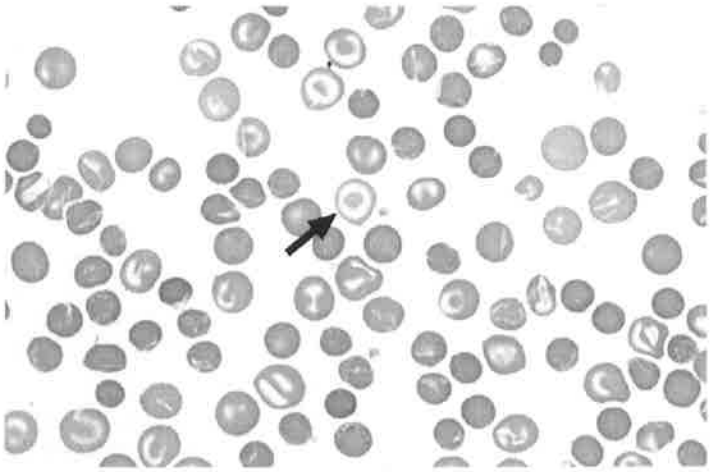
Cell Identification

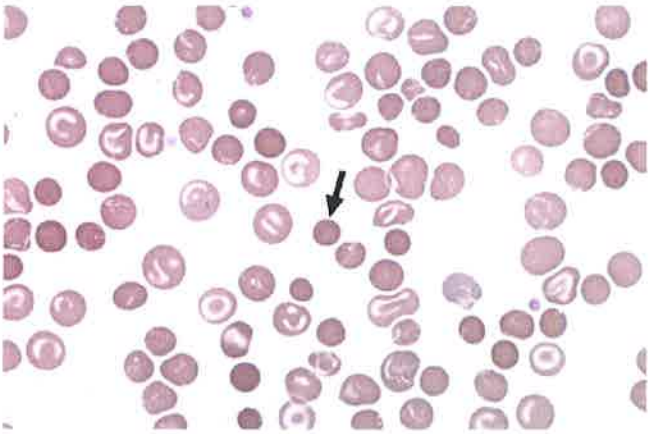
VPBS-02



Identification	Participants No.	Participants %	Evaluation
Target cell (codocyte)	1134	99.8	Educational
Neutrophil, promyelocyte, abnormal with/without Auer rod(s)	1	0.1	Educational
Stomatocyte	1	0.1	Educational

The arrowed cell is a target cell (codocyte), as correctly identified by 99.8% of participants. Target cells are red blood cells with central and peripheral hemoglobinized regions separated by a ring-shaped region of pallor. Often flattened out on peripheral smears, target cells may have a greater diameter than neighboring red blood cells. Target cells feature an increased surface membrane-to-volume ratio, and they are thought to result from disturbances in cell membrane cholesterol and lecithin content or from decreased cellular hemoglobin content. The presence of numerous target cells should prompt consideration of thalassemia, hemoglobinopathy, chronic liver disease, or the post-splenectomy state. While the mean corpuscular volume (MCV) is frequently increased in liver disease, target cells associated with hemoglobin C may be normal to slightly decreased in size, and those seen in association with hemoglobin E may be more conspicuously microcytic. Target cells may also arise as an artifact of slow drying of smears in humid conditions, and drying artifact usually result in the target cells being unevenly distributed across the smear.

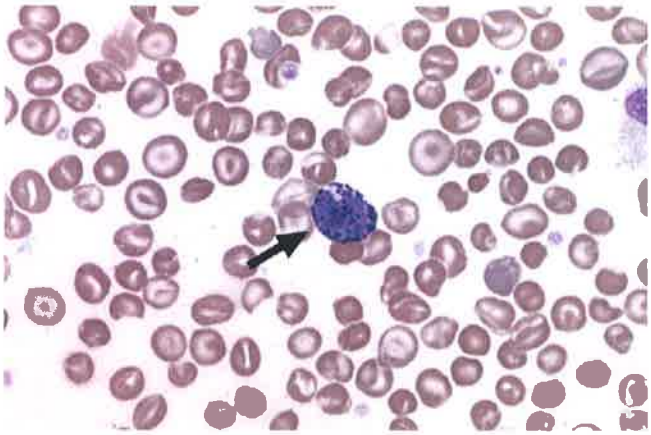
VPBS-03



Identification	Participants No.	%	Evaluation
Spherocyte	1116	98.2	Educational
Microcyte (with increased central pallor)	16	1.4	Educational
Acanthocyte (spur cell)	2	0.2	Educational
Erythrocyte, normal	1	0.1	Educational
Sickle cell (drepanocyte)	1	0.1	Educational

The arrowed cell is a spherocyte, as correctly identified by 98.2% of participants. Spherocytes are densely staining red blood cells that have smooth, round outlines, lack central pallor, and feature a decreased surface membrane-to-volume ratio. Loss of the biconcave architecture of normal red blood cells, with an associated increase in central thickness, results in their densely stained appearance. The finding of spherocytes raises the possibilities of hereditary spherocytosis and hemolytic anemia (microangiopathic or immune-mediated).

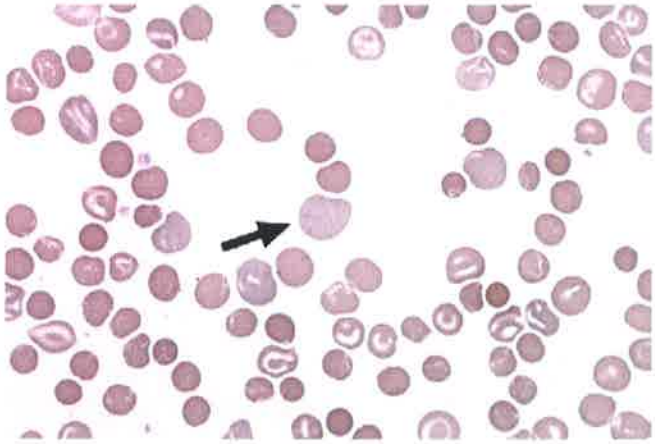
VPBS-04



Identification	Participants No.	%	Evaluation
Basophil, any stage	1129	99.4	Educational
Basophilic stippling (coarse)	3	0.3	Educational
Eosinophil, any stage	2	0.2	Educational
Basket cell/smudge cell	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 basophil, as correctly identified by 99.4% of participants. Basophils are comparable in size to neutrophils, but their distinctive blue-black cytoplasmic granules are not only densely staining but also coarser and larger than neutrophils' granules. The granules are frequently unevenly distributed within the cytoplasm, and they often obscure the nucleus. Basophilia may be encountered in the setting of myeloproliferative neoplasms or other hematologic malignancies as well as in association with hypothyroidism and hypersensitivity reactions.

VPBS-05

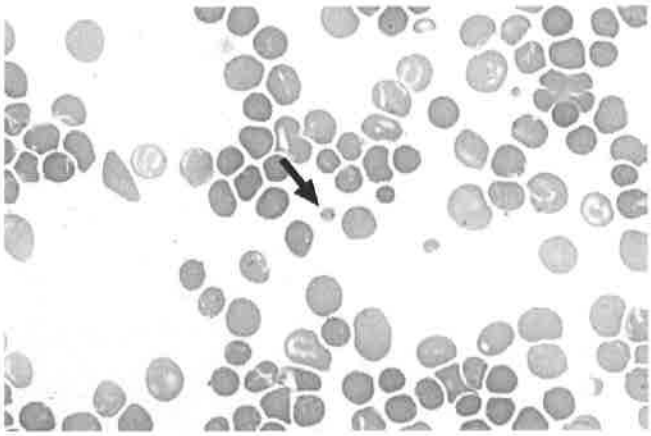


Identification	Participants No.	%	Evaluation
Polychromatophilic (non-nucleated) red blood cell	955	84.1	Educational
Macrocyte, oval round (excluding polychromatophilic red blood cell)	112	9.9	Educational
Pappenheimer bodies (iron or Wright stain)	42	3.7	Educational
Howell-Jolly body	10	0.9	Educational
Basophilic stippling (coarse)	4	0.3	Educational
Bite cell (degmacyte)	3	0.3	Eduational
Stain precipitate	2	0.2	Educational
Basket cell/smudge cell	1	0.1	Educational
Echinocyte (burr cell, crenated cell)	1	0.1	Educational
Erythrocyte , normal	1	0.1	Educational
Hypochromasia	1	0.1	Educational
Nucleated red blood cell, normal or abnormal morphology	1	0.1	Educational
Ovalocyte (elliptocyte)	1	0.1	Educational
Plasmodium spp. (malaria)	1	0.1	Educational
Teardrop cell (dacrocyte)	1	0.1	Educational

The arrowed cell is a polychromatophilic (non-nucleated) red blood cell, as correctly identified by 84.1% 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, which are round or ovoid and are larger than mature red blood cells, may stain as reticulocytes using new methylene blue or other supravital stains. The RNA present in polychromatophilic red blood cells lends the pink-gray or pale purple color seen upon staining with Romanowsky or Wright-Giemsa stains. Polychromasia should alert one to the possibility of increased red blood cell destruction for which the bone marrow is compensating with accelerated red blood cell production.

The arrowed cell was identified by 9.9% of participants as a macrocyte, oval/round (excluding polychromatophilic red blood cell). Macrocytes are abnormally large red blood cells (diameter > 8.5 µm). They may be oval or round. Importantly, the hemoglobin concentration in macrocytes is normal. If, as in the present case, polychromasia is observed, then the term polychromatophilic red blood cell is preferred for proficiency testing purposes.

VPBS-06



Identification	Participants No.	%	Evaluation
Platelet, normal	1110	97.7	Educational
Platelet, giant (macrothrombocyte)	25	2.2	Educational
Platelet, hypogranular	1	0.1	Educational

The arrowed object is a platelet, as correctly identified by 97.7% of participants. Platelets, also known as thrombocytes, originate as fragments of megakaryocytic cytoplasm. Most platelets measure 1.5 - 3 µm in diameter, though a few small (< 1.5 µm) and large (4 - 7 µm) platelets may also be seen in normal blood smears. Platelets typically appear blue-gray with fine purple-red granules, and while they vary in shape, most normal platelets are round or slightly elliptical.

Clinical Presentation:

This peripheral blood smear is from a 46-year-old African American man presenting with severe leg pain. Laboratory data include: WBC = 14.0 x 10E9/L; RBC = 4.02 x 10E12/L; HGB = 12.0 g/dL; HCT = 36.5%; PLT = 109 x 10E9/L; and MCV = 68 fL. Hemoglobin electrophoresis shows homozygous Hb C.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Hemoglobin C (HbC) disease

The CBC data in this case indicate a mild microcytic anemia in the context of mild leukocytosis and a mildly decreased platelet count. Target cells and spherocytes are numerous, there is polychromasia, and irregularly contracted red blood cells are also present. Hemoglobin electrophoresis was performed to address the possibility of a hemoglobinopathy or thalassemia underlying the peripheral blood findings, and the results indicated homozygosity for hemoglobin C (HbC).

HbC is a variant hemoglobin resulting from substitution of lysine for glutamic acid at position 6 in the beta globin chain. This position is the same one at which the substitution of valine for glutamic acid yields HbS. HbC is one of the most common variant hemoglobins worldwide. Numerous studies suggest a protective effect of HbC against severe forms of malaria, and an evolutionary advantage for people with HbC in malaria-endemic regions fits with the global distribution of HbC. The proportion of people with HbC trait is 20 - 40% in some West African countries, and the prevalence of HbC trait among African-Americans is estimated at 2 - 3%. Approximately 1 in 5000 African-Americans has HbC disease.

Red blood cells from patients with HbC disease exhibit higher viscosity that normal cells, and decreased deformability may result in a greater propensity for fragmentation and subsequent formation of spherocytes. HbC itself is relatively insoluble within red blood cells in comparison to HbA, and some investigators have attributed this to electrostatic interactions between the positive charges on variant HbC beta globin chains and negative charges on the adjacent alpha globin chains.

Laboratory findings in HbC disease typically include mild anemia, and the MCV is often moderately to markedly decreased. While not observed in the present case, an MCHC near the upper end of the reference range may be observed in HbC disease, and this finding is attributed to the propensity for dehydration of red blood cells in affected individuals. The reticulocyte count is often mildly increased.

Peripheral smear findings typically include target cells and irregularly contracted cells, along with polychromasia and variable numbers of spherocytes. Irregularly contracted cells are dense, hyperchromic cells featuring irregular outlines that contrast with the smooth round outlines of spherocytes. These cells lack the sharp edges and pointed projections typical of fragmented red blood cells (schistocytes). While nucleated red blood cells are not conspicuous in the present case, their presence is common in the peripheral blood of people with HbC disease. Hemoglobin C crystals, which are densely staining and elongate, are variably detectable.

Question 1. Which of the following peripheral smear findings is MOST closely associated with HbC disease?

A. Acanthocytes
B. Irregularly contracted cells
C. Schistocytes
D. Sickle cells (drepanocytes)

People who are heterozygous for HbC, ie, those who have HbC trait, typically have no clinically evident effects. Nonetheless, awareness of this carrier state is important for affected individuals with respect to reproductive decision-making, especially because of the possibility of HbSC disease (see below).

HbC disease is may be clinically silent, though a chronic mild hemolytic anemia is typical. Mild to moderate splenomegaly is common. Cholelithiasis with pigmented gallstones may result from persistent hemolytic anemia. While painful vaso-occlusive crises are much less common in HbC disease than in sickle cell disease, they have been reported in HbC disease and in other non-sickling hemoglobinopathies.

Question 2. Which of the following is MOST frequently associated with HbC disease?

A. Cholelithiasis
B. Decreased reticulocyte count
C. Macrocytosis
D. Painful vaso-occlusive crises

Newborn screening for variant hemoglobins, typically by isoelectric focusing (IEF) or high-performance liquid chromatography (HPLC), is a common means by which a diagnosis of HbC disease is now made in the United States. Asymptomatic individuals born prior to the initiation of such programs may come to medical attention upon review of a peripheral blood smear, at which point hemoglobin electrophoresis or HPLC for variant hemoglobin identification is typically performed. In people with HbC trait, the quantity of HbA slightly exceeds that of HbC. In HbC disease, HbC accounts for nearly all of the hemoglobin, though the HbF percentage is often mildly elevated.

Major differential diagnosis considerations include hemolytic anemias of other types, including sickle cell disease (HbS disease), HbSC disease and HbC-beta thalassemia. Microangiopathic hemolytic anemia is frequently associated with the presence of increased numbers of fragmented red blood cells (schistocytes) that are small, lack central pallor, and in contrast to the contracted cells seen in HbC disease feature pointed projections; target cells are not a common finding. Sickle cell disease, which results from homozygosity for HbS, is characterized by a moderate to marked chronic hemolytic anemia more severe than that of HbC disease along with distinctive clinical features (eg, painful vaso-occlusive crises) and peripheral blood smear findings (sickle cells resulting from HbS polymerization occurring in deoxygenated states). As the geographic distributions of HbS and HbC overlap, and HbS is considerably more common than HbC, compound HbS/HbC heterozygosity (HbSC disease) is more common than HbC disease. Because HbC is associated with red blood cell dehydration, the intracellular HbS concentration is increased, predisposing to sickling of red blood cells in people with HbSC. The clinical symptoms in HbSC disease are generally milder than in sickle cell disease, as is the degree of anemia, though there is increased risk of retinopathy in patients with HbSC disease in comparison to sickle cell disease. Compound heterozygosity for HbC and beta-thalassemia results in a degree of anemia corresponding to the severity of the thalassemia; a mild beta-thalassemia coinherited with HbC may yield anemia similar in degree to that of HbC disease.

Question 3. Which of the following conditions is typically associated with the MOST severe degree of anemia?

A. HbC disease (homozygosity for HbC)
B. HbS disease (homozygosity for HbS)
C. HbC/beta thalassemia (compound heterozygosity)
D. HbSC disease (compound heterozygosity)

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Hematology and Clinical Microscopy Resource Committee

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ANSWERS TO QUESTIONS:

Question 1: B. Irregularly contracted cells

Irregularly contracted cells are densely staining, hyperchromic cells featuring irregular outlines. While acanthocytes are likewise densely stained, they feature multiple thorn-like spicules and are classically associated with hereditary abetalipoproteinemia, not with HbC disease. Irregularly contracted cells lack the triangular shapes, pointed projections and/or irregular outlines typical of schistocytes, which result from intravascular fragmentation. Sickle cells that are crescentic with pointed ends are seen in sickle cell anemia (HbS disease) and in compound HbS/HbC heterozygosity (HbSC disease) but not in HbC disease.

Question 2: A. Cholelithiasis

Cholelithiasis with pigmented gallstones may result from chronic hemolytic anemia such as that which is encountered in HbC disease. HbC disease is often characterized by a mildly elevated reticulocyte count and by microcytosis that may be marked. While painful vaso-occlusive crises may occur in patients with non-sickling hemoglobinopathies, they are most frequently associated with sickle cell disease.

Question 3: B. HbS disease (homozygosity for HbS)

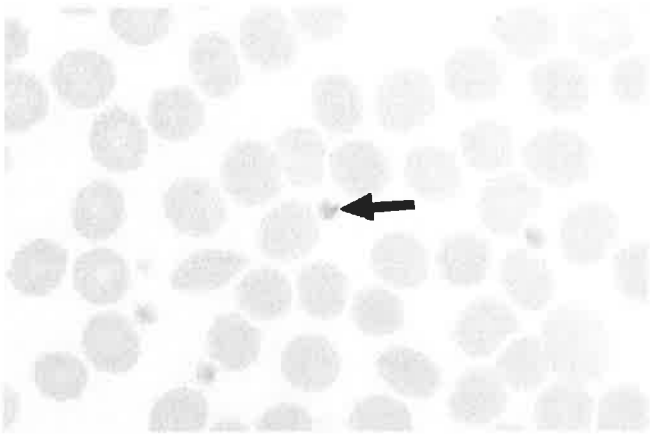
HbS disease (sickle cell disease) is typically associated with moderate to marked hemolytic anemia. While HbSC disease is also a sickling condition, the degree of anemia is typically milder than that of sickle cell disease. HbC disease is typically associated with a mild chronic hemolytic anemia. The degree of anemia encountered in HbC/beta-thalassemia corresponds to the severity of the thalassemic component and may be no more severe than that seen in HbC disease.

Committee Comments on Peripheral Blood Smear Whole Slide Image

The CBC data are indicative of a mild leukocytosis with unremarkable red blood cell indices and normal platelet count. Morphologic examination shows that the leukocytosis consists of numerous mature lymphocytes showing normal morphologic features with fewer neutrophils and monocytes. Occasional eosinophils and basophils are also present. Red blood cells and platelets are within normal limits.

Cell Identification

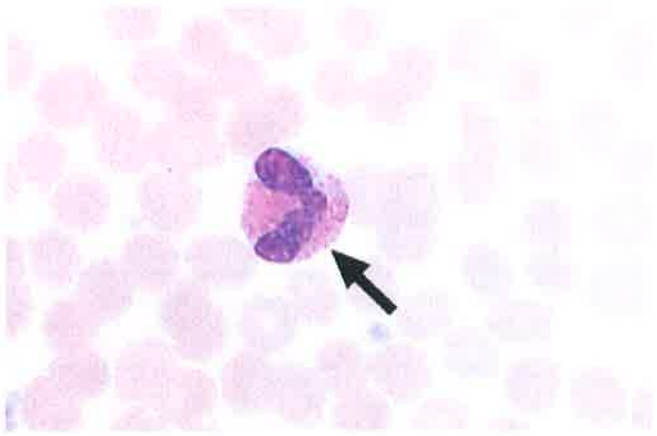
VPBS-08



Identification	Participants No.	%	Evaluation
Platelet, normal	1043	91.7	Educational
Plaletet, hypogranular	50	4.4	Educational
Platelet, giant (macrothrombocyte)	40	3.5	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	2	0.2	Educational
Blast cell	1	0.1	Educational
Target cell (codocyte)	1	0.1	Educational

The arrowed object is a platelet, as correctly identified by 91.7% of the participants. Platelets (also known as thrombocytes) are small, blue-gray fragments of megakaryocytic cytoplasm and typically measure 1.5 - 3 µm in diameter. Fine, purple-red granules are aggregated at the center or dispersed throughout the cytoplasm. Platelets play an essential role in primary hemostasis and normally circulate for 7 - 10 days before they are cleared by the spleen.

VPBS-09



Identification	Participants No.	%	Evaluation
Eosinophil, any stage	1130	99.4	Educational
Neutrophil, segmented or band	4	0.3	Educational
Spherocyte	2	0.2	Educational
Basophil, any stage	1	0.1	Educational

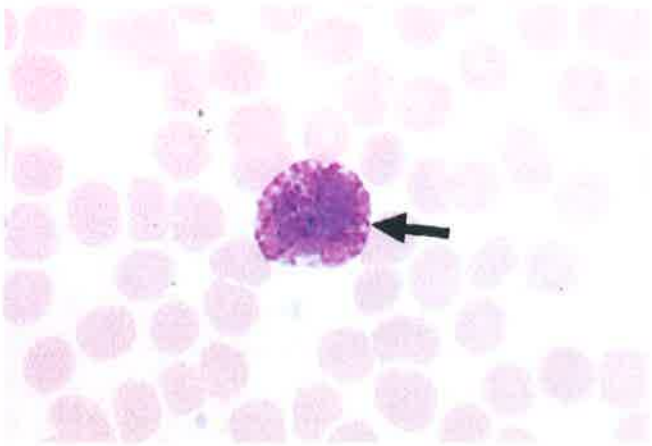
The arrowed cell is an eosinophil, as correctly identified by 99.4% of participants. Eosinophils are characterized by coarse, orange-red granules of uniform size and are similar to neutrophils in diameter (10 - 15 µm). Normally, the nucleus demonstrates condensed chromatin and nuclear segmentation with two or three nuclear lobes.

VPBS-10

Identification	Participants No.	%	Evaluation
Lymphocyte	1101	96.8	Educational
Lymphocyte, reactive (to include plasmacytoid and immunoblastic forms)	23	2.0	Educational
Lymphocyte, large granular	6	0.5	Educational
Acanthocyte (spur cell)	1	0.1	Educational
Basket cell/smudge cell	1	0.1	Educational
Basophil, any stage	1	0.1	Educational
Blast cell	1	0.1	Educational
Plasma cell	1	0.1	Educational
Platelet, hypogranular	1	0.1	Educational
Platelet satellitism	1	0.1	Educational

The arrowed cells are lymphocytes, as correctly identified by 96.8% of the participants. Further, this cell shows features of a mature, non-reactive lymphocyte and is a normal constituent of peripheral blood. The lymphocyte is slightly larger than a normal red blood cell with scant to moderate pale blue cytoplasm, round nuclear contours, and no visible nucleolus.

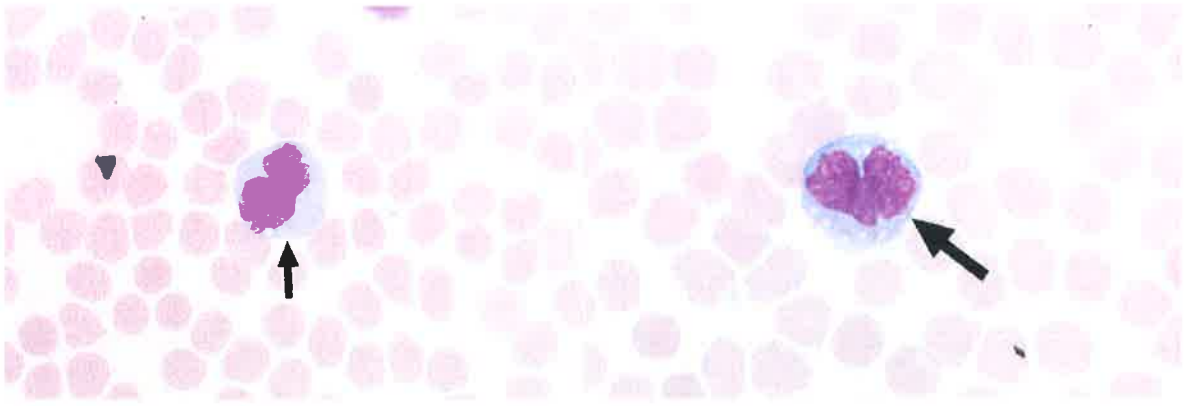
VPBS-11



Identification	Participants No.	%	Evaluation
Basophil, any stage	1063	93.5	Educational
Eosinophil, any stage	30	2.6	Educational
Neutrophil, toxic ((to include toxic granulation and or Döhle bodies, and/or toxic vacuolization)	20	1.8	Educational
Basophilic stippling (coarse)	4	0.3	Educational
Immature or abnormal cell, would refer	3	0.3	Educational
Neutrophil, segmented or band	3	0.3	Educational
Neutrophil, myelocyte	2	0.2	Educational
Neutrophil necrobiosis (degenerated neutrophil)	2	0.2	Educational
Echinocyte (burr cell, crenated cell)	1	0.1	Educational
Leukocyte containing fungi	1	0.1	Educational
Lymphocyte	1	0.1	Educational
Lymphocyte, reactive (to include plasmacytoid and immunoblastic forms)	1	0.1	Educational
Malignant lymphoid cell (other than blast)	1	0.1	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	1	0.1	Educational
Monocyte, immature (promonocyte, monoblast)	1	0.1	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational
Stain precipitate	1	0.1	Educational

The arrowed cell is a basophil, as correctly identified by 93.5% of the participants. Basophils are the least common circulating granulocytes. Unlike neutrophils with 3 - 5 lobed nuclei and fine pink or eosinophilic granules, basophils typically have only two prominent nuclear lobes and cytoplasm with numerous dense purple or basophilic granules, often obscuring the nuclear detail. Basophils are an important part of the allergic immune response, and infrequently circulate in appreciable number (typically representing < 0.3% of peripheral leukocytes).

VPBS-12



Identification	Participants No.	%	Evaluation
Monocyte	1123	98.8	Educational
Monocyte, immature (promonocyte, monoblast)	8	0.7	Educational
Platelet, giant (macrothrombocyte)	2	0.2	Educational
Immature or abnormal cell, would refer	1	0.1	Educational
Lymphocyte, reactive (to include plasmacytoid and immunoblastic forms)	1	0.1	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	1	0.1	Educational
Platelet, normal	1	0.1	Educational

The arrowed cells are monocytes, as correctly identified by 98.8% of participants. Monocytes are slightly larger than neutrophils, with a nuclear to cytoplasmic ratio of 2:1 to 4:1. The nuclear contours can vary from reniform to indented to folded. The chromatin is condensed, but is more open (ie, less condensed) compared to the chromatin of neutrophils or lymphocytes. Monocytes typically have smooth cytoplasmic margins, though some have pseudopod-like cytoplasmic extensions; this is a helpful feature in identifying monocytes, as seen in one of the arrowed cells in this case. Finally, the cytoplasm is abundant and gray to gray-blue and may contain fine azurophilic granules or vacuoles.

Clinical Presentation:

This peripheral blood smear is from a 27-year-old man with a history of polysubstance abuse and schizophrenia. He was admitted to the emergency room after snorting a crushed pill and becoming unresponsive. He was apneic (not breathing) and pulseless upon arrival. Cardiopulmonary resuscitation was administered. His pulse returned shortly thereafter and he was administered Narcan (naloxone hydrochloride). Laboratory data include: WBC = 15.6 x 10E9/L; RBC = 5.33 x 10E12/L; HGB = 16.0 g/dL; HCT = 48.0%; MCV = 88 fL; RDW = 12; and PLT = 322 x 10E9/L.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Stress Lymphocytosis

The CBC data in this case are indicative of a mild increase in white blood cell count (leukocytosis) with unremarkable red blood cell indices and a normal platelet count. Examination of the peripheral blood smear shows that the leukocytosis is accounted for in large part by mature, normal-appearing lymphocytes. The absolute lymphocyte count (ALC) as determined by automated instrument or by using the WBC count and leukocyte differential should be noted. Other normal leukocytes are present including scattered neutrophils and monocytes with fewer eosinophils and basophils.

Causes of lymphocytosis

Lymphocytosis is a commonly observed CBC abnormality in the hematology laboratory and often prompts peripheral blood smear review. In this context, it is helpful to keep in mind that there are numerous possible underlying causes in patients with lymphocytosis. These include both benign (reactive) as well as malignant processes. Malignant etiologies of lymphocytosis include B-cell lymphoproliferative disorders such as chronic lymphocytic leukemia/small lymphocytic lymphoma. T-cell lymphoproliferative disorders such as T-cell large granular lymphocytic leukemia are much less common and are typically accompanied by cytopenia(s). In adults, the most common cause of benign lymphocytosis in the hospital setting is stress lymphocytosis as is exemplified by the clinical vignette in this case. The ALC in stress lymphocytosis ranges from 4.0 – 11.0 x 10E9/L. This is a transient phenomenon and the lymphocyte count normalizes following resolution of physiologic stress. Other reactive causes of lymphocytosis include viral infection, autoimmune diseases, and drug reaction. In each of these situations, normal or resting lymphocytes predominate but a range of morphologic variants including occasional large granular, plasmacytoid, and immunoblast-like forms may be observed.

Question 1. In which of following clinical scenarios is reactive lymphocytosis most likely to be observed?

A. Chronic lymphocytic leukemia (CLL)
B. Iron deficiency
C. T-cell large granular lymphocytic (T-LGL) leukemia
D. Viral infection

Question 2. Which of the following best describes the clinical course of stress lymphocytosis?

A. Normalization of lymphocyte count coincident with resolution of physiologic stress
B. Oxidant hemolysis due to G6PD deficiency
C. Rapidly increasing lymphocyte count to over 100 x 10E9/L
D. Significantly increased risk of progression to acute lymphoblastic leukemia

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Diagnostic approach

Appropriate evaluation of patients with lymphocytosis begins with a complete clinical history. If a likely cause of secondary or reactive lymphocytosis is identified, additional investigation is usually not necessary. If there is suspicion for an underlying lymphoproliferative disorder based on clinical findings or atypical morphology, or the lymphocytosis is clinically unexplained, flow cytometry may be useful. Flow cytometry is an ancillary technique used to characterize leukocyte populations. Ultimately, close correlation of hematologic findings with clinical history, physical exam, radiologic, and other laboratory data is necessary for proper management of patients.

Question 3. Which of the following ancillary studies is the most appropriate tool for evaluation of a patient with suspected malignant lymphocytosis?

A. Erythrocyte sedimentation rate (ESR)
B. Flow cytometry
C. Leukocyte alkaline phosphatase (LAP) score
D. Serum lactose dehydrogenase (LDH)

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ANSWERS TO QUESTIONS:

Question 1: C. Viral infection

There are numerous possible causes of benign or reactive lymphocytosis including viral infection, autoimmune disease, physiologic stress, and drug effect. Both CLL and T-LGL are neoplastic conditions in which abnormal malignant lymphocytes are preset in the peripheral blood. Iron deficiency typically presents as microcytic hypochromic anemia with or without reactive thrombocytosis. Lymphocytosis is not common in iron deficient patients.

Question 2: A. Normalization of lymphocyte count coincident with resolution of physiologic stress

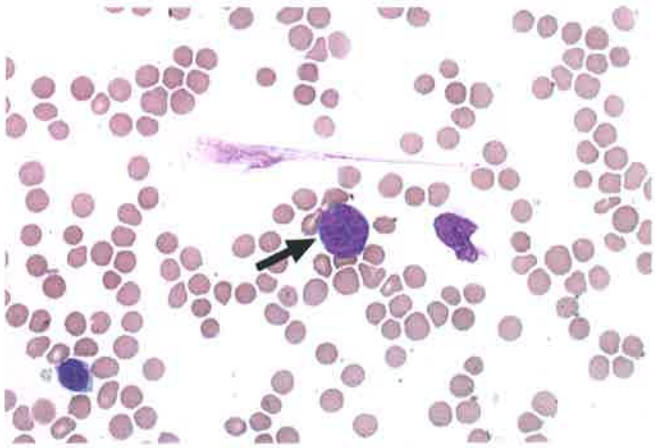
Stress lymphocytosis is transient in nature and resolves following resolution of the underlying physiologic event. There is no association with leukemia and there is only a mild or moderate increase in the ALC. An ALC of > 100 x 10E9/L should prompt concern for a malignant process and requires further investigation. There is no association with oxidative hemolysis.

Question 3: B. Flow cytometry

Flow cytometry allows for detailed characterization of lymphocyte subsets and is an appropriate ancillary study in patients with lymphocytosis if there is clinical suspicion for a neoplastic process and/or a clinical explanation for increased lymphocytes (eg, infection, stress response) is not readily identified. A LAP score may be helpful as a cost-effective surrogate for *BCR-ABL1* testing in patients with neutrophilic leukocytosis and a low index of suspicion for chronic myelogenous leukemia. ESR and/or LDH are relatively nonspecific findings and should not be used exclude malignancy in patients with lymphocytosis.

Cell Identification

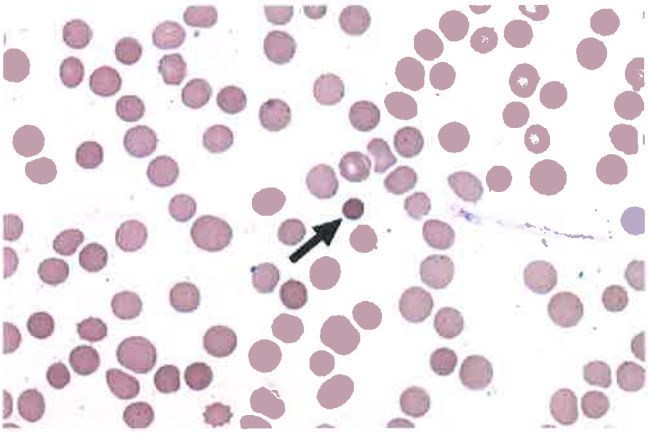
VPBS-14



Identification	Participants No.	%	Evaluation
Blast cell	970	85.2	Educational
Malignant lymphoid cell (other than blast)	91	8.0	Educational
Lymphocyte, reactive (to include plasmacytoid and immunoblastic forms)	25	2.2	Educational
Immature or abnormal cell, would refer	22	1.9	Educational
Lymphocyte	21	1.9	Educational
Lymphocyte, large granular	5	0.4	Educational
Platelet, normal	2	0.2	Educational
Acanthrocyte (spur cell)	1	0.1	Educational
Monocyte, immature (promonocyte, monoblast)	1	0.1	Educational

The arrowed cell is a blast, as correctly identified by 85.2% of participants. A blast is a large, round to oval cell, 10 - 20 µm in diameter. The nuclear-to-cytoplasmic ratio is high, varying from 7:1 - 5:1. The blast often has a round to oval nucleus, but sometimes it is indented or folded. It has fine, lacy, or reticular chromatin. One or more prominent nucleoli may be seen. The cytoplasm is variably basophilic and typically agranular. The morphologic features of a blast cell usually do not permit determination of the cell lineage (ie, myeloblast versus lymphoblast). The one exception is the presence of Auer rods, which are diagnostic of myeloid lineage. As blasts are quite variable in appearance, it is often impossible to correctly classify an individual cell based on the morphology alone. Specifically regarding lymphoblasts, 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, which closely resemble typical myeloblasts. For identification purposes, one should classify individual cells exhibiting this type of morphology as blast cells when additional confirmatory information is unavailable. In the absence of Auer rods, immunophenotyping by flow cytometry, immunohistochemistry on tissue sections, or, less commonly, cytochemical staining is required to determine the lineage of a given blast cell. Malignant lymphoid cell (other blast cell), as identified by 8.0% of participants, is an acceptable answer for this image.

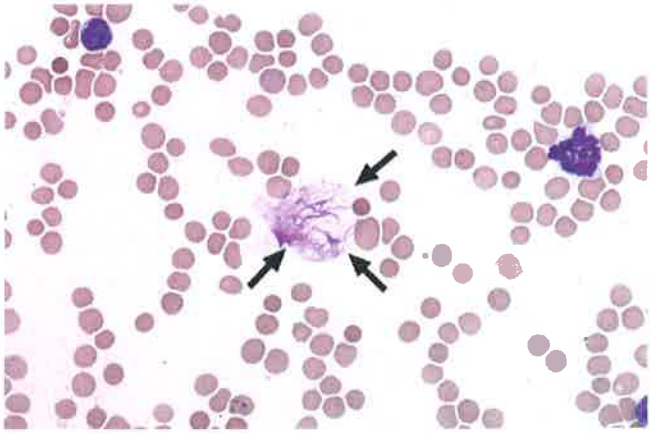
VPBS-15



Identification	Participants No.	%	Evaluation
Spherocyte	1096	96.3	Educational
Microcyte (with increased central pallor)	37	3.3	Educational
Eosinophil, any stage	2	0.2	Educational
Erythrocyte, normal	1	0.1	Educational
Immature or abnormal cell, would refer	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational

The arrowed cell is a spherocyte, as correctly identified by 96.3% of participants. Spherocytes are identified as densely staining, spherical, or globular red blood cells with normal or slightly reduced volume (MCV) and increased thickness (more than 3 µm), but with decreased diameter (usually less than 6.5 µm), and without central pallor. These cells appear denser than normal RBCs and are commonly found in hereditary spherocytosis and immune hemolytic anemias. Micro-spherocytes (spherocytes measuring 4 µm or less in diameter), frequently seen in severe burns or microangiopathies, probably represent rounded-up fragments of red blood cells.

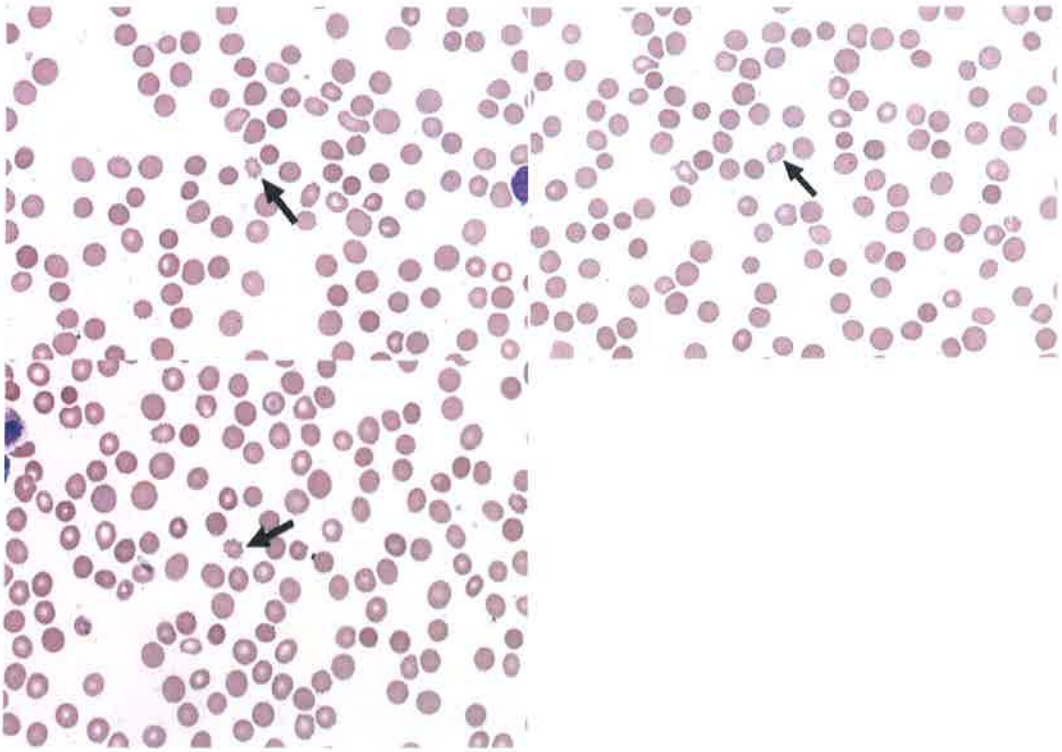
VPBS-16



Identification	Participants No.	%	Evaluation
Basket cell/smudge cell	1108	97.4	Educational
Stain precipitate	25	2.2	Educational
Lymphocyte	2	0.2	Educational
Immature or abnormal cell, would refer	1	0.1	Educational
Mast cell	1	0.1	Educational
Squamous epithelial cell/endothelial cell	1	0.1	Educational

The arrowed cell is a basket cell/smudge cell, as correctly identified by 97.4% of participants. Basket cells or smudge cells are most commonly associated with cells that are fragile and easily damaged in the process of making a peripheral blood smear. The nucleus may either be a non-descript chromatin mass or the chromatin strands may spread out from a condensed nuclear remnant, giving the appearance of a basket. Cytoplasm is either absent or indistinct. Smudge cells are usually lymphocytes, but there is no recognizable cytoplasm to give a clue to the origin of the cell. They are seen most commonly in disorders characterized by lymphocyte fragility, such as infectious mononucleosis and chronic lymphocytic leukemia. Basket cells should not be confused with necrobiotic neutrophils, which have enough cytoplasm to allow the cell to be classified.

VPBS-17



Identification	Participants No.	%	Evaluation
Echinocyte (burr cell, crenated cell)	1038	91.3	Educational
Acanthocyte (spur cell)	93	8.2	Educational
Basophil, any stage	2	0.2	Educational
Erythrocyte, normal	2	0.2	Educational
Immature or abnormal cell, would refer	1	0.1	Educational
Platelet, normal	1	0.1	Educational

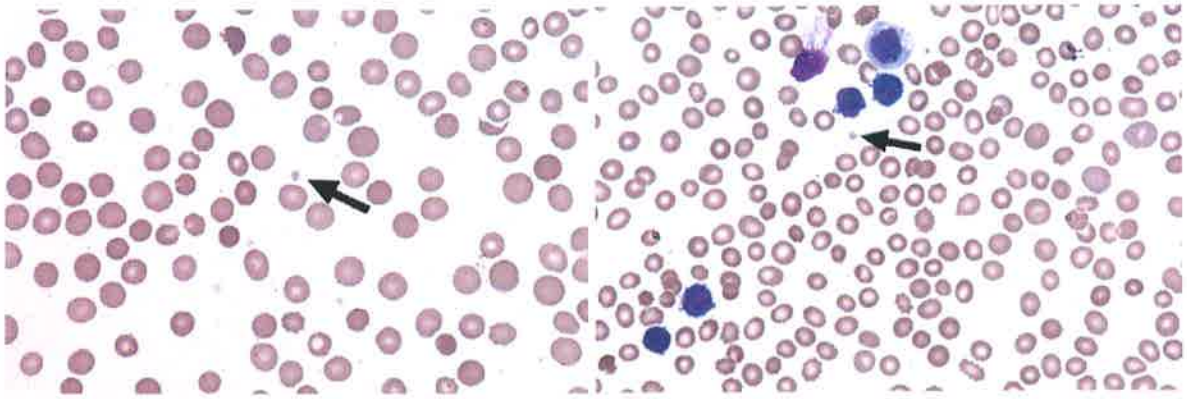
The arrowed cells are echinocytes (burr cells, crenated cells), as correctly identified by 91.3% of participants. Echinocytes are red blood cells with 10 - 30 uniform, short, blunt projections distributed evenly that impart a serrated appearance to the red blood cell surface. The red blood cells retain central pallor and are the same size or slightly smaller than normal red blood cells. Their appearance is often the result of an improperly prepared smear (slow drying, thick smears, aged blood, and pH alteration of glass slide). Echinocytes that are not artifacts may be indicative of disease, such as uremia or pyruvate kinase deficiency, and seen post splenectomy, in hepatitis of the newborn, and phospoglycerate kinase deficiency. Under such circumstances, they should be visible in wet preparations. In this case, the echinocytes are most likely artifactual in origin.

VPBS-17 Discussion, Cont'd:

Acanthocytes, which was selected by 8.2% of participants, are densely stained, spheroidal red blood cells that lack central pallor and have multiple (usually 3 to 20), irregularly distributed, thorn-like spicules of variable size, often with drumstick ends. Acanthocytes are classically described in association with hereditary abetalipoproteinemia (hereditary acanthocytosis). In addition, these cells are often seen in significant numbers in severe end-stage liver disease, post splenectomy, hepatorenal failure, infantile pyknocytosis, McLeod phenotype, anorexia nervosa, and chronic starvation. A small number of acanthocytes may be seen in other forms of severe hemolytic anemia, particularly after splenectomy. Acanthocytes are rarely encountered in otherwise normal blood smears (one or two per smear). In such smears, they represent older, senescent red blood cells approaching their extremes of life (120 days). Therefore, it is logical that acanthocytes should be more readily found in blood smears in the postsplenectomy state because of diminished splenic activity in removal of such poikilocytes.

Acanthocytes is an incorrect choice for the arrowed cells. The arrowed cells maintain central pallor and have uniform, blunt projections. Acanthocytes' projections, as mentioned above, are more irregularly distributed and variable in shape.

VPBS-18



Identification	Participants No.	%	Evaluation
Platelet, normal	1122	98.6	Educational
Platelet, hypogranular	8	0.7	Educational
Platelet, giant (macrothrombocyte)	3	0.3	Educational
Monocyte	2	0.2	Educational
Echinocyte (burr cell/crenated cell)	1	0.1	Educational
Immature or abnormal cell, would refer	1	0.1	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	1	0.1	Educational

The arrows objects are normal platelets, as correctly identified by 98.6% of participants. Platelets, also known as thrombocytes, are small, blue-gray fragments of megakaryocytic cytoplasm. Most are 1.5 - 3 µm in diameter. A few small platelets, less than 1.5 µm in diameter, and a few large platelets, 4 - 7 µm in diameter, can also be seen in normal blood films. Fine, purple-red granules are dispersed throughout the cytoplasm or are sometimes aggregated at the center. These granules are platelet alpha granules. Platelet delta granules (or dense granules) are not visible on light microscopy. Platelets may be variable in shape, but most normal platelets are round or very slightly elliptical. Some have short cytoplasmic projections or ruffled margins. They are typically single but may form aggregates, particularly in fresh (fingerstick) preparations.

Clinical Presentation:

This peripheral blood smear is from an 18-year-old woman with a recent history of T lymphoblastic leukemia/lymphoma of the mediastinum. Laboratory data include: WBC = 50.0 x 10E9/L; RBC= 3.57 x 10E12/L; HGB = 10.7 g/dL; HCT = 32.8%; and PLT = 49 x 10E9/L. Peripheral blood smear shows increased blasts.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: T lymphoblastic leukemia/lymphoma

The CBC data in this case are indicative of leukocytosis with accompanying anemia and thrombocytopenia. White blood cell differential reveals increased blasts in this patient with recently diagnosed T lymphoblastic leukemia/lymphoma (T-ALL/LBL) of the mediastinum. These findings reveal peripheral blood involvement by known leukemia/lymphoma.

T-ALL/LBL is a neoplasm of lymphoblasts, which are committed to the T-cell lineage. The blasts are typically small to medium sized with scant cytoplasm, moderately condensed to open chromatin, and inconspicuous nucleoli. The blasts are indistinguishable from those seen in B lymphoblastic leukemia/lymphoma (B-ALL/LBL). Moreover, the blasts are frequently indistinguishable from those of acute myeloid leukemia (AML). Specifically, only Auer rods would reliably distinguish AML from ALL by morphology alone as Auer rods are indicative of myeloid differentiation. T-ALL/LBL frequently presents with mediastinal involvement (ie, thymic involvement) as T-ALL/LBL is derived from thymocytes, precursor T-cells. However, a variable degree of involvement of lymph nodes, extranodal sites, and the 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 bone marrow involvement. However, if extensive peripheral blood and bone marrow involvement are noted, T-ALL is the appropriate term. Sometimes, the neoplasm involves both a tissue mass as well as the bone marrow/peripheral blood; therefore, the distinction between leukemia and lymphoma is defined by the degree of bone marrow involvement, defined in the WHO system as < 25% involvement of the bone marrow in cases of lymphoblastic lymphoma.

T-ALL/LBL comprises approximately 15% of childhood acute lymphoblastic leukemias. It is significantly less common than B-ALL. However, it comprises approximately 90% of all lymphoblastic lymphomas as B-ALL/LBL most frequently presents with peripheral blood and/or bone marrow disease without involvement of tissue mass.

When presenting as a mediastinal mass, T-ALL/LBL often shows rapid growth and may present as a respiratory emergency. Pleural effusions are common. When peripheral blood involvement is present, a high leukocyte count is frequent. However, T-ALL frequently shows relative sparing of normal bone marrow hematopoiesis when compared with B-ALL.

Question 1. Which is true of T lymphoblastic leukemia/lymphoma:

A. Blasts of T lymphoblastic leukemia are easily distinguished from blasts of B lymphoblastic leukemia by morphology
B. Pleural effusions are very rarely associated with T lymphoblastic lymphoma
C. T lymphoblastic leukemia is more common than B lymphoblastic leukemia
D. T lymphoblastic lymphoma comprises more cases of lymphoblastic lymphoma than B lymphoblastic lymphoma

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Immunophenotype of T lymphoblastic leukemia/lymphoma

As previously stated, T-ALL/LBL is committed to T-cell lineage. Therefore, the lymphoblasts express CD3 (either surface or cytoplasmic), which is lineage specific. In addition, the lymphoblasts often express TdT, a marker proving immature immunophenotype. In addition to TdT, other markers indicating immature phenotype are CD99, CD34, and CD1a. T-ALL/LBL shows variable expression of other pan-T-cell markers such as CD2, CD4, CD5, CD7, and CD8. Of these, CD7 is most often positive. CD4 and CD8 may be co-expressed or double negative, recapitulating

thymocyte maturation. CD79a, a marker of B-cell differentiation, has been reported in a minor subset of cases. Non-lineage defining myeloid markers, such as CD13 and CD33, are also positive in a subset of cases. Early T-cell precursor lymphoblastic leukemia, a provisional diagnosis in the 2016 WHO, is now recognized. This neoplasm has a unique immunophenotype and genetic makeup. By definition, the blasts in this entity are negative for CD1a and CD8, but express CD7 and one or more myeloid/stem cell markers including CD34, CD117, HLA-DR, CD13, CD33, CD11b, or CD65.

Question 2. Which case below would be consistent with early T-cell precursor lymphoblastic leukemia?

A. T lymphoblast population expressing cytoplasmic CD3, CD7, TdT, and CD33
B. T lymphoblast population expressing CD3, CD8, TdT, and CD33
C. T lymphoblast population expressing CD4, CD8, cytoplasmic CD3, and TdT
D. T lymphoblast population expressing CD4, surface CD3, and CD1a

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Genetics of T lymphoblastic leukemia/lymphoma

T-ALL/LBL usually shows clonal rearrangements of the T-cell receptor (*TCR*) genes. Interestingly, simultaneous *IGH* rearrangements (typical of B-cell neoplasms) are seen in a subset of cases. An abnormal karyotype is seen in approximately 50 - 70% of cases. The most common abnormalities involve the alpha and delta *TCR* loci, the beta locus, and the gamma locus with a variety of partner genes. Approximately 50% of cases show activating mutations of the *NOTCH1* gene, which encodes a protein that is necessary for early T-cell development. The direct downstream target of *NOTCH1* is *MYC*, which aids in growth of the neoplastic cells. However, *NOTCH1* mutations are not common 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 treated similar to other types of ALL. T-ALL/LBL is considered to have a poorer prognosis than the more common B-ALL/LBL. This is at least in part due to the presence of other high-risk clinical features such as older age and higher white blood cell count at presentation. Nonetheless, 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 a strong adverse prognostic factor, similar to B-ALL/LBL. The prognostic implication of early T-cell precursor lymphoblastic leukemia is currently controversial.

Question 3. Which of the following is true regarding the prognosis of T lymphoblastic leukemia/lymphoma?

A. B lymphoblastic leukemia more frequently shows isolated CNS 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 less frequently shows induction failure compared to B lymphoblastic leukemia

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Natasha M. Savage, MD
Hematology and Clinical Microscopy Committee

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Coustan-Smith E, Mullighan CG, Onciu M, et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. *Lancet Oncol.* 2009;10(2):147-156.

Gorczyca W, Tugulea S, Liu Z, Li X, Wong JY, Weisberger J. Flow cytometry in the diagnosis of mediastinal tumors with emphasis on differentiating thymocytes from precursor T Lymphoblastic lymphoma/leukemia. *Leuk Lymphoma.* 2004;45(3):529-538.

ANSWERS TO QUESTIONS:

Question 1: D. T lymphoblastic lymphoma comprises more cases of lymphoblastic lymphoma than B lymphoblastic lymphoma

T-ALL/LBL comprises approximately 15% of childhood acute lymphoblastic leukemia; therefore, it is less common than B-ALL. However, it comprises approximately 90% of all lymphoblastic lymphomas as B-ALL/LBL most frequently presents with peripheral blood and/or bone marrow disease without involvement of tissue mass.

Question 2: A. T lymphoblast population expressing cytoplasmic CD3, CD7, TdT, and CD33

Early T-cell precursor lymphoblastic leukemia has a unique immunophenotype. By definition, the blasts in this entity are negative for CD1a and CD8, but express CD7 and one or more myeloid/stem cell markers including CD34, CD117, HLA-DR, CD13, CD33, CD11b, or CD65.

Question 3: C. The prognosis is poorer than B lymphoblastic leukemia

T-ALL/LBL is considered to have a poorer prognosis than B-ALL/LBL. This is at least in part due to the presence of other high-risk clinical features such as older age and higher white blood cell count at presentation. In addition, 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.

COLLEGE of AMERICAN PATHOLOGISTS

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Participant	Date	Participant	Date

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

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