

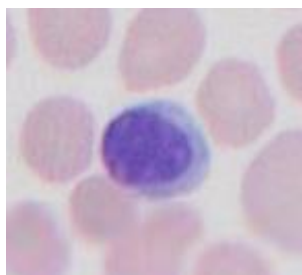
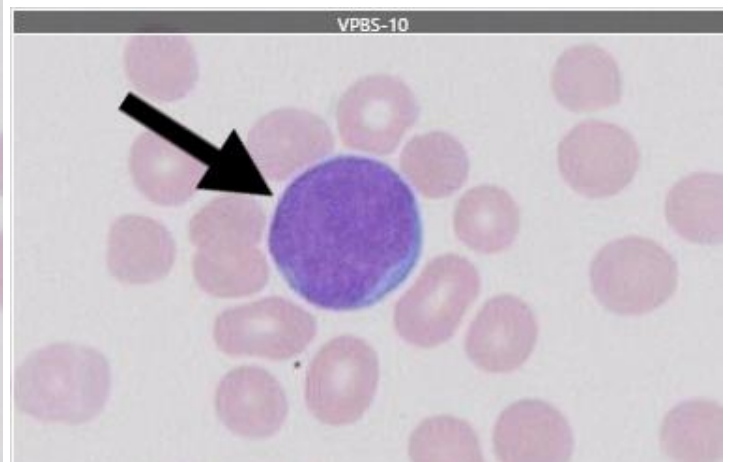
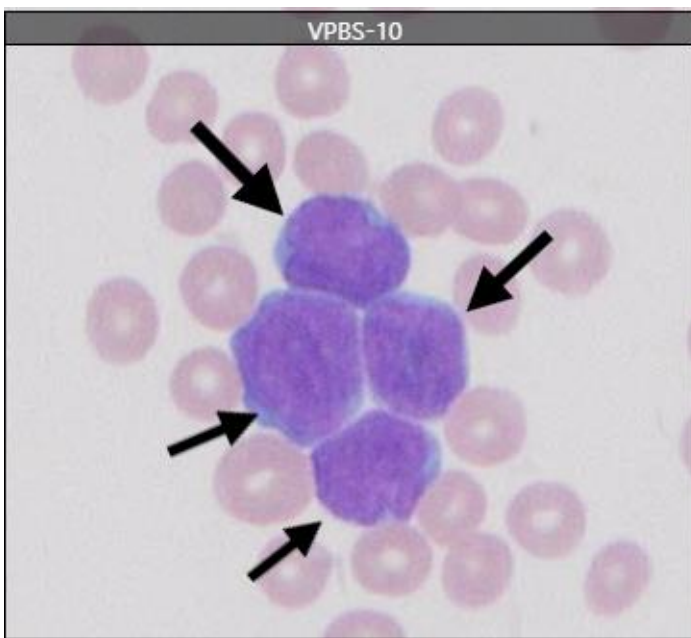
Clinical History for VPBS-07 – VPBS-12

This peripheral blood smear is from an 11-year-old boy who presented with fatigue and shortness of breath. Laboratory data include: WBC = $89.5 \times 10^9/L$; RBC = $2.11 \times 10^{12}/L$; HGB = 5.4 g/dL; HCT = 17.2%; MCV = 82 fL; and PLT = $17 \times 10^9/L$. Identify the arrowed object(s) on each whole slide image.

Is this going to be normal or abnormal? Why?

Yes, chances are this slide in general is going to be abnormal. Look at the WBC, RBC, and Platelet counts.

Therefore, Identify this cell.

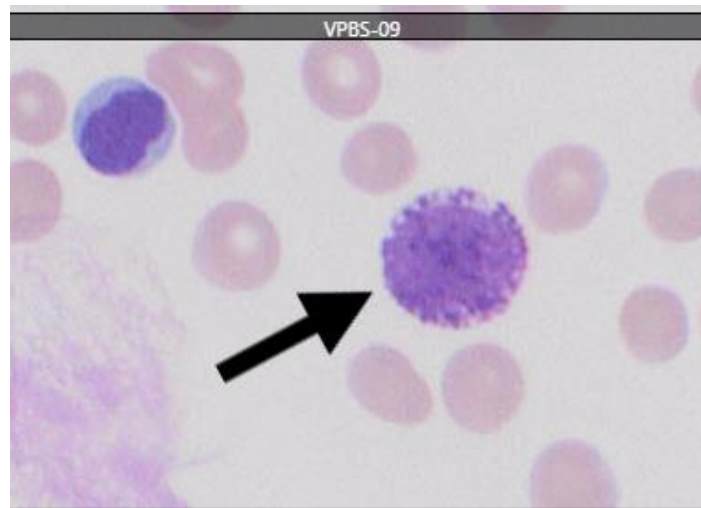


Normal Lymphocyte →

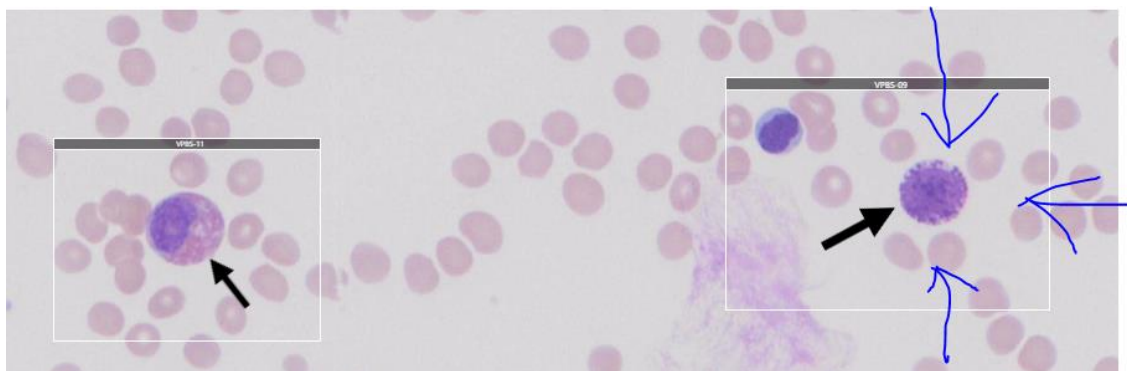
Yes, we got this image correct. Hopefully you were aware that these were blast cells. There is one normal lymphocyte shown for comparison purposes. Notice how much larger the blasts are compared to the normal lymphocyte. Notice, too, how abnormal the chromatin of the nucleus is, the presence of the nucleoli, and the dark blue cytoplasm. We are getting much better at identifying these cells.

Now, having a tiny bit of knowledge about this sample,
IDENTIFY THE FOLLOWING CELLS.

CELL TYPE 1



Does it help if I expand the image to include other cells?



What did you say? Be honest.

Did you call this a Large Granular Lymphocyte or did you call this a Basophil or did you call this a variant lymph or myelocyte or blast?

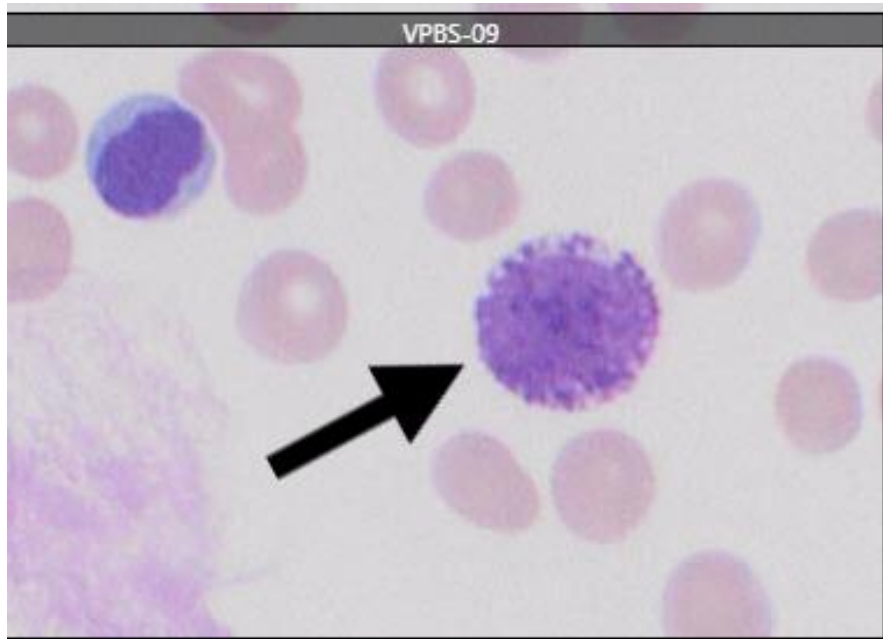
Here's a hint:

Just because the overall differential is abnormal does not mean every cell is meant to be abnormal.

Second hint:

Look beyond just the nucleus. Look at the cytoplasm. Look at the size and color of granules you can see in that cytoplasm.

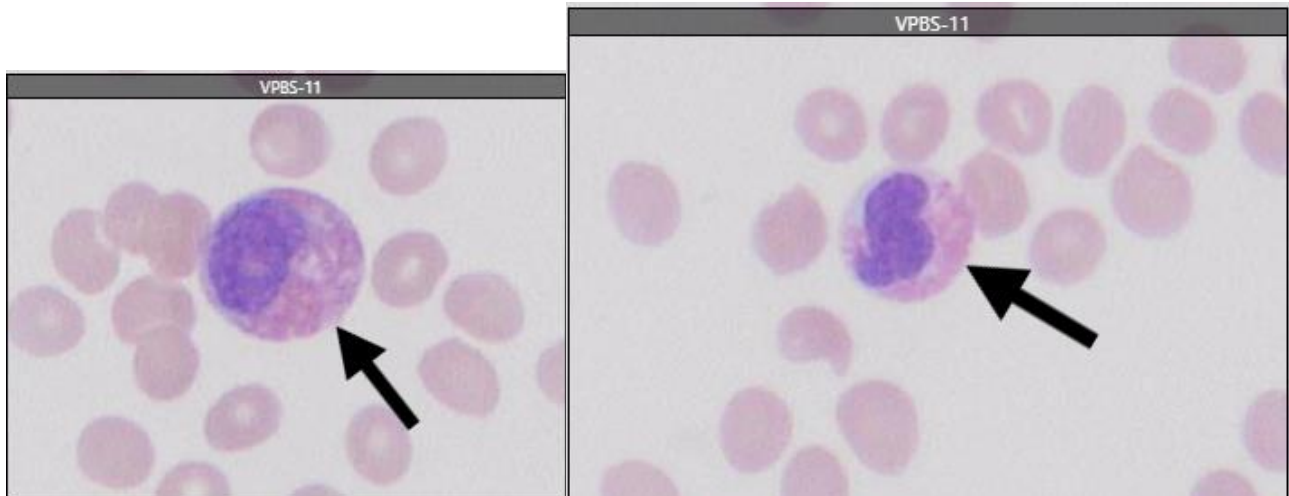
This cell is supposed to be a basophil.



If you look closely at the cytoplasm, the granules are very large and basophilic. However, you can also see some very fine azurophilic (pink) granules. The nucleus looks immature, yes. Without the granules, this would be classified as a myelocyte. However, because those large basophilic granules are formed and prominent, this cell is classified as a basophil.

Now, having seen that one, can you identify the next cells?

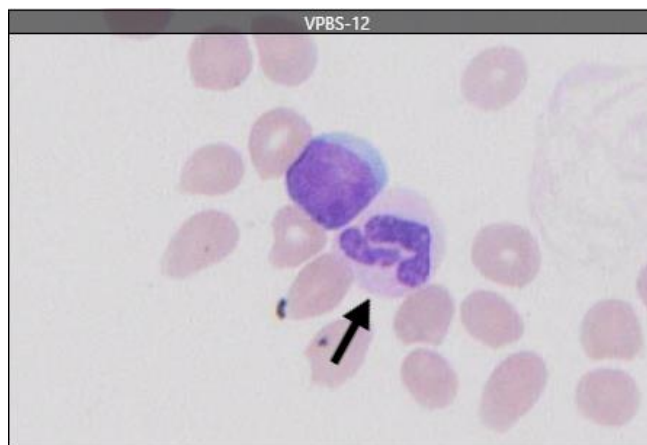
IDENTIFY THE FOLLOWING CELL
CELL TYPE 2



Hint: We made a similar mistake on this one in overlooking the cytoplasm and focusing too much on the nucleus of these cells.

Remember that the previous cell could have been a myelocyte if not for the prominent granules.

Here is an image of a normal segmented neutrophil as a bonus.



So what type of cells are the two above?

Hopefully you used the same logic as the first cell because the two cells for this one are **EOSINOPHILS!!!**

Even though the nuclei look to be immature (seemingly a band for one and a meta for the other), the bright orange/pink granules that are enlarged is the primary clue/feature. This makes even more sense when those cells are compared to the normal neutrophil at the bottom of the page.

Sometimes we can focus too closely on the single cell CAP is asking us to identify. We always must take the whole picture into account. We must find the cell types we know are “normal” and compare a questionable cell to the normal one. Just because the slide is abnormal overall, not every cell is necessarily abnormal. Also, even though the nucleus may be somewhat immature, we should be calling cells with prominent granules by their proper identification of basophil or eosinophil.

In general, I don't think this is a huge issue here. Hopefully you get the takeaways above, though.

There is a small quiz for this one. I made it quick. Let the granules be your guide! Please go into the quiz, 6 questions, and then mark that you have read and understand the contents of this document! The following pages are from the CAP summary for these images. The link to the slide is here:

<https://www.digitalscope.org/ViewerUI/?SlideId=d4b36b28-536a-4ba1-9bb6-7103320cc896>

Thank you for your time and effort!

VPBS-07

Clinical History for VPBS-07 – VPBS-12

This peripheral blood smear is from an 11-year-old boy who presented with fatigue and shortness of breath. Laboratory data include: WBC = 89.5 x 10E9/L; RBC = 2.11 x 10E12/L; HGB = 5.4 g/dL; HCT = 17.2%; MCV = 82 fL; and PLT = 17 x 10E9/L. Identify the arrowed object(s) on each whole slide image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Please click on the hyperlink below to view the DigitalScope images for this case.

<https://www.digitalscope.org/LinkHandler.axd?LinkId=0807f1f5-8064-4d98-814d-94b248652bbb>

To access the online Hematology Glossary, please click the hyperlink below:

<https://documents.cap.org/documents/cap-hematology-and-clinical-microscopy-glossary.pdf>

Summary of Participant Survey Results

The following is a statistical summary of all results submitted by participating laboratories. These are provided to allow participants to see their responses in the context of their peers. These results may identify findings or topics for further education or review. Survey results are not intended to represent the correct or desired responses for proficiency testing purposes and the SD and CV% should not be interpreted as acceptable reporting limits. Participants are encouraged to review discrepant results with their medical director.

WBC Differential - %	N	MEAN	SD	CV%	MEDIAN	MIN	MAX
Neutrophils (segmented or bands)	1206	2.3	1.2	54.0	2	0	6
Lymphocytes	1212	30.2	26.4	87.7	21	0	99
Lymphocytes, reactive	571	1.3	3.2	*	0	0	19
Monocytes	757	1.0	1.2	*	1	0	5
Eosinophils	681	0.5	0.6	*	0	0	2
Basophils	607	0.3	0.4	*	0	0	1
Metamyelocytes	507	0.0	0.2	*	0	0	1
Myelocytes	505	0.0	0.2	*	0	0	1
Promyelocytes	491	0.0	0.0	0.0	0	0	0
Blasts	1118	62.9	29.8	47.4	73	0	99
nRBC/100 WBC	579	0.2	0.4	*	0	0	1

WBC Differential - 10E9/L**	N	MEAN	SD	CV%	MEDIAN	MIN	MAX
Neutrophils (segmented or bands)	1107	1.944	0.997	51.3	1.79	0.00	4.50
Lymphocytes	1140	26.773	23.759	88.7	18.25	0.00	88.61
Lymphocytes, reactive	534	1.054	2.735	*	0.00	0.00	16.11
Monocytes	709	0.796	0.923	*	0.89	0.00	3.60
Eosinophils	644	0.417	0.541	*	0.00	0.00	1.80
Basophils	575	0.244	0.400	*	0.00	0.00	0.90
Metamyelocytes	478	0.032	0.167	*	0.00	0.00	0.90
Myelocytes	475	0.026	0.152	*	0.00	0.00	0.90
Promyelocytes	465	0.000	0.000	0.0	0.00	0.00	0.00
Blasts	1045	56.007	27.023	48.2	65.34	0.00	92.34

*When low results are reported on an analyte, a high coefficient of variance (CV%) may result. When the mean value is very low, the CV% may be exaggerated.

**Please see discussion on "Calculating Absolute Counts" that appears in this PSR.

VPBS-07, cont'd.

Other cells: All cells not listed on result form and cells not differentiated by your laboratory	N = 237
Cells not listed/differentiated	Freq
Blasts	73
Basket/smudge cell	27
Lymphoma/malignant lymphoid cell/immature lymphocyte	27
Myeloid precursors (promyelocyte, myelocyte, metamyelocyte)	16
Atypical/Reactive lymphocyte	2
Would refer for identification	92

Platelet Estimate	N = 1258	
Intended Response: Decreased platelets	Freq	%
Decreased platelets	1250	99.4
Adequate/normal platelets	6	0.5
Increased platelets	2	0.2

Note: For proficiency testing purposes only, platelet counts of < 140 x 10E9/L are considered decreased and > 450 x 10E9/L are considered increased.

Red Cell Morphology	Total Responses	Total Responses	Total Unique Kits
	N = 2511	N = 2511	N = 1147
	Freq	% Total Response	% Unique Kits
Microcyte (with increased central pallor)	464	18.5	40.5
Erythrocyte, normal	428	17.0	37.3
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	403	16.0	35.1
Spherocyte	335	13.3	29.2
Ovalocyte (elliptocyte)	242	9.6	21.1
Polychromatophilic (non-nucleated) red blood cell	160	6.4	13.9
Teardrop cell (dacrocyte)	156	6.2	13.6
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	149	5.9	13.0
Nucleated red blood cell, normal or abnormal morphology	40	1.6	3.5
Red blood cell agglutinates	26	1.0	2.3
Bite cell (degmacyte)	20	0.8	1.7
Howell-Jolly body	18	0.7	1.6
Stomatocyte	17	0.7	1.5
Target cell (codocyte)	15	0.6	1.3
Acanthocyte (spur cell)	12	0.5	1.0

Note: The "total number of responses" is how many responses received from all of the kits. The "unique kits" is how many kits were returned with any response for Red Cell morphology.

VPBS-07, cont'd.

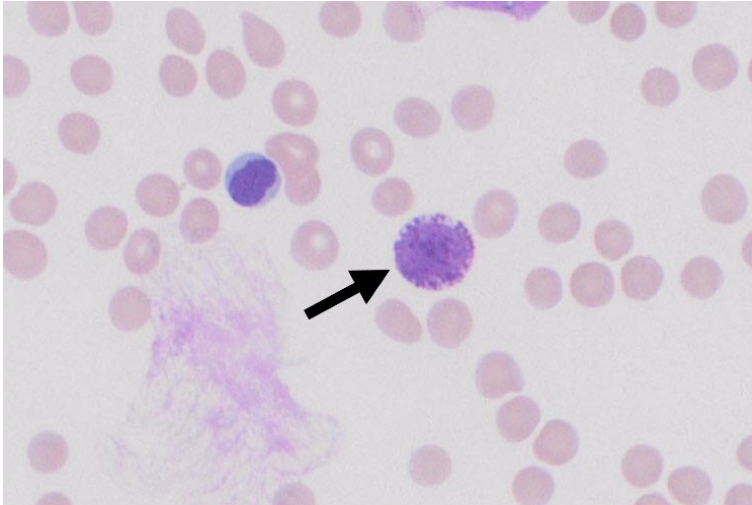
Red Cell Morphology, cont'd	Total Responses N = 2511	Total Responses N = 2511	Total Unique Kits N = 1147
	Freq	% Total Response	% Unique Kits
Erythrocyte with overlying platelet	8	0.3	0.7
Rouleaux	6	0.2	0.5
Blister cell/Prekeratocyte	4	0.2	0.3
Echinocyte (burr cell, crenated cell)	4	0.2	0.3
Basophilic stippling (coarse)	2	0.1	0.2
Immature or abnormal cell, would refer for identification	2	0.1	0.2

Note: The "total number of responses" is how many responses received from all of the kits. The "unique kits" is how many kits were returned with any response for Red Cell morphology.

Committee Comments on CBC and Peripheral Blood Whole Slide Image

Examination of the peripheral blood smear reveals a marked leukocytosis. The majority of the cells (> 95% of all leukocytes) are small to medium sized blasts. The blasts have fine chromatin, irregular nuclear contours, inconspicuous nucleoli, and scant agranular cytoplasm. Basket cells are present. Normal leukocytes are decreased in numbers. Red cells show anemia with mild anisopoikilocytosis. There is severe thrombocytopenia; platelets otherwise show normal size distribution and granulation.

VPBS-09



Identification	Participants		Evaluation
	Freq	%	
Basophil, any stage	1139	89.8	Educational
Platelet, giant (macrothrombocyte)	53	4.2	Educational
Neutrophil, promyelocyte	30	2.4	Educational
Neutrophil, myelocyte	11	0.9	Educational
Lymphocyte, large granular	6	0.5	Educational
Immature or abnormal cell, would refer for identification	5	0.4	Educational
Mast cell	5	0.4	Educational
Malignant lymphoid cell (other than blast)	4	0.3	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	4	0.3	Educational
Basophilic stippling (coarse)	2	0.2	Educational
Leukocyte containing Alder (Alder-Reilly) anomaly inclusion(s)	2	0.2	Educational
Stain precipitate	2	0.2	Educational
Eosinophil, any stage	1	0.1	Educational
Neutrophil, metamyelocyte	1	0.1	Educational
Neutrophil, promyelocyte, abnormal with/without Auer rod(s)	1	0.1	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	1	0.1	Educational
Platelet, hypogranular	1	0.1	Educational

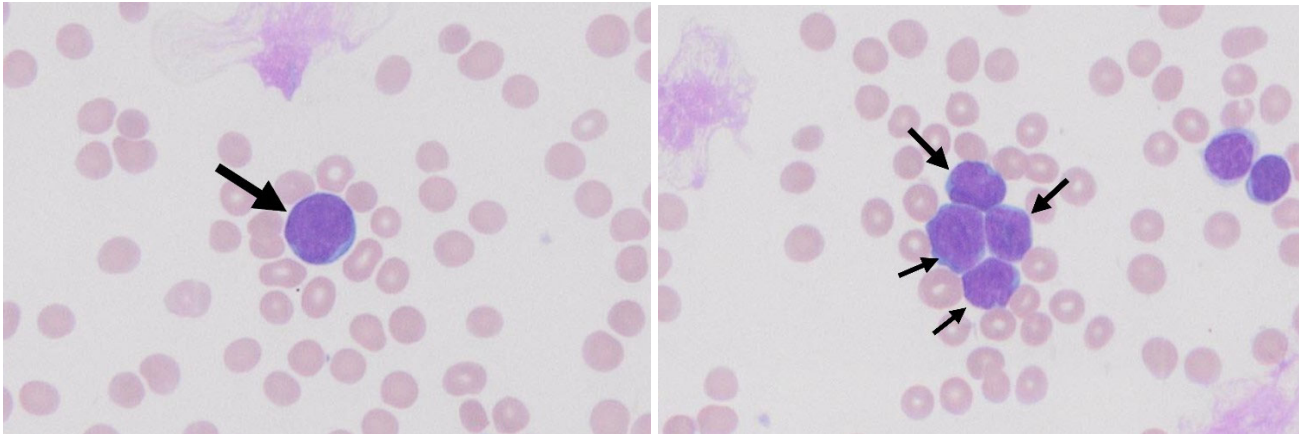
The arrowed cell is a basophil, as correctly identified by 89.8% of participants. Basophils have a maturation sequence analogous to neutrophils. At the myelocyte stage, when specific granules begin to develop, basophil precursors can be identified. All basophils, from the basophilic myelocyte to the mature segmented basophil, are characterized by the presence of numerous coarse and densely stained granules of varying sizes and shapes. The granules are larger than the granules of neutrophils and most are roughly spherical. The granules are typically blue-black, but some may be purple-red when stained using Wright-Giemsa preparations. The granules are unevenly distributed and frequently overlay and obscure the nucleus. Basophils are comparable in size to neutrophils, ie, 10 to 15 μm in diameter, and the nuclear-cytoplasm (N:C) ratio ranges from 1:2 to 1:3.

VPBS-09, cont'd

4.2% of participants incorrectly identified the arrowed cell as a giant platelet. The term giant platelet is used for a platelet that is larger than an average red blood cell. They have fine azurophilic granules, or the granules may fuse into giant forms. However, they do not have numerous blue-black coarse granules as seen in a basophil. Giant platelets, unlike basophils, do not have a nucleus.

2.4% of participants incorrectly identified the arrowed cell as a promyelocyte. A promyelocyte's N:C ratio usually ranges from 5:1 to 3:1. The nucleus is round to oval, has fine chromatin, and contains distinct nucleoli. The cytoplasm is basophilic and contains multiple distinct azurophilic (primary) granules. A paranuclear hof or cleared space is typically present. Unlike a promyelocyte, a basophil has numerous blue-black coarse granules, no paranuclear hof, and is smaller in size.

VPBS-10



Identification	Participants		Evaluation
	Freq	%	
Blast cell	1077	84.9	Educational
Malignant lymphoid cell (other than blast)	75	5.9	Educational
Immature or abnormal cell, would refer for identification	47	3.7	Educational
Lymphocyte	34	2.7	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	27	2.1	Educational
Lymphocyte, large granular	4	0.3	Educational
Metastatic tumor cell or tumor cell clump	2	0.2	Educational
Monocyte	1	0.1	Educational
Neutrophil, metamyelocyte	1	0.1	Educational

The arrowed cells are blast cells, as correctly identified by 84.9% of participants. Distinguishing between myeloid and lymphoblasts can only be done reliably by immunophenotyping. In this case, the blasts were determined to be T-lymphoblasts. Lymphoblasts are the most immature cells of the lymphoid series. They are most commonly seen in acute lymphoblastic leukemia (ALL) and lymphoid blast crisis of chronic myeloid leukemia (CML). These round-to-oval cells range in size from 10 to 20 μm . The N:C ratio varies from 7:1 to 4:1. Morphologically, lymphoblasts are variable in appearance, even at times within a single case. At one end of the spectrum, are small lymphoblasts with dense, but not clumped chromatin, inconspicuous or absent nucleoli, and extremely scanty cytoplasm. At the other end are large lymphoblasts with finely dispersed chromatin, variable numbers of distinct nucleoli, and moderate amounts of cytoplasm, closely resembling myeloblasts. The nuclear contours of lymphoblasts range from round to convoluted. The cytoplasm is typically slightly to moderately basophilic and is usually agranular. Auer rods are absent. As lymphoblasts are quite variable in appearance, it is often impossible to correctly classify an individual cell based on the morphology alone. Lymphoblasts can be indistinguishable from other types of blasts and lymphoma cells. For purposes of proficiency testing, one should identify individual cells exhibiting this immature type of morphology as blast cells.

VPBS-10, cont'd

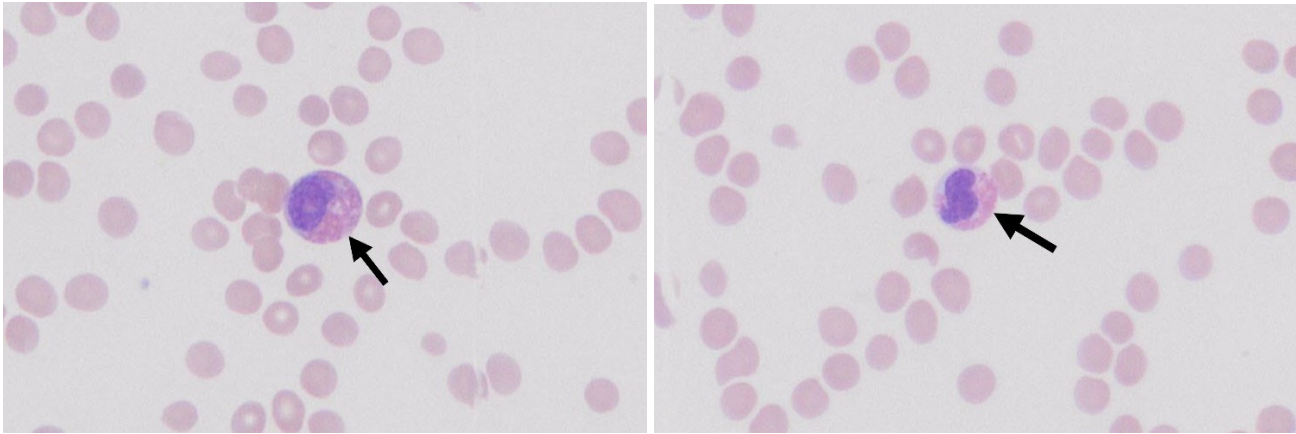
5.9% of participants incorrectly identified the arrowed cells as malignant lymphoid cells other than blasts. T-lymphoblastic lymphoma cells are considered blasts when circulating since the distinction between T-lymphoblastic leukemia and lymphoma is based on location. Other lymphoma cells can exhibit a variety of appearances, depending on the lymphoma subtype, and definitive diagnosis can be difficult. These cells can exhibit a variety of sizes, shapes, and nuclear and cytoplasmic characteristics. Cell size ranges from 8 to 30 μm , and the N:C ratio varies from 7:1 to 3:1. In children, the most likely circulating malignant lymphoid cell would be Burkitt lymphoma. Burkitt lymphoma cells are medium-to-large cells (10 to 25 μm) with a round-to-oval nucleus and moderately coarse chromatin with one or more prominent nucleoli. The cytoplasm is moderately abundant, deeply basophilic, and it often contains numerous small and uniformly round vacuoles.

3.7% of participants identified the arrowed cells as immature/abnormal cells would refer for identification. This is considered an acceptable answer if your laboratory routinely sends the cells in question to an outside laboratory with another CLIA number.

2.7% of participants incorrectly identified the arrowed cells as lymphocytes. Lymphocytes exhibit a range of normal morphology. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 μm with an N:C ratio ranging from 5:1 to 2:1. Most lymphocytes have round-to-oval nuclei that may be slightly indented or notched. The chromatin is diffusely dense or coarse and clumped. Nucleoli are not visible, although some cells may exhibit a small, pale chromocenter that may be mistaken for a nucleolus. Most lymphocytes have a scant amount of pale blue to moderately basophilic, agranular cytoplasm. The lymphoblasts in this case have fine chromatin, irregular nuclear contours, and scant agranular cytoplasm.

2.1% of participants incorrectly identified the arrowed cells as reactive lymphocytes. The most common type of reactive lymphocyte resembles a larger lymphocyte and corresponds to a Downey type II cell. Unlike lymphoblasts, these cells have round-to-oval nuclei, moderately condensed chromatin (giving it a smeared appearance), and absent or indistinct nucleoli. They contain abundant, pale gray-blue cytoplasm. Granules, if present, are usually small and few in number. Frequently, these reactive lymphocytes have an amoeboid cytoplasm that partially surrounds adjacent red cells and has a darker-staining, furled margin. Basophilia radiating out from the nucleus may also be present. The lymphoblasts in this case have fine chromatin, irregular nuclear contours, and scant agranular cytoplasm.

VPBS-11



Identification	Participants		Evaluation
	Freq	%	
Eosinophil, any stage	1229	96.9	Educational
Neutrophil, metamyelocyte	18	1.4	Educational
Neutrophil, segmented or band	6	0.5	Educational
Immature or abnormal cell, would refer for identification	3	0.2	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	3	0.2	Educational
Neutrophil, myelocyte	2	0.2	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	2	0.2	Educational
Basophil, any stage	1	0.1	Educational
Leukocyte containing Chediak-Higashi anomaly inclusion(s)	1	0.1	Educational
Lymphocyte	1	0.1	Educational
Myeloblast with Auer rod	1	0.1	Educational
Neutrophil necrobiosis (degenerated neutrophil)	1	0.1	Educational

The arrowed cells are eosinophils, as correctly identified by 96.9% of participants. Eosinophils are round-to-oval leukocytes that are recognizable by their characteristic coarse, orange-red granulation. They are comparable in size to neutrophils, ie, 10 to 15 μm in diameter in their mature forms, and 10 to 18 μm in diameter in immature forms. The eosinophil N:C ratio ranges from 1:3 for mature forms to 2:1 for immature forms. The eosinophil cytoplasm is generally evenly filled with numerous coarse, orange-red granules of uniform size. These granules rarely overlie the nucleus and are refractile by light microscopy due to their crystalline structure.

1.4% of participants incorrectly identified the arrowed cells as metamyelocytes. Metamyelocytes are approximately 10 to 18 μm in diameter. They are round to oval with a N:C ratio of 1.5:1 to 1:1. The nuclear chromatin is condensed, and the nucleus is indented to less than half of the maximal nuclear diameter (ie, the indentation is smaller than half of the distance to the farthest nuclear margin). The cytoplasm, has rare azurophilic or purple (primary) granules and many fine lilac or pale orange/pink specific granules. In this case, the arrowed cells have numerous orange-red granules, unlike a metamyelocyte.

Case Presentation:

This peripheral blood smear is from an 11-year-old boy presenting with fatigue and shortness of breath. Laboratory data include: WBC = $89.5 \times 10^9/L$; RBC = $2.11 \times 10^{12}/L$; HGB = 5.4 g/dL; HCT = 17.2%; MCV = 82 fL; and PLT = $17 \times 10^9/L$.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: T-lymphoblastic leukemia/lymphoma

T-lymphoblastic leukemia/lymphoma is a subtype of acute leukemia defined as a neoplastic proliferation of lymphoblasts committed to the T-cell lineage (expressing T-cell antigens, such as CD3, CD4, CD5, and CD8). The neoplastic blast cells are thought to arise from progenitor T-cells. The disease is referred to as leukemia/lymphoma because it can present either as a leukemia involving the blood and bone marrow (T-acute lymphoblastic leukemia, T-ALL) or as a mass lesion involving the thymus, lymph nodes, or extranodal tissues (T-acute lymphoblastic lymphoma, T-LBL). There is significant biological and clinical overlap between neoplasms diagnosed as T-ALL and T-LBL. In many cases, patients present with both blood involvement and a mass lesion, making the distinction between T-ALL and T-LBL arbitrary.

Question 1. A 5-year-old child presents to the Emergency Department and a CBC with differential reveals an elevated white count and 90% circulating blasts. By flow cytometry, the blasts express strong CD5. A chest X-ray reveals a large mediastinal mass. What is the most likely diagnosis?

- A. Acute myeloid leukemia (AML)
- B. B-lymphoblastic leukemia (B-ALL)
- C. Early T-cell precursor ALL (ETP -ALL)
- D. T-lymphoblastic leukemia (T-ALL)

Clinical Features

The median age at diagnosis of T-lymphoblastic leukemia/lymphoma is 25 - 30 years. There is a male predominance (M:F = 2:1). T-ALL comprises about 15% of childhood ALL and about 25% of adult ALL. In adults, acute myeloid leukemia (AML) is more common than ALL, while in children the opposite is true. Most ALL cases are of B-cell lineage (B-ALL) (**Table 1**). In contrast, the majority (90%) of lymphoblastic lymphomas are of T-cell lineage (T-LBL), with only 10% being B-cell lineage (B-LBL). There is also a less common type of T-ALL that has a unique immunophenotype that does not include expression of T-cell markers CD5, CD8, or CD1a and expresses stem cell and/or myeloid antigens. These cases are classified as early T-cell precursor ALL (ETP-ALL).

Table 1: Approximate frequency of acute leukemias by type and age

	Children	Adults
Lymphoblastic leukemia	80%	20%
• T-lymphoblastic leukemia	20%	25%
• B-lymphoblastic leukemia	80%	75%
Acute myeloid leukemia, all types	20%	80%

T-LBL most commonly involves the mediastinum as a rapidly enlarging mediastinal mass; it is presumed to arise from the thymus. Other common sites of involvement include lymph nodes, skin, tonsil, liver, spleen, and central nervous system, but any site can be involved. Approximately 50% of patients with T-LBL have symptoms of fever, night sweats, and weight loss ("B symptoms"). Most have elevated serum LDH levels.

Patients presenting with T-ALL often have a high WBC count, anemia, and thrombocytopenia. The bone marrow is involved in essentially 100% of T-ALL cases.

Morphology

The lymphoblasts in T-ALL are morphologically indistinguishable from those in B-ALL. In blood smears and bone marrow aspirate smears, lymphoblasts are often larger than mature lymphocytes, but their size can be quite variable (**Figure 1**). Their nuclei are predominantly round and sometimes deeply clefted, and less commonly may be more irregular. Chromatin immaturity can be subtle in smaller blasts but is typically smoother and less dense than a mature lymphocyte. Nucleoli are usually indistinct but can also be prominent. The nuclear:cytoplasmic (N:C) ratio is often higher than a mature lymphocyte, with only a very scant rim of cytoplasm. Cytoplasm is basophilic and usually without granules or vacuoles. Auer rods, which are diagnostic of acute myeloid leukemia, are never present.

Smaller lymphoblasts with subtle immaturity can be mistaken for mature lymphocytes. However, lymphoblasts are typically larger and may have distinct nucleoli compared to mature lymphocytes. If the peripheral blood smear staining is too dark, chromatin immaturity may be obscured, making distinction from lymphocytes difficult. Occasionally, lymphoblasts are larger, have more cytoplasm, or have prominent nucleoli, resembling myeloblasts. In the absence of Auer rods, immunophenotyping (eg, flow cytometry) is required to differentiate lymphoblasts from myeloblasts. Immunophenotyping is also required to distinguish T-ALL from B-ALL and is helpful to exclude leukemic presentations of mantle cell lymphoma and Burkitt lymphoma, which can also have blast-like morphologic features.

In T-LBL, the normal tissue architecture is effaced by sheets of round to irregular blast cells with scant cytoplasm. Commonly, there are many mitotic figures. Flow cytometry can be performed on a fresh sample of tissue, or immunohistochemistry can be performed on paraffin-embedded tissue to determine the lineage of the cells.

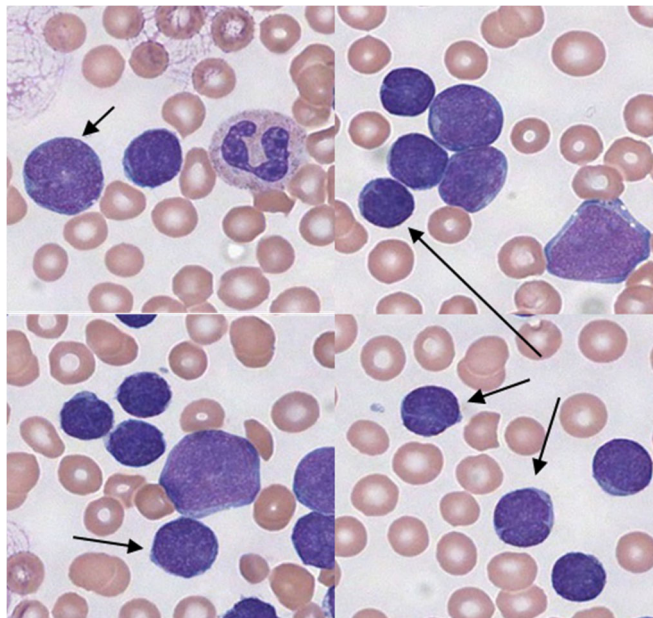


Figure 1. Representative blasts from the presented case of T-ALL (all images are at the same magnification). With the exception of the single neutrophil, all cells in these images are lymphoblasts. Note the wide variety of cell sizes and variety of nuclear features of the blasts. The immature, dispersed chromatin is much more apparent in the large blasts compared to the smaller ones. Some of the larger blasts have subtle nucleoli. The small blasts may be difficult to distinguish from normal lymphocytes. Clues to aid in the distinction are the presence of accompanying medium and large blasts and the fact that some of the small blasts have very scant cytoplasm, cleaved nuclei, or irregular nuclear contours.

Flow cytometry in cases of T-ALL/LBL will demonstrate variable expression of normal T-cell antigens such as CD2, CD3, CD4, CD5, CD7, and CD8. Of these, only cytoplasmic CD3 is considered lineage-specific. Surface CD3 expression, typical of mature T-cells, is often absent. Expression of the other T-cell antigens varies from case to case, although CD7 is present in most cases. Frequently, there is coexpression of CD4 and CD8 (“double positive” T-cells) or absence of both CD4 and CD8 (“double negative” T-cells), a finding not seen in normal T-cells outside of the thymus (the site of normal T-cell development). TdT is expressed on T-ALL but is non-specific since it can be expressed in B-ALL and AML.

In flow cytometry samples obtained from a mediastinal mass, distinguishing T-LBL from thymoma or normal thymus tissue may be problematic. In contrast to thymoma or normal thymus, T-LBL often demonstrates aberrant loss of T-cell antigens. In addition, T-LBL cases usually show homogeneous antigen expression, producing a tight cluster of dots on flow scatter plots, whereas normal thymus produces a more variable pattern of T-cell antigen expression that reflects normal T-cell maturation.

Question 2. A 5-year-old woman has numerous blasts on her peripheral blood smear. The blasts are small to intermediate in size, have scant cytoplasm, and no Auer rods. Flow cytometry was performed, and the diagnosis of T-lymphoblastic leukemia (T-ALL) was established. The expression of which marker is specific for T-ALL?

- A. CD4
- B. Cytoplasmic CD3
- C. Myeloperoxidase
- D. TdT (terminal deoxynucleotidyl transferase)

Genetics

Under the International Consensus Classification (ICC), some T-ALL subtypes are defined by cytogenetic and molecular findings and are considered provisional entities. The 5th edition of the World Health Organization (WHO) does not subclassify by genetic/molecular characteristics. The most common recurrent cytogenetic abnormalities seen in T-ALL/LBL are translocations involving the various T-cell receptor genes (TCR) and a variety of partner genes. Involved TCR loci are located at 14q11.2 (TCR alpha and delta), 7q35 (TCR beta), and 7p14-15 (TCR gamma). In most cases, the translocations lead to dysregulation of the partner gene, resulting in maturation block at an immature developmental stage and uncontrolled proliferation of the blast cells. Frequently, these translocations cannot be detected by karyotyping but can be identified by molecular genetic studies.

The most common chromosomal deletion (seen in 30% of cases by karyotyping) is del(9p), which results in loss of an important tumor suppressor gene (CDKN2A). About 50% of cases show activating mutations in the NOTCH1 gene, which encodes a protein critical for early T-cell development. Molecular studies using PCR will show clonal rearrangement of the TCR genes in almost all cases. Approximately 20% of cases will show concurrent clonal rearrangement of immunoglobulin heavy chain genes (IGH). This latter finding highlights the fact that gene rearrangement studies are useful to demonstrate clonality but are not reliable for lineage assignment.

Prognosis

T-cell acute lymphoblastic leukemia/lymphoma are aggressive but curable diseases, particularly in children. The prognosis of T-LBL is dependent on the age of the patient, stage of the disease, and serum LDH levels, similar to factors important in the prognosis of other lymphomas. ETP-ALL is generally associated with a poor prognosis; however, recent studies in children indicated that outcomes may be similar to T-ALL.

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REFERENCES:

1. Alaggio R, Amador C, Anagnostopoulos I, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid neoplasms. *Leukemia*. 2022;36(7):1720-1748.
2. Arber DA, Orazi A, Hasserjian RP, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Arber DA, Orazi A, Hasserjian RP, et al. *Blood*. 2022;140(11):1200-1228.
3. Cherian S, Wood B. *Flow cytometry in evaluation of hematopoietic neoplasms*. College of American Pathologists; 2012.
4. Cortelazzo S, Ferreri A, Hoelzer D, Ponzoni M. Lymphoblastic lymphoma. *Crit Rev Oncol Hematol*. 2017;113:304-317.
5. Glassy EF. *Color Atlas of Hematology: An Illustrated Field Guide Based on Proficiency Testing*. 2nd Ed. College of American Pathologists; 2018.
6. Li S, Juco J, Mann KP, Holden JT. Flow cytometry in the differential diagnosis of lymphocyte-rich thymoma from precursor T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma. *Am J Clin Pathol*. 2004;121:268-274.
7. Wood B, Devidas M, Summers RJ, et al. Prognostic significance of ETP phenotype and minimal residual disease in T-ALL: A children's oncology group study. *Blood*. 2023;142:2069-2078.

ANSWERS TO QUESTIONS:

Question 1: D. T-lymphoblastic leukemia (T-ALL)

A mediastinal mass with circulating blasts expressing CD5 is associated with T-ALL. Both AML and B-ALL can be found in tissues outside the blood or bone marrow (extramedullary involvement) but is uncommon. ETP-ALL comprises 25% of all T-ALL cases. Strong CD5 expression is not seen in ETP-ALL.

Question 2: B. Cytoplasmic CD3

Cytoplasmic CD3 expression defines the lineage of T-lymphoblasts. The T-cell marker CD4 may be seen in T-ALL but does not define the T-cell lineage and can be seen in other acute leukemias, especially AML with monocytic differentiation. Myeloperoxidase defines the myeloid lineage. Although TdT (terminal deoxynucleotidyl transferase) is seen in many cases of T-ALL, it is not lineage specific and is frequently expressed in B-ALL and some cases of AML.