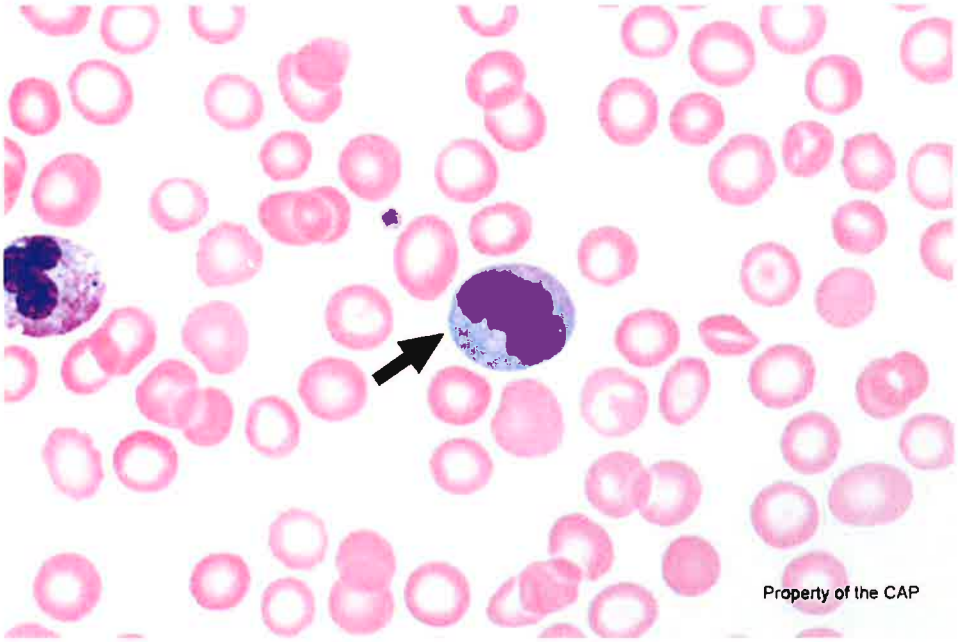
Blood Cell Identification – Graded

Case History
This peripheral blood smear is from an 81-year-old woman with septic shock. Laboratory data includes: WBC = 15.6 x 10E9/L; RBC = 3.01 x 10E12/L; HGB = 9.3 g/dL; HCT = 28.5%; MCV = 95 fL; MCHC = 32.6 g/dL; PLT = 32 x 10E9/L; and RDW = 16.0%; Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

To access the online Hematology Glossary, please click the hyperlink below:
https://documents.cap.org/documents/2020-hematology-clinical-microscopy-glossary.pdf

BCP-11



Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Monocyte	161	94.7	4746	89.4	Good
Neutrophil, metamyelocyte	4	2.4	266	5.0	Unacceptable
Monocyte, immature (promonocyte, monoblast)	2	1.2	120	2.3	Unacceptable
Neutrophil, segmented or band	2	1.2	47	0.9	Unacceptable

The arrowed cell is a monocyte, as correctly identified by 94.7% of the referees and 89.4% of the 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 nuclear-to-cytoplasmic (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 usually less dense than that of a neutrophil or lymphocyte. Nucleoli are generally absent, but occasional monocytes may contain a small, inconspicuous nucleolus.

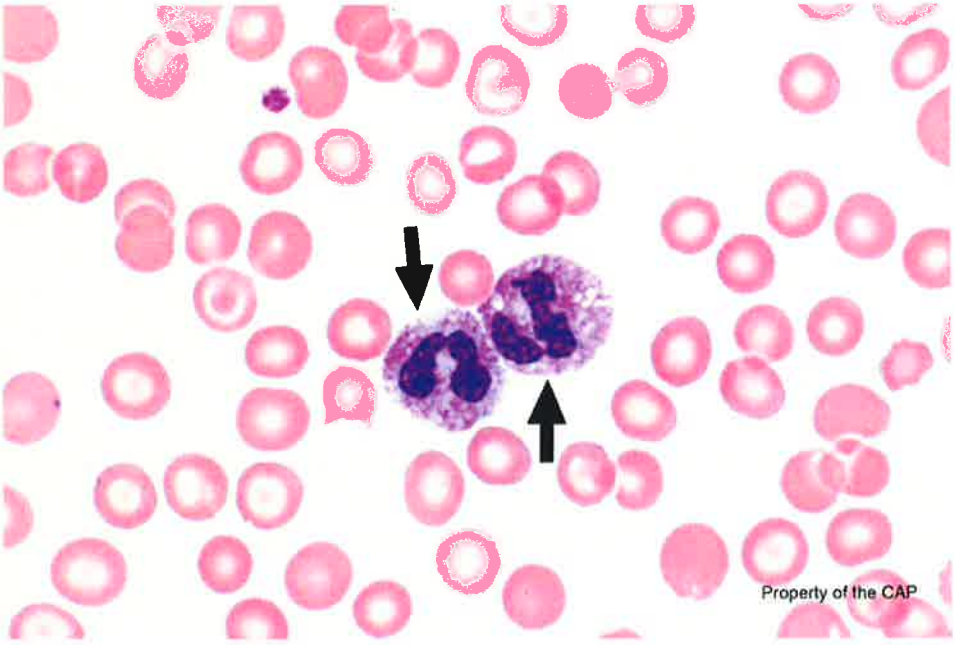
BCP-11, cont'd

The arrowed cell was incorrectly identified as a neutrophil, metamyelocyte by 2.4% of the referees and 5.0% of the 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. The cell in question here does not have the nuclear shape or the many specific granules in the cytoplasm characteristic of a metamyelocyte.

The arrowed cell was incorrectly identified as a monocyte, immature (promonocyte, monoblast) by 1.2% of the referees and 2.3% of the participants. For the purposes of proficiency testing, selection of the response "monocyte, immature (promonocyte, monoblast)" should be reserved for malignant cells in the context of acute and has finely dispersed chromatin and distinct nucleoli. The cytoplasm is blue to gray-blue and may contain small, scattered azurophilic granules. Some monoblasts cannot be distinguished morphologically from other blast forms. Promonocytes have nuclear and cytoplasmic characteristics that are between those of monoblasts and mature monocytes. They are generally larger than mature monocytes, but they have similar-appearing gray-blue cytoplasm that often contains uniformly distributed, fine azurophilic granules. Cytoplasmic vacuolization is not a typical feature. The nuclei show varying degrees of lobulation, usually characterized by delicate folding or creasing of the nuclear membrane, in contrast to the rounder nuclear profile of monoblasts. Nucleoli are present but may not be as distinct as in monoblasts. The chromatin pattern of the arrowed cell is not fine, and therefore, not consistent with promonocytes or monoblasts.

Blood Cell Identification – Graded

BCP-12



Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	124	72.9	3898	73.4	Good
Neutrophil, segmented or band	44	25.9	1355	25.5	Acceptable
Neutrophil with Pelger-Huët nucleus (acquired or congenital)	2	1.2	31	0.6	Unacceptable

The arrowed cells are toxic neutrophils, as correctly identified by 72.9% of the referees and 73.4% of the participants. Toxic changes in neutrophils include toxic granulation, toxic vacuolization, and Döhle bodies. Toxic granulation and Döhle bodies each may be present in an individual cell without the other finding, and either change alone is sufficient to designate a neutrophil as toxic. The arrowed cells show mild toxic granulation and cytoplasmic vacuoles without distinct Döhle bodies. Classification as neutrophils (non-toxic), as identified by 25.9% of referees and 25.5% of participants, was also deemed acceptable given that the granulation is only mildly exaggerated and that definite Döhle bodies are not present.

Toxic granulation is defined by the presence of large, purple or dark blue cytoplasmic granules in neutrophils, bands, and metamyelocytes. Vacuoles within the cytoplasm of these same cells also define toxic vacuolization. The vacuoles are variable in size and may coalesce, sometimes distorting the neutrophil cytoplasm to form pseudopodia. Ethylenediaminetetraacetic acid (EDTA) blood collection may produce degenerative vacuolization; in this context, only a few, small, punched-out-appearing vacuoles may be found. However, as it may be difficult to distinguish toxic from degenerative vacuoles, neutrophil vacuoles should not be labeled as toxic vacuoles unless accompanied by other toxic changes.

BCP-12, cont'd

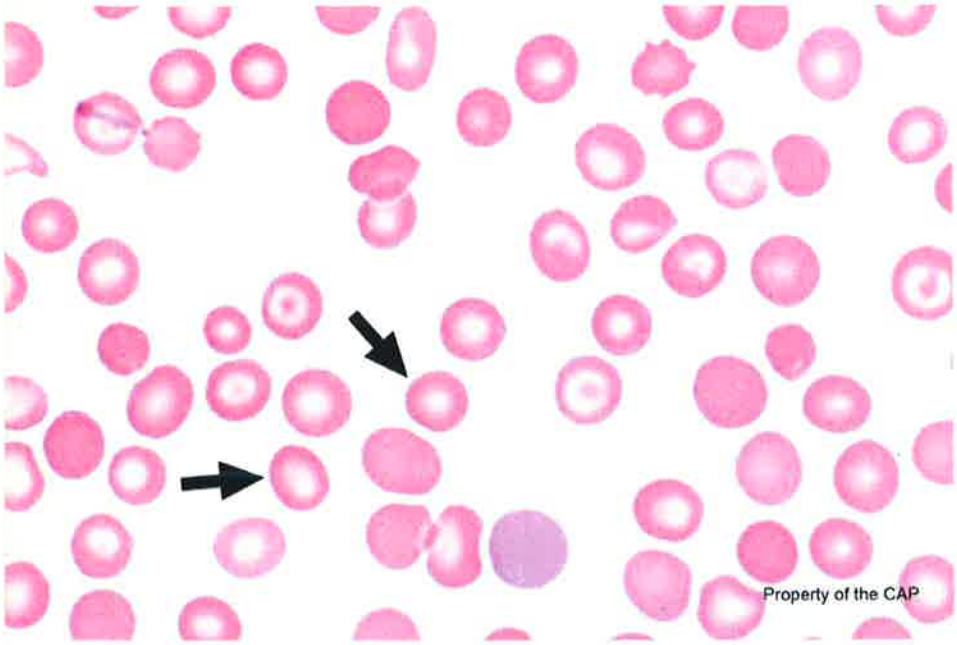
Döhle bodies appear as single or multiple blue or gray-blue inclusions of variable size (0.1 to 5.0 µm) and shape (round or elongated or crescent-shaped) in the cytoplasm of neutrophils, bands, or metamyelocytes. They are often found at the periphery of the cytoplasm, near the cell membrane. These inclusions represent parallel strands of rough endoplasmic reticulum.

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.

In the May-Hegglin anomaly, inclusions that resemble Döhle bodies are seen. Unlike Döhle bodies, however, the May-Hegglin inclusion is due to aggregates of non-muscle myosin heavy chain IIA. Also seen in concert with neutrophil abnormalities are thrombocytopenia and giant platelets. The May-Hegglin anomaly is inherited in an autosomal dominant fashion, owing to mutations in *MYH9*.

Blood Cell Identification – Graded

BCP-13



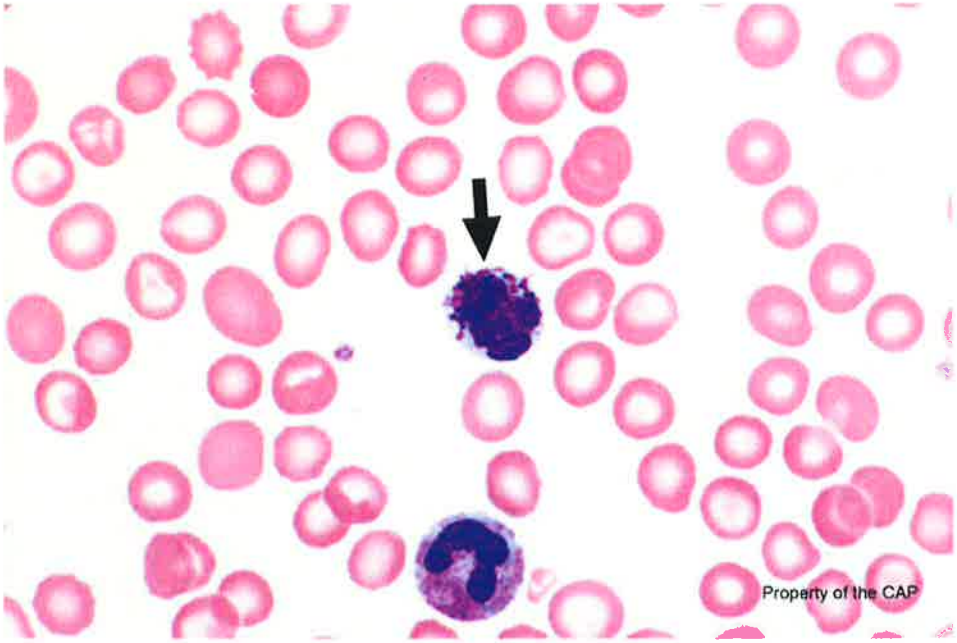
Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Erythrocyte, normal	155	91.2	4892	92.2	Good
Hypochromasia	10	5.9	277	5.2	Unacceptable
Microcyte (with increased central pallor)	5	2.9	127	2.4	Unacceptable

The arrowed cells are normal erythrocytes, as correctly identified by 91.2% of the referees and 92.2% of the participants. An erythrocyte is a mature, non-nucleated biconcave disc-shaped cell of fairly uniform diameter (6.7 to 7.8 µm) with a uniform round area of central pallor. It contains hemoglobin and stains uniformly pink red. The zone of central pallor is due to the biconcavity of the cell and occupies approximately one third (2 to 3 µm) of the cell diameter. Normal erythrocytes circulate in the peripheral blood for approximately 120 days before they undergo catabolism or destruction in the spleen.

The arrowed cells have normal central pallor. Hypochromasia, identified by 5.9% of the referees and 5.2% of the participants, is characterized by increased pallor (usually greater than 50% of the cell diameter) and is thus incorrect. Similarly, microcytes also often have increased pallor and are also smaller the normal erythrocytes (less than 6 microns). For these reasons, microcyte (with increased central pallor) identified by 2.9% of the referees and 2.4% of the participants, is also incorrect.

Blood Cell Identification – Graded

BCP-14

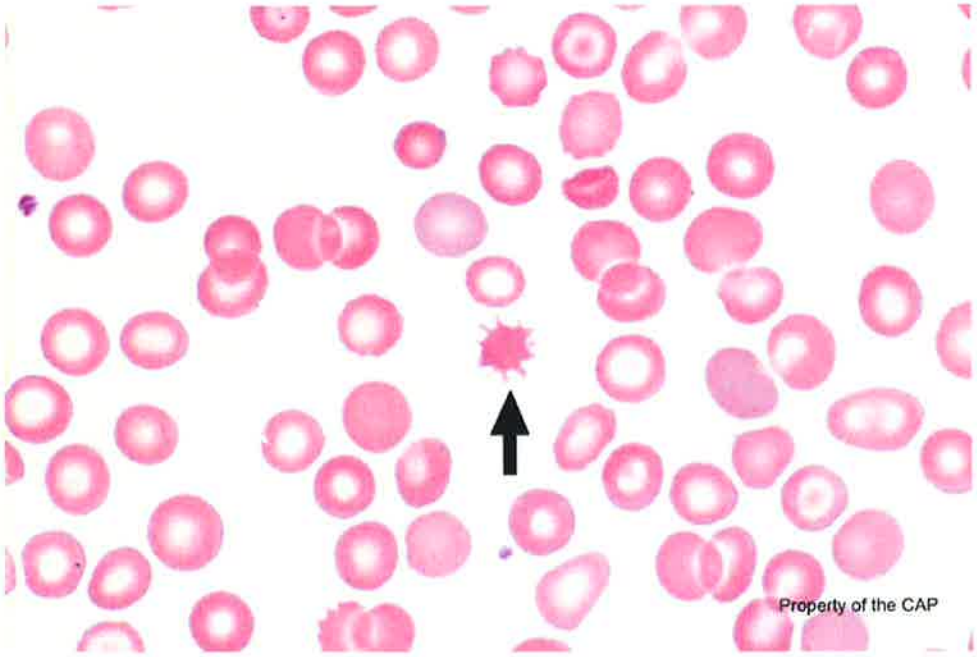


Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Basophil, any stage	168	98.8	5210	98.2	Good
Leukocyte with intracellular bacteria	1	0.6	10	0.2	Unacceptable
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic acuolization)	1	0.6	21	0.4	Unacceptable

The arrowed cell is a basophil, as correctly identified by 98.8% of the referees and 98.2% of the participants. Basophils have a maturation sequence analogous to neutrophils. The earliest basophil precursors can be identified in bone marrow at the myelocyte stage, when specific granules begin to develop. All basophils, from the basophilic myelocyte to the mature segmented basophil, are characterized by the presence of numerous coarse and densely stained granules of varying sizes and shapes. The granules are larger than the granules of neutrophils and most are roughly spherical. The granules are typically blue-black, but some may be purple-red when stained using Wright-Giemsa preparations. The granules are unevenly distributed and frequently overly and obscure the nucleus. Basophils are comparable in size to neutrophils, ie, 10 to 15 µm in diameter, and the N:C ratio ranges from 1:2 to 1:3. Basophilia may be seen in several contexts, including in association with myeloproliferative neoplasms, hypersensitivity reactions, hypothyroidism, iron deficiency, and renal disease.

Blood Cell Identification – Graded

BCP-15



Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Acanthocyte (spur cell)	147	86.5	4587	86.4	Good
Echinocyte (burr cell, crenated cell)	23	13.5	708	13.3	Unacceptable

The arrowed cell is an acanthocyte, as correctly identified by 86.5% of the referees and 86.4% of the participants. Acanthocytes are densely stained, spheroidal red blood cells that lack central pallor and have multiple (usually three to 20), irregularly distributed, thorn-like spicules of variable size, often with drumstick ends. Spicules may occasionally have branches. Acanthocytes are classically described in association with hereditary abetalipoproteinemia (hereditary acanthocytosis). In addition, these cells are often seen in significant numbers in end-stage liver disease, post splenectomy, hepatorenal failure, infant pyknocytosis, McLeod phenotype, anorexia nervosa, and chronic starvation. In the latter two disorders, they appear as irregularly shaped erythrocytes with multiple blunt projections imparting an "animal cracker-like" appearance. A small number of acanthocytes may be seen in forms of severe hemolytic anemia, particularly after splenectomy. Acanthocytes are rarely encountered in otherwise normal blood smears (one or two per smear). In such smears, they represent older, senescent red blood cells approaching their end of life (120 days). It is logical, therefore, that acanthocytes should be more readily found in blood smears in the post-splenectomy state because of diminished splenic activity in removal of such poikilocytes.

The arrowed cell was incorrectly identified as an echinocyte by 13.5% of the referees and 13.3% of the participants. Echinocytes are red blood cells with 10 - 30 uniform, short, blunt projections distributed evenly that impart a serrated appearance to the red blood cell surface. The red blood cells retain central pallor and are the same size or slightly smaller than normal red blood cells. The irregular distribution of the projections as well as lack of central pallor are not compatible with echinocytes, and therefore excludes this identification.

BCP-15, cont'd

Echinocyte appearance is often the result of an improperly prepared smear (slow drying, thick smears, aged blood and pH alteration of glass slide). Echinocytes that are not artifacts may be indicative of disease, such as uremia or pyruvate kinase deficiency, and are seen post splenectomy, in hepatitis of the newborn, and phosphoglycerate kinase deficiency. Under such circumstances, they should be visible in wet preparations.

Clinical Presentation:

This peripheral blood smear is from an 81-year-old woman with septic shock. Laboratory data includes: WBC = 15.6 x 10E9/L; RBC = 3.01 x 10E12/L; HGB = 9.3 g/dL; HCT = 28.5%; MCV = 95 fL; MCHC = 32.6 g/dL; PLT = 32 x 10E9/L; and RDW = 16.0%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Bacterial Sepsis

Septic shock refers to low blood pressure and dysfunction of multiple organs caused by full-body inflammatory response to an infection. Typically caused by bacteria in the bloodstream, septic shock may also be triggered by infection with other organisms (eg, viruses, fungi, parasites) in other organs and tissues (eg, kidney, heart, brain, skin and soft tissue). Occasionally certain organisms, including bacteria, may be visible on a peripheral blood smear or other infected body fluids.

Complete blood count (CBC) values from the patient in this case demonstrate mild leukocytosis, thrombocytopenia, and anemia that is normocytic (normal MCV). The leukocytosis is characterized by increased neutrophils, many of which show toxic changes including toxic granulation, toxic vacuolization, and Döhle bodies. This combination of features is often seen in patients with septic shock: neutrophils are "activated" to help fight the infection, leading to the morphologic changes referred to as "toxic" features. Bone marrow production of other hematopoietic elements may be suppressed by the infection and/or the body's inflammatory response, resulting in anemia and thrombocytopenia.

Leukocytosis in septic shock and other reactive conditions may be so high as to mimic leukemia, then referred to as a leukemoid reaction. Infection, trauma (eg, burn, tissue injury), underlying cancer (termed a paraneoplastic reaction), and drug effects (eg, granulocyte-colony stimulating factor, G-CSF, other bone marrow growth factors, certain chemotherapies) may cause leukemoid reactions. The differential diagnosis of neutrophilia also includes some myeloproliferative neoplasms, including chronic myeloid leukemia (CML) and the rare atypical CML (aCML) and chronic neutrophilic leukemia (CNL). Careful attention to cytologic characteristics of the neutrophils (eg, toxic features) and presence of an accompanying left shift as well as to clinical and laboratory features (eg, infection, trauma, neoplasm, drug effect) is required to arrive at the correct interpretation of the peripheral smear findings.

Alexandra E. Kovach, MD
Hematology and Clinical Microscopy Committee

References:

1. Glassy EF, ed. *Color Atlas of Hematology: An Illustrated Field Guide Based on Proficiency Testing*, 2nd ed. Peripheral Blood. College of American Pathologists; 2018.
2. George TI. Malignant or benign leukocytosis. *Am Soc Hematol Ed Program.* 2012:475-484.
3. Chabot-Richards DS, George TI. Leukocytosis. *Int J Lab Hematol.* 2014;36:279-288.

Blood Cell Identification – Ungraded

Case History
This peripheral blood smear is from a 15-year-old boy who presented with fevers, headaches, night sewats, and pancytopenia. He underwent a bone marrow biopsy and was subsequently diagnosed with acute megakaryoblastic leukemia. Laboratory data include: WBC = 0.5 x 10E9/L; RBC = 2.24 x 10E12/L; HGB = 6.3 g/dL; HCT = 18.1%; MCV = 81 fL; PLT = 7 x 10E9/L; and RDW = 18%. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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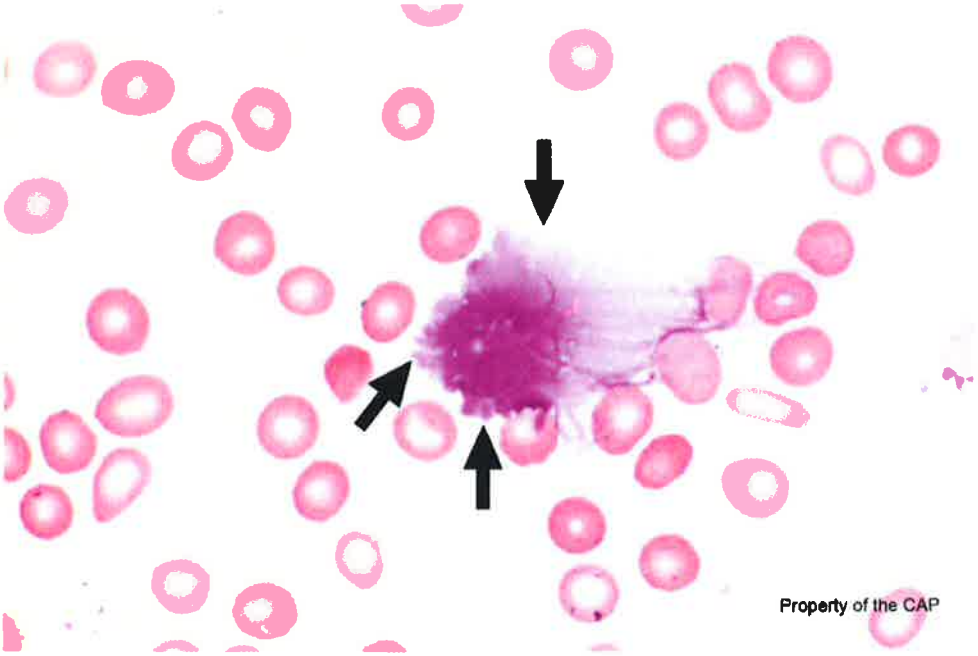
BCP-16

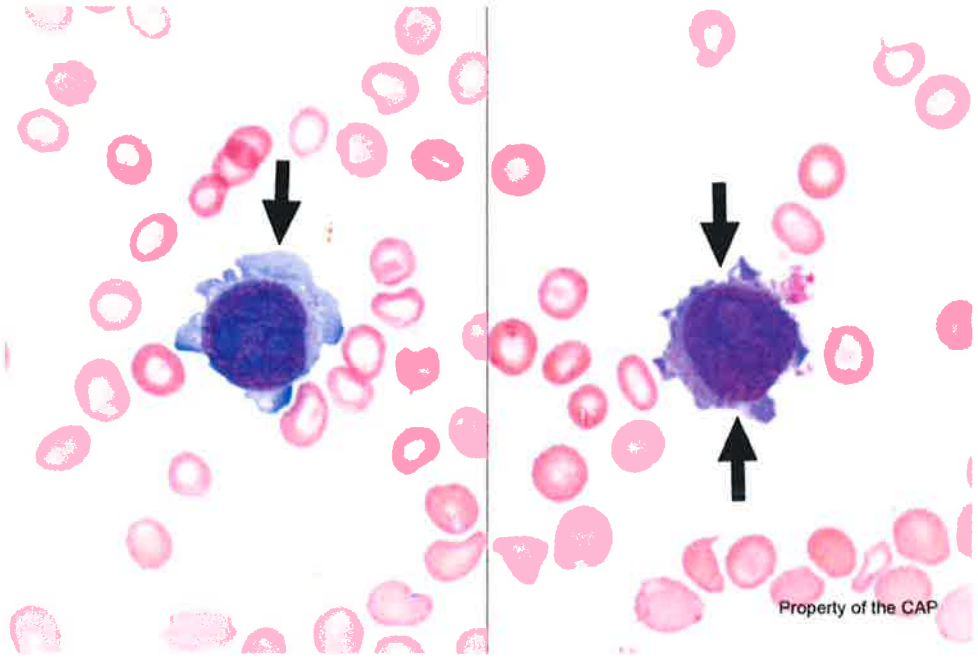


Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Basket cell/smudge cell	167	98.8	5182	98.5	Educational
Stain precipitate	1	0.6	27	0.5	Educational
Immature or abnormal cell, would refer for identification	1	0.6	12	0.2	Educational

The arrowed cell is a basket cell or smudge cell, as correctly identified by 98.8% of referees and 98.5% of participants. A basket cell or smudge cell is most commonly associated with cells that are fragile and easily damaged in the process of making a peripheral blood smear. The basket cell cytoplasm is indistinct, and chromatin strands spread out from a condensed nuclear remnant, giving the appearance of a basket.

Blood Cell Identification – Ungraded

BCP-17



Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Blast cell	68	40.2	1721	33.2	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	71	42.0	2420	46.6	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	9	5.3	377	7.3	Educational
Malignant lymphoid cell (other than blast)	4	2.4	312	6.0	Educational
Lymphocyte	2	1.2	30	0.0	Educational
Monocyte, immature (promonocyte, monoblast)	2	1.2	29	0.0	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	2	1.2	27	0.0	Educational
Immature or abnormal cell, would refer for identification	11	6.5	252	4.9	Educational

The arrowed cells are blasts, as correctly identified by 40.2% of the referees and 33.2% of participants. Blasts are large, round-to-oval cells, with high nuclear-to-cytoplasmic ratios, often with large nuclei demonstrating lacy or reticular (immature) chromatin.

The arrowed cells were incorrectly identified as a megakaryocyte (normal, abnormal or nuclear fragment) by 42.0% of the referees and 46.6% of the participants. While the arrowed cells do demonstrate features suggestive of megakaryocytic lineage, namely the cytoplasmic coloration and blebbing, the immaturity of the nuclear

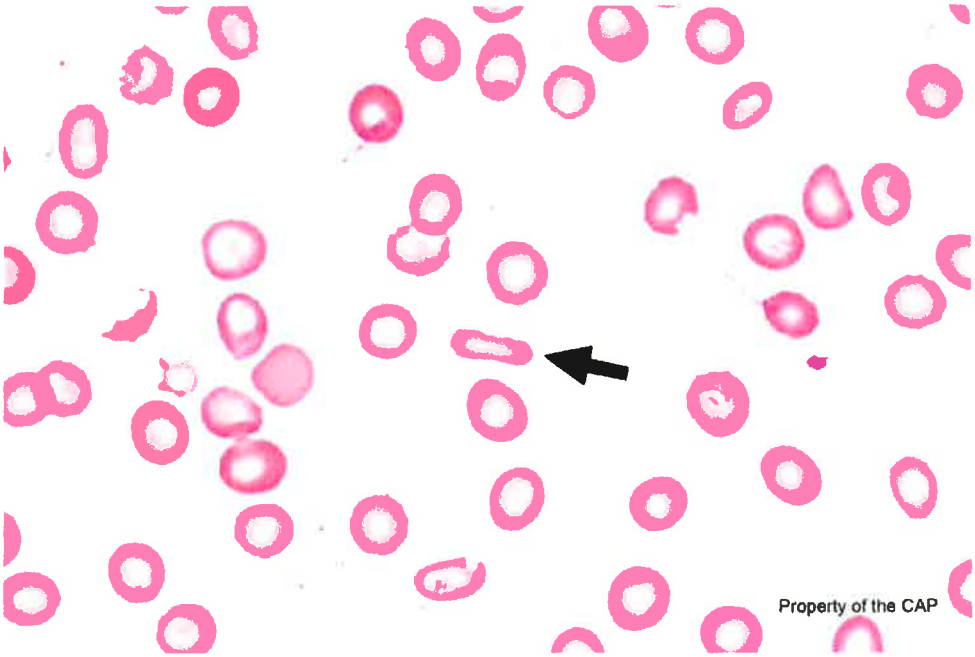
BCP-17, cont'd
chromatin and prominence of the nucleoli suggest immaturity. As such, the classification as blast is more appropriate than megakaryocyte (normal, abnormal or nuclear fragment).

The arrowed cells were incorrectly identified as a lymphocyte, reactive (includes plasmacytoid and immunoblastic forms) by 5.3% of the referees and 7.3% of the participants. Reactive lymphocytes tend not to demonstrate such prominent blebbing or features of nuclear immaturity, making this selection an inappropriate one.

Finally, the arrowed cells were incorrectly identified a malignant lymphoid cell (other than blast) by 2.4% of the referees and 6.0% of the participants. Indeed, some malignant lymphoid cells can demonstrate prominent blebbing (as, for example, in some T-cell lymphomas), however, the nuclear features are not suggestive of a mature lymphoid process, rendering this selection inappropriate.

Blood Cell Identification – Ungraded

BCP-18

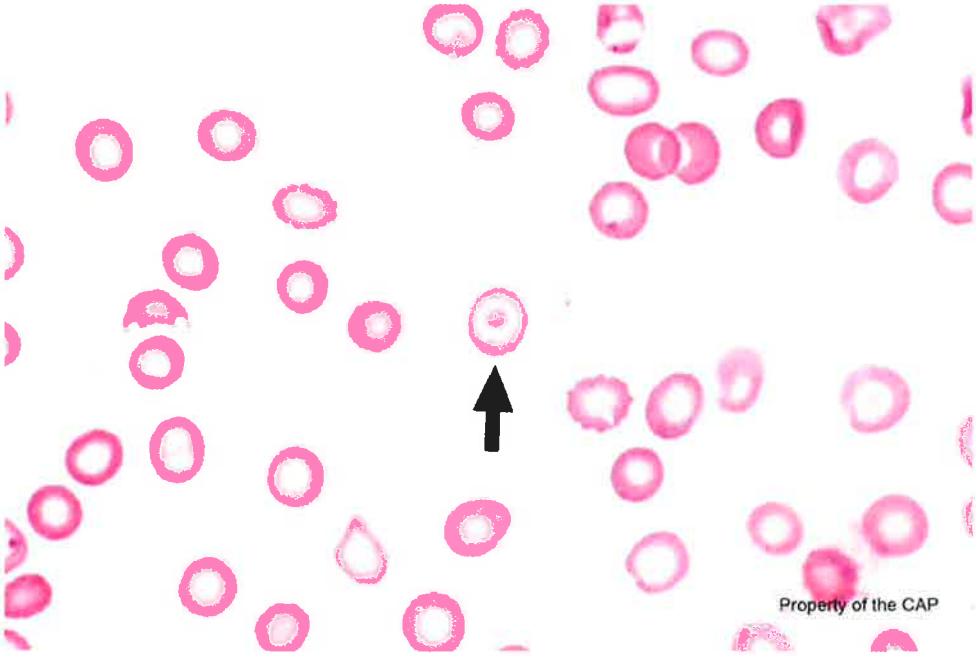


Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Ovalocyte (elliptocyte)	167	98.8	5158	99.4	Educational
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	1	0.6	3	0.1	Educational
Stomatocyte	1	0.6	13	0.3	Educational

The arrowed cell is an elliptocytes/ovalocytes, as correctly identified by 98.8% of referees and 99.4% of participants. The terms ovalocyte and elliptocyte are interchangeably used to describe elongated red blood cells with blunt ends and parallel sides. A small number of elliptocytes/ovalocytes may be present on the smears of normal individuals (< 1%), whereas a moderate to marked elliptocytosis/ovalocytosis (> 25%) is observed in patients with hereditary elliptocytosis, an abnormality of erythrocyte cytoskeletal proteins. Elliptocytes are also commonly increased in number in iron deficiency and in the same states in which teardrop cells may be seen.

Blood Cell Identification – Ungraded

BCP-19

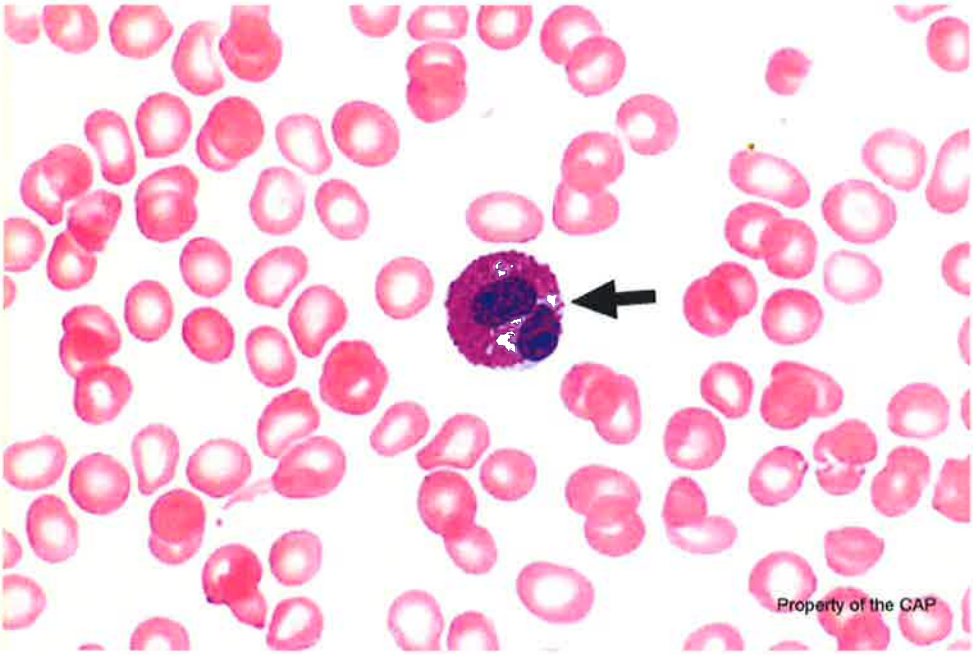


Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Target cell (codocyte)	169	100.0	5174	99.7	Educational

The arrowed cell is a target cell (codocyte), as correctly identified by 100.0% of referees and 99.7% of participants. Target cells have a characteristic appearance, consisting of a ring of pallor encircling a central zone of hemoglobinization, together reminiscent of a "bull's-eye." The target cell appearance results from an abnormally increased cell membrane-to-volume ratio, purported to result either from abnormal cell membrane lipid content or reduced cytoplasmic hemoglobin content. Relating to the former context, target cells may be seen in patients with biliary or liver disease, in which abnormal cholesterol metabolism may be a contributing factor. Relating to the latter, target cells may be seen in patients with iron deficiency or thalassemia. Of note, target cells may also be encountered post-splenectomy and rarely as an artifact of slide preparation (in the latter context, target cells are usually very few in number).

Blood Cell Identification – Ungraded

BCP-20



Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Eosinophil, any stage	168	99.4	5116	98.6	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	1	0.6	20	0.4	Educational

The arrowed cell is an eosinophil, as correctly identified by 99.4% of referees and 98.6% of participants. Eosinophils are comparable in size to neutrophils, but demonstrate characteristic coarse, orange-red granules of uniform size; these granules are generally refractile under light microscopy. Eosinophils exhibit the same nuclear characteristics and the same stages of development as neutrophils; in contrast to neutrophils, however, eosinophils demonstrate a bilobed nuclear appearance in the vast majority of cases.

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FH(1-4, 6, 9-10, 13)-B 2020: Acute Megakaryoblastic Leukemia

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Disclosure Statement

The following authors/planners have no financial relationships to disclose:
Etienne Mahé, MD, MSc, FRCPC, FCAP; Stephanie A. Salansky, MEd, MS, MT(ASCP)

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Learning Objectives

Upon completing the reading and answering the learning assessment questions, you should be able to:

1. Describe the salient aspects of classification of acute myeloid leukemia (AML) with megakaryocytic features.
2. Describe some of the epidemiological features of acute megakaryoblastic leukemia (AMKL).
3. Describe the characteristic morphological features of AMKL and other myeloid entities with megakaryocytic features.
4. Describe the utility of common ancillary tests used to confirm and further investigate cases of potential AMKL.

FH(1-4, 6, 9-10, 13)-B 2020: Acute Megakaryoblastic Leukemia

Case Presentation

This peripheral blood smear is from a 15-year-old boy who presented with fevers, headaches, night sweats, and pancytopenia. He underwent a bone marrow biopsy and was subsequently diagnosed with acute megakaryoblastic leukemia. Laboratory data include: WBC = 0.5 × 10E9/L; RBC = 2.24 × 10E12/L; HGB = 6.3 g/dL; HCT = 18.1%; MCV = 81 fL; PLT = 7 × 10E9/L; and RDW = 17.6%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

INTRODUCTION

Acute megakaryoblastic leukemia (AMKL) is a specific subtype of acute myeloid leukemia (AML), classified according to the World Health Organization's (WHO) classification of tumours of haematopoietic and lymphoid tissues under the larger category of AML, not otherwise specified.[1] The criteria for classification in this subcategory are quite strict, requiring a sufficient number of blasts (at least 20%) of megakaryoblastic lineage (at least 50%), an absence of other class-defining recurrent genetic abnormalities, an absence of features diagnostic of AML with myelodysplasia-related changes, and an absence of an underlying diagnosis of Down syndrome.

Published data relating to AMKL are limited, owing in part to the rarity of this entity, but also to changes in the accepted AML classification standards. While early AML classification systems were centered mainly around morphological and cytochemical features, the more recent WHO classification system includes a far more detailed assessment that includes immunophenotypic and molecular genetic parameters. As such, data gathered under the aegis of earlier classification systems, in particular the French-American-British (FAB), may not provide a complete picture of disease-specific characteristics relative to current standards (see, for example, a comparison of the various acute leukemia classification systems by Behm).[2] This is indeed the case in AMKL, and part of the reason for the narrow criteria required for its diagnosis.

EPIDEMIOLOGY & CLINICAL FEATURES

A handful of high-quality studies of AMKL and morphologically related AML subtypes are available. A recent Surveillance, Epidemiology, and End Results (SEER) registry study suggested that AMKL accounted for only 0.7% of AML cases.[3] Comparable low relative incidence rates have been recognized in other trial-based cohorts.[4,5] SEER data suggest a broad adult age range at diagnosis, with a median age of 67.5 years.[3] Published SEER-informed data are limited to patients of adult age range, however.

When cases are considered based solely on the presence of megakaryocytic features (ie, excluding cytogenetic features, myelodysplasia-related changes, and Down syndrome-related cases), the incidence of acute leukemia with megakaryocytic features demonstrates substantial early-age bias. In their fairly rigorous retrospective study, Duchayne et al. reported 57% of cases of acute leukemia with megakaryocytic features were identified in patients under age 18, with a median childhood age of only 12 months.[6] When they further segregated their cohort based on cytogenetic parameters, only 20% of their cohort met the criteria for AMKL, with most cases (64%) identified in the pediatric age range.[6] Indeed, in stark contrast to the SEER-informed adult dataset, when a broad age range is

considered, the data from Duchayne et al. suggest a median age of two years for AMKL patients, even when cases of Down syndrome and t(1;22) subtypes are excluded.

Available data do not suggest an AMKL-specific bias of sex or ethnic background.[3,4,6] Some studies suggest a relatively high proportion of cases presenting with organomegaly.[4,6] There are also reports of a peculiar association between AMKL and mediastinal germ cell tumors in young adult males.[1,7]

Diagnosis & Laboratory Parameters

As with all cases of putative acute leukemia, a complete bone marrow study (including peripheral smear review), flow cytometric assessment, cytogenetic analysis, and molecular profiling are strongly recommended.[1]

The complete blood count (CBC) & peripheral blood evaluation
Patients typically present with cytopenia(s). The observation of thrombocytopenia is typical, but thrombocytosis and leukocytosis have also been documented.[1,4,6] Dysplastic features may be identified, typified by neutrophil dysplasia, but also by atypia of platelets and (if present) erythroid precursors.[1] Circulating megakaryocytes (including dysplastic forms) may be seen, but are not considered blast equivalents.[1] Circulating blasts may be identified.[4,6]

Bone marrow evaluation
Although not specifically required according to the WHO rubric, most cases will demonstrate a preponderance of blasts with megakaryocytic morphological features.[1,6] Megakaryoblasts are relatively large-sized blasts, with immature nuclear features (often with prominent nucleoli), with typically basophilic cytoplasm and distinct cytoplasmic blebs.[1]

In their exhaustive cytological study of acute leukemias with megakaryocytic features, Duchayne et al. reported three general categories of megakaryoblasts: blast cells with clear and typical megakaryocytic features (eg, large size, cytoplasmic blebs); an immature cell contingent including an admixture of typical megakaryoblasts and otherwise morphologically undifferentiated blasts; and cases consisting entirely of morphologically undifferentiated blasts, for which ancillary immunophenotypic studies were required for lineage confirmation.[6] This study serves to highlight the need for rigorous immunophenotypic analysis to ascertain blast lineage.

The presence of micromegakaryocytes and/or other dysplastic features may be present but should not be of sufficient prominence for a diagnosis of AML with myelodysplasia-related changes. AMKL cases may demonstrate extensive bone marrow fibrosis, which often limits the availability or quality of aspirate materials.

Cytochemistry, flow cytometry, & immunophenotyping
Although infrequently required, it bears noting that certain cytochemical studies can be informative in the workup of a putative AMKL. In particular, AMKL cases should be myeloperoxidase negative in the blast cells of interest.[1]

For confirmation of megakaryocytic lineage, otherwise undifferentiated blasts must express at least one of the lineage-defining platelet glycoprotein markers CD41, CD61, and/or CD42b.[1,8] Owing to the potential for nonspecific positivity due to platelet-adherence to blasts, identification of intracytoplasmic CD41, CD61, and/or

CD42b positivity is considered more specific.[1] Other nonspecific myeloid markers such as CD13 and CD33 may also be positive; CD45, CD34, and HLA-Dr are typically negative, but CD117 is reported to be variably positive.[1,8] MPO and other markers of granulocytic lineage should be negative.[1] CD36 is characteristically positive, but is not specific, as this marker can be positive in erythroid leukemia.[1]

If aspirate materials are limited, as may be the case in situations of extensive fibrosis, immunohistochemical stains with the above (or alternative) antibodies can be performed. FVIII antibodies are typically readily available for immunohistochemical techniques.

Cytogenetic analysis

AMKL demonstrates no specific cytogenetic features. Other megakaryoblastic acute leukemias have been described with specific cytogenetic features. AML (megakaryoblastic) with t(1;22)(p13.3;q13.1) is considered by the WHO as its own subtype of AML with recurrent genetic abnormalities. Patients with Down syndrome should also not be classified as AMKL, but rather under the category of myeloid proliferations associated with Down syndrome. Finally, while the initial FAB M7 category included many cases with complex cytogenetic features, or other cytogenetic aneuploidies or partial aneuploidies, the current WHO classification would subclassify the majority of these cases as AML with myelodysplasia-related changes.

Differential Diagnosis

While the differential diagnosis of AMKL includes a number of specific subtypes of other AML, many can be excluded by way of careful review of the clinical history and with the aid of a robust cytogenetic workup. Cases of AMKL can show some morphologic resemblance to acute panmyelosis with myelofibrosis, especially if the available materials are limited. This equally rare entity, in contrast, is characterized by a proliferation of admixed marrow elements from all three myeloid lineages, rather than one biased toward megakaryoblastic proliferation. The differential diagnosis may also include AML with myelodysplasia-related changes. If the latter cannot be excluded by way of cytogenetic studies, careful review of the clinical history for evidence of myelodysplasia, as well as a careful assessment of the overall burden of dysplasia, is required.

Therapy and Prognosis

The aggressive nature of AMKL is highlighted in all published studies. In their SEER-informed dataset, for example, Giri, et al. compared AMKL to other non-AMKL cases, demonstrated an inferior survival, even after excluding cytogenetically good-risk cases (such as core-binding factor subtypes and cases of acute promyelocytic leukemia).[3] Similar data, albeit centered around the FAB M7 classification (ie, acute leukemia with megakaryocytic features), have been demonstrated in other retrospective datasets, including a relatively robust cohort from MD Anderson.[9] The latter study suggests that the mere presence of predominant megakaryocytic features is an adverse overall survival parameter.[9] It should be noted, however, that AML with t(1;22) and Down syndrome associated myeloid neoplasms do fare much better than cases of AMKL.[1]

Most studies reporting treatment outcome data in adults with AMKL record approximately 50% response rates to aggressive chemotherapy, but with subsequent rapid relapses and relatively brief overall survival.[4-6,9] The GIMEMA dataset reports a median survival of only 40 weeks, with a five-year overall survival rate of only 10%.[4]

In stark contrast, however, children with AML t(1;22) or Down syndrome-associated myeloid neoplasms demonstrate far better outcomes. Patients with Down syndrome associated myeloid neoplasms are typically treated with reduced intensity regimens (sometimes only requiring supportive care); children with AML t(1;22) treated with intensive chemotherapy respond well to treatment and demonstrate relatively long disease-free survival.[6]

SUMMARY

AMKL is a rare subtype of AML, NOS, characterized by a predominance of megakaryoblasts (or blasts of megakaryocytic lineage). This entity is often a diagnosis of exclusion, once other more common (and biologically and prognostically distinct) entities are excluded. The optimal workup in cases putative AMKL is extensive and should include morphological, immunophenotypic and cytogenetic studies.

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