimal peripheral blood and/or bone marrow involvement. However, T-ALL is the appropriate designation if there is extensive peripheral blood and/or bone marrow involvement. When the neoplasm involves both a tissue mass and the bone marrow or peripheral blood, the distinction between leukemia and lymphoma is defined by the degree of bone marrow involvement. In these instances, a case is classified as T-ALL if there is more than 25% bone marrow involvement.

T-ALL/LBL is significantly less common than B-ALL, as it comprises approximately 15% of childhood lymphoblastic leukemias and 20 - 25% of adult cases. However, it constitutes 90% of lymphoblastic lymphoma cases. T-ALL typically presents with a high leukocyte count and often with a large mediastinal mass or other tissue mass. T-LBL frequently presents with a mass in the anterior mediastinum, which often shows rapid growth and may present as a respiratory emergency.

Question 1. Which of the following is true of T lymphoblastic leukemia/lymphoma?

A. Morphologic characteristics are usually sufficient to classify a case as T lymphoblastic leukemia/lymphoma
B. T lymphoblastic leukemia is more common than B lymphoblastic leukemia
C. T lymphoblastic lymphoma frequently presents with an anterior mediastinal mass
D. T lymphoblastic lymphoma more commonly presents with isolated bone marrow involvement

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Immunophenotype of T lymphoblastic leukemia/lymphoma

Since T-ALL/LBL is a T-cell neoplasm, lymphoblasts express CD3 (either surface or cytoplasmic) and other pan-T-cell markers such as CD2, CD5, and CD7. Only CD3 is considered lineage-specific. In addition, the lymphoblasts often express TdT and variably express CD99, CD34, and/or CD1a - markers that help show the precursor nature of T-lymphoblasts. CD4 and CD8 may be co-expressed, singly expressed, or absent, recapitulating thymocyte maturation. Non-lineage defining myeloid markers, such as CD13 and CD33, can be positive in a subset of cases, specifically in the cases now classified as early T-cell precursor lymphoblastic leukemia (ETP-ALL). ETP-ALL is now a provisional category under the current 2016 WHO classification, which is characterized by T-lymphoblasts with a unique immunophenotype and genetic makeup indicating early T-cell differentiation. By definition, the blasts in this entity express CD7 and one or more myeloid/stem cell markers, including CD34, CD117, HLA-DR, CD13, CD33, CD11b, or CD65 and are negative for CD1a and CD8.

Genetics of T lymphoblastic leukemia/lymphoma

T-ALL/LBL shows clonal rearrangements of the T-cell receptor (*TCR*) genes in most cases. Simultaneous *IGH* rearrangements (typical of B-cell neoplasms) are seen in a subset of patients. An abnormal karyotype is seen in approximately 50 - 70% of cases. The most common genetic abnormalities involve the *TCR* gene, with a variety of partner genes. About 50% of cases show mutations of the *NOTCH1* gene, which encodes a necessary protein for early T-cell development. *NOTCH1* mutations are uncommon in early T-cell precursor lymphoblastic leukemia, whereas myeloid-associated gene mutations, including *FLT3*, are frequent.

Prognosis of T lymphoblastic leukemia/lymphoma

T-ALL/LBL is an aggressive disease that is treated like other types of ALL. T-ALL/LBL is considered to have a poorer prognosis than the more common B-ALL/LBL. Compared to B-ALL/LBL patients, T-ALL/LBL patients have an increased risk for induction failure, early relapse, and isolated central nervous system relapse. The presence of minimal residual disease following therapy is an adverse prognostic factor. The prognostic significance of ETP-ALL is currently controversial.

Question 2. Which of the following is true regarding the prognosis of T lymphoblastic leukemia/lymphoma?

A. B lymphoblastic leukemia more frequently presents with early relapse compared to T lymphoblastic leukemia.
B. Minimal residual disease testing has no utility in T lymphoblastic leukemia.
C. The prognosis is poorer than B lymphoblastic leukemia.
D. T lymphoblastic leukemia is an indolent disorder that does not often require therapy.

Catalina Amador, MD
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Hematology and Clinical Microscopy Committee

REFERENCES:

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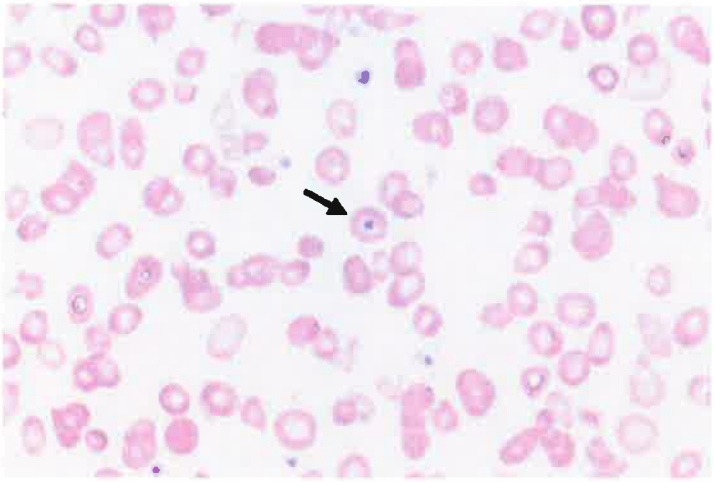
ANSWERS TO QUESTIONS:

Question 1: C. T lymphoblastic lymphoma frequently presents with an anterior mediastinal mass
T-LBL frequently presents with a mass in the anterior mediastinum, which often shows rapid growth and may present as a respiratory emergency. By definition, the diagnosis of T lymphoblastic lymphoma requires a mass lesion to be present.

Question 2: C. The prognosis is worse than B lymphoblastic leukemia
T-ALL/LBL is considered to have a worse prognosis than B-ALL/LBL. Compared to B-ALL/LBL patients, T-ALL/LBL patients have an increased risk for induction failure, early relapse, and isolated central nervous system relapse.

Cell Identification

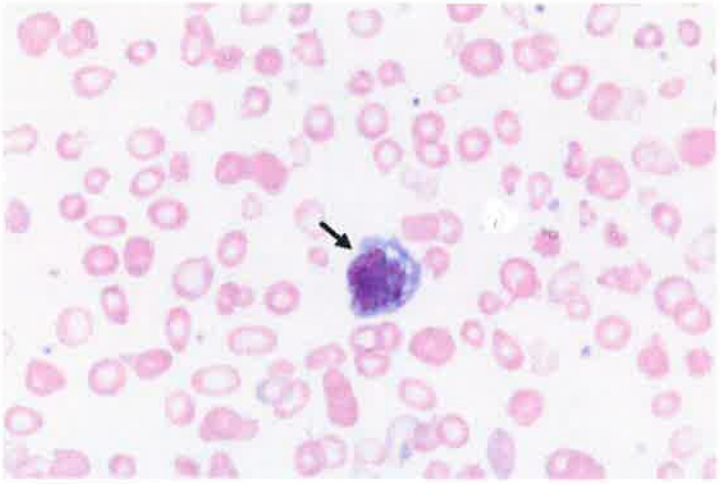
VPBS-14



Identification	Participants Labs	%	Evaluation
Erythrocyte with overlying platelet	1179	98.6	Educational
Platelet, normal	8	0.7	Educational
Erythrocyte, normal	4	0.3	Educational
Howell-Jolly body	2	0.2	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	2	0.2	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational

The arrowed cell is an erythrocyte with overlying platelet, as correctly identified by 98.6% of participants. In preparing a peripheral blood smear, platelets may adhere to or overlap red blood cells, suggesting a red blood cell inclusion or parasite. A correct interpretation depends on carefully examining the morphology of the platelet and comparing the size, staining characteristics, and granularity with known platelets in the same field as well as determining if the platelet is in the same plane of focus as the red blood cell. Many times, the platelet is surrounded by a thin clear zone or halo (as noted in this arrowed cell), which is not a feature of most genuine red blood cell inclusions.

VPBS-15



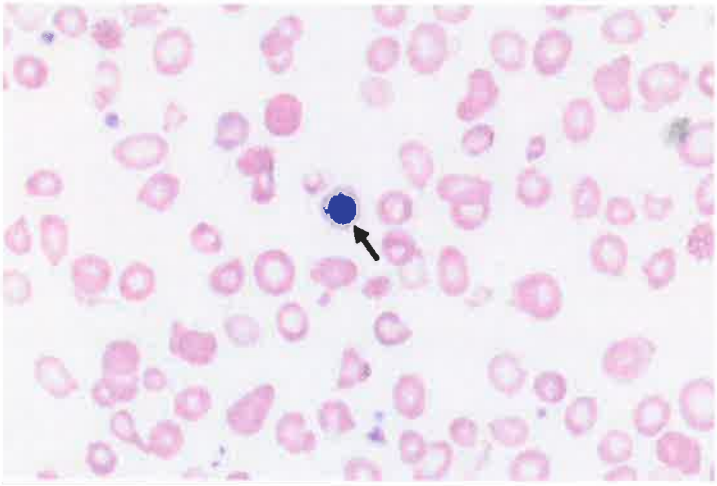
Identification	Participants Labs	Participants %	Evaluation
Monocyte	1110	92.8	Educational
Monocyte, immature (promonocyte, monoblast)	47	3.9	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	10	0.8	Educational
Immature or abnormal cell, would refer for identification	8	0.7	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	4	0.3	Educational
Platelet, giant (macrothrombocyte)	4	0.3	Educational
Lymphocyte, large granular	3	0.3	Educational
Lymphocyte	2	0.2	Educational
Blast cell	1	0.1	Educational
Malignant lymphoid cell (other than blast)	1	0.1	Educational
Neutrophil, metamyelocyte	1	0.1	Educational
Neutrophil, myelocyte	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
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	1	0.1	Educational

The arrowed cell is a monocyte, as correctly identified by 92.8% of participants. Monocytes are slightly larger than neutrophils, ranging from 12 to 20 µm in diameter. Most 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.

VPBS-15, cont'd.

3.9% of participants identified this arrowed cell as a monocyte, immature (promonocyte, monoblast). 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. The malignant monoblast is a large cell, usually 15 to 25 µm in diameter. It has relatively more cytoplasm than a myeloblast with the nuclear to cytoplasmic ratio ranging from 7:1 to 3:1. The monoblast nucleus is round or oval and has finely dispersed chromatin and distinct nucleoli. The cytoplasm is blue to gray-blue and may contain small, scattered azurophilic granules. Some monoblasts cannot be distinguished morphologically from other blast forms; in these instances, additional tests (eg, cytochemistry and/or flow cytometry) are required to accurately assign blast lineage. Promonocytes have nuclear and cytoplasmic characteristics that are between those of monoblasts and mature monocytes. They are generally larger than mature monocytes, but they have similar-appearing gray-blue cytoplasm that often contains uniformly distributed, fine azurophilic granules. Cytoplasmic vacuolization is not a typical feature. The nuclei show varying degrees of lobulation, usually characterized by delicate folding or creasing of the nuclear membrane, in contrast to the rounder nuclear profile of monoblasts. Nucleoli are present but may not be as distinct as in monoblasts. The mature chromatin pattern in the arrowed cell excludes a promonocyte or monoblast and instead confirms its "non-blast" identity.

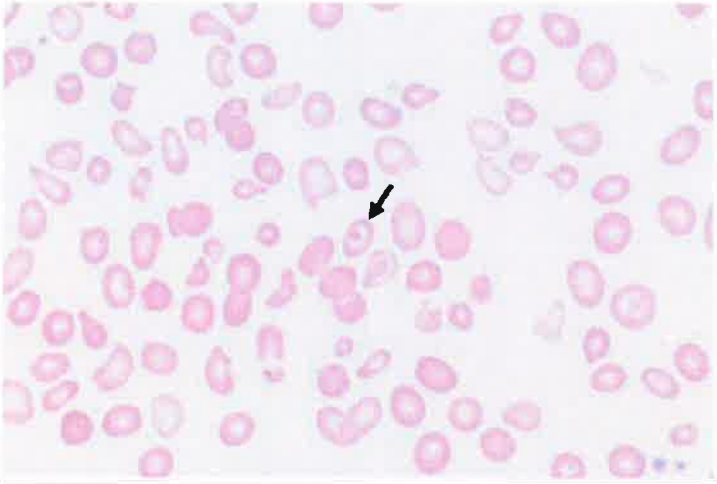
VPBS-16



Identification	Participants Labs	%	Evaluation
Nucleated red blood cell, normal or abnormal morphology	1180	98.7	Educational
Lymphocyte	4	0.3	Educational
Polychromatophilic (non-nucleated) red blood cell	3	0.3	Educational
Blast cell	2	0.2	Educational
Neutrophil necrobiosis (degenerated neutrophil)	2	0.2	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	2	0.2	Educational
Howell-Jolly body	1	0.1	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	1	0.1	Educational
Pappenheimer bodies (iron or Wright stain)	1	0.1	Educational

The arrowed cell is a nucleated red blood cell (nRBC), normal or abnormal morphology, as correctly identified by 98.7% of participants. The term nucleated red blood cell is used to state the presence of normoblasts in the peripheral blood and includes all normoblasts regardless of the stage of maturation. Typically, the nRBC is at the orthochromic stage of differentiation. Caution should be used in classifying a circulating nucleated red blood cell as dysplastic on the basis of abnormal nuclear shape (lobated or fragmented), as these changes may occur during their egress from the marrow space and may not be present in the maturing erythroid precursors present in the 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, be it normal or abnormal (ie, exhibits megaloblastic or dysplastic changes).

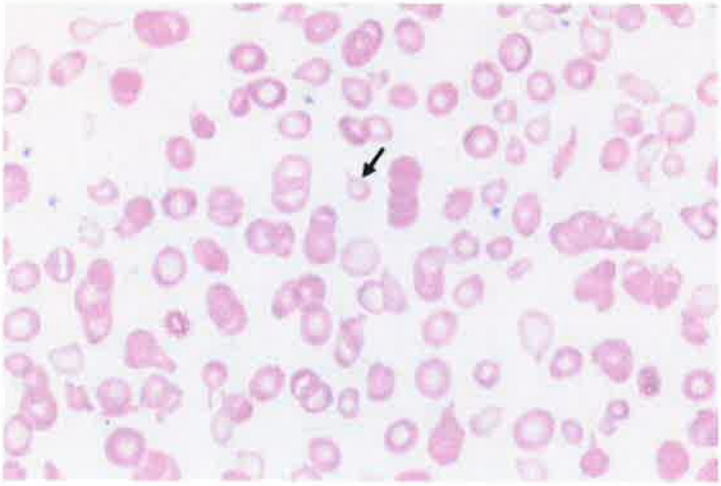
VPBS-17



Identification	Participants Labs	Participants %	Evaluation
Target cell (codocyte)	1192	99.7	Educational
Basket cell/smudge cell	1	0.1	Educational
Erythrocyte with overlying platelet	1	0.1	Educational
Spherocyte	1	0.1	Educational
Stomatocyte	1	0.1	Educational

The arrowed cell is a target cell (codocyte), as correctly identified by 99.7% of participants. Target cells, also known as codocytes, are thin red blood cells with an increased surface membrane-to-volume ratio. They are often flattened out on the smears and may appear macrocytic. Target cells are believed to arise from disturbances in red blood cell membrane cholesterol and lecithin content or decreased cytoplasmic hemoglobin content. Target cells are characterized by a central hemoglobinized area within the surrounding area of pallor, which in turn is surrounded by a peripheral hemoglobinized zone giving target cells the appearance of a bullseye. Target cells associated with hemoglobin C may have a slightly reduced or normal MCV, whereas those associated with hemoglobin E disorders or hemoglobin H disease exhibit microcytosis of varying degree. Target cells are usually seen in thalassemias (as in our case), iron deficiency anemia, following splenectomy, and in patients who are jaundiced or who have chronic liver disease; in the latter two conditions, the MCV may be normal or increased. Target cells may also appear as artifacts from slowly drying the slides in a humid environment or from specimens anticoagulated with excessive EDTA. The drying artifact results in the presence of numerous target cells in some fields, but none or few in other fields.

VPBS-18



Identification	Participants Labs	Participants %	Evaluation
Teardrop cell (dacrocyte)	1191	99.6	Educational
Acanthocyte (spur cell)	1	0.1	Educational
Blast cell	1	0.1	Educational
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	1	0.1	Educational
Ovalocyte (elliptocyte)	1	0.1	Educational
Pappenheimer bodies (iron or Wright stain)	1	0.1	Educational

The arrowed cell is a tear drop cell (dacrocyte), as correctly identified by 99.6% 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 or dacrocytes. These are commonly seen in patients with bone marrow fibrosis, but may also be seen in pernicious anemia, anemia of renal disease, hemolytic anemias (as in our case), and other forms of severe anemia. These cells are often associated with an abnormal spleen or bone marrow. Bone marrow infiltration with hematologic and non-hematologic malignancies may also be accompanied by dacrocytosis. Also, teardrop cells may be an artifact of slide preparation; such dacrocytes are usually easily recognized because their "tails" all point in the same direction.

Case Presentation:

This peripheral blood smear is from a 46-year-old woman with a history of beta-thalassemia. Laboratory data include: WBC = 4.1 x 10E9/L; RBC = 4.20 x 10E12/L; HGB = 9.4 g/dL; HCT = 22.0%; MCV = 75 fL; PLT = 149 x 10E9/L; and RDW = 33%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Beta thalassemia

Thalassemia in general

Thalassemia is derived from "Thalassa" and "Haema", Greek words for sea and blood, respectively. Thalassemias, which are among the most common monogenetic disorders worldwide, are disorders due to defective synthesis of alpha or beta globin subunits of hemoglobin A, which is made up of two alpha and two beta subunits. These anemias are inherited due to mutations in these corresponding genes on chromosomes 16 (alpha) and 11 (beta). Many mutations are described; therefore, the phenotype or clinical findings are diverse. However, the mutations result in decreased hemoglobin production, decreased red blood cell survival, and excess amounts of unaffected globin chains. This decreased survival and ineffective erythropoiesis results in the need for increased erythropoiesis as evidenced by distortion of bones in the cranium and other sites. Moreover, extramedullary hematopoiesis may occur. The mutations are generally divided into two major groups, those that produce no globin (ie, alpha^0 or beta^0) and those that produce reduced globin (ie, alpha$^+$ or beta$^+$). As decreased or no affected globins are produced and an excess of unaffected globins are made, increased amounts of other hemoglobins may be noted, such as increased hemoglobin A2 (comprised of two alpha globins and two delta globins) or fetal hemoglobin (HbF; comprised of two alpha globins and two gamma globins). As heterozygosity for thalassemia results in some resistance to malaria infection, there is an increased incidence in certain areas of the world prone to malaria such as the Mediterranean, Middle East, Africa, and Asia.

Question 1. Which statement below concerning thalassemia is CORRECT?

A. Thalassemia is commonly due to mutations in the delta and gamma globins.
B. Thalassemia is a rare genetic disorder seen only in Africa.
C. Thalassemia provides no survival advantage against malaria.
D. Thalassemia may result in extramedullary hematopoiesis due to decreased RBC survival.

Beta thalassemia in general

Beta thalassemia is due to decreased production of the beta globin chain and is a result of more than 200 point mutations, deletions, or insertions and (rarely) gross deletions in beta globin gene. Again, given these variable mutations, the clinical features are heterogeneous. Patients with beta thalassemia trait carry one beta globin gene mutation resulting in mild or no anemia and are therefore asymptomatic. They have variable microcytosis with an MCV ranging from 60 fL to normal. Beta thalassemia intermedia is due to two beta globin gene mutations where at least one mutation results in only partial reduction of the beta globin. These patients will have mild to moderate anemia without substantial transfusion requirements. They have variable splenomegaly and bone deformities due to increased erythropoiesis demands. Based on the quantity of beta globin production, they may be asymptomatic or severely symptomatic. Finally, thalassemia major, also known as Cooley anemia, is due to two beta globin genes carrying significant mutations with marked reduction in beta globin production. These patients have severe transfusion dependent anemia, splenomegaly, and bone deformities. Due to their transfusion needs, they may develop iron overload. Beta thalassemia may be inherited with other disorders such as sickle cell trait, hemoglobin E, hereditary persistence of fetal hemoglobin, and alpha thalassemia, which will alter the clinical and laboratory findings.

Question 2. Which statement is CORRECT?

A. Beta thalassemia is typically due to large deletions in the beta globin gene.
B. Beta thalassemia trait is due to one deletion resulting in significant reduction in beta globin production and moderate microcytic anemia.
C. Beta thalassemia major may be associated with iron overload due to significant transfusion needs.
D. Beta thalassemia intermedia is asymptomatic without splenomegaly, anemia, or bone deformities.

Beta thalassemia laboratory findings

As already mentioned, the degree of anemia and microcytosis will vary in beta thalassemia based on mutation and other clinical features. The finding of microcytosis and anemia raise the differential of iron deficiency, which is a more common disorder than thalassemia. Typical laboratory findings are expected, however, which can help distinguish between these two. First, decreased hemoglobin with normal to increased red blood cell count is common in beta thalassemia, whereas the red cell count is frequently reduced in iron deficiency. Microcytosis is frequent and target cells may be seen due to decreased hemoglobin within red blood cells in beta thalassemia. Both microcytosis and target cells can be noted in iron deficiency, but the RDW is frequently increased in iron deficiency (but normal in thalassemia) due to more pronounced anisocytosis associated with iron deficiency.

Hemoglobin electrophoresis and high-performance liquid chromatography (HPLC) will reveal an increase in hemoglobin A2 in patients with beta thalassemia due to decreased beta globin production resulting in a compensatory increase in delta globin. This will not be seen in iron deficiency. Other findings may also be seen on HPLC or electrophoresis such as increased HbF in beta thalassemia; moreover, other disorders may be detected such as concordant sickle cell trait, etc. It is important to note that iron deficiency may suppress hemoglobin A2 concentration, confounding results. Therefore, in a patient with suspected beta thalassemia and/or iron deficiency, repeat HPLC may be needed after iron is replaced if microcytosis persists to confirm the diagnosis of beta thalassemia.

Question 3. Which of the below would be MORE TYPICAL of beta thalassemia than iron deficiency?

A. Elevated RDW
B. Decreased red blood cell count
C. Elevated hemoglobin A2
D. Normocytic anemia

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Hematology and Clinical Microscopy Committee

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2. Rund D, Rachmilewitz E. Beta-thalassemia. *N Engl J Med.* 2005;353(11):1135-1146.
3. Hoyer JD, Kroft SH, eds. Color Atlas of Hemoglobin Disorders: A Compendium Based on Proficiency Testing. College of American Pathologists. 2003.

ANSWERS TO QUESTIONS:

Question 1: D. Thalassemia may result in extramedullary hematopoiesis due to decreased RBC survival. Thalassemia is due to mutations in beta and alpha globins. It is not a rare disorder and is seen in many places especially those with high incidence of malaria as it provides a survival advantage. Thalassemia can be associated with extramedullary hematopoiesis due to decreased red blood cell survival.

Question 2: C. Beta thalassemia major may be associated with iron overload due to significant transfusion needs. Beta thalassemia is frequently due to point mutations, and large deletions are rare. Beta thalassemia trait results in no or mild anemia. Beta thalassemia intermedia has variable phenotype associated with variable splenomegaly, anemia, or bone deformities. Cooley anemia, or beta thalassemia major, is a transfusion dependent hemolytic anemia which may result in iron overload.

Question 3: C. Elevated hemoglobin A2. Beta thalassemia and iron deficiency both can result in microcytic anemia. However, iron deficiency typically has lower red blood cell count and higher RDW. Finally, iron deficiency will decrease hemoglobin A2 whereas beta thalassemia will increase it.

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Participant Summary/Final Critique report and can self-report this activity towards fulfilling education and maintenance of certification (MOC) requirements.

Participant	Date	Participant	Date
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_____	_____	_____	_____
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_____ _____

Director (or Designee) Signature - I have verified that the individuals listed above have successfully participated in this activity. Date

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