Case History for BMD-01 – BMD-06

This bone marrow aspirate smear is from a 25-year-old woman being seen in the emergency room for fatigue, dehydration, and complaining of nausea and vomiting. Laboratory peripheral blood data includes: WBC = 120.3 x 10E9/L; RBC = 3.86 x 10E12/L; HGB = 9.6 g/dL; HCT = 30.0%; MCV = 78 fL; PLT = 73 x10E9/L; and RDW = 18%. Additional laboratory data: Flow cytometry show a T-lymphoblastic phenotype with blasts expressing partial CD34, CD38, CD7, CD2, CD3, partial TdT, co-expressing CD4 and CD8. Normal karyotype. Identify the arrowed object(s) on each whole slide image.

(BONE MARROW, WRIGHT-GIEMSA)

Please click on the hyperlink below to view the DigitalScope images for this case.
http://www.digitalscope.org/LinkHandler.axd?LinkId=8654606f-2e59-43c0-b402-f25629e1e9b1

To access the online Hematology Glossary, please click the hyperlink below:
https://cap.objects.frb.io/documents/2019-hematology-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.

Bone Marrow Differential – %

		NO. LABS	MEAN	S.D.	C.V.*	Median	Low Value	High Value
BMD-01	Blasts	297	76.99	20.89	27.1	85.0	5.0	98.0
	Promyelocytes	268	0.19	0.34	*	0.0	0.0	1.0
	Myelocytes	285	0.68	0.58	85.4	0.6	0.0	2.5
	Metamyelocytes	285	0.71	0.56	78.9	0.8	0.0	2.0
	Band/Segmented neutrophils	291	1.30	0.90	69.4	1.0	0.0	4.0
	Eosinophils (all stages)	275	0.24	0.35	*	0.0	0.0	1.0
	Basophils	249	0.00	0.01	*	0.0	0.0	0.1
	Monocytes	268	0.19	0.35	*	0.0	0.0	1.5
	Lymphocytes	286	14.42	17.24	*	6.9	0.0	69.0
	Plasma cells (normal and abnormal)	273	0.19	0.32	*	0.0	0.0	1.0
	Erythroid precursors (all stages)	297	2.95	1.95	66.0	2.2	0.0	10.0
	Other	203	0.07	0.48	*	0.0	0.0	6.0

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

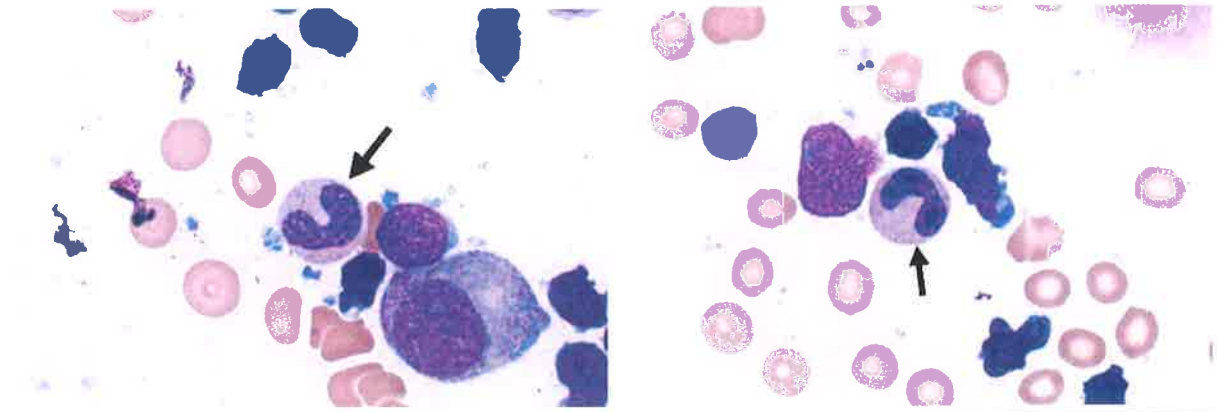
	Other cells not listed:	Total (N=9)
BMD-01	Immature mononuclear cell	2
	Malignant lymphoid cell (other than blast)	2
	Lymphoblast	2
	Basket/smudge cell	1
	Mast cell	1
	Megakarocyte	1

Committee Comments on the Bone Marrow Differential and Aspirate

This bone marrow aspirate smear is from a 25-year-old woman being seen in the emergency room for fatigue, dehydration, and complaining of nausea and vomiting. Laboratory peripheral blood data includes: WBC = 120.3 x 10E9/L; RBC = 3.86 x 10E12/L; HGB = 9.6 g/dL; HCT = 30.0%; MCV = 78 fL; PLT = 73 x 10E9/L; and RDW = 18%. Numerous blasts (over 50% of cells) are seen on the aspirate smear that are small to intermediate in size with smooth chromatin, indistinct nucleoli, and very scant agranular cytoplasm. Residual trilineage hematopoiesis is present with maturation.

Cell Identification

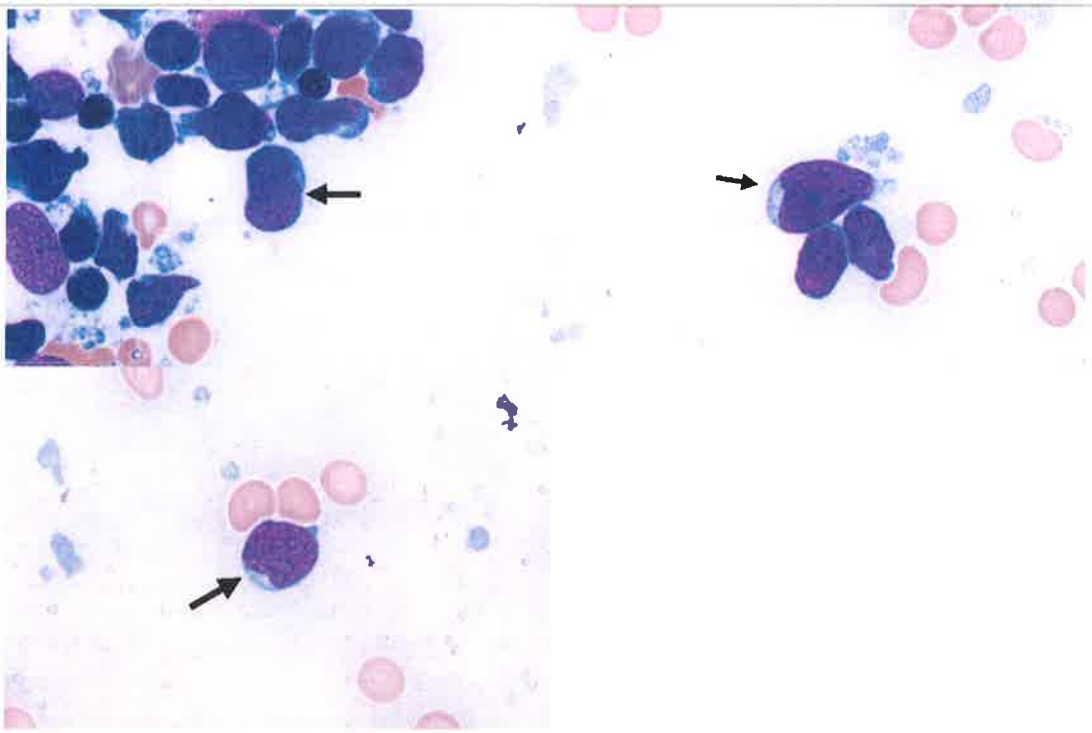
BMD-02



Identification	Participants No.	Participants %	Evaluation
Neutrophil, segmented or band	299	97.4	Educational
Neutrophil, metamyelocyte	5	1.6	Educational
Neutrophil, giant band or giant metamyelocyte	3	1.0	Educational

The arrowed cells are neutrophil, band forms, as correctly identified by 97.4% of participants. Together, band neutrophils and segmented neutrophils constitute 12% to 25% of the nucleated cells in the bone marrow. Both segmented and band neutrophils have specific granules and mature chromatin. Unlike the segmented neutrophil, however, the band neutrophil does not show nuclear condensation to a thin filament. Also, unlike the next most immature neutrophil form (the metamyelocyte), the band neutrophil nucleus is indented to *more* than half the distance to the farthest nuclear margin. The nucleus can assume many shapes: it can be band-like; sausage-like; S-, C-, or U-shaped; or twisted and folded on itself. For further description of a metamyelocyte, please refer to BMD-06.

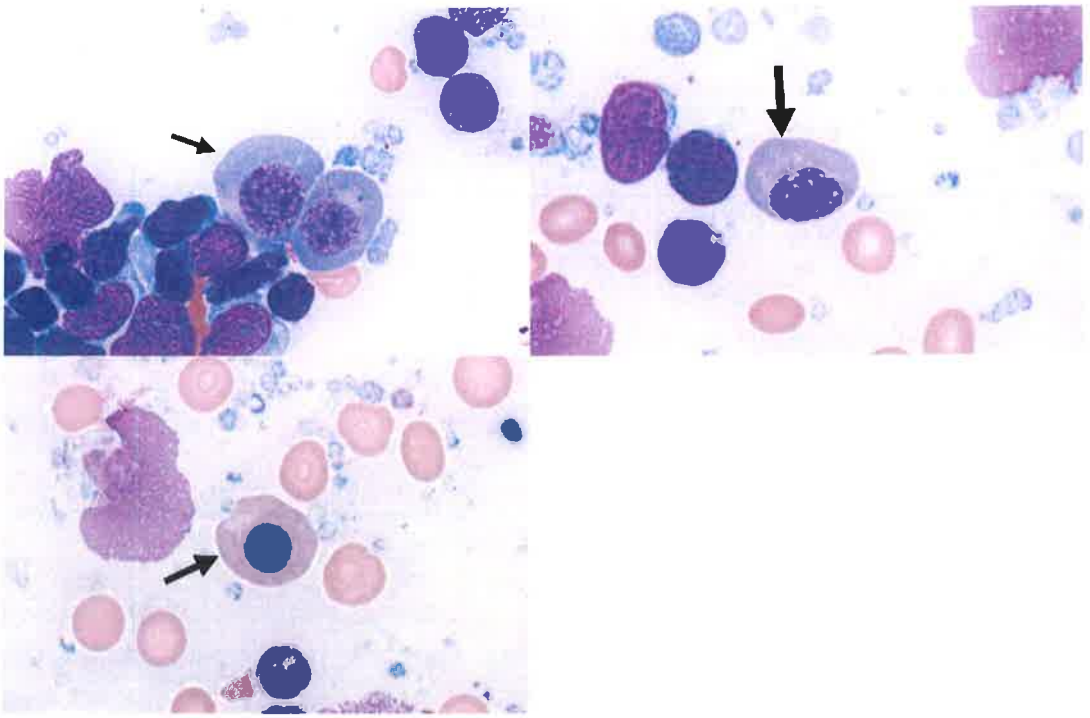
BMD-03



Identification	Participants No.	%	Evaluation
Blast cell (including lymphoblast)	301	98.0	Educational
Lymphocyte	4	1.3	Educational
Malignant lymphoid cell (other than blast)	2	0.7	Educational

The arrowed cells are blasts, as correctly identified by 98.0% of participants. A blast is a large, round to oval cell, 10 to 20 µm in diameter. In the blood film, the cell may appear flattened or compressed by adjacent red cells. The nuclear-to-cytoplasmic ratio is high, varying from 7:1 to 5:1. The blast often has a round to oval nucleus, but sometimes it is indented or folded; and it has fine, lacy or reticular chromatin. One or more prominent nucleoli may be seen. The cytoplasm is variably basophilic and typically agranular. Lineage assignment is not possible by morphology alone unless an Auer rod is present, which is only seen in myeloblasts. Auer rods in blast are only present in a minority of cases of acute myeloid leukemias and rarely in myelodysplastic syndrome (MDS) cases, where presence would signify a high grade MDS even when the blast percentage is low. Blast lineage may be assigned by flow cytometric immunophenotyping, often in conjunction with cytochemical staining for myeloperoxidase, sudan black, and/or nonspecific esterase.

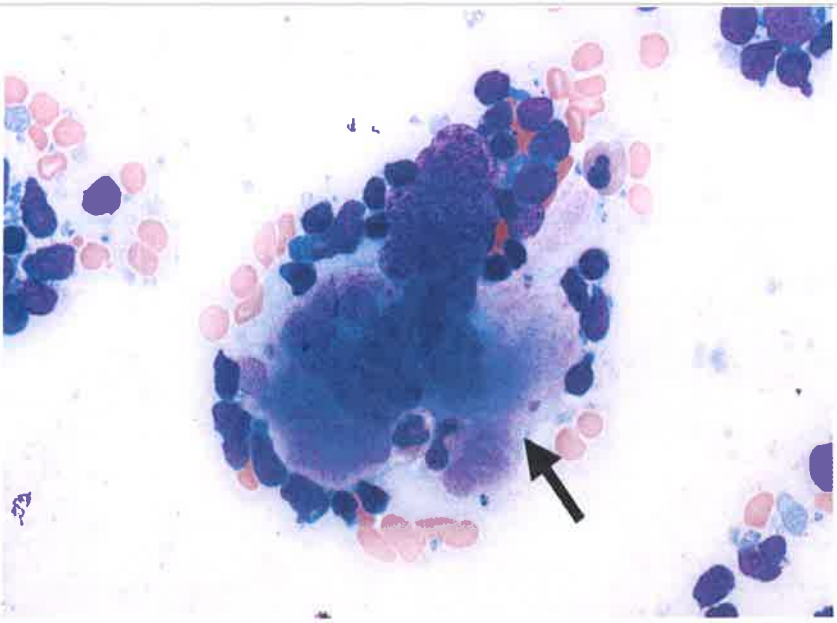
BMD-04



Identification	Participants No.	%	Evaluation
Erythrocyte precursor, normal (includes pronormoblast, basophilic, polychromatophilic, and orthochromic normoblasts)	217	70.7	Educational
Erythrocyte precursor with megaloblastic changes/maturation	56	18.2	Educational
Erythrocyte precursor, abnormal/dysplastic nuclear features (includes pronormoblast, basophilic, polychromatophilic, and orthochromic normoblasts)	34	11.1	Educational

The arrowed cells are normal erythrocyte precursors at various stages of maturation, as correctly identified by 70.7% participants. Mature erythrocytes are derived from erythrocyte precursors in the bone marrow. The earliest recognizable erythroid precursor is the pronormoblast (proerythroblast, erythroblast). These are the largest erythroid precursors, measuring 17 - 24 μm in diameter with an 8:1 N:C ratio, and contain finely reticulated chromatin and basophilic cytoplasm. A perinuclear halo can be seen. From this stage, the maturation sequence progresses through the basophilic (10 - 17 μm, N:C ratio 6:1, basophilic cytoplasm), polychromatophilic (10 - 15 μm, N:C ratio 4:1, gray-blue to gray-pink cytoplasm), and orthochromic normoblast (8 - 12 μm, N:C ratio 1:2, pink-orange cytoplasm) stages. The nuclear chromatin becomes progressively condensed or clumped. It is very dense and pyknotic in the most mature forms of orthochromic normoblasts. In this case some of the erythroid precursors show subtle dysplastic (slight nuclear border irregularity) or megaloblastoid features, while many are normal. The mild dysmorphology seen in this case is likely a manifestation of "stress erythropoiesis" since this bone marrow is being replaced by T-lymphoblastic leukemia. Thus, responses of erythrocyte precursor normal, erythrocyte precursor abnormal, and erythrocyte precursor with megaloblastic changes are considered acceptable.

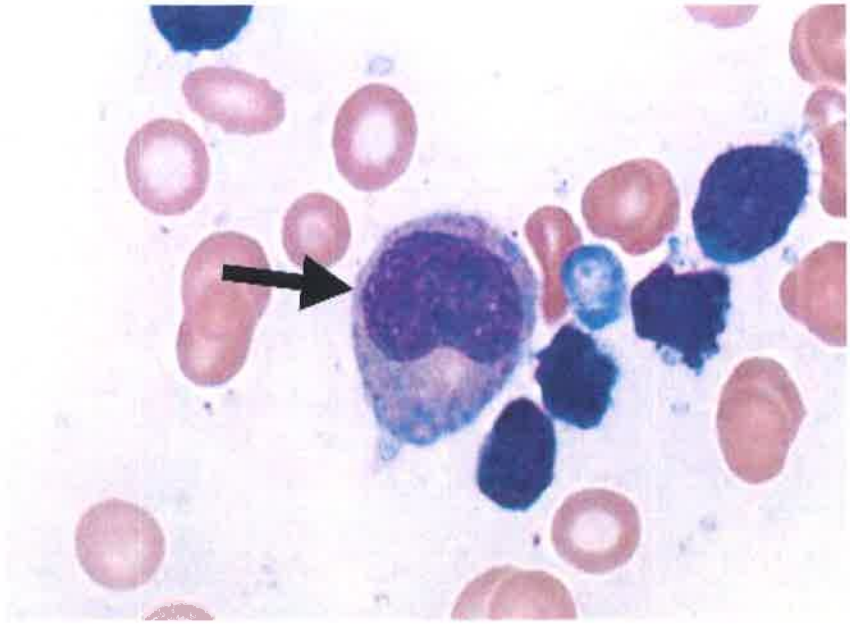
BMD-05



Identification	Participants No.	%	Evaluation
Megakaryocyte or precursor, normal	255	83.1	Educational
Megakaryocyte or precursor, abnormal	49	16.0	Educational
Macrophage containing cell (hemophagocytosis)	1	0.3	Educational
Metastatic tumor cell or tumor cell clump	1	0.3	Educational
Osteoclast	1	0.3	Educational

The arrowed cell is a normal megakaryocyte, as correctly identified by 83.1% of participants. Megakaryocytes are the largest bone marrow hematopoietic cells. They are derived from bone marrow stem cells and are responsible for platelet production. During development, the cell does not divide, but instead the nucleus undergoes nuclear replication without cell division (endoreduplication) giving rise to a hyperdiploid nucleus with several lobes and each lobe roughly containing a normal complement of chromosomes. Typically, the mature megakaryocyte measures at least 25 to 50 µm in diameter. The numerous nuclear lobes are of various sizes, connected by large bands or fine chromatin threads. The chromatin is coarse and clumped to pyknotic. The abundant cytoplasm stains pink or wine-red and contains fine azurophilic granules that may be clustered. The image clearly identifies megakaryocytes but does include a degenerated or disrupted megakaryocyte nucleus overlying at least one other normal megakaryocyte. For this reason, responses of both megakaryocyte normal and abnormal are considered acceptable.

BMD-06



Identification	Participants No.	%	Evaluation
Neutrophil, metamyelocyte	283	92.2	Educational
Neutrophil, myelocyte	18	5.9	Educational
Neutrophil, promyeolocyte	3	1.0	Educational
Neutrophil, giant band or giant metamyelocyte	2	0.7	Educational
Megakaryocyte, abnormal	1	0.3	Educational

The arrowed cell is a metamyelocyte, as correctly identified by 92.2% participants. Metamyelocytes are the first of the postmitotic myeloid precursors. They constitute 15% to 20% of nucleated cells in the bone marrow. They are approximately 10 to 18 µm in diameter. They are round to oval with a nuclear-to-cytoplasmic ratio of 1.5:1 to 1:1. The nuclear chromatin is condensed, and the nucleus is indented to less than half of the potential round nucleus (ie, the indentation is smaller than half of the distance to the farthest nuclear margin). This feature helps distinguish metamyelocytes from band neutrophils (see BMD-02). The cytoplasm is amphophilic, containing rare azurophilic (primary) granules and many fine pink specific granules.

Case Presentation:

This bone marrow aspirate smear is from a 25-year-old woman being seen in the emergency room for fatigue, dehydrated, and complaining of nausea and vomiting. Laboratory peripheral blood data includes: WBC = 120.3 x 10E9/L; RBC = 3.86 x 1Ee12/L; HGB = 9.6 g/dL; HCT = 30.0%; MCV = 78 fL: PLT = 73 x 10E9/L; and RDW = 18%. Additional laboratory data: Flow cytometry show a T-lymphoblastic phenotype with blasts expressing partial CD34, CD38, CD7, CD2, CD3, partial TdT, co-expressing CD4 and CD8. Normal karyotype.

(BONE MARROW, WRIGHT-GIEMSA)

Case Discussion: T-lymphoblastic leukemia

The bone marrow shows a prominent blast population, most of which are small with very high nuclear:cytoplasmic ratios. The nuclear chromatin pattern is fine and indistinct nucleoli are present. There is very scant agranular basophilic cytoplasm. While not clinically relevant, these features correspond to the old French-American-British "L1" cytologic category and remains useful descriptor that quickly conveys the morphologic appearance. Flow cytometric phenotyping showed expression of CD34 (subset), CD38, CD7, CD2, cytoplasmic CD3, partial TdT, CD4, and CD8. The cells were negative for CD34, CD19, CD20, CD13, CD33, CD117, and myeloperoxidase. The karyotype was normal. Since the marrow is involved (> 25% blasts), the case, by definition, is considered as a T-lymphoblastic leukemia (T-ALL) in the World Health Organization (WHO) classification.

T-ALL is a neoplasm of precursor T-cells. It accounts for approximately 15% of childhood acute lymphoblastic leukemias and presents commonly in adolescents as opposed to young children. Patients present commonly with leukocytosis due to high numbers of circulating blasts. Extramedullary presentation as a rapidly growing mediastinal mass is also common, although presentation in nodal and other extranodal sites may occur. Respiratory compromise from a large mediastinal mass can be a medical emergency. The blasts can be variable ranging from small "L1" type blasts as in this case to numerous blasts that are intermediate-to-large containing fine chromatin, relatively prominent nucleoli, and moderate amounts of agranular cytoplasm (previously referred to as "L2" blasts).

Question 1: Common presenting features in T-ALL include:

A. Gum enlargement
B. Leukopenia
C. Mediastinal mass
D. 10% bone marrow blasts

Immunophenotyping by flow cytometry is critical for lineage assignment. CD7 and cytoplasmic CD3 (cCD3) are most commonly expressed, with the latter being important for definitive T-cell lineage assignment in the updated WHO classification. Expression of other pan-T-cell markers such as CD2, CD5, as well as CD4 and/or CD8 is common. Expression of CD1a along with CD4 and CD8 as in this case is associated with the cortical thymocyte stage of differentiation. Expression of myeloid lineage markers such as CD13, CD33, or CD117 can be seen in a minority of cases but does not preclude a T-ALL diagnosis. Schema that placed T-ALL phenotypes in context of normal precursor T-cell development exist but are not clinically relevant. A recently recognized subtype of T-ALL is the so-called early T-cell precursor lymphoblastic leukemia (ETP-ALL). These are felt to represent a T-ALL with limited/early T-cell differentiation. These cases can be recognized by expression of CD7 and one or more of the myeloid/stem cell markers CD117, CD13, CD33, CD34, HLA-DR, CD11b, and CD65. They express cCD3, may express CD2 or CD4, weakly express or lack CD5, and typically lack CD1a and CD8.

Question 2: Which of the following immunophenotypes would be consistent with T-ALL?

A. CD13+/CD33+/CD2+/CD64+/CD34+/MPO+/TdT+
B. cCD3+/Myeloperoxidase (MPO)-/CD4-/CD8-/CD13+/CD33-/CD5-/CD7+/CD117+
C. cCD3+/MPO+/CD4-/CD8-/CD13+/CD33+/CD5-/CD7+/CD34+
D. surface CD3+/CD4+/CD8-/CD5+/CD7+/CD34-/CD117-/TdT-

Cytogenetic abnormalities are commonly seen in T-ALL and commonly involve translocations of transcription factors such as homeobox (*HOX*) genes, LIM only domain (*LMO*) family member genes, and basic helix-loop-helix (bHLH) genes. Partner genes for these transcription factor translocations often involve T-cell receptor (*TCR*) genes such that their enhancer regions drive the aberrant gene expression. *IGH* and *IGL* loci are not involved. An important signaling pathway that is activated in T-LBL is the NOTCH pathway that include activating mutations in *NOTCH1* as well as inactivating mutations of inhibitors of this pathway such as *FBXW7*.

Question 3: Common cytogenetic or molecular abnormalities in T-ALL include:

A. Loss of function mutations in the NOTCH signaling pathway
B. Translocations in *RARA*
C. Translocations involving *IGH* locus
D. Translocation involving *TCR* genes

Prognosis of T-ALL is poor relative to B-ALL and cases are generally considered high risk during treatment selection. The clinical features often seen in T-ALL such as male gender, older age, and high WBC are in and of themselves also considered high risk. The ETP phenotype was initially considered as poor prognosis/very high risk but subsequent clinical studies of large numbers of patients with modern therapeutic strategies showed no substantial difference in outcomes for ETP ALL when compared to other types of T-ALL.

The differential diagnosis of T-ALL in the bone marrow revolves around other acute leukemias including B-ALL and acute myeloid leukemias (AML). These are usually readily distinguished by the combination of morphologic and immunophenotypic features by flow cytometry. B-ALLs will express B-cell markers such as CD19 without cCD3 and typically without other T-cell associated markers. AMLs express numerous myeloid associated markers such as CD13, CD33, and MPO and lack cCD3. AML blasts may contain granules and/or Auer rods not typically seen in ALLs. When tissue biopsy of a mediastinal mass is done and a T-lymphoblastic population is detected, a thymoma is in the differential diagnosis. This is an epithelial neoplasm that may be rich in non-neoplastic thymocytes (immature T-cells) at varying stages of maturation. Thus, detection of numerous keratin-positive cells (thymic epithelial cells) in the tumor and heterogeneous, non-neoplastic immature T-cell populations help identify a thymoma and differentiate it from T-lymphoblastic lymphoma that lacks these features. Additionally, the lack of cytogenetic or molecular abnormalities is characteristic of thymoma but is less likely in cases of T-lymphoblastic lymphoma.

Question 4: The differential diagnosis of a mediastinal mass demonstrating T-lymphoblasts includes a thymoma. Features that favor a thymoma include:

A. Expression of CD1a
B. Notch1 mutations
C. Expression of TdT
D. Heterogeneous expression of CD4 and CD8 by flow cytometry

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Hematology and Clinical Microscopy Committee

References:

1. Swerdlow SH, Campo E., Harris N. L., Jaffe, E. S., Pileri, S. A., Stein, H., Thiele, J., Arber, D. A.,Hasserjian, R. P., Le Beau, M. M., Orazi, A.,Siebert, R. ed WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (ed Revised 4th). Lyon, France: IARC; 2017.

2. You MJ, Medeiros LJ, Hsi ED. T-lymphoblastic leukemia/lymphoma. *Am J Clin Pathol.* 2015;144(3):411-422.

3. Jegalian AG, Bodo J, Hsi ED. NOTCH1 intracellular domain immunohistochemistry as a diagnostictool to distinguish T-lymphoblastic lymphoma from thymoma. *Am J Surg Pathol.* 2015;39(4):565-572.

Answers to Questions:

Question 1: Common presenting features in T-ALL include: **C. Mediastinal mass**

T-ALL is generally defined as bone marrow involvement by > 25% bone marrow T lymphoblasts. Patients present most commonly with leukocytosis and may have a mediastinal mass, hepatosplenomegaly, and/or lymphadenopathy. Gum enlargement is often a feature of acute monocytic leukemias rather than lymphoblastic leukemias.

Question 2: Which of the following immunophenotypes would be consistent with T-ALL? **B. cCD3+/MPO-/CD4-/CD8-/CD13+/CD33-/CD5-/CD7+/CD117+**

By definition, T-ALL expresses cytoplasmic CD3 (cCD3) and is usually positive for one or more pan-T-cell markers such as CD2, CD7, or CD5. CD4 and/or CD8 may be expressed. Aberrant myeloid marker expression such as CD13 or CD33 can be seen. However, expression of myeloperoxidase (MPO) indicates myeloid lineage differentiation. When MPO is expressed along with cCD3 (a rare occurrence), a mixed lineage leukemia diagnosis is appropriate according to WHO criteria. A particular immunophenotype in which T-lymphoblasts express cCD3 along with myeloid associated surface markers such as CD13, CD33, and CD117, but lack CD8 and CD5 are consistent with the early T-precursor (ETP) lymphoblastic leukemia. This has been associated with poor prognosis on many studies but may, with modern therapy, fare similarly to non-ETP phenotype cases.

Question 3: Common cytogenetic or molecular abnormalities in T-ALL include**: D. Translocation involving *TCR* genes**

There are heterogenous but characteristic genetic abnormalities in T-ALL that are reviewed in the reference list. Translocations in transcription factor family genes such as HOX genes, LMO family genes, and basic helix-loop-helix (bHLH) genes are common in T-ALL, with partner genes often being T-cell receptor (TCR) genes. IGH is not a partner gene in T-ALL translocations, but it is often rearranged in B-cell malignancies. A commonly activated pathway is the NOTCH pathway and approximately 50% - 80% of cases have activating mutations in genes within this signaling

COLLEGE of AMERICAN PATHOLOGISTS

Attestation of Participation of Self-Reported Training*

We the participants below have completed the review of the CAP BMD-A 2019 (Product Mailing, Year) Participant Summary/Final Critique report and can self-report the recommended 0.5 (Education Hours) hours towards fulfilling education and certification of maintenance requirements.

Participant	Date	Participant	Date

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

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