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Laboratory Quality Solutions

Surveys and Anatomic Pathology Education Programs

Virtual Peripheral Blood Smear VPBS-B 2022

Participant Summary

1.0 Credit of Continuing Education Available

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PARTICIPANT SUMMARY

Program Update

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With your participation in CAP's Surveys programs, *every member of your team* can take part in education activities that may earn Continuing Education (CE) credits or receive Self-Reported Training* at no additional charge.

This Survey mailing includes an online education activity to earn **1.0** CE credit. To access the activity, see page 39.

**CAP Self-Reported Training activities do not offer CE credit but can be used towards fulfilling requirements for maintenance of certification (MOC) by agencies such as the American Society of Clinical Pathology (ASCP). Please verify with your certifying agency to determine your education requirements.*

Evaluation Criteria

Results for the VPBS Survey are **not** formally evaluated; however statistics will appear in the participant summary for your information.

The quantitative data tables provided in this participant summary include multiple statistical values which may include the median, MIN, and MAX values reported for each peer group. The MIN and MAX values are not the limits of acceptability. The acceptable limits are located on your participant evaluation report.

To provide a timely evaluation of your results, statistics presented in this participant summary reflect participant data received by the due date.

In the event a result is not graded, a numeric code will appear next to your result. A definition of the code will appear on the first page of your evaluation. Please see "Actions Laboratories Should Take when a PT Result is Not Graded" on page 37. Laboratories should perform a self-evaluation. For more information, go to cap.org.

1. Hover over Laboratory Improvement and click **Proficiency Testing.**
2. Under Proficiency Testing (PT) Programs, Surveys, click **PT Resources.**
3. Under Existing Customers, click **Performing a Self-Evaluation When PT is Not Graded.**

VPBS-19

Clinical History for VPBS-20 – VPBS-24

This peripheral blood smear is from a 21-month-old boy with a history of lethargy and easy bruising. Laboratory data include: WBC = 58.1 x 10E9/L; RBC = 1.63 x 10E12/L; HGB = 4.1 g/dL; HCT = 13.1%; MCV = 84 fL; PLT = 7 x 10E9/L; and RDW = 18%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Please click on the hyperlink below to view the DigitalScope images for this case.
https://www.digitalscope.org/ViewerUI/?SlideId=8643e7c4-3867-4fe1-96aa-5fdcf5f9399e

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)	1238	2.0	1.1	55.4	2	0	5
Lymphocytes	1294	32.9	25.2	76.6	24	0	108
Lymphocytes, reactive	528	1.1	3.3	*	0	0	23
Monocytes	735	0.9	1.1	*	1	0	6
Eosinophils	518	0.0	0.2	*	0	0	1
Basophils	513	0.0	0.0	0.0	0	0	0
Metamyelocytes	661	0.5	0.6	*	0	0	2
Myelocytes	500	0.0	0.2	*	0	0	1
Promyelocytes	488	0.0	0.1	*	0	0	1
Blasts	1003	55.2	32.1	58.2	69	0	108
nRBC/100 WBC	592	0.1	0.5	*	0	0	3

WBC Differential - 10E9/L**	N	MEAN	SD	CV%	MEDIAN	MIN	MAX
Neutrophils (segmented or bands)	1145	1.145	0.643	56.1	1.16	0.00	3.40
Lymphocytes	1194	18.909	14.576	77.1	13.94	0.00	58.10
Lymphocytes, reactive	487	0.707	2.180	*	0.00	0.00	16.27
Monocytes	665	0.541	0.668	*	0.58	0.00	3.49
Eosinophils	468	0.013	0.085	*	0.00	0.00	0.60
Basophils	462	0.000	0.000	0.0	0.00	0.00	0.00
Metamyelocytes	604	0.286	0.330	*	0.00	0.00	1.70
Myelocytes	440	0.000	0.000	0.0	0.00	0.00	0.00
Promyelocytes	447	0.004	0.047	*	0.00	0.00	0.58
Blasts	911	32.223	18.006	57.7	40.67	0.00	56.36

*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-19, cont'd.

Other cells: *All cells not listed on result form and cells not differentiated by your laboratory*

Cells not listed/differentiated	N = 220 Freq
Lymphoma/malignant lymphoid cell/immature lymphocyte (other than blast)	57
Basket/smudge cell	34
Immature/abnormal blast-like/blast cell (includes blast/lymphoblast/monoblast)	20
Abnormal/atypical mononuclear cell	11
Abnormal/atypical/reactive lymphocyte	7
Myeloid precursors	3
Sezary cell	3
Plasma cell	2
Would refer for identification	83

Platelet Estimate

Intended Response: Decreased platelets	N = 1293 Freq	%
Decreased platelets	1291	99.8
Adequate/normal platelets	2	0.2
Increased platelets	-	-
Unable to quantitate - platelet clumps present	-	-

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 N = 2830 Freq	Total Responses N = 2830 % Total Response	Total Unique Kits N = 1181 % Unique Kits
Microcyte (with increased central pallor)	552	19.5	46.7
Ovalocyte (elliptocyte)	399	14.1	33.8
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	388	13.7	32.9
Polychromatophilic (non-nucleated) red blood cell	299	10.6	25.3
Rouleaux	286	10.1	24.2
Erythrocyte, normal	213	7.5	18.0
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	211	7.5	17.9
Stomatocyte	108	3.8	9.1
Spherocyte	104	3.7	8.8
Echinocyte (burr cell, crenated cell)	53	1.9	4.5
Teardrop cell (dacrocyte)	38	1.3	3.2
Erythrocyte with overlying platelet	36	1.3	3.0
Bite cell (degmacyte)	31	1.1	2.6
Acanthocyte (spur cell)	27	0.9	2.3
Red blood cell agglutinates	26	0.9	2.2
Target cell (codocyte)	17	0.6	1.4
Howell-Jolly body	16	0.6	1.4

VPBS-19, cont'd.

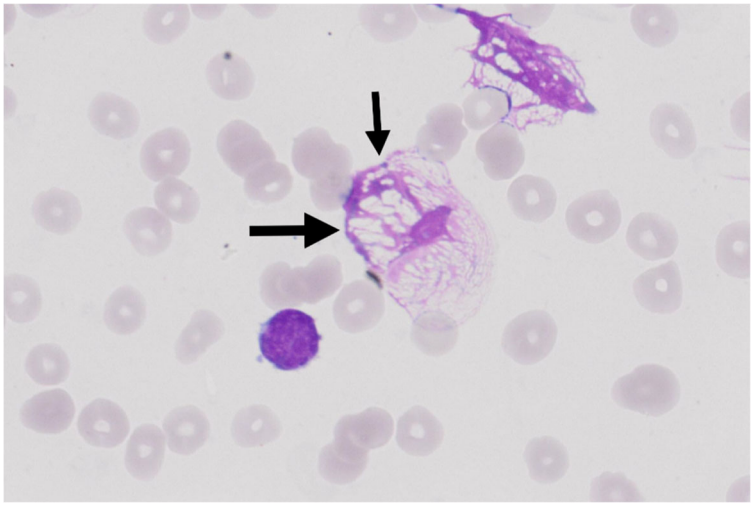
Red Cell Morphology	Total Responses N = 2830 Freq	Total Responses N = 2830 % Total Response	Total Unique Kits N = 1181 % Unique Kits
Nucleated red blood cell, normal or abnormal morphology	14	0.5	1.2
Immature or abnormal cell, would refer for identification	4	0.1	0.3
Basophilic stippling (coarse)	3	0.1	0.3
Blister cell/Prekeratocyte	3	0.1	0.3
Pappenheimer bodies (iron or Wright stain)	2	0.1	0.2

Committee Comments on the CBC and Peripheral Blood Whole Slide

The peripheral smear findings confirm the reported leukocytosis with an increased population of immature cells, consistent with blast forms. They demonstrate a spectrum of morphologic features, ranging from small to medium sized cells with round nuclear contours, nuclear folds/clefting, dispersed chromatin, distinct nucleoli and scant deep blue cytoplasm. Segmented neutrophils and monocytes are scant. The red blood cells show hypochromic anemia, mild anisopoikilocytosis, and polychromasia. Platelets are few but appear normal in size and granularity.

Cell Identification

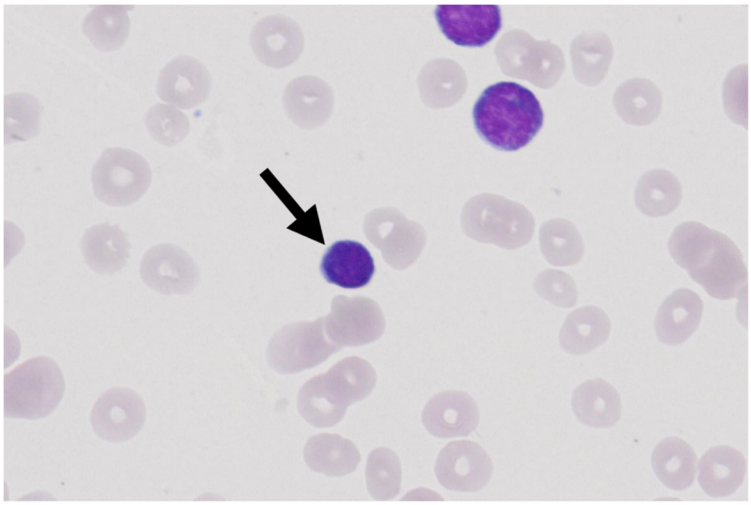
VPBS-20



Identification	Participants Freq	%	Evaluation
Basket cell/smudge cell	1285	98.9	Educational
Stain precipitate	7	0.5	Educational
Blast cell	2	0.1	Educational
Lymphocyte	1	0.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.1	Educational
Monocyte	1	0.1	Educational
Neutrophil necrobiosis (degenerated neutrophil)	1	0.1	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	1	0.1	Educational

The arrowed cell is a basket cell or smudge cell, as correctly identified by 98.9% of participants. Basket/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.

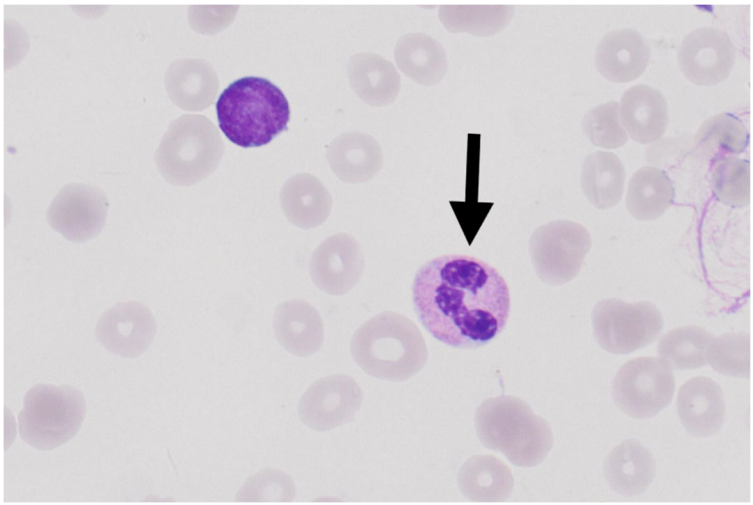
VPBS-21



Identification	Participants Freq	%	Evaluation
Lymphocyte	1280	98.5	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	7	0.5	Educational
Blast cell	4	0.3	Educational
Nucleated red blood cell, normal or abnormal morphology	3	0.2	Educational
Malignant lymphoid cell (other than blast)	2	0.1	Educational
Erythrocyte with overlying platelet	1	0.1	Educational
Immature or abnormal cell, would refer for identification	1	0.1	Educational
Lymphocyte, large granular	1	0.1	Educational

The arrowed cell is a lymphocyte, as correctly identified by 98.5% of participants. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 µm with an N:C ratio ranging from 5:1 to 2:1. Most lymphocytes have round to oval nuclei that may be slightly indented or notched. The chromatin is 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. Occasionally, the edges may be slightly frayed or pointed due to artifacts induced during smear preparation. Occasional lymphocytes will have a small clear zone, or hof, adjacent to one side of the nucleus. While most normal lymphocytes are fairly homogeneous, they may exhibit a range of normal morphology.

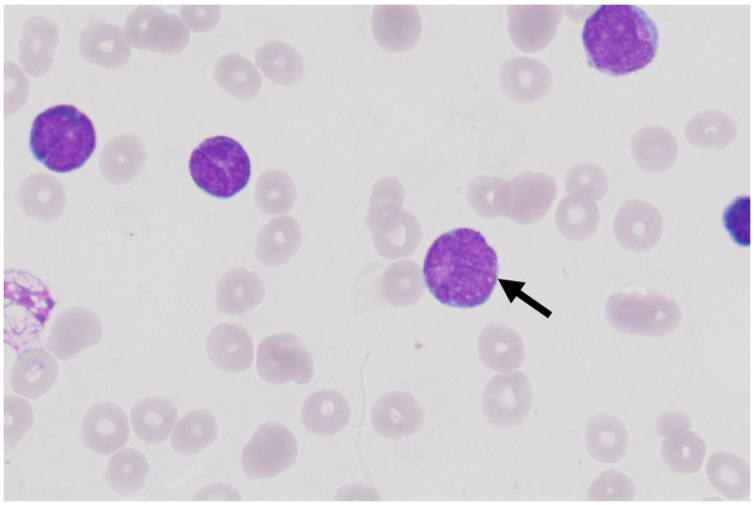
VPBS-22



Identification	Participants Freq	%	Evaluation
Neutrophil, segmented or band	1285	98.9	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	4	0.3	Educational
Neutrophil with Pelger-Huët nucleus (acquired or congenital)	3	0.2	Educational
Basophil, any stage	1	0.1	Educational
Eosinophil, any stage	1	0.1	Educational
Neutrophil, giant band or giant metamyelocyte	1	0.1	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	1	0.1	Educational
Neutrophil with hypersegmented nucleus	1	0.1	Educational
Neutrophil necrobiosis (degenerated neutrophil)	1	0.1	Educational
Platelet, hypogranular	1	0.1	Educational

The arrowed cell is a segmented neutrophil, as correctly identified by 98.9% of participants. The segmented neutrophil is the predominant blood leukocyte. It has a similar size to a band neutrophil (ie, 10 to 15 µm in diameter), as well as comparable shape (round to oval), and cytoplasmic appearance (pale pink cytoplasm with specific granules). The nucleus is segmented into two to five lobes that are connected by a thin filament of chromatin. The abundant, pale pink cytoplasm contains many fine, lilac-colored granules. The nuclear lobes may appear eccentric and the cytoplasm may be vacuolated. Nuclear pyknosis and fragmentation in degenerating neutrophils can make recognition difficult.

VPBS-23



Identification	Participants Freq	%	Evaluation
Blast cell	814	62.7	Educational
Malignant lymphoid cell (other than blast)	339	26.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	64	4.9	Educational
Immature or abnormal cell, would refer for identification	51	3.9	Educational
Lymphocyte	15	1.1	
Monocyte	10	0.8	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	3	0.2	Educational
Lymphocyte, large granular	1	0.1	Educational
Monocyte, immature (promonocyte, monoblast)	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational

The arrowed cell is a blast, as correctly identified by 62.7% of participants. A blast is a large, round to oval cell, 10 to 20 µm in diameter, with a high nuclear-to-cytoplasmic ratio. The blast often has a round to oval nucleus, but it is sometimes indented or folded. The nuclear chromatin is typically fine, lacey, or granular, and one or more nucleoli may be present. Nucleoli are more prominent in cytocentrifuged slides. The cytoplasm is basophilic and often agranular; however, when cytoplasmic granules occur, they are more easily visualized in the cytocentrifuge slide than in peripheral blood or bone marrow smears. In the absence of lineage-associated findings, such as Auer rods, cytochemical data, or cell surface marker data, it is not possible to further characterize a given blast cell morphologically. In addition to blasts, immature cells also include promyelocytes and myelocytes. Promyelocytes are slightly larger than blasts, with more plentiful cytoplasm and contains multiple distinct azurophilic (primary granules). A paranuclear hof or cleared space is typically present and nucleus is folded, bilobed, or reniform (overlapping nuclear lobes). Myelocytes also have abundant cytoplasm with azurophilic and specific granules. Their nuclei are slightly eccentric, lack nucleoli, and demonstrate more condensed chromatin in comparison to blasts.

3.9% of participants identified the arrowed cell as “Immature, would refer”. This is an acceptable answer for laboratories that always refer abnormal cell identification to an outside laboratory with a different CLIA number.

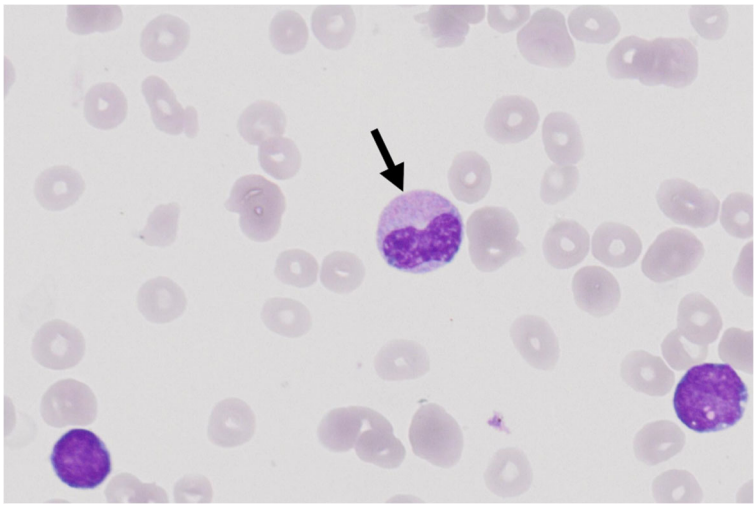
26.1% of participants incorrectly identified the arrowed cell as a malignant lymphoid cell (other than blast). Lymphoma cells can have variable morphology, depending on lymphoma subtype. The arrowed cells have higher N:C ratios and finer chromatin than would be typical for malignant lymphoid cells other than blasts.

The morphologic characteristics of some of the common malignant lymphoid cells, compared to blasts are described below. Prolymphocytes can be similar in size to blasts, however, they have more abundant cytoplasm than blasts and shows somewhat condensed chromatin. The nuclei of follicular lymphoma have more mature, dense chromatin and are usually clefted, indented, folded, convoluted, or even lobulated. Hairy cells have moderate to abundant cytoplasm and cell borders are often indistinct, secondary to the presence of fine (hairy) cytoplasmic projections. In Burkitt lymphoma, the cytoplasm is moderately abundant, deeply basophilic and often contains numerous small and uniformly round vacuoles. Sezary cells have folded, grooved or convoluted nuclear membranes, giving them a cerebriform appearance. The chromatin is dark and hyperchromatic without visible nucleoli. Large cell lymphoma can be easily confused with blasts and additional immunophenotyping are often necessary to make the correct diagnosis. They are large (20 - 30 µm) and have scant to moderate amounts of basophilic cytoplasm. For identification purposes, one should classify individual cells exhibiting this type of morphology as blast cells when additional confirmatory information is unavailable.

4.9% of participants incorrectly identified the arrowed cell a lymphocyte, reactive. Reactive lymphocytes are distinguished by their wide range of cellular sizes and shapes. Generally, the N:C ratio is not as high as in blasts, and the chromatin is more condense. Immunoblasts and immunoblastic-like reactive lymphocytes are large cells (15 to 20 µm) with prominent nucleoli that may look like blasts, however, their cytoplasm is more abundant than blasts.

1.1% of participants incorrectly identified the arrowed cell as a lymphocyte. Lymphocytes are relatively smaller cells with a high N:C ratio. However, their chromatin is diffusely dense or coarse and clumped and their nucleoli are not visible, in contrast to blasts.

VPBS-24



Identification	Participants Freq	%	Evaluation
Neutrophil, metamyelocyte	1155	88.9	Educational
Neutrophil, segmented or band	63	4.8	Educational
Neutrophil, giant band or giant metamyelocyte	24	1.9	Educational
Monocyte	16	1.2	Educational
Immature or abnormal cell, would refer for identification	9	0.7	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	8	0.6	Educational
Neutrophil, myelocyte	7	0.5	Educational
Neutrophil with Pelger-Huët nucleus (acquired or congenital)	7	0.5	Educational
Malignant lymphoid cell (other than blast)	2	0.1	Educational
Monocyte, immature (promonocyte, monoblast)	2	0.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.1	Educational
Myeloblast with Auer rod	1	0.1	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	1	0.1	Educational
Neutrophil necrobiosis (degenerated neutrophil)	1	0.1	Educational
Target cell (codocyte)	1	0.1	Educational
Teardrop cell (dacrocyte)	1	0.1	Educational

The arrowed cell is a neutrophil, metamyelocyte, as correctly identified by 88.9% of participants. Metamyelocytes are the first of the postmitotic myeloid precursors. They constitute 15% to 20% of nucleated cells in the bone marrow and may be seen in the blood in pathologic states and in response to stress. They are approximately 10 to 18 µm in diameter. They are round to oval with a N:C ratio of 1.5:1 to 1:1. The nuclear chromatin is condensed, and the nucleus is indented to less than half of the maximal nuclear diameter (ie, the indentation is smaller than half of the distance to the farthest nuclear margin). The cytoplasm is amphophilic containing rare azurophilic or purple (primary) granules and many fine lilac or pale orange/pink specific granules.

4.8% of participants incorrectly identified the arrowed cell as a neutrophil (segmented or band). Both segmented or band neutrophils may be similar in size to metamyelocyte. However, the band nucleus is indented to more than half the distance to the farthest nuclear margin, compared to metamyelocyte where

the indentation is less than half of the distance. In segmented neutrophils, the nucleus is completely segmented or lobated with a normal range of three to five lobes that are connected by a thin filament.

1.9% of participants incorrectly identified the arrowed cell as a neutrophil, giant band. These cells have diameters 1.5 times those of normal metamyelocytes and the nucleus is indented to more than half of the maximal diameter.

1.2% of participants incorrectly identified the arrowed cell as a monocyte. The nuclei of monocytes are usually indented or folded when compared to a metamyelocyte. The cytoplasm of monocytes typically demonstrates a gray or gray-blue ground-glass appearance, and may contain vacuoles or fine, evenly distributed azurophilic granules, unlike the more coarse granules in this metamyelocyte.

Case Presentation:

This peripheral blood smear is from a 21-month-old boy with a history of lethargy and easy bruising. Laboratory data include: WBC = 58.1 x 10E9/L; RBC = 1.63 x 10E12/L; HGB = 4.1 g/dL; HCT = 13.1%; MCV = 84 fL; PLT = 7 x 10E9/L; and RDW = 18%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case discussion: B-Lymphoblastic leukemia

B-lymphoblastic leukemia/lymphoma (B-ALL/LBL) is a neoplastic proliferation of precursor B-cells. Acute lymphoblastic leukemia is the most common form of leukemia in pediatric populations, accounting for about 74% of pediatric leukemia cases. B-lymphoblastic leukemias represent ~ 80% of ALL. B-ALL is also encountered in adult populations and the estimated global incidence of B-ALL is around one to five per 100,000 persons per year. Constitutional genetic disorders, including Down syndrome, increases the risk. Unlike acute myeloid leukemias, there is no lower limit of blasts required, to establish the diagnosis of lymphoblastic leukemias, however, cases frequently present with high blast counts. Clinically, patients frequently present with leukocytosis due to circulating blasts, or cytopenias due to displacement of normal hematopoietic elements. Extramedullary involvement is common and thus lymphadenopathy, hepatomegaly and splenomegaly are also frequent.

Question 1. Which of the following represents the lower threshold of blast count for making the diagnosis of B-LBL?

A. No lower limit
B. 5%
C. 10%
D. 20%

The malignant cells are precursor lymphoid cells (ie, lymphoblasts) that are arrested at an early stage of development. Morphologically, they present as undifferentiated blasts that may vary in size and can exhibit scant to moderate cytoplasm, with occasional vacuolation and coarse azurophilic granules in rare cases. Some cases exhibit cytoplasmic pseudopods, also referred to as ‘hand-mirror’ cells, however, this finding is not specific to B-ALL/LBL. Nuclear features may also show a spectrum of condensed to dispersed chromatin and indistinct to prominent nucleoli.

Hematogones are normal precursor B-cells that reside in the bone marrow that may simulate B-LBLs, necessitating flow cytometry immunophenotyping to differentiate them from leukemic cells. Their cytomorphologic features may be indistinguishable from those of B lymphoblasts. Hematogones are most often seen in the bone marrow aspirates of pediatric population in a variety of hematologic and non-hematologic disorders. When present in peripheral blood in rare instances, they typically constitute a small fraction.

Given the undifferentiated nature of the blasts, the morphologic differential diagnosis of B-ALL/LBL includes T-LBL and acute myeloid leukemia, requiring lineage assessment by ancillary testing such as flow cytometry and immunohistochemical and/or cytochemical stains for definitive diagnosis.

Question 2. B-lymphoblasts have distinct morphologic features and do not require ancillary testing for diagnosis.

A. True
B. False

B-ALL/LBL has a good prognosis in children (Clinical Remission > 95%) and fair prognosis in adults (Clinical Remission in ~ 80%). Multidrug chemotherapy is used in different combinations and various phases based on risk stratification of patients. Induction therapy is the initial 4 - 6 weeks of treatment that achieves remission in over 95% of patients. Post-induction chemotherapy is used to remove any residual leukemic cells.

Improved outcomes are seen in those with low initial leukocyte count (< 50 x 10E9/L), ages between 2 and 10 years old, female gender, and complete remission following induction therapy. Chromosomal abnormalities are common in leukemic cells with certain abnormalities having prognostic significance. Hypodiploidy (< 46 chromosomes) is associated with a relatively poor prognosis, compared to those with hyperdiploid (> 50 chromosomes) status. The most common structural abnormality in B-ALL is **t(9;22)(q34;q11); *BCR::ABL1***, an unfavorable finding. However, treatment with tyrosine kinase inhibitors has improved survival in these patients.

Question 3. Which of the following is considered a poor prognostic factor in B-LBL patients?

A. Age between 2 - 10 years
B. Female gender
C. Hypodiploidy (< 46 chromosomes)
D. Initial WBC count of 25 x 10E9/L

Aadil Ahmed, MD
Philipp Raess, MD, PhD
Hematology and Clinical Microscopy Committee

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2. *Cancer Facts and Figures 2021*. American Cancer Society. Available at https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2021/cancer-facts-and-figures-2021.pdf. Accessed March29th, 2022.
3. Mais DD. *Practical Clinical Pathology*. 2nd ed. ASCP Press. 2017.

ANSWERS TO QUESTIONS:

Question 1: A. No lower limit
There is no lower limit of blast count needed to establish the diagnosis of B-LBL. Although, most cases present with high blast count.
Question 2: B. False
B-lymphoblasts may appear similar to blasts of any lineage and require ancillary studies for immunophenotyping and differentiation.
Question 3: C. Hypodiploidy (< 46 chromosomes)
Hypodiploidy is considered as a poor prognostic factor whereas the other features portend a better prognosis in B-LBL patients.

VPBS-25

Clinical History for VPBS-26 – VPBS-30

This peripheral blood smear is from a 76-year-old woman presenting with fatigue. Laboratory data include: WBC = 3.1 x 10E9/L; RBC = 2.67 x 10E12/L; HGB = 8.3 g/dL; HCT = 27.1%; MCV = 108 fL; PLT = 122 x 10E9/L; and RDW = 16%.

(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=d62c08c2-5391-4a75-a1ae-e13329f6993f

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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)	1243	54.6	5.1	9.3	55	38	71
Lymphocytes	1288	30.2	9.0	29.7	31	4	56
Lymphocytes, reactive	771	5.8	6.3	*	4	0	26
Monocytes	1225	7.6	4.2	54.7	7	0	20
Eosinophils	1204	1.7	0.7	39.8	2	0	4
Basophils	496	0.0	0.0	0.0	0	0	0
Metamyelocytes	476	0.0	0.2	*	0	0	1
Myelocytes	477	0.0	0.1	*	0	0	1
Promyelocytes	478	0.0	0.0	0.0	0	0	0
Blasts	489	0.0	0.2	*	0	0	1
nRBC/100 WBC	605	0.4	1.0	*	0	0	6

WBC Differential - 10E9/L**	N	MEAN	SD	CV%	MEDIAN	MIN	MAX
Neutrophils (segmented or bands)	1184	1.692	0.184	10.9	1.70	0.84	2.57
Lymphocytes	1182	0.943	0.284	30.1	0.99	0.06	1.80
Lymphocytes, reactive	713	0.187	0.208	*	0.12	0.00	0.93
Monocytes	1135	0.241	0.135	56.3	0.22	0.00	0.69
Eosinophils	1111	0.055	0.025	45.9	0.06	0.00	0.16
Basophils	451	0.000	0.000	0.0	0.00	0.00	0.00
Metamyelocytes	460	0.004	0.015	*	0.00	0.00	0.06
Myelocytes	447	0.002	0.010	*	0.00	0.00	0.09
Promyelocytes	439	0.000	0.000	0.0	0.00	0.00	0.00
Blasts	459	0.003	0.015	*	0.00	0.00	0.15

*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.

Other cells: *All cells not listed on result form and cells not differentiated by your laboratory*

Cells not listed/differentiated	N = 105 Freq
Plasma/plasmacytoid cell	31
Abnormal/atypical/reactive lymphocyte	13
Basket/smudge cell	5
Atypical mononuclear cell	4
Myeloid precursors	2
Lymphoma/malignant lymphoid cell/immature lymphocyte (other than blast)	1
Mitotic figure	1
Would refer for identification	48

Platelet Estimate

Intended Response: Decreased platelets	N = 1285 Freq	%
Decreased platelets	1082	84.2
Adequate/normal platelets	133	10.3
Increased platelets	-	-
Unable to quantitate - platelet clumps present	70	5.5

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 N = 3876 Freq	Total Responses N = 3876 % Total Response	Total Unique Kits N = 1272 % Unique Kits
Spherocyte	1060	27.4	83.3
Ovalocyte (elliptocyte)	908	23.4	71.4
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	712	18.4	56.0
Polychromatophilic (non-nucleated) red blood cell	484	12.5	38.0
Teardrop cell (dacrocyte)	160	4.1	12.6
Microcyte (with increased central pallor)	150	3.9	11.8
Erythrocyte, normal	111	2.9	8.7
Howell-Jolly body	99	2.5	7.8
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	90	2.3	7.1
Target cell (codocyte)	27	0.7	2.1
Erythrocyte with overlying platelet	21	0.5	1.6
Stomatocyte	17	0.4	1.3
Nucleated red blood cell, normal or abnormal morphology	14	0.4	1.1
Pappenheimer bodies (iron or Wright stain)	8	0.2	0.6
Blister cell/Prekeratocyte	5	0.1	0.4
Basophilic stippling (coarse)	3	0.1	0.2

VPBS-25, cont'd.

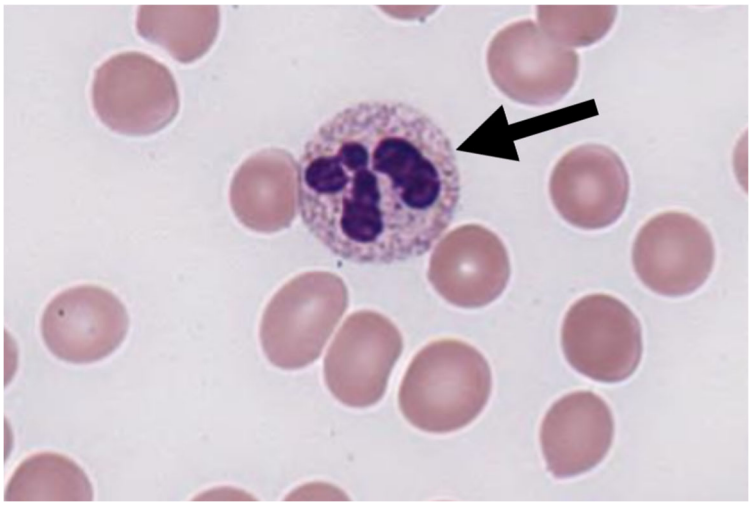
Red Cell Morphology	Total Responses N = 3876 Freq	Total Responses N = 3876 % Total Response	Total Unique Kits N = 1272 % Unique Kits
Rouleaux	3	0.1	0.2
Red blood cell agglutinates	2	0.1	0.2
Acanthocyte (spur cell)	1	0.0	0.1
Bite cell (degmacyte)	1	0.0	0.1

Committee Comments on CBC and Peripheral Blood Whole Slide Image

The slide image shows macrocytic anemia in an elderly patient presenting with fatigue. The presence of ovalocytes and spherocytes (poikilocytes) suggests some marrow production dysfunction. The differential in this case is relatively broad and includes myelodysplasia (MDS), bone metastasis, and plasma cell myeloma, among other entities. Evaluation of white blood cell morphology, vitamin B12 and folate levels, and likely a bone marrow biopsy would be integral in arriving at an accurate diagnosis.

Cell Identification

VPBS-26



Identification	Participants Freq	%	Evaluation
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	289	22.2	Educational
Neutrophil, segmented or band	913	70.2	Educational
Neutrophil with hypersegmented nucleus	84	6.5	Educational
Neutrophil, polyploid	4	0.3	Educational
Eosinophil, any stage	3	0.2	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	3	0.2	Educational
Neutrophil necrobiosis (degenerated neutrophil)	3	0.2	Educational
Basket cell/smudge cell	1	0.1	Educational

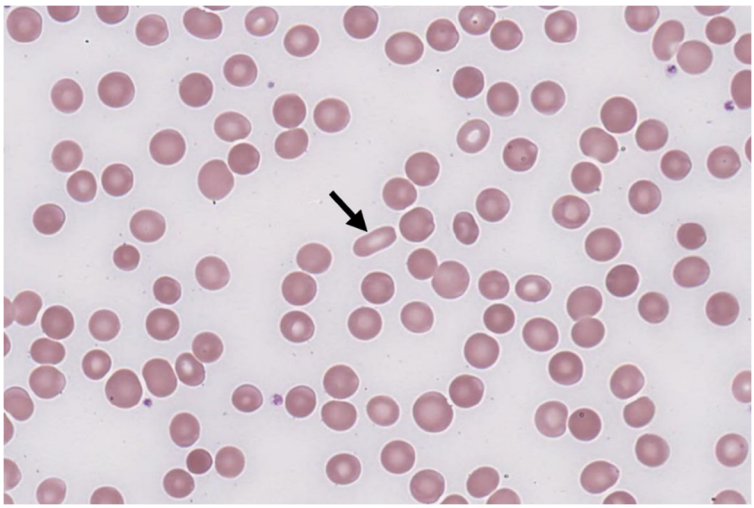
The arrowed cell is a toxic neutrophil, as correctly identified by 22.2% of participants. Toxic changes in neutrophils include toxic granulation, Döhle bodies, and toxic vacuolization. Toxic granulation is defined by the presence of large, purple or dark blue cytoplasmic granules in neutrophils, bands, and metamyelocytes. Toxic granules are typically larger and darker staining than normal neutrophil granules. Döhle bodies represent parallel strands of rough endoplasmic reticulum and appear as single or multiple blue or gray-blue cytoplasmic inclusions of various sizes, typically located near the plasma membrane. Vacuoles within the cytoplasm of these same cells define toxic vacuolization. The vacuoles are variable in size and may coalesce, sometimes distorting the neutrophil cytoplasm to form pseudopodia. Toxic granulation and Döhle bodies each may be present in an individual cell without the other finding. Either change alone is sufficient to designate a neutrophil as toxic. Isolated vacuolation should not be considered evidence of toxic change, as it may be the result of degeneration artifact and should not be labeled as toxic vacuoles unless accompanied by other toxic changes. Toxic changes result from the action of cytokines released in response to infection, burns, trauma, and granulocyte colony stimulating factor (G-CSF), and they indicate a shortened maturation time and activation of post-mitotic neutrophil precursors.

The arrowed cell was incorrectly identified as a neutrophil, segmented or band by 70.2% of participants. Neutrophils should have a pale pink cytoplasmic appearance, unlike the arrowed cell in this case which has purplish granules signifying toxic change. In addition, there is a small gray-blue inclusion at the periphery of

the cytoplasm, which represents a Döhle body. The distinction between normal neutrophils and one with toxic changes can be challenging, however, close morphologic evaluation should aid in this distinction.

This cell was incorrectly identified as a neutrophil with hypersegemented nucleus by 6.5% of participants. Hypersegmentation of a neutrophil is defined as a nucleus with six or more lobes. These lobes should be separated by a thin filament that contains no internal chromatin, giving it the appearance of a solid, thread-like line. The arrowed cell demonstrates only three distinct lobes, and thus should not be considered hypersegmented.

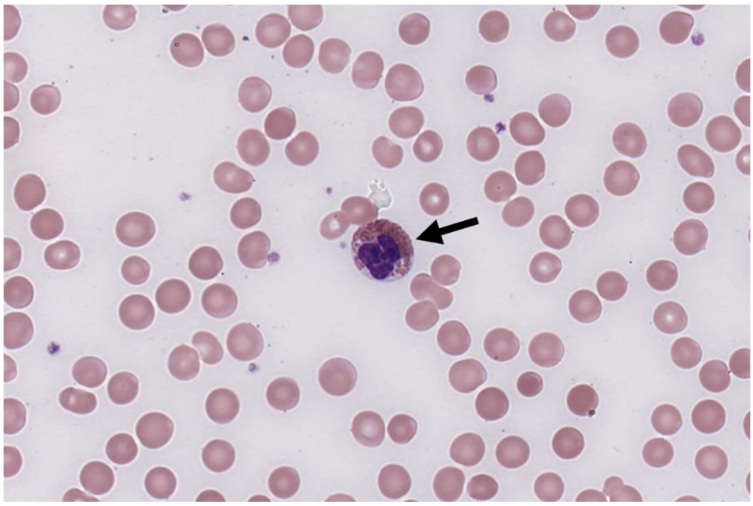
VPBS-27



Identification	Participants Freq	%	Evaluation
Ovalocyte (elliptocyte)	1294	99.5	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	3	0.2	Educational
Erythrocyte, normal	1	0.1	Educational
Lymphocyte	1	0.1	Educational
Microcyte (with increased central pallor)	1	0.1	Educational

The arrowed cell is an ovalocyte (elliptocyte), as correctly identified by 99.5% of participants. The terms elliptocytes and ovalocytes are used to describe red blood cells appearing in the shape of a pencil or thin cigar, with blunt ends and parallel sides. Hemoglobin is often concentrated at the ends, producing a dumbbell appearance. 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 are prominent (eg, myelophthisic processes). Some ovalocytes may superficially resemble oval macrocytes but are not as large as macrocytes and tend to be less oval with sides that are nearly parallel. The ends of ovalocytes are always blunt and never sharp, unlike those of sickle cells.

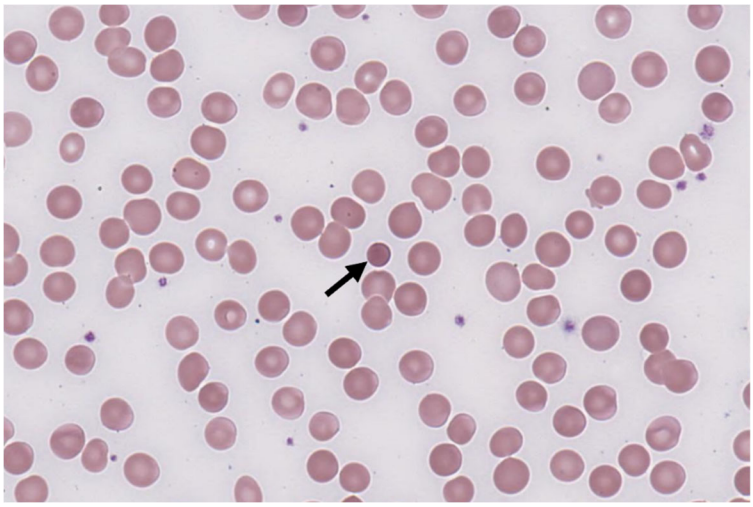
VPBS-28



Identification	Participants Freq	%	Evaluation
Eosinophil, any stage	1290	99.2	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	4	0.3	Educational
Basophil, any stage	3	0.2	Educational
Neutrophil, segmented or band	2	0.1	Educational
Neutrophil with Pelger-Huët nucleus (acquired or congenital)	1	0.1	Educational

The arrowed cell is an eosinophil, as correctly identified by 99.2% of participants. Eosinophils are generally easily 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 their 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. This refractile appearance is not apparent in photomicrographs or pictures, however. Due to inherent problems with color rendition on photomicrographs, which is sometimes imperfect, eosinophil granules may appear lighter or darker than on a freshly stained blood film. Discoloration may give the granules a blue, brown, or pink tint. Nonetheless, the uniform, coarse nature of eosinophil granules is characteristic and differs from the smaller, finer granules of neutrophils. Occasionally, eosinophils can become degranulated, with only a few orange-red granules remaining visible within the faint pink cytoplasm. About 80% of segmented eosinophils will have the classic two-lobed appearance. Typically, these lobes are of equal size and round to ovoid or potato shaped with dense, compact chromatin. The remainder of segmented eosinophils will have 3 lobes and an occasional cell will exhibit 4 to 5 lobes.

VPBS-29

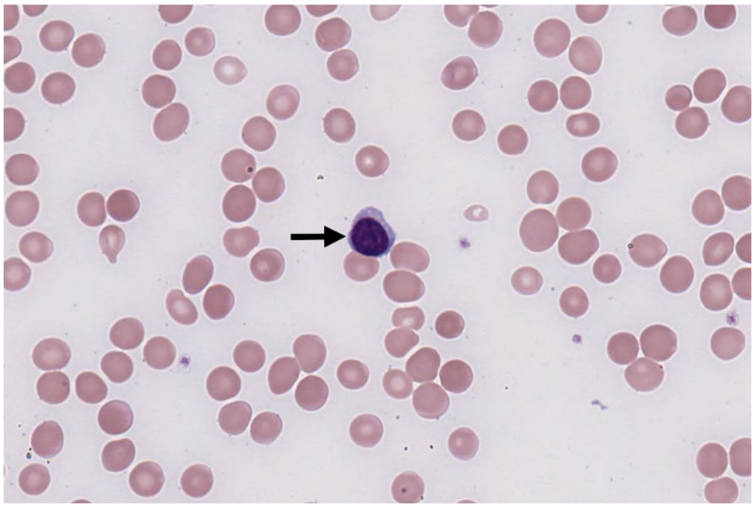


Identification	Participants Freq	%	Evaluation
Spherocyte	1255	96.5	Educational
Microcyte (with increased central pallor)	36	2.8	Educational
Erythrocyte, normal	4	0.3	Educational
Stomatocyte	3	0.2	Educational
Blast cell	1	0.1	Educational
Ovalocyte (elliptocyte)	1	0.1	Educational

The arrowed cell is a spherocyte, as correctly identified by 96.5% of participants. Spherocytes are identified as densely staining, spherical, or globular red blood cells with normal or slightly reduced volume (ie, normal or low MCV) and increased thickness (more than 3 µm), but with decreased diameter (usually less than 6.5 µm) and usually without central pallor. These cells appear denser than normal RBCs and are commonly found in hereditary spherocytosis and immune hemolytic anemias.

2.8% of participants incorrectly identified the arrowed cell as a microcyte with increased central pallor. Microspherocytes (spherocytes measuring 4 µm or less in diameter) are frequently seen in severe burns or microangiopathies and represent rounded-up fragments of red blood cells. Spherocytes which can be very small in size, are distinguished from microcytes by their lack central pallor and should be specifically identified rather than classified as “microcytes.” Microcytes are commonly seen in iron deficiency anemia, thalassemia, lead poisoning and some cases of anemia of chronic disease, retain central pallor.

VPBS-30



Identification	Participants Freq	%	Evaluation
Lymphocyte	1165	89.6	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	69	5.3	Educational
Nucleated red blood cell, normal or abnormal morphology	42	3.2	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	12	0.9	Educational
Lymphocyte, large granular	4	0.3	Educational
Monocyte	2	0.1	Educational
Neutrophil, metamyelocyte	2	0.1	Educational
Malignant lymphoid cell (other than blast)	1	0.1	Educational
Microcyte (with increased central pallor)	1	0.1	Educational
Monocyte, immature (promonocyte, monoblast)	1	0.1	Educational
Platelet, hypogranular	1	0.1	Educational

The arrowed cell is a lymphocyte, as correctly identified by 89.6% of participants. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 µm with an N:C ratio ranging from 5:1 to 2:1. Most lymphocytes have round to oval nuclei that may be slightly indented or notched. The chromatin is 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. Occasionally, the edges may be slightly frayed or pointed due to artifacts induced during smear preparation. Occasional lymphocytes will have a small clear zone, or hof, adjacent to one side of the nucleus.

The arrowed cell was incorrectly identified as a lymphocyte, reactive by 5.3% of participants. Reactive lymphocytes can demonstrate a variety of morphologic features, however, they contain more abundant cytoplasm than lymphocytes and nucleoli are sometimes present. The arrowed cell has scant cytoplasm and condensed chromatin, consistent with the intended response of lymphocyte.

The arrowed cell was incorrectly identified as a nucleated red blood cell by 3.2% of participants. Circulating nucleated red blood cells are typically at the orthochromatic stage of development. Orthochromic normoblasts are 8 to 12 µm in diameter and the nucleus is very small, often pyknotic. The cytoplasm is typically pinkish orange with little or absent basophilia. While the arrowed cell is similar in size, the coarse, clumped chromatin and cytoplasmic basophilia are consistent with the intended response of lymphocyte.

Case Presentation:

This peripheral blood smear is from a 76-year-old woman presenting with fatigue. Laboratory data include: WBC = 3.1 x 10E9/L; RBC = 2.67 x 10E12/L; HGB = 8.3 g/dL; HCT = 27.1%; MCV = 108 fL; PLT = 122 x 10E9/L; and RDW = 16%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Macrocytic anemia

The patient is presenting with macrocytic anemia, which, according to the World Health Organization (WHO) classification, is defined as a decrease in hemoglobin concentration of less than 12 g/dL in females and less than 13 g/dL in males. Etiologies of anemia are plentiful, especially in an older patient population, but can be largely divided into abnormalities in red blood cell (RBC) production (eg, reduced or ineffective erythropoiesis) and RBC survival (eg, increased loss or decreased RBC life span). Occasionally, anemia may be relative due to dilutional effects of peripheral volume expansion (eg, aggressive fluid resuscitation) or pregnancy.

Several approaches to anemia classification are offered though none completely encompass the idiosyncrasies of the disease. Nonetheless, a commonly employed classification is based on the average size of RBC—measured by the mean corpuscular volume (MCV). Using this framework, anemia can be broadly divided into microcytic (MCV < 80 fL), normocytic (MCV 80 - 100 fL) or macrocytic (MCV > 100 fL) (Table 1). Reticulocyte count and RBC size variation (measured by red cell distribution width (RDW)) are analyzed in conjunction with the MCV to assist in determining whether anemia is due to RBC production versus RBC survival.

Table 1: Anemia Classification based on MCV with the most common causes

Microcytic (MCV < 80 fL)	Thalassemia Iron deficiency Intravascular hemolysis
Normocytic (MCV 80 - 100 fL)	Anemia of chronic disease Aplastic anemia Hemoglobinopathies Hemorrhage Myelophthisic process
Macrocytic (MCV > 100 fL)	Aplastic anemia Vitamin B12 or folate deficiency Hemolysis Cold agglutinin disease Liver disease Myelodysplastic syndrome

*MDS: Myelodysplastic syndrome; AIHA: autoimmune hemolytic anemia

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Question 1. Anemia is broadly subclassified into microcytic, normocytic and macrocytic based on what CBC parameter?

A. Hemoglobin concentration
B. Mean corpuscular volume (MCV)
C. Red cell distribution width (RDW)
D. White blood cell (WBC) count

Plasma cell neoplasms, including multiple myeloma, commonly present with anemia and patients need to undergo an extensive examination that includes laboratory, radiologic, molecular testing, as well as bone marrow

biopsy, if needed. Bone marrow examination of this patient revealed greater than 60% infiltration by neoplastic plasma cells that in conjunction with the other testing resulted in a clinical diagnosis of multiple myeloma.

Plasma cell neoplasms are a diverse group of disorders originating from terminally differentiated B-cells which typically secrete homogenous immunoglobulin. Serum protein electrophoresis (SPEP) is used to detect a monoclonal ("M") protein, or paraprotein. A M-protein is a monoclonal immunoglobulin secreted by neoplastic cells indicating the presence of an underlying clonal plasma cell disorder. Although not seen in this case, microscopic examination of the peripheral blood smear on a patient with a plasma cell neoplasm may reveal rouleaux formation—at least four RBCs organized in a linear array that resembles a stack of coins—due to increased serum proteins.

Clonal plasma cell proliferations are divided into three main categories according to the WHO: 1) monoclonal gammopathy of undetermined significance (MGUS), 2) plasma cell myeloma (PCM), and 3) plasmacytoma (Table 2). MGUS is a precursor plasma cell lesion that affects approximately 3% of persons older than 50 years of age. MGUS is defined by low-level M-protein, < 10% marrow clonal plasma cells and no evidence of end-organ damage. PCM (symptomatic multiple myeloma) requires at least 10% marrow clonal plasma cells (or the presence of a plasmacytoma), an M-protein concentration of at least 30 g/dL (or urine M-protein of at least 0.5 g/24 hrs), and evidence of end-organ damage or tissue impairment, the latter caused by deposition of excess immunoglobulin. Hypercalcemia, renal insufficiency, anemia, and lytic bone lesions are features of organ damage. Non-secretory PCM and asymptomatic (smoldering) PCM are clinical variants diagnosed based on the M-protein concentration, frequency of marrow plasma cells, and presence or absence of tissue impairment. Clonal plasma cell proliferations may also present localized to the bone or soft tissue, solitary plasmacytoma of bone and extraosseous plasmacytoma, respectively. Osseous plasmacytomas are slightly more common than extraosseous tumors. By definition, these localized tumors have no other clinical or radiographic evidence or physical manifestations of PCM.

Table 2: Classification of Plasma Cell Neoplasms

	Serum M protein	Urine M protein	Plasma cell frequency	Evidence of end organ damage
MGUS	< 30 g/L	< 0.5 g/24 hrs	< 10% in BM	No
Plasma cell myeloma (PCM)	≥ 30 g/L^	≥ 0.5 g/24 hrs^	> 10% in BM	Yes
Smoldering PCM	≥ 30 g/L	≥ 0.5 g/24 hrs	> 10% in BM	No
Non-secretory	None	None	> 10% in BM	Yes
Plasma cell leukemia (PCL)	≥ 30 g/L^	≥ 0.5 g/24 hrs^	PB: > 20% of WBCs, or absolute count > 2.0 x 10E9/L	Yes
Solitary plasmacytoma of bone	None	None	Solitary lesion (no plasma cell infiltrate in random BM biopsy)	No
Extraosseous plasmacytoma	None	None	None	None

*MGUS: monoclonal gammopathy of undetermined significance; BM: bone marrow; PB: peripheral blood
^ Though commonly reported above the provided values, increased M-protein concentrations are not required for classification or diagnosis of symptomatic PCM and PCL.

Question 2. Plasma cell myeloma (PCM) is diagnosed based on which of the following:

A. At least 10% of clonal plasma cells in the bone marrow and lytic bone lesions
B. Low hemoglobin concentration and high mean corpuscular volume (MCV)
C. Rouleaux formation and reticulocytosis
D. The presence of M-protein and fatigue

The pathophysiologic mechanism behind myeloma-associated anemia may include anemia of chronic disease (altered cytokine production), relative erythropoietin (EPO) deficiency (due partly to renal impairment) and myelosuppressive effects of marrow infiltration by plasma cells. Impaired iron metabolism is also seen in myeloma patients. As such, anemia in PCM patients may be normocytic, macrocytic or microcytic.

Despite numerous attempts to classify anemia, and regardless of the underlying etiology, the condition is complex and often multifactorial. Evaluation of anemia thus requires comprehensive clinical and laboratory investigation in order to treat patients appropriately.

Julie Rosser, DO, FCAP
Hematology and Clinical Microscopy Committee

REFERENCES:

1. Swerdlow SH, Campo E, Harris NL, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. International Agency for Research on Cancer; 2017.
2. König C, Kleber M, Ihorst G, et al. Prevalence of iron overload vs iron deficiency in multiple myeloma: resembling or different from MDS--and stem cell transplant (SCT)-patients?. *Clin Lymphoma Myeloma Leu*k. 2013;13(6):671-680.e3. doi:10.1016/j.clml.2013.06.001

ANSWERS TO QUESTIONS:

Question 1: (B) Mean corpuscular volume (MCV)
Anemia is typically broadly classified based on RBC cell size into microcytic (MCV < 80 fL), normocytic (MCV 80 - 100 fL) or macrocytic (MCV > 100 fL).

A is incorrect. The amount of hemoglobin, or hemoglobin concentration, allows for designation of anemia. It does not provide clarification of subclassification.

C is incorrect. RDW provides an idea of how variable the size is within a sample of RBCs.

D is incorrect. White blood cell (WBC) count does not influence classification of anemia.

Question 2: (A) At least 10% of clonal plasma cells in the bone marrow and lytic bone lesions
PCM (symptomatic multiple myeloma) requires at least 10% marrow clonal plasma cells (or the presence of a plasmacytoma), and/or evidence of end-organ damage or tissue impairment.

B is incorrect. Low hemoglobin concentration and high mean corpuscular volume (MCV) is diagnostic of macrocytic anemia. Macrocytic anemia may be related to abnormal RBC development, abnormal RBC membrane composition, increased reticulocyte count or a combination of these. Myeloma-associated anemia may be microcytic (MCV < 80 fL), normocytic (MCV = 80 - 100 fL) or macrocytic (MCV > 100 fL).

C is incorrect. Rouleaux formation is caused by an increase in serum proteins, such as immunoglobulins and fibrinogen. Although this is characteristically seen with PCM, other causes occur more frequently, such as acute and chronic infections, connective tissue diseases, and chronic liver disease. Reticulocytosis, or increase in circulating immature red blood cells, indicates active marrow regeneration of red blood cells, typically as a response to anemia.

D is incorrect. An M-protein is a monoclonal immunoglobulin secreted by neoplastic plasma cells. Low-levels of serum M-protein (< 30 g/dL) with < 10% clonal plasma cells in the marrow, and in the absence of end-organ damage or tissue impairment, defines monoclonal gammopathy of undetermined significance (MGUS).

VPBS-31

Clinical History for VPBS-32 – VPBS-36

This peripheral blood smear is from a 29-year-old Asian woman with lifelong history of microcytic anemia. Laboratory data include: WBC = 8.8 x 10E9/L; RBC = 4.22 x 10E12/L; HGB = 9.4 g/dL; HCT = 30.9%; MCV = 70 fL; and PLT = 199 x 10E9/L; and RDW = 28%.

(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=0747f136-0674-42b0-a565-cfb327baa3af

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)	1284	44.3	4.5	10.2	45	31	58
Lymphocytes	1270	43.6	6.3	14.3	44	25	61
Lymphocytes, reactive	780	3.7	4.1	*	2	0	17
Monocytes	1250	6.4	3.2	49.5	6	0	16
Eosinophils	1013	1.1	0.7	64.7	1	0	3
Basophils	851	0.7	0.6	81.7	1	0	2
Metamyelocytes	598	0.5	0.8	*	0	0	3
Myelocytes	618	0.6	0.9	*	0	0	3
Promyelocytes	473	0.0	0.0	0.0	0	0	0
Blasts	604	0.4	0.7	*	0	0	2
nRBC/100 WBC	1228	9.7	4.3	44.0	10	0	22

WBC Differential - 10E9/L**	N	MEAN	SD	CV%	MEDIAN	MIN	MAX
Neutrophils (segmented or bands)	1186	3.891	0.409	10.5	3.90	2.46	5.28
Lymphocytes	1171	3.831	0.561	14.6	3.87	2.11	5.37
Lymphocytes, reactive	713	0.315	0.354	*	0.18	0.00	1.50
Monocytes	1178	0.585	0.317	54.3	0.53	0.00	1.85
Eosinophils	923	0.101	0.066	64.9	0.09	0.00	0.30
Basophils	774	0.062	0.052	82.7	0.09	0.00	0.20
Metamyelocytes	545	0.045	0.069	*	0.00	0.00	0.30
Myelocytes	574	0.059	0.085	*	0.00	0.00	0.35
Promyelocytes	436	0.000	0.000	*	0.00	0.00	0.01
Blasts	559	0.042	0.065	*	0.00	0.00	0.26

*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.

Other cells: *All cells not listed on result form and cells not differentiated by your laboratory*

Cells not listed/differentiated	N = 60 Freq
Myeloid precursors	6
Abnormal/atypical/reactive lymphocyte	4
Basket/smudge cell	5
Hairy cell	5
Immature/blast cell	3
Would refer for identification	37

Platelet Estimate

Intended Response: Adequate/normal platelets	N = 1282 Freq	%
Adequate/normal platelets	1238	96.0
Decreased platelets	41	3.2
Increased platelets	3	0.2
Unable to quantitate - platelet clumps present	-	-

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 N = 4957 Freq	Total Responses N = 4957 % Total Response	Total Unique Kits N = 1289 % Unique Kits
Teardrop cell (dacrocyte)	1205	24.3	93.5
Polychromatophilic (non-nucleated) red blood cell	915	18.5	71.0
Target cell (codocyte)	903	18.2	70.0
Microcyte (with increased central pallor)	623	12.6	48.3
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	533	10.8	41.4
Stomatocyte	209	4.2	16.2
Ovalocyte (elliptocyte)	127	2.6	9.8
Nucleated red blood cell, normal or abnormal morphology	117	2.4	9.1
Basophilic stippling (coarse)	104	2.1	8.1
Macrocyte, oval or round (excluding polychromatophilic red bood cell)	92	1.9	7.1
Erythrocyte with overlying platelet	40	0.8	3.1
Howell-Jolly body	26	0.5	2.0
Acanthocyte (spur cell)	16	0.3	1.2
Rouleaux	16	0.3	1.2
Spherocyte	11	0.2	0.9
Bite cell (degmacyte)	7	0.1	0.5
Erythrocyte, normal	5	0.1	0.4
Pappenheimer bodies (iron or Wright stain)	4	0.1	0.3
Blister cell/Prekeratocyte	2	0.0	0.2
Echinocyte (burr cell, crenated cell)	2	0.0	0.2

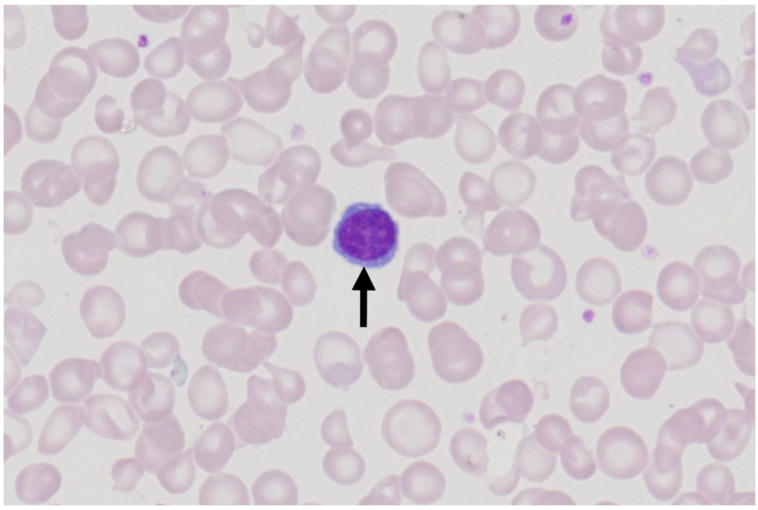
VPBS-31, cont'd.

Committee Comments on the CBC and Peripheral Blood Whole Slide Image

The morphologic findings show microcytic anemia with significant anisopoikilocytosis including target cells, teardrop cells, and hypochromia. A few irregularly contracted cells are identified. There is slight polychromasia, reflective of an increased reticulocyte count. The white blood cell count and differential are within normal limits, and the platelets have normal granularity and size. The combined findings suggest a thalassemic hemoglobinopathy with some hemoglobin instability. Clues to the diagnosis are the spectrum of morphologic changes in the red cells, microcytosis, and elevated red cell distribution width.

Cell Identification

VPBS-32



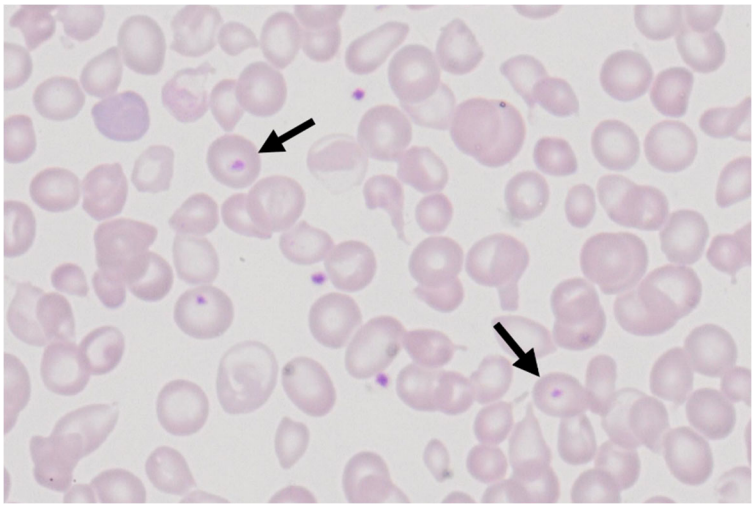
Identification	Participants Freq	%	Evaluation
Lymphocyte	1232	94.8	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	43	3.3	Educational
Malignant lymphoid cell (other than blast)	16	1.2	Educational
Blast cell	2	0.1	Educational
Lymphocyte, large granular	2	0.1	Educational
Nucleated red blood cell, normal or abnormal morphology	2	0.1	Educational
Immature or abnormal cell, would refer for identification	1	0.1	Educational
Neutrophil, promyelocyte	1	0.1	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	1	0.1	Educational

The arrowed cells cell is a lymphocyte, as correctly identified by 94.8% of participants. While most normal lymphocytes are fairly homogeneous, they do 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. Occasionally, the edges may be slightly frayed or pointed due to artifacts induced during smear preparation. Occasional lymphocytes will have a small clear zone, or hof, adjacent to one side of the nucleus.

3.3% of participants incorrectly identified the arrowed cell is a lymphocyte, reactive (including plasmacytoid and immunoblastic forms). The arrowed cells are relatively uniform in appearance and small in size. Reactive lymphocytes are identified by their wide range of cellular sizes and shapes. Frequently, reactive lymphocytes are larger (10 to 25 µm) in size than the arrowed cells. Plasmacytoid reactive lymphocytes have more abundant cytoplasm than the arrowed cells, and possibly a perinuclear clear zone, or hof. Immunoblastic reactive lymphocytes are larger than the arrowed cells and have deeply basophilic cytoplasm and prominent nucleoli.

1.2% of the participants incorrectly identified the arrowed cell as a malignant lymphoid cell (other than blast). While malignant lymphoid cells can have a variety of appearances, the arrowed cells do not show morphologic features typical of any subtype of malignant lymphoid cells. Furthermore, the clinical history (lifelong microcytic anemia) and CBC (no leukocytosis is present) do not suggest a malignant process.

VPBS-33



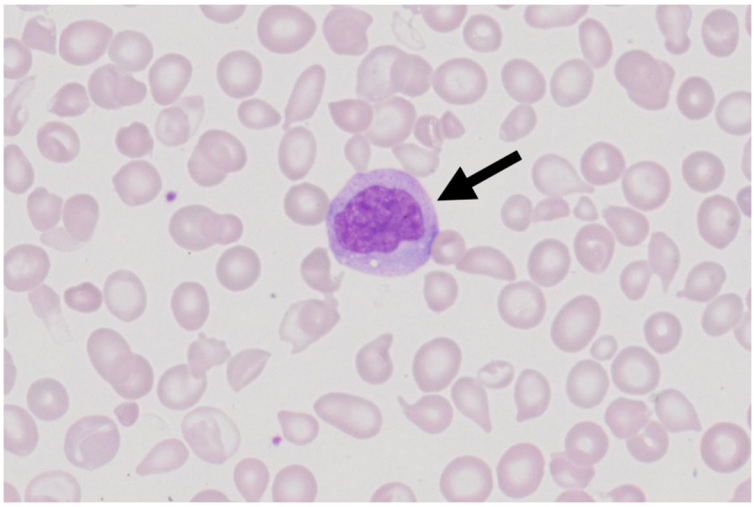
Identification	Participants Freq	%	Evaluation
Erythrocyte with overlying platelet	1237	95.2	Educational
Howell-Jolly body	27	2.1	Educational
Platelet, normal	25	1.9	Educational
Parasite(s) seen, referred for definitive identification	4	0.3	Educational
Erythrocyte, normal	2	0.1	Educational
Pappenheimer bodies (iron or Wright stain)	2	0.1	Educational
Nucleated red blood cell, normal or abnormal morphology	1	0.1	Educational
Ovalocyte (elliptocyte)	1	0.1	Educational
Platelet satellitism	1	0.1	Educational

The arrowed cells are red blood cells with overlying platelets, as correctly identified by 95.2% of participants. In preparing a peripheral blood smear, platelets may adhere to or overlap red blood cells, suggesting a red blood cell inclusion or parasite. A correct interpretation depends on carefully examining the morphology of the platelet and comparing the size, staining characteristics, and granularity with known platelets in the same field as well as determining if the platelet is in the same plane of focus as the red blood cell. Many times the platelet is surrounded by a thin clear zone or halo, which is not a feature of most genuine red blood cell inclusions.

2.1% of participants incorrectly identified the arrowed cells as Howell-Jolly bodies. Howell-Jolly bodies are characterized by a distinct border and dark purple homogeneous color, as opposed to the thin clear zone or halo around the platelet and the irregular granular nature of the platelet seen in the arrowed cells.

1.9% of participants identified the arrowed cells as normal platelets. While these participants correctly identified these as platelets, for proficiency testing purposes the more precise answer of ‘red blood cell with overlying platelet’ is correct.

VPBS-34

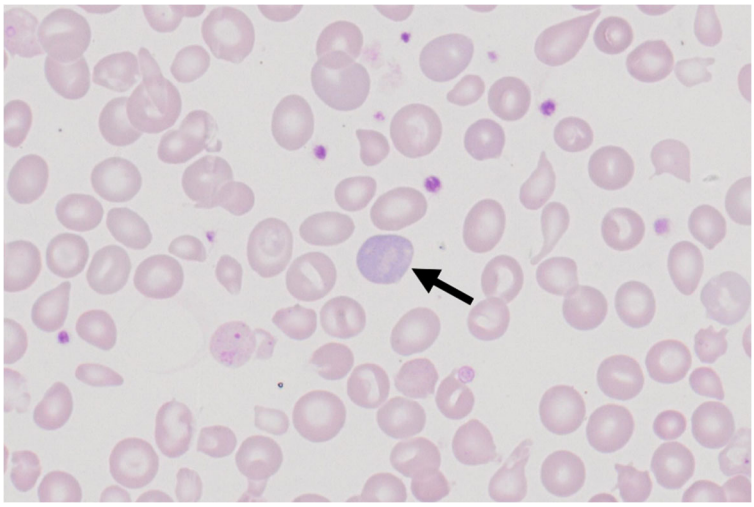


Identification	Participants Freq	%	Evaluation
Monocyte	1242	95.5	Educational
Monocyte, immature (promonocyte, monoblast)	25	1.9	Educational
Neutrophil, metamyelocyte	9	0.7	Educational
Neutrophil, myelocyte	6	0.5	Educational
Neutrophil, segmented or band	4	0.3	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	3	0.2	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	2	0.1	Educational
Neutrophil, giant band or giant metamyelocyte	2	0.1	Educational
Eosinophil, any stage	1	0.1	Educational
Erythrocyte with overlying platelet	1	0.1	Educational
Immature or abnormal cell, would refer for identification	1	0.1	Educational
Lymphocyte, large granular	1	0.1	Educational
Malignant lymphoid cell (other than blast)	1	0.1	Educational
Neutrophil, promyelocyte	1	0.1	Educational
Polychromatophilic (non-nucleated) red blood cell	1	0.1	Educational

The arrowed cell is a monocyte, as correctly identified by 95.5% of participants. Monocytes are slightly larger than neutrophils, ranging from 12 to 20 µm in diameter. The majority of monocytes are round with smooth edges, but some may have pseudopod-like cytoplasmic extensions. The cytoplasm is abundant, with a gray or gray-blue ground-glass appearance, and may contain vacuoles or fine, evenly distributed azurophilic granules. The N:C ratio ranges from 4:1 to 2:1. The nucleus is usually indented, often resembling a three-pointed hat, but it can also be folded or band-like. The chromatin is condensed but is monocytes may contain a small, inconspicuous nucleolus.

1.9% of participants incorrectly identified the arrowed cell as an immature monocyte (promonocyte or monoblast). For proficiency testing purposes, this answer is reserved for malignant cells in the context of acute monocytic/monoblastic leukemia, acute myelomonocytic leukemia, chronic myelomonocytic leukemia, or myelodysplastic syndromes; the peripheral smear and CBC do not support these diagnosis. In terms of morphologic identification, immature monocytes are generally larger than the arrowed cell and do not typically show cytoplasmic vacuolization. The nuclei of immature monocytes demonstrate finer chromatin and show delicate folding or creasing, unlike the arrowed cell.

VPBS-35

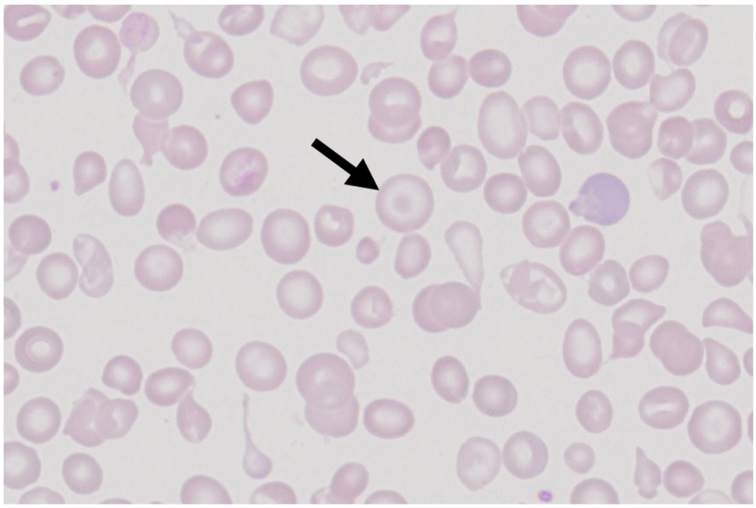


Identification	Participants Freq	%	Evaluation
Polychromatophilic (non-nucleated) red blood cell	1276	98.2	Educational
Basophilic stippling (coarse)	16	1.2	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	2	0.1	Educational
Monocyte	2	0.1	Educational
Erythrocyte, normal	1	0.1	Educational
Metastatic tumor cell or tumor cell clump	1	0.1	Educational
Spherocyte	1	0.1	Educational
Stomatocyte	1	0.1	Educational

The arrowed cell is a polychromatophilic red blood cell, as correctly identified by 98.2% of participants. A polychromatophilic red blood cell is a non-nucleated, round or ovoid red blood cell that represents the final stage of red blood cell maturation after exiting the bone marrow. It is larger than a mature erythrocyte and usually lacks central pallor. It primarily contains hemoglobin with a small amount of RNA, and thereby stains pale purple to pink-gray with Romanowsky or Wright-Giemsa stain. These cells can be stained as reticulocytes and enumerated by using supravital stains, such as new methylene blue. With supravital staining, reticulocytes reveal deep blue granular and/or filamentous structures. This reticulin network is called the “substantia reticulofilamentosa.” The amount of precipitated RNA and intensity of polychromasia varies inversely with the age of the reticulocyte. The intensity of the polychromasia will vary with the amount of RNA and the age of the cell, with younger cells (ie, earlier polychromatophilic red cells) appearing more purple or blue and relatively more mature cells (ie, later polychromatophilic red cells) appearing more pink-gray. Automated technologies for assessing reticulocytes improve the accuracy and precision of determining reticulocyte numbers.

1.2% of participants incorrectly identified the arrowed cell as containing coarse basophilic stippling. Fine basophilic stippling can be seen in reticulocytes, as in the arrowed cell (which could also be classified as a reticulocyte if stained with a supravital dye). However, coarse basophilic stippling is more easily identified on routine preparations, is more prominent than in the arrowed cells, and shows more distinguishable blue-gray granules than in fine basophilic stippling.

VPBS-36



Identification	Participants Freq	%	Evaluation
Target cell (codocyte)	1296	99.7	Educational
Teardrop cell (dacrocyte)	2	0.1	Educational
Lymphocyte	1	0.1	Educational
Stomatocyte	1	0.1	Educational

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

Calculating Absolute Counts:

The absolute count (ABS) for any leukocyte subset is calculated utilizing the following formula:

$$\text{ABS (× 10E9/L)} = \frac{\text{\% × WBC (× 10E9/L)}}{100}$$

where % is the percentage of the leukocyte (neutrophils, lymphocytes, etc. obtained from the manual differential), WBC represents the total white blood cell count (×10E9/L) for the particular case, and ABS represents the absolute count for the subset in question (neutrophils, lymphocytes, etc.). For example, if the total white count (WBC) is 8.0 × 10E9/L and lymphocytes are 25% of the differential, then the absolute lymphocyte count (ABS) is obtained as follows:

$$\text{ABS (× 10E9/L)} = \frac{\text{25\% × 8.0 (× 10E9/L)}}{100}$$

$$= 2.00$$

REFERENCE:

1. CAP Hematology checklist item HEM.36820

NOTE: For WBC differential counts, the CAP recommends that laboratories report absolute cell counts, along with their corresponding reference intervals. The CAP discourages the reporting of percent cell counts without absolute counts on WBC differentials. Laboratories reporting only percent cell counts must provide laboratory established reference intervals.

Actions Laboratories Should Take when a PT Result is Not Graded

The CAP uses exception reason codes that signify the proficiency testing (PT) for an analyte has not been graded. The exception reason code is located on the evaluation report in brackets to the right of the result. Your laboratory must identify all analytes with an exception reason code, review, and document the acceptability of performance as outlined below and retain documentation of review for at least 2 years. The actions laboratories should take include, but are not limited to:

Code	***Exception Reason Code Description***	***Action Required***
11	Unable to analyze	Document why the specimens were not analyzed (eg, instrument not functioning or reagents not available). Perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
20	Response was not formally graded due to insufficient peer group data. Please see the participant summary for additional information.	Applies to a response that is not formally evaluated when a peer group is not established due to fewer than 10 laboratories reporting. Document that the laboratory performed a self-evaluation using the data presented in the participant summary and compared its results to a similar method, all method, all participant statistics, or data tables for groups of 3-9 laboratories, if provided. Perform and document the corrective action of any unacceptable results. If self-evaluation is not possible, it is up to the laboratory director/designee to determine an alternative performance assessment.
21	Specimen problem	Document that the laboratory has reviewed the proper statistics supplied in the participant summary. Perform and document alternative assessment for the period that commercial PT was not tested to the same level and extent that would have been tested. Credit is not awarded in these cases.
22	Result is outside the method/ instrument reportable range	Document the comparison of results to the proper statistics supplied in the participant summary. Verify detection limits. Perform and document the corrective action of any unacceptable results.
24	Incorrect response due to failure to provide a valid response code	Document the laboratory's self-evaluation against the proper statistics and evaluation criteria supplied in the participant summary. Perform and document the corrective action of any unacceptable results. Document corrective action to prevent future failures.
25	Inappropriate use of antimicrobial	Document the investigation of the results as if they were unacceptable and review the proper reference documents to gain knowledge of the reason your response is not appropriate.
26	Educational challenge	Review participant summary for comparative results and document performance accordingly. Evaluation criteria are not established for educational challenges. Laboratories should determine their own evaluation criteria approved by their laboratory director for self-evaluation.
27,31	Lack of participant or referee consensus	Document that the laboratory performed a self-evaluation and compared its results to the intended response when provided in the participant summary. If comparison is not available, perform and document alternative assessment (ie, split samples) for the period that commercial PT reached non-consensus to the same level and extent that would have been tested.
28	Response qualified with a greater than or less than sign; unable to quantitate	Applies to a response that is not formally evaluated when a less than or greater than sign is reported. Document that the laboratory performed a self-evaluation and compared its results to the proper statistics supplied in the participant summary. Verify detection limits. Perform and document the corrective action of any unacceptable results.
30	Scientific committee decision	Applies to a response that is not penalized based on scientific committee decision. Document that the laboratory has reviewed the proper statistics supplied in the participant summary.

Actions Laboratories Should Take when a PT Result is Not Graded

The CAP uses exception reason codes that signify the proficiency testing (PT) for an analyte has not been graded. The exception reason code is located on the evaluation report in brackets to the right of the result. Your laboratory must identify all analytes with an exception reason code, review and document the acceptability of performance as outlined below and retain documentation of review for at least 2 years. The actions laboratories should take include but are not limited to:

Code	***Exception Reason Code Description***	***Action Required***
33	Specimen determined to be unsatisfactory after contacting the CAP	Document that the laboratory has contacted the CAP and no replacements specimens were available. Perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
40 41	Results for this kit were not received. Results for this kit were received past the evaluation cut-off date.	Document why results were not received, corrective action to prevent recurrence and the laboratory's self-evaluation of the results by comparing results to the proper statistics and evaluation criteria supplied in the participant summary. If PT specimens were not analyzed, perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
42	No credit assigned due to absence of response	The participant summary indicates which tests are graded (see evaluation criteria) and which tests are not evaluated/educational. Updates to grading will also be noted. If a test is educational, the laboratory is not penalized for leaving a result(s) blank. If a test is graded (regulated and non-regulated analytes) and your laboratory performs that test, results cannot be left blank. The laboratory is required to submit results for **all** challenges within that test or use an appropriate exception code or indicate test not performed/not applicable/not indicated. Exceptions may be noted in the kit instructions and/or the result form. Document corrective actions to prevent future failures.
44	This drug is not included in our test menu. Use of this code counts as a correct response.	Verify that the drug is not tested on patient samples and document to ensure proper future reporting.
45	Antimicrobial agent is likely ineffective for this organism or site of infection	Document that the laboratory performed a self-evaluation of written protocols and practices for routine reporting of antimicrobial susceptibility reports to patient medical records. Document that routine reporting of this result to clinicians for patient care is compliant with specific recommendations of relevant medical staff and committees (eg, infectious diseases, pharmacy and therapeutics, infection control).
77	Improper use of the exception code for this mailing	Document the identification of the correct code to use for future mailings.
91	There was an insufficient number of contributing challenges to establish a composite grade.	Document the investigation of the result as if it were an unacceptable result. Perform and document the corrective action if required.
35, 43, 46, 88, 92	Various codes	No action required.

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For assistance, call the Customer Contact Center at 800-323-4040 or 847-832-7000 (Country code: 001) option 1.

VPBS-B 2022: Hemoglobin E/Beta Thalassemia

Case Presentation

This peripheral blood smear is from a 29-year-old Asian woman with a lifelong history of microcytic anemia. Laboratory data include: WBC = 8.8 x 10E9/L; RBC = 4.22 x 10E12/L; HGB = 9.4 g/dL; HCT = 30.9%; MCV = 70 fL; PLT = 199 x 10E9/L; and RDW = 28%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

INTRODUCTION

Hemoglobin E/beta thalassemia (HbE/b-thal) is a compound heterozygous state resulting from mutations or abnormalities in both beta globin genes. Hemoglobin E is caused by a substitution of lysine for glutamate at position 26. A mutation or deletion in or near the other beta globin gene causes beta thalassemia through absent or reduced rates of synthesis of beta globin (giving rise to a β^+ or β^0 allele, respectively). Hemoglobin E results in a structurally abnormal hemoglobin in which the mutated beta globin is produced at a decreased rate and is considered a thalassemic hemoglobinopathy with mild clinical effects as a single abnormality, whether it is inherited as HbE trait or as HbEE homozygous state. However, when HbE trait is paired with beta thalassemia trait, there is wider variability in clinical symptoms and typically, moderately severe anemia. The clinical picture depends on whether hemoglobin A is produced, and whether other additional hemoglobin abnormalities are inherited.[1-3]

Epidemiology

The HbE/b-thal compound state is the most common beta thalassemia phenotype in the world and represents approximately half of patients affected by clinically severe beta thalassemia disease. HbE/b-thal occurs throughout a large part of Southeast Asia including Thailand, Indonesia, Sri Lanka, northeast India, southern China, and Bangladesh. The frequency of HbE approaches 60% in many regions of Thailand, Laos, and Cambodia. While HbE is most prevalent in and around Thailand, it is increasingly common in North America and Europe. Beta thalassemia mutations are diverse, with over 200 recognized mutations in a wide range of ethnic groups. Beta thalassemia is common around the Mediterranean, in India, Southeast Asia, and in individuals of African descent. The World Health Organization expects high estimates of HbE/b-thal in areas with a high carrier frequency in upcoming decades, and a rising prevalence of HbE/b-thal in the United States due to demographic changes.[2]

Pathogenesis

HbE/b-thal results from co-inheritance of a beta thalassemia allele from one parent and a mutated beta allele (HbE) from the other parent. The mutation that causes hemoglobin E results in an amino acid substitution, which activates a cryptic splice site that competes with the normal ribonucleic acid (RNA) splice site, resulting in decreased production of the normally processed RNA. The abnormally spliced mRNA is non-functional, and HbE is produced at a reduced rate and behaves like a thalassemic disorder. A globin chain imbalance results in an excess of alpha chains, in contrast to the normal hemoglobin molecule that contains two alpha and two beta globins. HbE is slightly unstable as a laboratory specimen, a state that does not appear to occur *in vivo*, with the exception of febrile patients who may experience accelerated hemolysis.[1,4]

The clinical manifestations of HbE/b-thal result from the globin chain imbalance, ineffective erythropoiesis, oxidative damage, and shortened red blood cell survival.

Clinical Features

There is marked clinical variability, but the most common phenotypes are thalassemia intermedia or thalassemia major. A small subset of HbE/b-thal individuals (approximately 14%) may experience mild symptoms and a thalassemia minor clinical picture, but the majority have a disease that is at least moderately severe. Factors that influence the severity of the disease course include the type of beta thalassemia mutation co-inherited with HbE, the presence of polymorphisms associated with increased amounts of fetal hemoglobin, and the possibility of co-inheritance of alpha thalassemia with HbE/b-thal. Table 1 provides a list of factors that ameliorate or worsen the HbE/b-thal clinical state.

The most severely affected patients are blood transfusion-dependent and have hepatosplenomegaly, jaundice, delayed or stunted growth and sexual maturation, and facial deformity associated with expansion of the bone marrow cavity. In patients who have received a therapeutic splenectomy, there is a high rate of thromboembolism. The effects of iron overload from frequent transfusions include liver fibrosis and diabetes mellitus. Patients who are less severely affected may have splenomegaly and chronic anemia but may not require transfusions. Stressors such as pregnancy, severe infection, or other illness can temporarily result in more severe disease requiring transfusion.[1,5]

Table 1. Impact of co-inherited or other factors on the severity of hemoglobin E/b thalassemia

Variable	Impact
Co-inheritance of alpha thalassemia	Improves disease
Co-inheritance of Hemoglobin Constant Spring	Improves disease
Homozygous non-deletional hereditary persistence of fetal hemoglobin	Improves disease
Polymorphisms in the HbF promotor region leading to increased HbF	Improves disease
Triplicated alpha genes	Worsens disease
Decreased or absent quantity of hemoglobin A	Worsens disease
Higher proportion of alternatively spliced mRNA	Worsens disease

Adapted from Vichinsky[2]

DIAGNOSIS

Laboratory features, complete blood count, and peripheral blood smear evaluation

Patients with HbE/b-thal have moderate anemia, averaging 7 - 8 g/dL with an elevated red cell distribution width (RDW) and decreased mean corpuscular volume (MCV) of approximately 60 fL. The hemoglobin concentration is lower than in HbE trait or HbEE homozygous state. The mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) are also reduced. The reticulocyte count is increased. The peripheral blood smear demonstrates anisocytosis and poikilocytosis, with target cells, irregularly contracted cells, hypochromia, and nucleated red blood cells.

Patients with severe phenotypes of HbE/b-thal may undergo therapeutic splenectomy. In addition to the changes noted above, the peripheral blood smear may show an increase in circulating nucleated red blood cells, Pappenheimer bodies, and Heinz body-like alpha chain precipitates.

Hydroxyurea therapy is associated with a slight rise in the hemoglobin concentration and a rise in the MCV.

Individuals with related disorders (HbE trait, HbEE homozygosity, and beta thalassemia trait) have some overlapping features with HbE/b-thal but can be distinguished based on a combination of peripheral blood findings and hemoglobin electrophoresis. Patients with HbEE homozygosity have microcytosis and variable numbers of target cells with a normal hemoglobin concentration or mild anemia. HbE trait as an isolated abnormality may show a normal peripheral blood smear or have hypochromia, microcytosis, and target cells. Beta thalassemia trait as an isolated abnormality varies depending upon the phenotype, and may appear indistinguishable from normal, may have microcytosis, or may appear markedly abnormal with microcytosis, anisocytosis, hypochromia, and poikilocytosis.[1,2]

Hemoglobin electrophoresis

A combination of laboratory techniques is required to make a diagnosis of HbE/b-thal. For most purposes, a combination of at least two laboratory methods should be performed and interpreted in combination with the clinical features, the ancestry and ethnicity of the patient, and complete blood count and peripheral blood smear review. All methods will show hemoglobins E, A2, and F in the case of HbE/β^0 thalassemia and hemoglobins E, A2, F, and A in the case of HbE/β^+ thalassemia. **Capillary zone electrophoresis** will show an abnormal hemoglobin migrating in the HbE zone. The HbA2 percentage may appear slightly increased due to the reduced production of HbA caused by the HbE mutation. A variable amount of HbA is present in the HbA zone (zone 8), depending upon the nature of the beta thalassemia mutation. Hemoglobin F is significantly elevated and ranges from 30% - 60% in HbE/β^0 thalassemia and shows a variable elevation in HbE/β^+ thalassemia. **Cellulose acetate and agarose agar at acid pH** are used by laboratories to confirm abnormal bands with specific migration patterns. On alkaline electrophoresis, HbE migrates in the HbC/HbA2/HbO position, while on acid agar electrophoresis HbE migrates with HbA. **High performance liquid chromatography** will show a variable amount of hemoglobin A and F, and HbE will be visible as a variant hemoglobin with a retention time in the A2 window. **Isoelectric focusing** is another method of hemoglobin electrophoresis, which separates hemoglobins based on their isoelectric point along a pH gradient and will show an abnormal band representing HbE at a position slightly anodal to HbA2.[3,4]

DNA-based methods including sequencing of the *HBB* gene can identify beta globin gene defects, but these are not typically used as screening tests and are not necessary for most cases.

Differential Diagnosis

The differential diagnosis of HbE/b-thal includes other hemoglobin disorders that occur with a high prevalence in Southeast Asia. These can be differentiated based on the pattern and quantity of hemoglobin typing along with clinical and laboratory data. See Table 2.

Table 2. Differential diagnosis of hemoglobin E/b thalassemia and corresponding characteristics

	Clinical	Laboratory	Hb typing
HbE trait	Asymptomatic	Microcytosis; MCV ~ 75 fL, no anemia	HbE 30% - 35%, HbA 65% - 70%, HbF < 2%
Homozygous HbE	Asymptomatic	Microcytosis; MCV ~ 67 fL, no anemia	HbE 99%, some HbF
He/β^+ thalassemia	Variable, but typically moderately severe thalassemia	Microcytosis; MCV ~ 60 fL, anemia with average hemoglobin 7 - 8 g/dL	HbE 40% - 50%, HbA ~ 10%, HbA2 slightly elevated, and HbF ~ 30% - 40%
HbE/β^0 thalassemia	Variable, but typically moderately severe thalassemia	Microcytosis; MCV ~ 60 fL, anemia with average hemoglobin 7 - 8 g/dL	HbE 40% - 60%, HbA2 slightly elevated, and HbF 30% - 60%
HbE/alpha thalassemia	Usually asymptomatic, use serum ferritin to differentiate from HbE trait + iron deficiency	Microcytosis 77 - 79 fL, usually no anemia	HbE 25% - 30% (1 alpha deletion) or 20% - 25% (2 alpha deletions), remainder HbA, HbF normal
HbE/HbH disease (also called HbAE Bart's disease)	Moderately severe thalassemic disorder with hemolytic anemia	Microcytic anemia; MCV ~ 67 fL; hemoglobin ~ 9 g/dL	HbE 10% - 15%, HbF 3% - 13%, small amount of Hb Bart's (less than the amount of HbE) and the rest is HbA
Homozygous HbE/HbH disease	Moderately severe thalassemic disorder with hemolytic anemia	Microcytic anemia; MCV ~ 61 fL; hemoglobin ~ 7.5 g/dL	HbE 80%, Hb Bart's avg 10%, and HbF 1% - 7% (some cases have no HbF or Barts closely mimicking HbEE homozygosity)
HbE/HPFH (deletional)	Asymptomatic	Microcytosis	HbE, HbF, no HbA
HbE/Hb Lepore	Mild thalassemic phenotype	Microcytic anemia	HbE ~ 55%, Hb Lepore ~ 10%, HbF ~ 28%, the rest HbA2
HbE/alpha thalassemia/Hb Constant Spring	Moderately severe thalassemic disorder with hemolytic anemia	Microcytic anemia	Hb Constant Spring 1.1 +/- 0.4%, HbE ~ 15%, HbBarts ~ 5%, the rest HbA
Sickle cell/HbE	Mild compensated hemolysis, splenomegaly	Hemoglobin concentration and MCV normal or reduced	HbS 60%, HbE 30%, HbF normal or elevated

Adapted from Bain[1], Vichinsky[2], and Rees, et al[4]

THERAPY AND PROGNOSIS

Management of the compound heterozygous state of HbE/b-thal is highly variable, since the clinical presentation varies from lack of symptoms in a minor group of patients to complete transfusion dependency in another group of patients.

Observations of a large HbE/b-thal study group found that the time of onset of symptoms was more predictive of the long-term clinical phenotype than the small differences that were found in hemoglobin levels between populations of differing clinical severity. Severe anemia with symptoms beginning in infancy predicted a thalassemia major phenotype, while a stable clinical condition at five years of age usually indicated a thalassemia intermedia phenotype. As patients aged, transfusion requirements increased.

Splenectomy as a therapeutic measure for splenomegaly and hemolytic anemia was routinely performed in the past, but awareness of the increased risk of thromboembolism in these patients after splenectomy has made this intervention less common.

Patients with HbE/b-thal are at an increased risk of iron overload, and quantitative liver iron measurements can help identify patients with a high burden of storage iron even in the absence of having received transfusions. Monitoring for the development of cardiopulmonary disease is also important, since these patients have a risk of cardiomyopathy secondary to iron overload, pulmonary hypertension, and right heart failure.

The current strategy for most patients who require therapy involves agents directed at elevating HbF production, which increases the steady-state hemoglobin level without significant toxicity.[6]

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NOTES

This concludes the report.

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