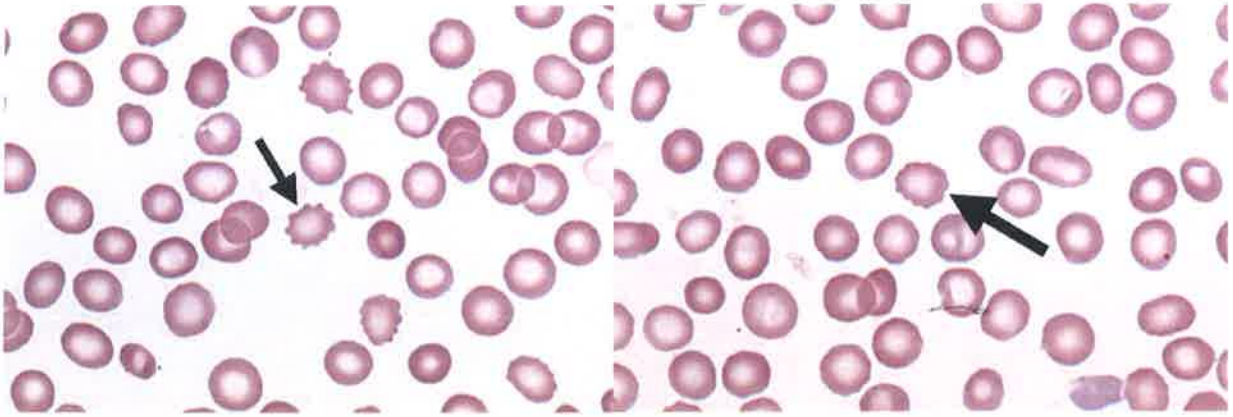
Cell Identification

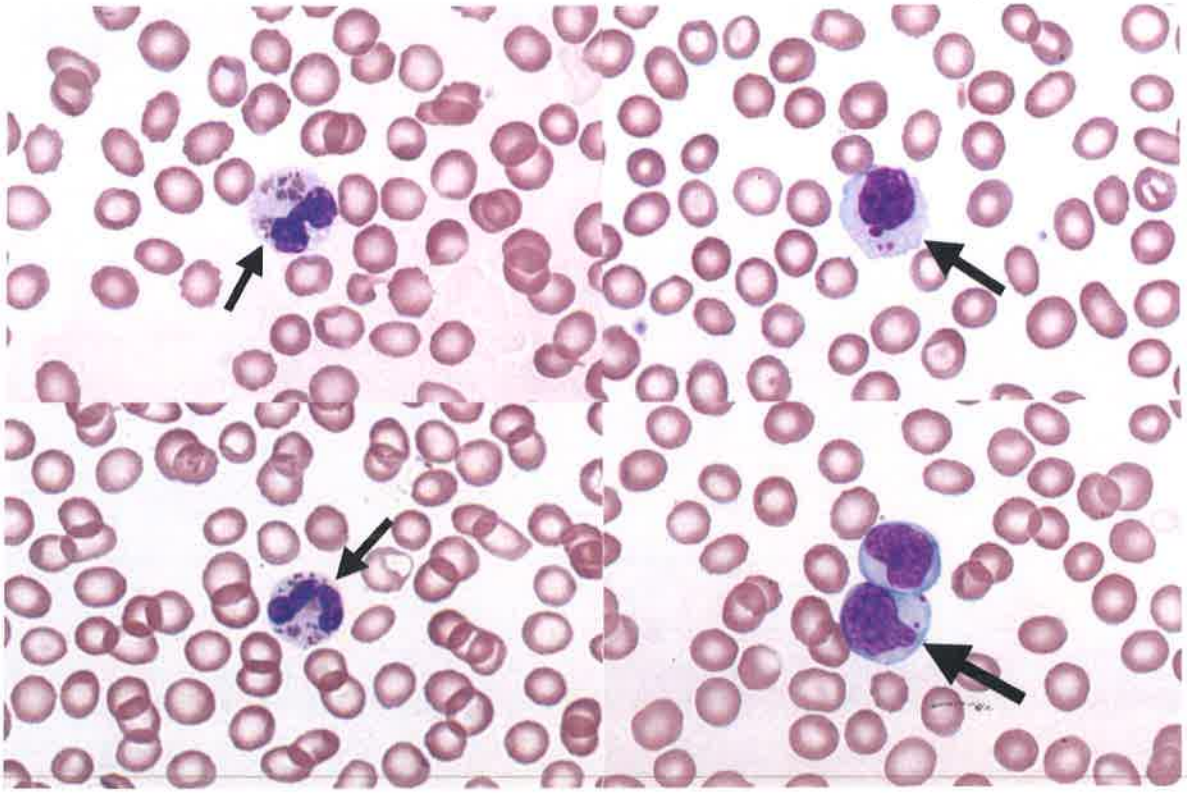
VPBS-20



Identification	Participants No.	Participants %	Evaluation
Echinocyte (burr cell, crenated cell)	1210	98.9	Educational
Acanthocyte (spur cell)	13	1.1	Educational
Neutrophil, segmented or band	1	0.1	Educational

The arrowed cells are echinocytes, as correctly identified by 98.9% of the participants. Echinocytes are red blood cells with uniform, short projections imparting a serrated appearance to the cell surface. 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, pyruvate kinase or phosphoglycerate kinase deficiency. They may also be seen post splenectomy and in hepatitis of the newborn.

VPBS-21



Identification	Participants No.	%	Evaluation
Leukocyte containing Chediak-Higashi anomaly inclusions(s)	1053	86.0	Educational
Leukocyte with intracellular *Anaplasma/Ehrlichia*	42	3.4	Educational
Leukocyte with intracellular bacteria	24	2.0	Educational
Lymphocyte, large granular	19	1.6	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	17	1.4	Educational
Immature or abnormal cell, would refer for identification	14	1.0	Educational
Monocyte	13	1.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	7	0.6	Educational
Neutrophil, segmented or band	7	0.6	Educational
Monocyte, immature (promonocyte, monoblast)	4	0.3	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	4	0.3	Educational
Howell-Jolly body	3	0.3	Educational
Leukocyte containing Alder (Alder-Reilly) anomaly inclusion(s)	3	0.3	Educational
Lymphocyte	3	0.3	Educational
Parasite(s) seen, referred for definitive identification	2	0.2	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	2	0.2	Educational
Eosinophil, any stage	1	0.1	Educational
Leukocyte with intracellular fungi	1	0.1	Educational

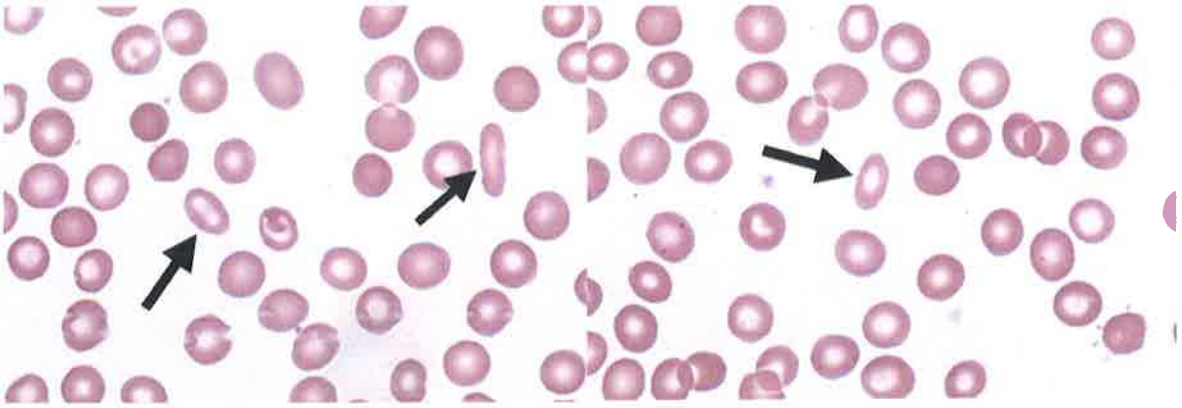
VPBS-21 (cont.)

Identification	Participants No.	%	Evaluation
Megakaryocyte	1	0.1	Educational
Neutrophil, metamyelocyte	1	0.1	Educational
Neutrophil with Pelger-Huët nucleus (acquired or congenital)	1	0.1	Educational
Ovalocyte (elliptocyte)	1	0.1	Educational
Pappenheimer bodies (Iron or Wright stain)	1	0.1	Educational

The arrowed cells are leukocytes containing Chediak-Higashi anomaly inclusions, as correctly identified by 86.0% of the participants. Chediak-Higashi inclusions are characterized as large round granules that may impart a variety of colors (including red, blue, or green-gray). These may be seen within the cytoplasm of otherwise normal leukocytes including granulocytes, lymphocytes, and monocytes. These granules are attributed to a lysosomal trafficking abnormality that characterizes the inherited disease. Affected leukocytes also demonstrate poor function in killing phagocytosed organisms.

Chediak-Higashi inclusions may be confused with intracellular organisms (including parasites such as *Anaplasma/Ehrlichia*, or intracellular bacteria) or other intracellular inclusions. One of the key distinctive features in this case is the identification of inclusions across the spectrum of leukocytes, rather than in a single lineage; intracellular organisms, by contrast, are not identifiable in lymphocytes. Intracellular bacteria, despite the relative commonality of bacteremia, are rarely identifiable, and typically only in overwhelming infections, which the provided CBC data do not suggest. Intracellular organisms are also typically more uniform in size and shape, whereas the inclusions of Chediak-Higashi are more typically irregular and variable in size from cell-to-cell. In some cases, intracellular organisms can be seen surrounded by a clear halo, representative to their localization within an intracellular vacuole (this is frequently the case for intracellular fungal elements). A halo is also typical of the Alder-Reilly anomaly inclusions, surrounding azurophilic granules, in contrast to Chediak-Higashi inclusions which are not surrounded by a halo.

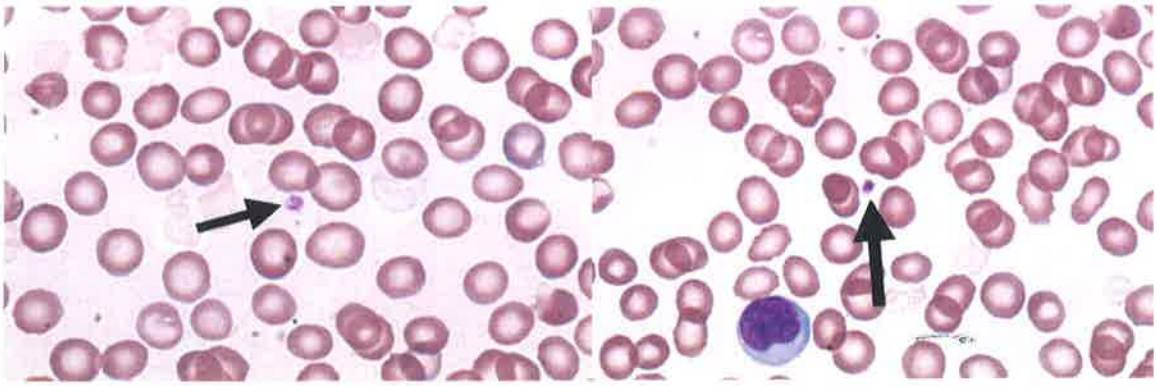
VPBS-22



Identification	Participants No.	%	Evaluation
Ovalocyte (elliptocyte)	1217	99.4	Educational
Stomatocyte	2	0.2	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	2	0.2	Educational
Leukocyte containing Chediak-Higashi anomaly inclusions(s)	1	0.1	Educational
Nucleated red blood cell, normal or abnormal morphology	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational

The arrowed cells are ovalocytes, as correctly identified by 99.4% of the participants. The terms ovalocyte and elliptocyte are interchangeably used to describe elongated red blood cells with blunt ends and parallel sides. A small number of elliptocytes/ovalocytes may be present on the smears of normal individuals (< 1%), whereas a moderate to marked elliptocytosis/ovalocytosis (> 25%) is observed in patients with hereditary elliptocytosis, an abnormality of erythrocyte skeletal membrane proteins. Elliptocytes are also commonly increased in number in iron deficiency and in the same states in which teardrop cells may be seen.

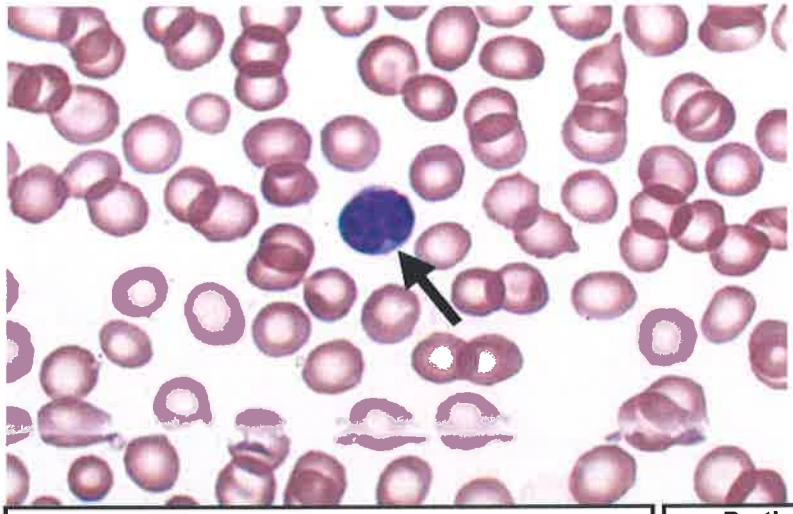
VPBS-23



Identification	Participants No.	%	Evaluation
Platelet, normal	1208	98.7	Educational
Platelet, giant (macrothrombocyte)	6	0.5	Educational
Platelet, hypogranular	3	0.3	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	2	0.2	Educational
Erythrocyte with overlying platelet	1	0.1	Educational
Leukocyte with intracellular bacteria	1	0.1	Educational
Lymphocyte	1	0.1	Educational
Mast cell	1	0.1	Educational
Platelet satellitism	1	0.1	Educational

The arrowed cells are normal platelets, as correctly identified by 98.7% of the participants. Platelets are small, blue-gray fragments of megakaryocytic cytoplasm, most are 1.5 to 3 µm in diameter. A few small platelets, less than 1.5 µm in diameter, and a few large platelets, 4 to 7 µm in diameter, can also be seen in normal blood films. Fine, purple-red alpha granules are dispersed throughout the cytoplasm or are sometimes aggregated at the center. Platelets may be variable in shape, but most normal platelets are round or very slightly elliptical.

VPBS-24



Identification	Participants No.	Participants %	Evaluation
Lymphocyte	1189	97.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	18	1.5	Educational
Malignant lymphoid cell (other than blast)	6	0.5	Educational
Monocyte	3	0.3	Educational
Immature or abnormal cell, would refer for identification	2	0.2	Educational
Lymphocyte, large granular	2	0.2	Educational
Acanthocyte (spur cell)	1	0.1	Educational
Basophil, any stage	1	0.1	Educational
Blast cell	1	0.1	Educational
Platelet, normal	1	0.1	Educational

The arrowed cell is a lymphocyte, as correctly identified by 97.1% of the participants. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 µm with an N:C ratio ranging from 5:1 to 2:1. Most lymphocytes have round to oval nuclei that may be slightly indented or notched (as in this case). The chromatin is diffusely dense or coarse and clumped. Nucleoli are not typically visible, although some cells may exhibit a small, pale chromocenter that may be mistaken for a nucleolus. Most lymphocytes have a scant amount of pale blue to moderately basophilic, agranular cytoplasm.

Clinical Presentation:

This peripheral blood smear is from a 3-year-old girl with oculocutaneous albinism, presenting with fever, splenomegaly, and lymphadenopathy. Laboratory data include: WBC = 5.2 x 10E9/L; RBC = 3.34 x 10E12/L; HGB = 9.9 g/dL; and PLT = 43 x 10E9/L.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Chediak-Higashi Syndrome

The provided CBC data report anemia and thrombocytopenia. Red cells are generally normochromic but demonstrate echinocytosis and ovalocytosis. Platelets are morphologically unremarkable. The majority of leukocytes demonstrate large, cytoplasmic red-purple granules, in keeping with the Chediak-Higashi anomaly.

Chediak-Higashi syndrome is a rare autosomal recessive disease originally described in 1943 by Cesar (1). The disease was later eponymously named for Chediak and Higashi who both independently further characterized the disease in the 1950s (1). Initially identified by an autosomal recessive coincidence of partial albinism, a susceptibility to infection, increased bleeding tendency, and the presence of characteristic giant cytoplasmic leukocyte granules, the disease was later attributed to defective lysosomal trafficking (1). Modern diagnosis continues to rely on clinical suspicion, although recent studies have identified mutations in the *LYST* gene as the likely underlying cause of the disease (1).

Epidemiology & Clinical Features

Although the exact prevalence of Chediak-Higashi is unknown, a recent review suggests that fewer than 500 cases have been reported since the disease was initially described, with the notable caveat that this is probably an underestimate (2). The disease typically presents in childhood, often first coming to attention due to abnormal skin pigmentation or abnormally light colored hair (2). Of perhaps greater concern, Chediak-Higashi patients have a tendency toward frequent bacterial infections, most commonly affecting the skin, respiratory tract, and mucous membranes (2). Neurological features are often common, including motor and sensory neuropathies, ataxia, and low cognitive functioning (2). Although historically fatal due to infection (1), patients surviving into their early adulthood may develop progressive neurologic deterioration; some patients may also experience a so-called "accelerated phase" characterized by a hemophagocytic-like syndrome (2).

Question 1: Patients with Chediak-Higashi may be brought to clinical attention due to:

A. Abnormally dark colored hair and skin pigmentation
B. Clotting factor abnormalities
C. Increased susceptibility to infection, especially of the upper respiratory tract
D. Neutrophil inclusions, predominantly centered in the nucleus

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Laboratory Features

Routine peripheral smear evaluation is generally required for diagnosis, and may represent the first clinical laboratory indication of the disease during an initial presentation (2). Microscopic examination of hair and skin might also assist the diagnosis (although typically not required), potentially showing clumped melanotic pigment and giant melanosomes, respectively (2). The immune cell defects can also be identified by way of impaired functional assays (2). Evidence of a platelet storage pool disorder is also typical of patients with Chediak-Higashi syndrome (3).

Question 2: Which statement is correct?

A. All cases of Chediak-Higashi syndrome come to clinical attention with an hemophagocytic-like syndrome.
B. Leukocyte morphologic evaluation is central to the diagnosis of Chediak-Higashi syndrome
C. Most patients with the Chediak-Higashi syndrome are expected to have a normal life expectancy
D. Platelets in the Chediak-Higashi syndrome demonstrate normal platelet storage characteristics

The *LYST* Gene

The causative genetic mutations in Chediak-Higashi syndrome have been attributed to the lysosome trafficking regulation gene *LYST* (1,2). The specific function of the LYST protein product is uncertain, however studies have demonstrated that the gene has homologues in nearly all eukaryotes, underscoring its likely evolutionary significance (1). Also of interest, genotype-phenotype studies of Chediak-Higashi patients suggest that many early onset cases have predicted protein truncation mutations (2).

Treatment

Modern treatment for patients with Chediak-Higashi focuses on supportive care, with hematopoietic stem cell transplant where possible. Supportive care generally addresses the direct sequelae of disease (ie, frequent infections, bleeding tendency, and neurologic symptoms) as well as the potential for "accelerated disease," with the latter often requiring treatment regimens comparable to those employed to treat hemophagocytic syndrome (2). Hematopoietic stem cell transplant offers the potential to overcome the immunodeficiency and bleeding tendencies of Chediak-Higashi, although neurologic degeneration is largely felt to be insurmountable even with successful stem cell transplantation (2).

Question 3: Which statement is correct?

A. Supportive treatment strategies, including those employed in other diseases such as hemophagocytic syndrome, are indicated in the treatment of Chediak-Higashi syndrome .
B. The gene attributed to cause Chediak-Higashi syndrome is unique to humans among studied eukaryotes.
C. The neurologic decline seen in patients with Chediak-Higashi syndrome is generally considered to respond well to stem cell transplantation.
D. There are currently no treatments available to address the immunodeficiency characteristic of Chediak-Higashi syndrome.

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

References:

1. Kaplan J, De Domenico I, Ward DM. Chediak-Higashi syndrome: *Current Opinion in Hematology*. 2008 Jan;15(1):22–9.

2. Lozano ML, Rivera J, Sánchez-Guiu I, Vicente V. Towards the targeted management of Chediak-Higashi syndrome. *Orphanet Journal of Rare Diseases* [Internet]. 2014 Dec [cited 2019 Mar 29];9(1). Available from: http://ojrd.biomedcentral.com/articles/10.1186/s13023-014-0132-6

3. Buchanan GR, Handin RI. Platelet function in the Chediak-Higashi syndrome. *Blood.* 1976 Jun;47(6):941–8.

Answers to Questions:

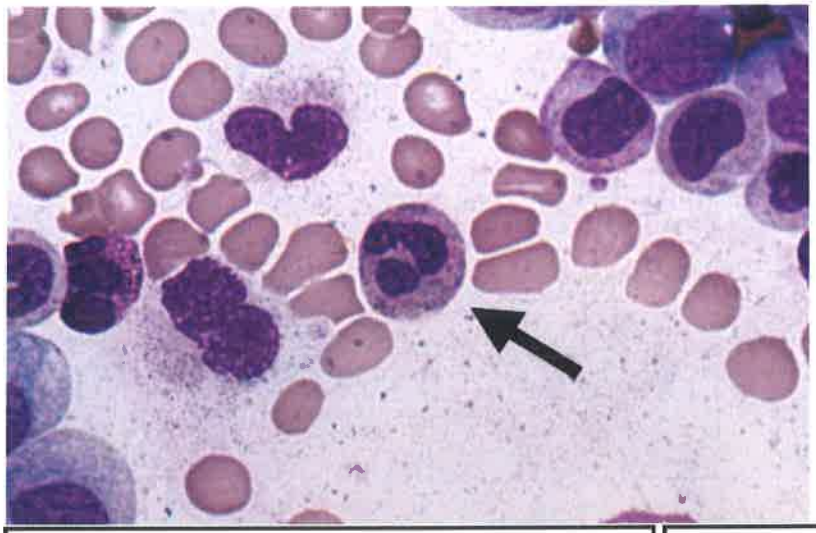
Question 1: C. Increased susceptibility to infection, especially of the upper respiratory tract
Owing to defective leukocyte function, patients with Chediak-Higashi syndrome demonstrate an increased susceptibility to infections, usually bacterial in nature, and often involving the upper respiratory tract.

Question 2: B. Leukocyte morphologic evaluation is central to the diagnosis of Chediak-Higashi syndrome
The morphologic features of leukocytes in Chediak-Higashi syndrome, namely giant intracellular granules, are central to the diagnosis of the disease.

Question 3: A. Supportive treatment strategies, including those employed in other diseases such as hemophagocytic syndrome, are indicated in the treatment of Chediak-Higashi syndrome
Aggressive supportive treatment, including treatments similar to those employed in hemophagocytic syndrome if/when the disease progresses to "accelerated phase," may be indicated in the treatment of Chediak-Higashi syndrome.

Cell Identification

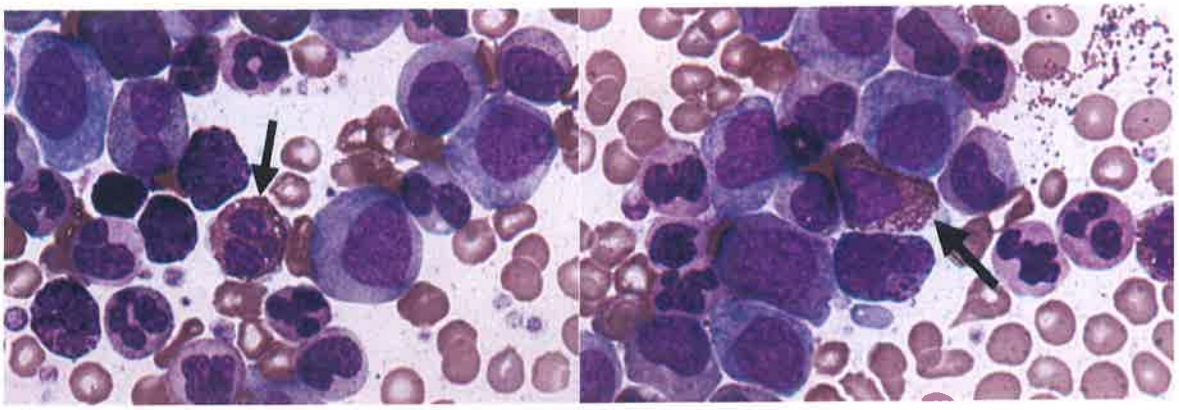
VPBS-26



Identification	Participants No.	Participants %	Evaluation
Neutrophil, segmented or band	1115	91.2	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	93	7.6	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	6	0.5	Educational
Neutrophil, polyploid	3	0.3	Educational
Immature or abnormal cell, would refer for identification	2	0.2	Educational
Neutrophil necrobiosis (degenerated neutrophil)	2	0.2	Educational
Lymphocyte	1	0.1	Educational
Neutrophil, metamyelocyte	1	0.1	Educational

The arrowed cell is a segmented neutrophil, as correctly identified by 91.2% 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. The arrowed neutrophil is normally-granulated, lacks both Döhle bodies and cytoplasmic vacuoles, and therefore is not a toxic neutrophil.

VPBS-27

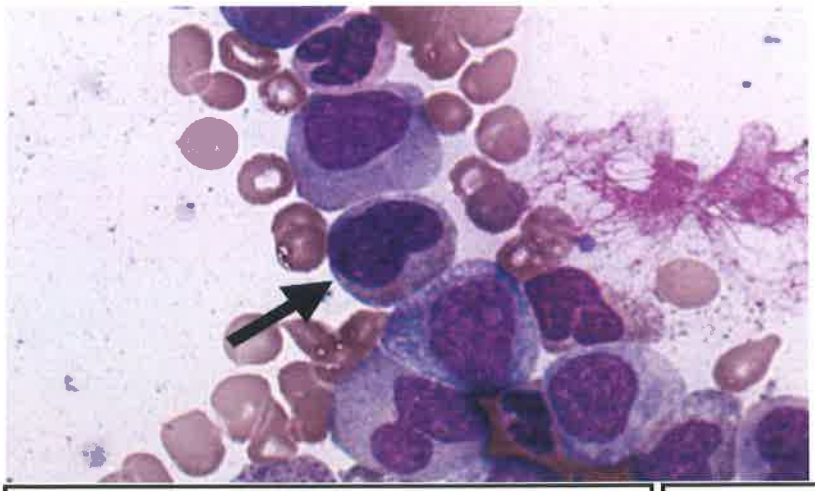


Identification	Participants No.	%	Evaluation
Eosinophil, any stage	1214	99.3	Educational
Immature or abnormal cell, would refer for identification	2	0.2	Educational
Basophil, any stage	1	0.1	Educational
Leukocyte containing Alder (Alder-Reilly) anomaly inclusion(s)	1	0.1	Educational
Mast cell	1	0.1	Educational
Neutrophil, metamyelocyte	1	0.1	Educational
Neutrophil necrobiosis (degenerated neutrophil)	1	0.1	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational

The arrowed cells are eosinophils, as correctly identified by 99.3% of participants. Eosinophils are similar in size to neutrophils (10 - 15 µm in diameter) but are readily distinguishable from neutrophils due to their characteristic coarse, orange-red cytoplasmic granules. Mature eosinophils typically have two or three nuclear lobes with condensed chromatin. Immature eosinophils (eg, eosinophil myelocytes) are rarely encountered in the blood but may be seen in some myeloid neoplasms. The arrowed cells include one eosinophil myelocyte with a round to ovoid nucleus (image on right) and one mature eosinophil with three nuclear lobes (image of left).

Note the presence of two basophils immediately adjacent to the arrowed mature eosinophil (see above image on left). In addition to a prominent left-shifted neutrophilia, increased basophils (absolute basophilia) is a near constant finding in untreated chronic myeloid leukemia.

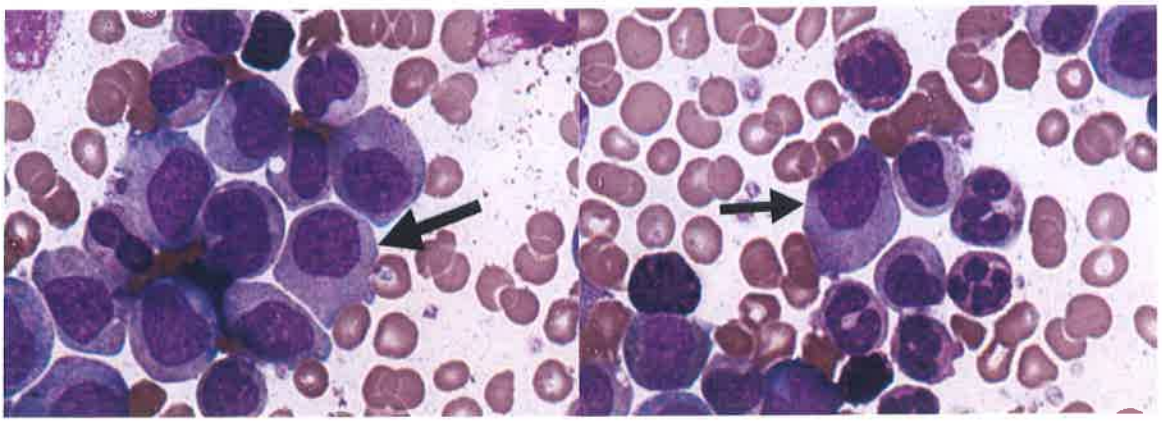
VPBS-28



Identification	Participants No.	%	Evaluation
Neutrophil, metamyelocyte	1083	88.5	Educational
Neutrophil, giant band or giant metamyelocyte	33	2.7	Educational
Neutrophil, segmented or band	24	2.0	Educational
Immature or abnormal cell, would refer for identification	19	1.6	Educational
Monocyte	19	1.6	Educational
Monocyte, immature (promonocyte, monoblast)	12	1.0	Educational
Neutrophil, myelocyte	10	0.8	Educational
Blast cell	3	0.3	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	3	0.3	Educational
Neutrophil with Pelger-Huët nucleus (acquired or congenital)	3	0.3	Educational
Lymphocyte, large granular	2	0.2	Educational
Malignant lymphoid cell (other than blast)	2	0.2	Educational
Neutrophil, promyelocyte, abnormal with/without Auer rod(s)	2	0.2	Educational
Lymphocyte	1	0.1	Educational
Mast cell	1	0.1	Educational
Megakaryocyte (normal, abnormal, or unclear fragment)	1	0.1	Educational
Metastatic tumor cell or tumor cell clump	1	0.1	Educational
Myeloblast with Auer rod	1	0.1	Educational
Neutrophil, polyploid	1	0.1	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	1	0.1	Educational
Nucleated red blood cell, normal or abnormal morphology	1	0.1	Educational

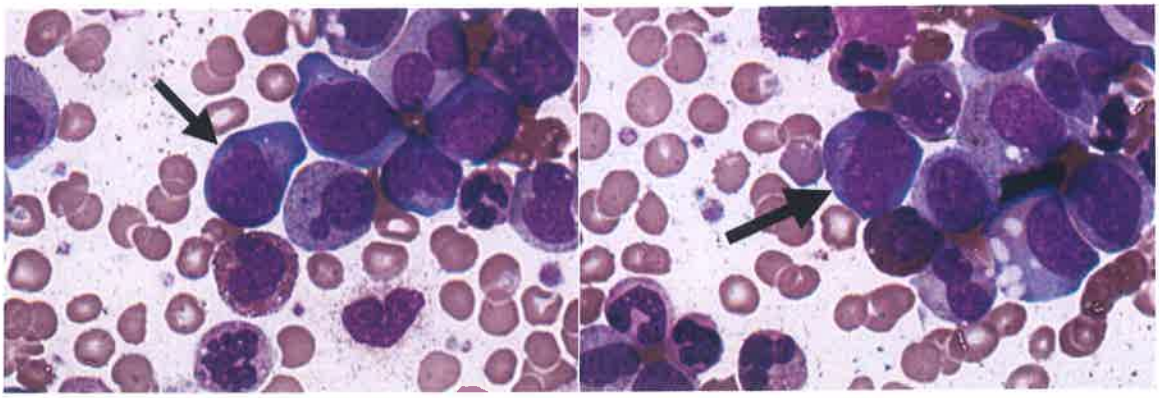
The arrowed cell is a metamyelocyte, as correctly identified by 88.5% of participants. Metamyelocytes are neutrophil precursors that are approximately 10 - 18 µm in diameter, which is slightly larger than mature neutrophils. They are round to oval with a nuclear-to-cytoplasmic ratio of 1.5:1 to 1:1. The cytoplasm contains numerous pale pink specific granules with few, if any, azurophilic primary granules. The nuclear chromatin is condensed and the nucleus is indented to less than half of the potential round nucleus (ie, the indentation is smaller than half of the distance to the farthest nuclear margin). Metamyelocytes may be seen in the blood in pathologic states or in response to stress or other reactive cause for a neutrophilic left shift.

VPBS-29



Identification	Participants No.	%	Evaluation
Neutrophil, myelocyte	1053	86.1	Educational
Neutrophil, promyelocyte	44	3.6	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	26	2.1	Educational
Monocyte, immature (promonocyte, monoblast)	24	2.0	Educational
Immature or abnormal cell, would refer for identification	23	1.9	Educational
Blast cell	13	1.1	Educational
Monocyte	10	0.8	Educational
Lymphocyte	6	0.5	Educational
Malignant lymphoid cell (other than blast)	6	0.5	Educational
Neutrophil, metamyelocyte	6	0.5	Educational
Neutrophil, promyelocyte, abnormal with/without Auer rod(s)	6	0.5	Educational
Lymphocyte, large granular	2	0.2	Educational
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	1	0.1	Educational
Metastatic tumor cell or tumor cell clump	1	0.1	Educational
Myeloblast with Auer rod	1	0.1	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	1	0.1	Educational

The arrowed cells are myelocytes, as correctly identified by 86.1% of participants. Myelocytes are neutrophil precursors at a maturational stage between promyelocytes and metamyelocytes. They are usually 10 - 18 µm in diameter and have a nuclear-to-cytoplasmic ratio of 2:1 to 1:1. The nucleus is often eccentric, lacks a nucleolus, and begins to demonstrate chromatin clumping. The side of the nucleus that is farther from the cell membrane often shows slight flattening, as seen in these two examples. Sometimes a clear space or hof is seen adjacent to the nucleus, indicating the location of the Golgi apparatus. The cytoplasm is relatively more abundant than in promyelocytes and contains both azurophilic and specific granules.



Identification	Participants No.	%	Evaluation
Blast cell	659	53.9	Educational
Neutrophil, promyelocyte	395	32.3	Educational
Myeloblast with Auer rod	61	5.0	Educational
Neutrophil, promyelocyte, abnormal with/without Auer rod(s)	52	4.3	Educational
Immature or abnormal cell, would refer for identification	26	2.1	Educational
Monocyte, immature (promonocyte, monoblast)	12	1.0	Educational
Lymphocyte, reactive lincludes plasmacytoid and immunoblastic forms)	5	0.4	Educational
Malignant lymphoid cell (other than blast)	4	0.3	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body	2	0.2	Educational
Babesia sp.	1	0.1	Educational
Basket cell/smudge cell	1	0.1	Educational
Lymphocyte, large granular	1	0.1	Educational
Metastatic tumor cell or tumor cell clump	1	0.1	Educational
Monocyte	1	0.1	Educational
Neutrophil, myelocyte	1	0.1	Educational
Neutrophil, segmented or band	1	0.1	Educational

The arrowed cells are immature myeloid cells with dispersed nuclear chromatin, visible nucleoli, moderately abundant cytoplasm, and cytoplasmic granules, best considered by the committee members to be examples of promyelocytes, as correctly identified by 32.3% of participants, in this patient with chronic myeloid leukemia. Additional answers deemed acceptable in this case include blasts (whether with or without Auer rods), as identified by 5.0% and 53.9% of participants, respectively, and abnormal promyelocytes, as identified by 4.3% of participants, though these were not the preferred responses. Immature or abnormal, would refer for identification, as identified by 2.1% of participants, is also an acceptable answer.

The possibility that these immature myeloid cells represent blasts was deemed acceptable in this case since blasts may occasionally contain a few granules in their cytoplasm. For proficiency testing purposes, "Neutrophil, Promyelocyte, Abnormal with/without Auer rod(s)" should be reserved specifically for the neoplastic cell in acute promyelocytic leukemia. Similarly, "Myeloblast with Auer rod", should be reserved for blast cells that show distinct rod-shaped cytoplasmic inclusions. However, the

VPBS-30, (cont.)

VPBS-30 Discussion, Cont'd:

committee members acknowledge that distinction between clustered granules and true Auer rod formation may be difficult to discern on a virtual blood smear and would accept this answer in this case. A small percentage of participants recognized the arrowed cells as immature or abnormal and appropriately recommended referral to a pathologist. Collectively, the arrowed cells were identified as normal or abnormal promyelocytes, blasts, or immature/abnormal cells and would refer by 97.6% of participants.

Promyelocytes are neutrophil precursors at a maturational stage between myeloblasts and myelocytes. They are round to oval cells that are typically 12 - 24 µm in diameter, which is slightly larger than myelocytes or myeloblasts. Promyelocytes have a nuclear-to-cytoplasmic (N:C) ratio of approximately 5:1 to 3:1, whereas the N:C ratio in myeloblasts is typically 7:1 to 5:1 (ie, promyelocytes usually have more cytoplasm then myeloblasts). The promyelocyte nucleus is round to oval, has fine chromatin, and often contains distinct nucleoli, similar to the nucleus of a myeloblast. However, promyelocytes characteristically have multiple distinct azurophilic (primary) granules, some of which are seen to overlie the nucleus. In this virtual smear it is difficult to evaluate for the presence or absence of granules overlying the nucleus, which contributes to the challenge in distinguishing whether these cells are promyelocytes or blasts. A paranuclear hof or cleared space may be present in promyelocytes. Promyelocytes are normally confined to bone marrow and are typically encountered in the blood only in pathologic states.

Case Presentation:
This peripheral blood smear is from a 58-year-old man now presenting with fever and fatigue. Laboratory data include: WBC = 535.0 x 10E9/L; RBC = 2.18 x 10E12/L; HGB = 6.5 g/dL; HCT = 19.5%; and PLT = 654 x 10E9/L.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Chronic Myeloid Leukemia

Epidemiology and Clinical Features

Chronic myeloid leukemia (CML) is the most common of the myeloproliferative neoplasms, with an incidence of 1 - 2 cases per 100,000 individuals per year. Although CML can occur at any age, it most commonly presents in adults in the age range of 20 - 60 years. Presenting clinical symptoms often include fatigue, malaise, weight loss, and/or night sweats, although nearly 50% of patients are asymptomatic and the diagnosis discovered when a CBC is performed for an unrelated reason. If untreated, the disease has a triphasic natural history, beginning with a chronic phase and then progressing through an accelerated phase into blast phase, which is transformation into acute leukemia. Approximately 95% of patients are in the chronic phase at the time of diagnosis.

Genetic Basis for CML

The key initiating event in all cases of CML is creation of a *BCR-ABL1* fusion gene in a bone marrow stem cell. In 90 - 95% of cases this abnormal gene arises due to a t(9;22) translocation between the *BCR* gene on chromosome 22 and the *ABL1* gene on chromosome 9, t(9;22)(q34;q11) resulting in formation of an abnormal chromosome 22 referred to as the Philadelphia (Ph) chromosome. In the remaining 5 - 10% of cases, the *BCR-ABL1* fusion gene is created through other mechanisms. The *BCR-ABL1* fusion gene results in production of an abnormal BCR-ABL1 protein that promotes unregulated proliferation of myeloid cells in the marrow, resulting in marked leukocytosis in the blood. Molecular analysis and/or fluorescence in situ hybridization (FISH) analysis are commonly used to detect the *BCR-ABL1* fusion gene. Chromosome analysis (karyotyping) can be used to detect the abnormal Ph chromosome or other structural chromosomal abnormalities.

Question 1: Which of the following abnormal fusion genes is present in all cases of CML?

A. *RUNX1-RUNX1T1*
B. *BCR-ABL1*
C. *PML-RARA*

Peripheral Blood Findings

The hallmark of chronic phase CML is marked neutrophilia with a prominent left shift. The WBC count typically ranges from 12 – 1,000 x 10E9/L with a median WBC at presentation of 80 x 10E9/L. The left shift includes a much larger proportion of earlier precursors than is typically seen in a reactive left shift, including frequent myelocytes (so-called "myelocyte bulge") and promyelocytes in addition to a small proportion of blasts. Blasts typically comprise less than 2% of WBCs in chronic phase and, by definition, must comprise < 10% of WBCs. The presence of 10 - 19% blasts is one criterion for accelerated phase, and ≥ 20% blasts defines blast phase. Absolute basophilia is present in most cases of CML, and there is sometimes eosinophilia, which can be helpful in the distinction from a reactive neutrophilia. There may be a mild monocytosis, but monocytes typically comprise < 3% of WBCs. Leukocytes at all stages of maturation are morphologically normal and lack evidence of dysplasia. The platelet count is usually normal or increased and may be markedly increased (> 1,000 x 10E9/L.

Question 2: In addition to increased numbers of left-shifted neutrophils, which of the following is seen in most cases of chronic phase CML at the time of initial diagnosis?

A. Absolute basophilia
B. Granulocytic dysplasia
C. Increased blasts (> 5%)
D. Monocytosis (> 5%)
E. Thrombocytopenia

Treatment and Prognosis

Essentially all patients diagnosed with CML are treated with a class of drugs known as tyrosine kinase inhibitors (TKIs), which function to block the activity of the mutant BCR-ABL1 protein. Examples of TKIs used for initial therapy are imatinib, dasatinib, and nilotinib. Targeted therapy with TKIs has revolutionized the management of CML and has replaced use of combination chemotherapy because TKIs substantially prolong the overall survival of CML patients and have fewer adverse side effects. Patients with CML treated with TKIs often have a lifespan comparable to non-CML patients, although the treatment is not curative. In addition, there are small numbers of patients who either do not respond to TKI therapy or develop drug resistance and progress to accelerated or blast phase. For these patients, conventional chemotherapy and allogeneic stem cell transplantation may be used to control or cure the leukemia.

Question 3: What is the typical treatment for a patient with newly diagnosed chronic phase CML?

A. Allogeneic stem cell transplantation
B. Combination chemotherapy
C. Tyrosine kinase inhibitor (TKI) therapy

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1. Saglio G, Jabbour E. First-line therapy for chronic phase CML: selecting the optimal BCR-ABL1-targeted TKI. *Leuk Lymphoma*. 2018;59(7):1523-1538.

2. Vardiman JW, Melo JV, Baccarini M, et al. Chronic myeloid leukemia, *BCR-ABL1*-positive. In Swerdlow SH, et al, (Eds.) *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, revised 4th ed. (pp. 30-36). IARC, 2017.

Answers to questions:

Question 1: B. *BCR-ABL1*

All cases of CML have an abnormal *BCR-ABL1* fusion gene, and in most cases this occurs due to a t(9;22) translocation between the *BCR* gene on chromosome 22 and the *ABL1* gene on chromosome 9. The *BCR-ABL1* fusion gene can be detected in CML patients using molecular or FISH studies. Chromosome analysis (karyotyping) can also be used to demonstrate the structural chromosomal rearrangement seen in most patients. Translocation t(15;17), *PML-RARA* is characteristic of acute promyelocytic leukemia. Translocation t(8;21), *RUNX1-RUNX1T1* is seen as a recurrent genetic abnormality in a subset of acute myeloid leukemia cases.

Question 2: A. Absolute basophilia

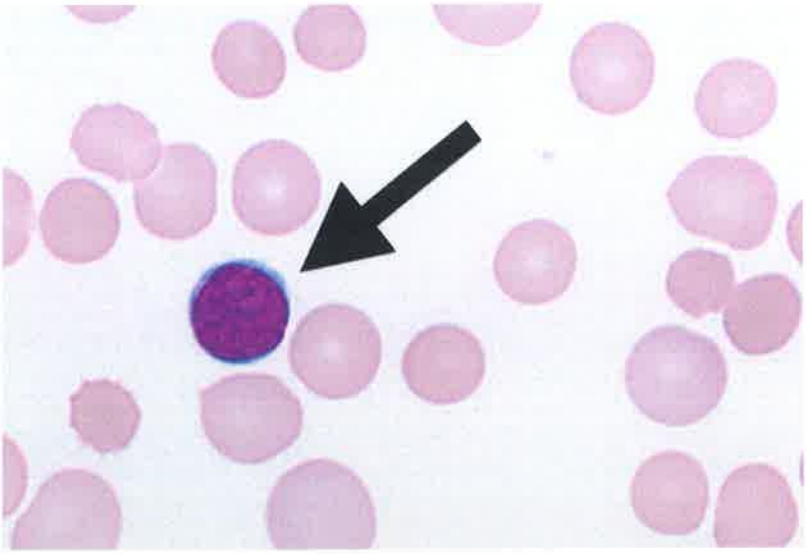
Most patients with CML have absolute basophilia at the time of diagnosis, and this can be a helpful clue to the diagnosis of CML because significant basophilia is typically absent in reactive neutrophilia with left shift. The diagnosis of CML requires demonstration of the *BCR-ABL1* fusion gene, which will be absent in reactive conditions. CML usually lacks granulocytic dysplasia and has a low proportion of monocytes (usually < 3%). Although blasts are often present, they typically comprise < 2% of leukocytes and, by definition, must comprise < 10% of leukocytes in chronic phase. Most patients have normal or increased platelet counts rather than thrombocytopenia.

Question 3: C. Tyrosine kinase inhibitor (TKI) therapy

Treatment with BCR-ABL1-directed tyrosine kinase inhibitors is the standard first-line therapy for all CML patients. This group of drugs has revolutionized the clinical management of CML such that, on therapy, many patients have normal or near-normal lifespans. Combination chemotherapy and allogeneic stem cell transplantation remain alternatives for patients who are unable to take TKIs, do not respond to TKI therapy, or develop drug resistance.

Cell Identification

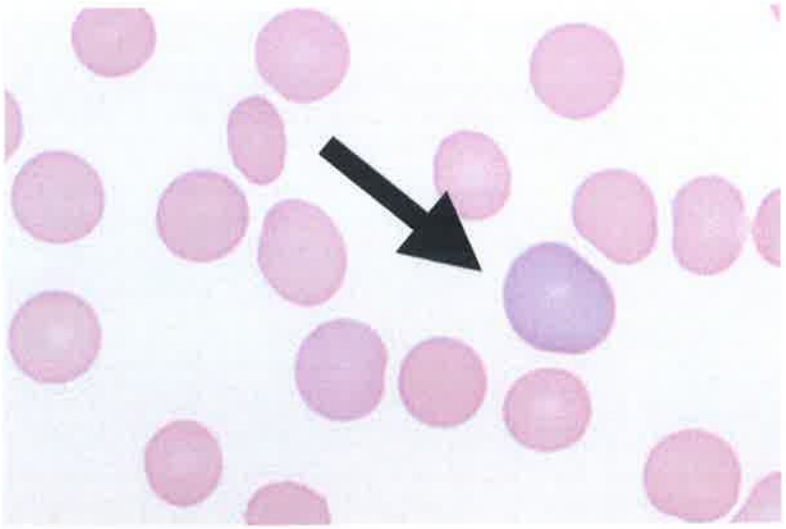
VPBS-32



Identification	Participants No.	Participants %	Evaluation
Lymphocyte	1200	98.0	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	11	0.9	Educational
Blast cell	6	0.5	Educational
Nucleated red blood cell, normal or abnormal morphology)	4	0.3	Educational
Bite cell (degmacyte)	1	0.1	Educational
Lymphocyte, large granular	1	0.1	Educational
Malignant lymphoid cell (other than blast)	1	0.1	Educational
Neutrophil, segmented or band	1	0.1	Educational

The arrowed cell is a normal lymphocyte, as correctly identified by 98.0% of the participants. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 µm with an N:C ratio ranging from 5:1 to 2:1. Most lymphocytes have round to oval nuclei that may be slightly indented or notched. The chromatin is diffusely dense or coarse. Nucleoli are not visible, although some cells may exhibit a small, pale chromocenter that may be mistaken for a nucleolus. Most lymphocytes have a scant amount of pale blue to moderately basophilic, agranular cytoplasm.

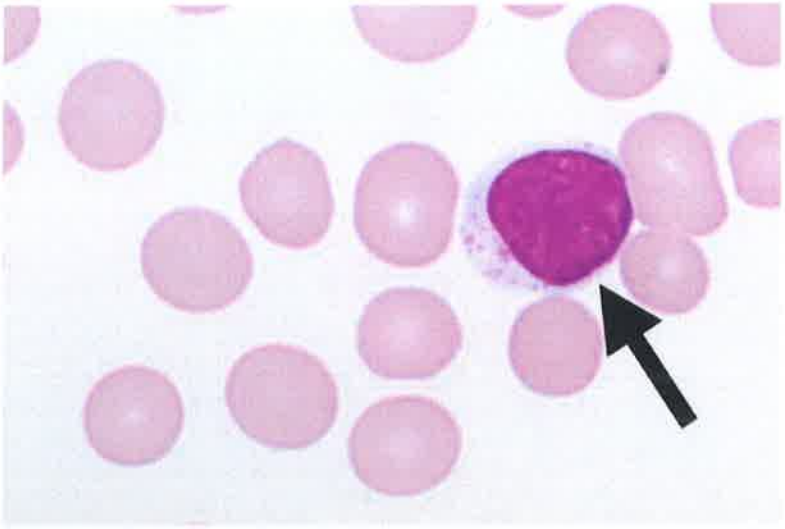
VPBS-33



Identification	Participants No.	%	Evaluation
Polychromatophilic (non-nucleated) red blood cell	1206	98.5	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	16	1.3	Educational
Hypochromasia	2	0.2	Educational
Eosinophil, any stage	1	0.1	Educational

The arrowed cell is a polychromatophilic erythrocyte, as correctly identified by 98.5% of the participants. These are non-nucleated red cells that have been recently released from the bone marrow. They are larger than a typical mature erythrocyte and stain homogeneously pink-gray or pale purple with Romanowsky or Wright-Giemsa stain. This is due to the presence of small amounts of RNA in the cytoplasm, in addition to the abundant hemoglobin. These cells can be stained as reticulocytes and enumerated by using supravital stains, such as new methylene blue. Presence of many polychromatophilic red blood cells may raise the MCV as well as the RDW in the CBC data, as in this case.

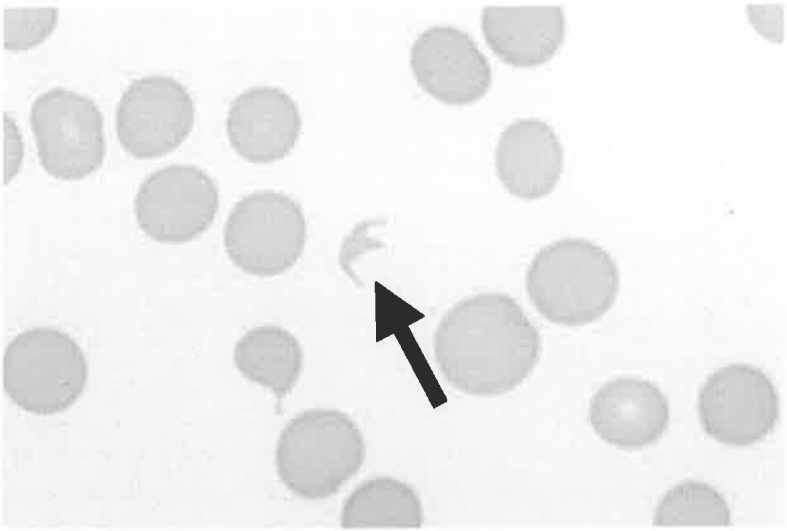
VPBS-34



Identification	Participants No.	%	Evaluation
Lymphocyte, large granular	1085	88.6	Educational
Lymphocyte	92	7.5	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	24	2.0	Educational
Neutrophil, myelocyte	9	0.7	Educational
Immature or abnormal cell, would refer for identification	3	0.2	Educational
Leukocyte containing Alder (Alder-Reilly) anomaly inclusion(s)	2	0.2	Educational
Monocyte	2	0.2	Educational
Monocyte, immature (promonocyte, monoblast)	2	0.2	Educational
Neutrophil, promyelocyte	2	0.2	Educational
Blast cell	1	0.1	Educational
Myeloblast with Auer rod	1	0.1	Educational
Neutrophil, metamyelocyte	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 large granular lymphocyte (LGL), as correctly identified by 88.6% of the participants. LGLs are medium to large cells with round nuclei and dense chromatin that lack nucleoli. The cytoplasm is moderate to abundant and clear or lightly basophilic, and contains several coarse, unevenly distributed, small azurophilic granules. These cells are found in small numbers in blood smears (10 - 15%) from normal individuals. Cell surface marker studies show that these cells are natural killer cells or suppressor/cytotoxic T lymphocytes. LGLs may be increased in association with reactive conditions such as viral infection or autoimmune disease; However, they may also be neoplastic, particularly when seen as a large proportion of lymphocytes and associated with cytopenias, particularly neutropenia. Special studies (cell surface markers and molecular genetic studies) may be required to distinguish reactive from neoplastic conditions. 7.5% of participants identified the cell as a lymphocyte and while this is correct, the more specific choice and accurate identification as an LGL is the preferred response.

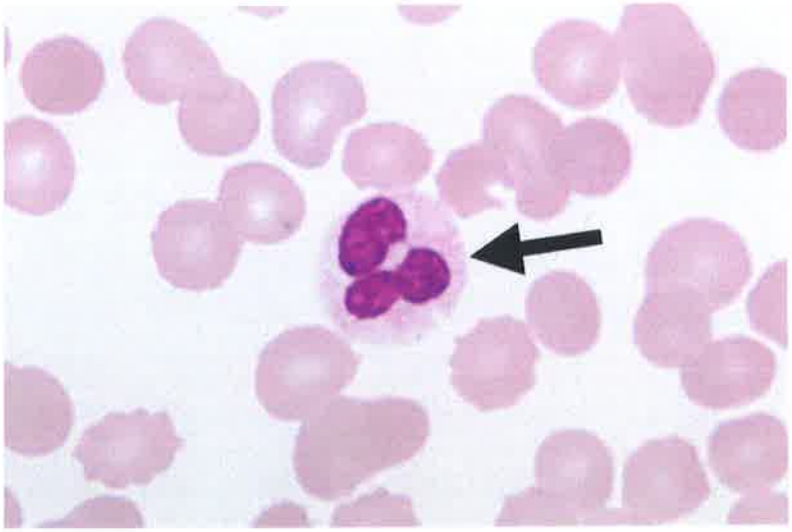
VPBS-35



Identification	Participants No.	%	Evaluation
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	1179	96.2	Educational
Acanthocyte (spur cell)	28	2.3	Educational
Bite cell (degmacyte)	15	1.2	Educational
Bacteria (cocci or rod), extracellular	1	0.1	Educational
Echinocyte (burr cell, crenated cell)	1	0.1	Educational
Neutrophil, myelocyte	1	0.1	Educational

The arrowed cell is a fragmented red blood cell or schistocyte, as correctly identified by 96.2% of the participants. Fragmented red cells have undergone rips and tears when obstructed by fibrin strands in the microcirculation or undergo fragmentation against unyielding structures in the macrocirculation. Fragments resulting from such trauma reseal by fusion of opposing ends and persist in the circulation, presumably for a short time. Fragmented red cells include helmet cells, keratocytes (horn cells), triangulocytes and the more general term, schistocytes. A zone of central pallor is rarely present in fragmented cells. Fragmented cells are seen in severe burns, microangiopathic hemolytic anemias, in patients with prosthetic cardiac valves or severe valvular stenosis, malignant hypertension, or other mechanical trauma to the cell (march hemoglobinuria). When present in large numbers, they may cause the MCV to fall into the microcytic range or interfere with platelet enumeration.

VPBS-36



Identification	Participants No.	%	Evaluation
Neutrophil, segmented or band	1172	95.7	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	23	1.9	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	10	0.8	Educational
Neutrophil with Pelger-Huët nucleus (acquired or congenital)	9	0.7	Educational
Neutrophil with hypersegmented nucleus	3	0.2	Educational
Neutrophil, polyploid	2	0.2	Educational
Eosinophil, any stage	1	0.1	Educational
Lymphocyte	1	0.1	Educational
Lymphocyte, large granular	1	0.1	Educational
Monocyte, immature (promonocyte, monoblast)	1	0.1	Educational
Neutrophil, promyelocyte	1	0.1	Educational
Platelet satellitism	1	0.1	Educational

The arrowed cell is a segmented neutrophil, as correctly identified by 95.7% of the participants. The neutrophil is the most mature cell in the neutrophil series and the predominant white cell in blood. Neutrophils are 10 to 15 µm in size, are round to oval in shape, and have pale pink cytoplasm with specific granules. The N:C ratio is 1:3 and the nuclear chromatin is condensed. The nucleus is segmented or lobated (two to five lobes normally). The lobes are connected by a thin filament that contains no internal chromatin, giving it the appearance of a solid, thread-like dark line. The presence of these thread-like filaments is the basis for distinguishing the segmented neutrophil from the band neutrophil.

Case Presentation:

This peripheral blood smear is from a 62-year-old man presenting with anemia and iron overload syndrome. Laboratory data include: WBC = 8.6 x 10E9/L; RBC = 2.83 x 10E12/L; HGB = 8.5 g/dL; HCT = 25.5%; MCV = 107 fL; MCHC = 33.3 g/dL; PLT = 40 x 10E9/L; and RDW = 27%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: T-Large granulocytic leukemia (T-LGL)

T-LGL leukemia is a clonal T-cell lymphoproliferative disorder presenting in adults with a median age at diagnosis of 60 years. It is associated with autoimmune diseases such as rheumatoid arthritis. Patients often present with cytopenias, such as neutropenia, and splenomegaly. Recently, an association between T-LGL proliferations and B-cell lymphoproliferative disorders and plasma cell dyscrasias has been reported.

Question 1: T-LGL leukemia patients present with:

A. Fever
B. Lymphadenopathy
C. Neutropenia
D. Skin rash
E. Thrombocytosis

Lymphocytosis is usually, but not always, present and LGLs are increased in the peripheral blood. These are cells with mature chromatin and moderate amounts of cytoplasm containing coarse azurophilic granules. Bone marrow examination shows an interstitial lymphocytosis with a sinusoidal pattern. Flow cytometry shows the cells most frequently have a cytotoxic T-cell phenotype, with expression of CD2, CD3, CD5, CD7, CD8, CD16, and CD57. Cells are usually αβ T-cells but cases of γδ T-LGL leukemia do occur.

Question 2: An appropriate immunophenotype for T-LGL leukemia is:

A. CD2+/CD3+/CD4+/CD5+/CD8-/TIA1-
B. CD2+/CD3+/CD4-/CD8+/CD16+/TIA1+
C. CD3-/CD19-/CD4-/CD8+/CD16+/CD56
D. CD3+/CD19+/CD4-/CD8-/CD16+/CD57+

Molecular genetic studies will demonstrate T-cell clonality. Activating mutations in *STAT3* (up to 70% of cases) and *STAT5B* have been identified, confirming the neoplastic mature of T-LGL leukemia. *STAT3* mutations have also been associated with rheumatoid arthritis and response to methotrexate. T-LGL leukemia follows an indolent course and morbidity is related to cytopenias such as neutropenia and less commonly red cell aplasia. The degree of cytopenias drives decisions on initiating immunosuppressive therapy.

Question 3: Common recurrent molecular genetic alterations in T-LGL include:

A. *ALK* translocation
B. *BCL2* translocation
C. *RHOA* mutation
D. *STAT1* mutation
E. *STAT3* mutation

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5. Rajala HL, Porkka K, Maciejewski JP, Loughran TP Jr, Mustjoki S. Uncovering the pathogenesis of large granular lymphocytic leukemia-novel STAT3 and STAT5b mutations. *Ann Med.* 2014 May;46(3):114-22.

Answers to Questions:

Question 1: C. Neutropenia.
Patients with T-LGL leukemia present with cytopenias such as neutropenia and splenomegaly, often with a history of autoimmune disease. They do not typically have fever, skin rash, lymphadenopathy or thrombocytosis.

Question 2: B. CD2+/CD3+/CD4-/CD8+/CD16+/TIA1+.
T-LGL leukemia is a neoplasm of cytotoxic CD8+ T-cells, with expression of pan-T-cell markers such as CD2, CD3, CD5 and CD7 and the NK-associated marker CD16. CD57 is also expressed but CD56 is usually not. The cytotoxic molecule TIA-1 is often expressed. B-cell markers such as CD19 are, of course, not expressed.

Question 3: E. *STAT3* mutation.
Recent molecular genetic studies have identified activating mutations in *STAT3*. *STAT1* is not mutated in T-LGL leukemia. *RHOA* mutation, *ALK* translocation, and *BCL2* translocation are characteristically seen in angioimmunoblastic T-cell lymphoma, anaplastic large cell lymphoma, and follicular lymphoma, respectively.

COLLEGE of AMERICAN PATHOLOGISTS

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