Blood Cell Identification – Graded

Case History

This peripheral blood smear is from a 41-year-old man who presents with 1 day of abdominal pain and jaundice. His past medical history is significant for Evan syndrome (autoimmune hemolytic anemia and thrombocytopenia). Laboratory data includes: WBC = 5.3 x 10E9/L; RBC = 2.55 x 10E12/L; HGB = 7.7 g/dL; HCT = 24.3%; MCV = 82 fL; PLT = 73 x 10E9/L; RDW = 18.0%; and absolute reticulocyte count = 200 x 10E9/L (reference range 25 – 140 x 10E9/L). Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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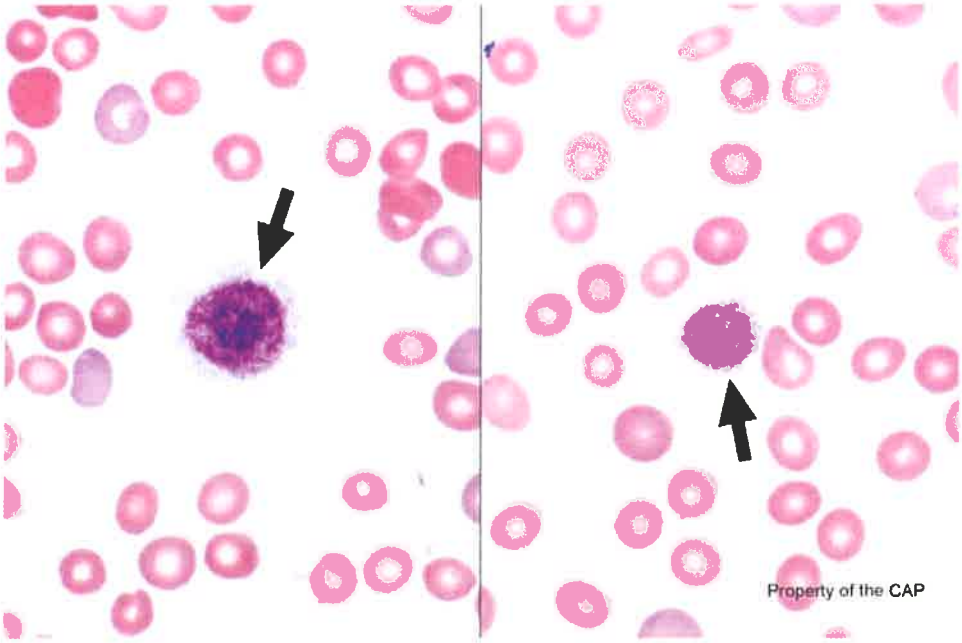
BCP-01

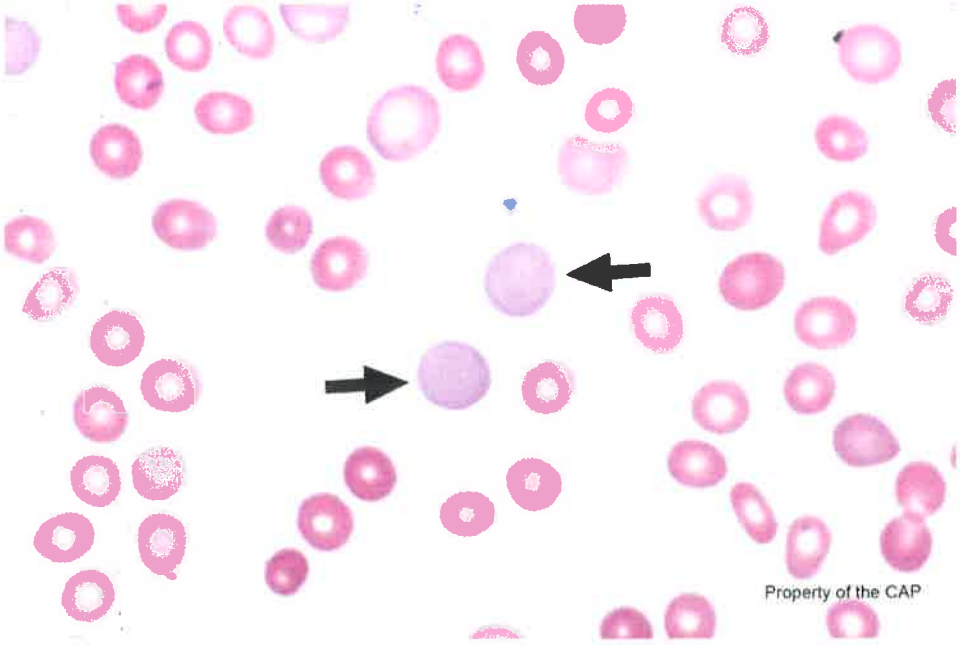


Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Platelet, giant (macrothrombocyte)	162	98.2	5324	97.5	Good
Megakaryocyte (normal, abnormal, or nuclear fragment)	2	1.2	58	1.1	Unacceptable
Neutrophil necrobiosis (degenerated neutrophil)	1	0.6	10	0.2	Unacceptable

The arrowed objects are giant platelets, as correctly identified by 98.2% of referees and 97.5% of participants. Platelets (also known as thrombocytes) are small, blue-gray fragments of megakaryocytic cytoplasm and typically measure 1.5 - 3 µm in diameter. Fine, purple-red granules are aggregated at the center or dispersed throughout the cytoplasm. Platelets play an essential role in primary hemostasis and normally circulate for 7 - 10 days before they are cleared by the spleen. In contrast, giant platelets (or macrothrombocytes), as highlighted in the photomicrograph, usually measure from 10 – 20 µm in diameter and are larger than background erythrocytes. Giant platelets may show variation in cytoplasmic appearance with coarse granules seen in some instances.

Blood Cell Identification – Graded

BCP-02

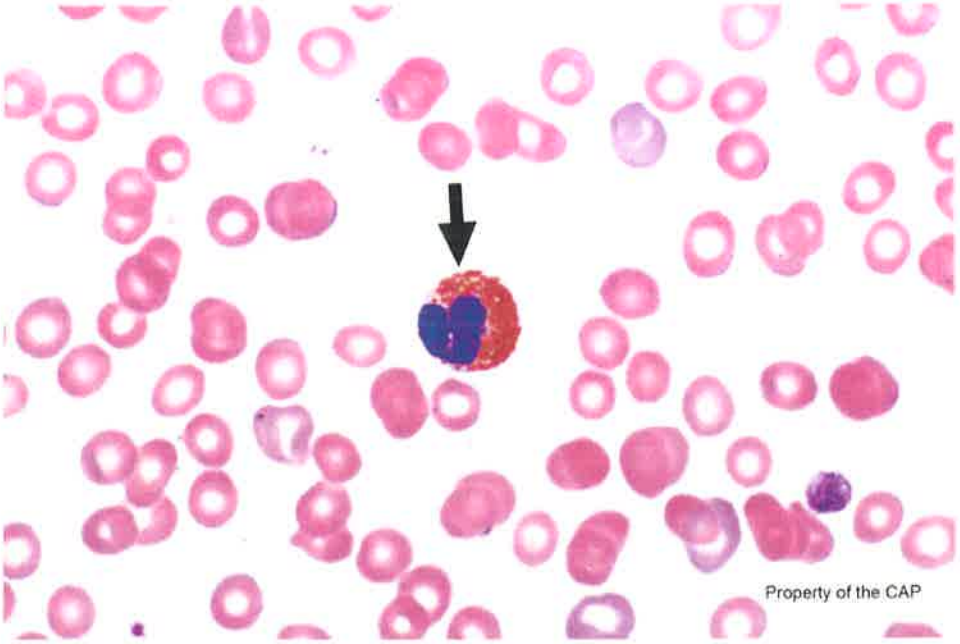


Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Polychromatophilic (non-nucleated) red blood cell	162	98.2	5288	96.8	Good
Spherocyte	2	1.2	98	1.8	Unacceptable
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	1	0.6	57	1.0	Unacceptable

The arrowed cells are polychromatophilic red blood cells, as correctly identified by 98.2% of referees and 96.8% of participants. Polychromatophilic red blood cells are slightly larger than mature erythrocytes, lack central pallor, and contain a small amount of residual RNA, thereby appearing uniformly pink-gray or pale purple. These reticulocytes represent the stage of erythrocyte maturation that emerges from the bone marrow and are physiologically increased in the peripheral blood in response to anemia (as in this case). Morphologic features can be seen in the photomicrograph, particularly in comparison to mature, background erythrocytes.

Blood Cell Identification – Graded

BCP-03

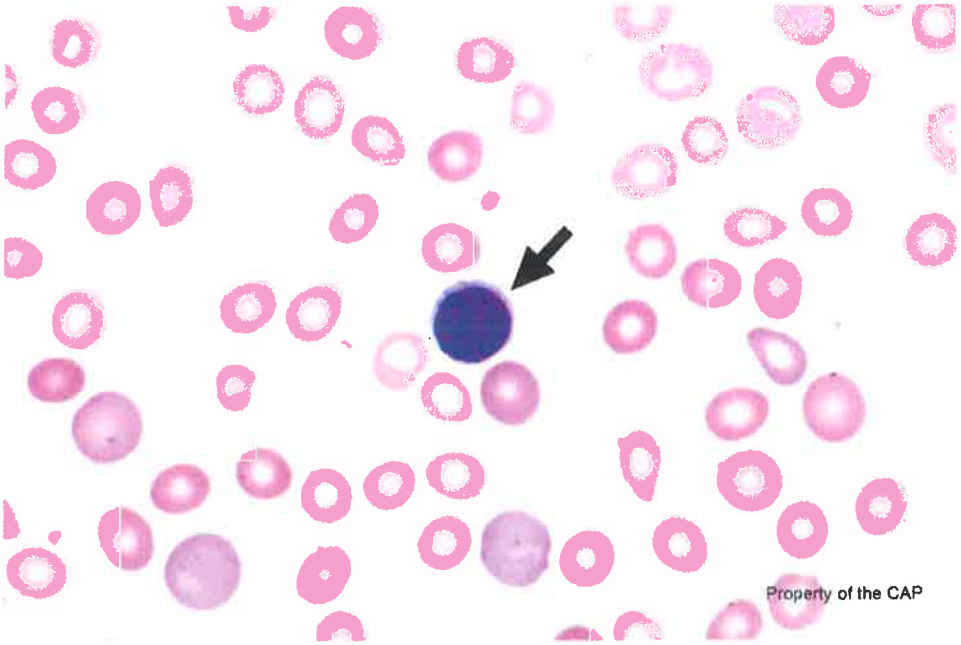


Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Eosinophil, any stage	165	100.0	5459	100.0	Good

The arrowed cell is an eosinophil, as correctly identified by 100.0% of referees and 100.0% of participants. Eosinophils demonstrate characteristic coarse, orange-red granules of uniform size; these granules are generally refractile under light microscopy. Eosinophils are similar to neutrophils in diameter (10 - 15µm), and the nucleus demonstrates condensed chromatin and nuclear segmentation with two or three nuclear lobes.

Blood Cell Identification – Graded

BCP-04

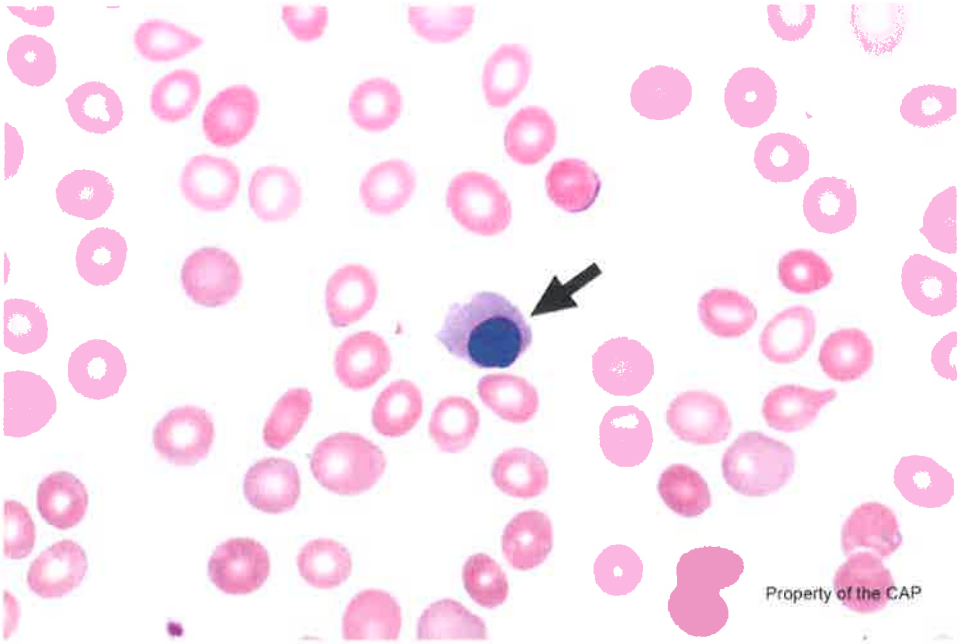


Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Lymphocyte	162	98.2	5329	97.6	Good
Nucleated red blood cell, normal or abnormal morphology	2	1.2	42	0.8	Unacceptable
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.6	26	0.5	Unacceptable

The arrowed cell is a lymphocyte, as correctly identified by 98.2% of referees and 97.6% of participants. Further, this cell shows features of a mature, non-reactive lymphocyte and is a normal constituent of peripheral blood. The lymphocyte is slightly larger than a normal red blood cell with scant to moderate pale blue cytoplasm, round nuclear contours, and no visible nucleolus.

Blood Cell Identification – Graded

BCP-05



Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Nucleated red blood cell, normal or abnormal morphology	164	100.0	5425	99.3	Good

The arrowed cell is a nucleated red blood cell (nRBC), as correctly identified by 100.0% of referees and 99.3% of participants. Nucleated RBCs are usually readily recognizable, with characteristic central nuclei, typically uniformly basophilic, and with cytoplasmic coloration demonstrating variable levels of hemoglobinization (depending on the stage of maturation) ranging from basophilic (usually infrequently seen) to orthochromatic (most frequently encountered). Less mature forms may demonstrate coarse-trabecular chromatin patterns. Occasionally, nRBCs can appear similar to lymphocytes (BCP-04), however the degree of nuclear condensation and cytoplasmic color can aid in accurate identification.

Clinical Presentation:

This peripheral blood smear is from a 41-year-old man who presents with 1 day of abdominal pain and jaundice. His past medical history is significant for Evans syndrome (autoimmune hemolytic anemia and thrombocytopenia). Laboratory data includes: WBC = 5.3 x 10E9/L; RBC = 2.55 x 10E12/L; HGB = 7.7 g/dL; HCT = 24.3%; MCV = 82 fL; PLT = 73 x 10E9/L; RDW = 18.0%; and absolute reticulocyte count = 200 x 10E9/L (reference range 25 - 140 x 10E9/L).

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Evans Syndrome

Evans Syndrome (ES) is an autoimmune disease defined by the constellation of autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia (ITP). ES can present in adult or pediatric patients with other immune-mediated diseases, infection, leukemias, lymphomas, and other cancers, however an underlying cause or event is not identified in many cases. The clinical manifestations of disease reflect hemolytic anemia and thrombocytopenia, and include jaundice, fatigue, easy bruising, and epistaxis. Detailed clinical and laboratory evaluations are necessary, as ES can be difficult to distinguish from other life-threatening disorders that warrant specific treatment, including hemolytic uremic syndrome and thrombotic thrombocytopenic purpura.

The underlying mechanism of ES remains unclear, however, immune dysregulation with antibodies targeting erythrocytes and platelets is central to the pathophysiology of disease. The peripheral blood typically shows a reticulocytosis with spherocytes, characteristic of AIHA, however schistocytes and red cell fragments are *not* characteristic. Laboratory findings include a positive direct antibody test, increased indirect bilirubin, and increased lactate dehydrogenase. As the bone marrow attempts to respond to hemolysis and thrombocytopenia, polychromatophilic red blood cells (BCP-02), nucleated red blood cells (BCP-04), and large platelets (BCP-01) can be seen on peripheral blood smears.

Clinical outcomes and treatment for ES are largely dependent on whether an underlying cause can be identified. In most cases, immunosuppressive regimens including high-dose steroids and/or intravenous immunoglobulins are the mainstay of therapy. The course of disease is variable, however relapses and exacerbations are common.

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Hematology and Clinical Microscopy Committee

References:

1. Evans R, Takahashi K, Duane RT, et al. Primary thrombocytopenic purpura and acquired haemolytic anemia: evidence of a common etiology. *AMA Arch Intern Med.* 1951;87(1):48-65.
2. Michel M, Chanet V, Dechartres A, et al. The spectrum of Evans syndrome in adults: new insight into the disease based on the analysis of 68 cases. *Blood.* 2009;114(15):3167-3172.
3. Hansen DL. Evans syndrome in adults - incidence, prevalence, and survival in a nationwide cohort. *American Journal of Hematology.* 10/2019;94(10):1081-1090.

Blood Cell Identification – Ungraded

Case History
This peripheral blood smear is from a 44-year-old woman was recently diagnosed with acute myeloid leukemia with t(9;11), *KMT2A-MLLT3 (MLL-MLLT3)* and persistent/refractory disease. Laboratory data include: WBC = 30.8 x 10E9/L; RBC = 3.22 x 10E12/L; HGB = 9.4 g/dL; HCT = 29.0%; MCV = 90 fL; MCHC = 32.4 g/dL; PLT = 134 x 10E9/L; and RDW = 23%. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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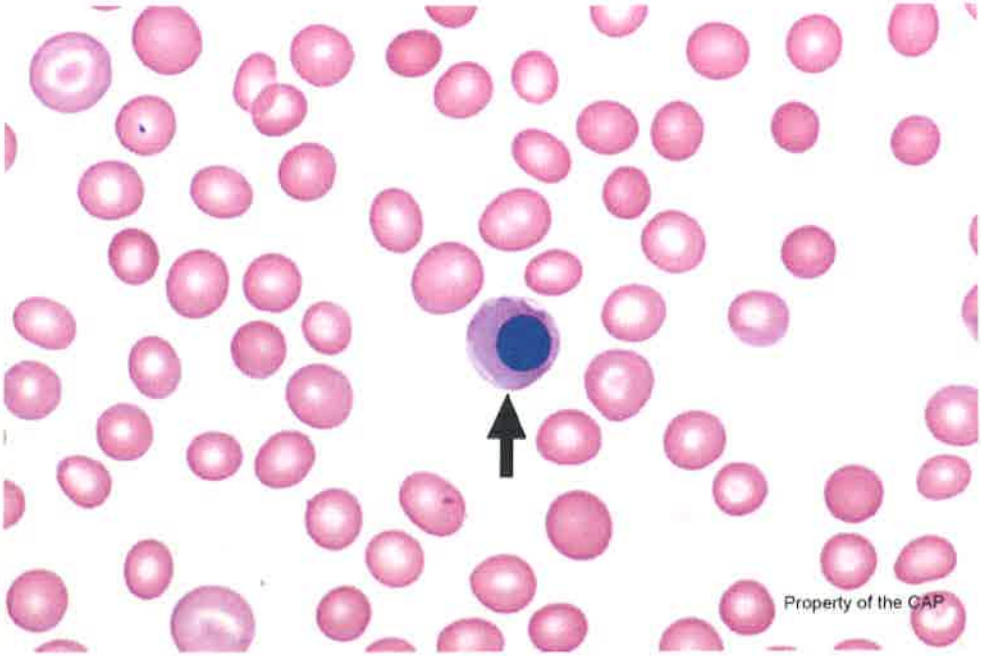
BCP-06

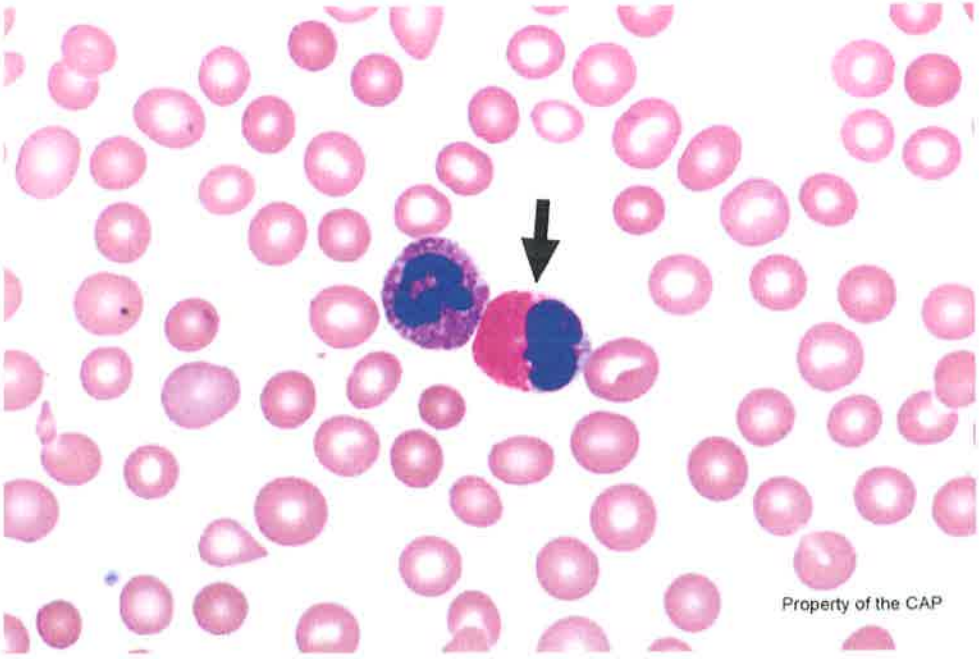


Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Nucleated red blood cell, normal or abnormal morphology	164	99.4	5373	99.2	Educational
Immature or abnormal cell, would refer for identification	1	0.6	21	0.4	Educational

The arrowed cell is a nucleated red blood cell (RBC), as correctly identified by 99.4% of referees and 99.2% of participants. For the purposes of cell identification, both normal or abnormal nucleated RBCs are considered together, irrespective of the stage of maturation. Nucleated RBCs are usually readily recognizable, with characteristic central nuclei, typically uniformly basophilic, and with cytoplasmic coloration demonstrating variable levels of hemoglobinization (depending on the stage of maturation) ranging from basophilic (usually infrequently seen) to orthochromatic (most frequently encountered). Less mature forms may demonstrate coarse-trabecular chromatin patterns.

Blood Cell Identification – Ungraded

BCP-07

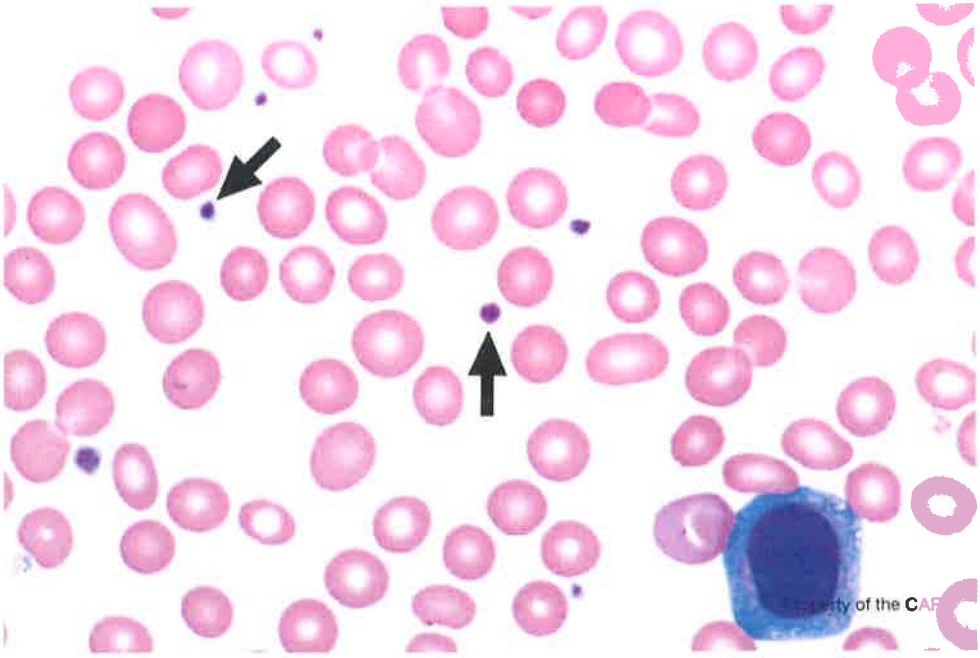


Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Eosinophil, any stage	165	100.0	5322	99.5	Educational

The arrowed cell is an eosinophil, as correctly identified by 100.0% of referees and 99.5% of participants. In this image, the eosinophil is noted in direct contrast to a neutrophil. 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.

Blood Cell Identification – Ungraded

BCP-08

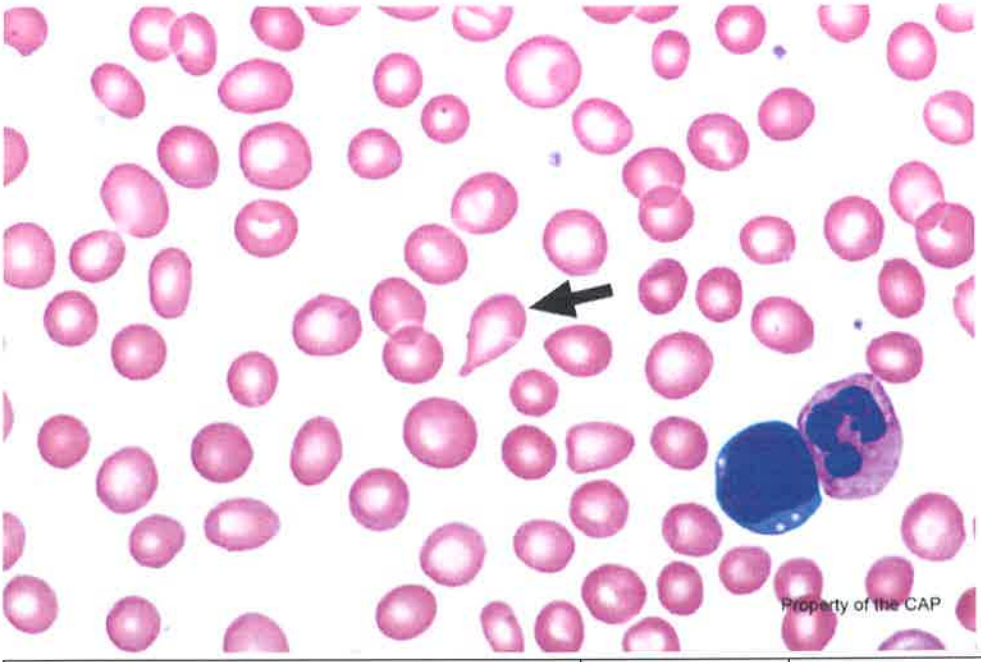


Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Platelet, normal	165	100.0	5331	99.7	Educational

The arrowed objects are normal platelets, as correctly identified by 100.0% of referees and 99.7% of participants. Platelets are small gray-blue anucleate fragments of megakaryocyte cytoplasm. Adequately granulated platelets are typified by fine purple-red (alpha) granules dispersed in the platelet cytoplasm, often centrally located. To this end, the platelets highlighted herein are not hypogranular. Most normal platelets are 1.5 to 3 µm in diameter, strictly smaller in size than red blood cells. To this end, the platelets highlighted herein are not giant forms.

Blood Cell Identification – Ungraded

BCP-09

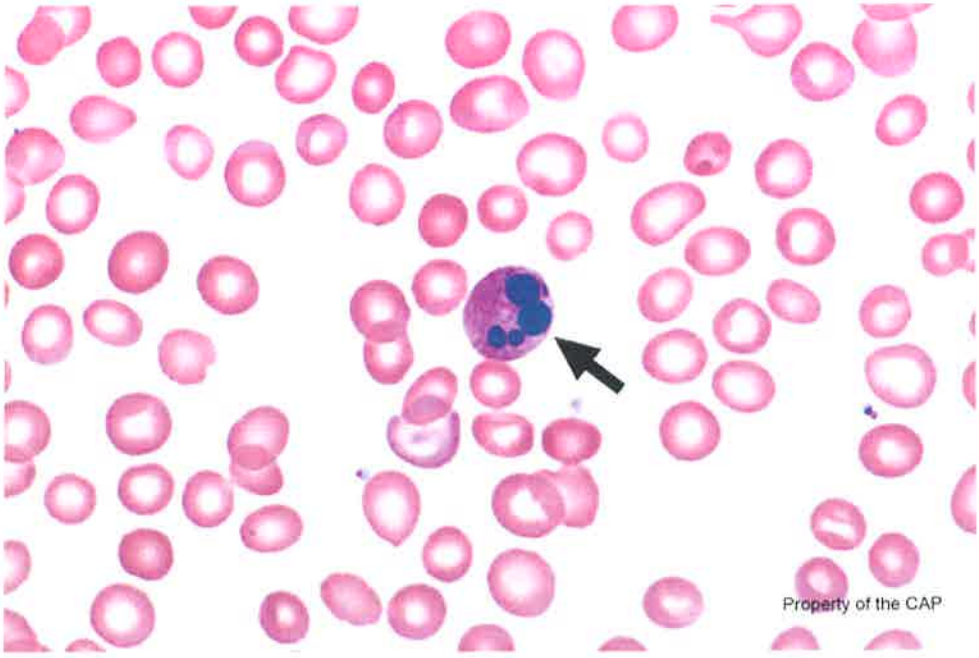


Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Teardrop cell (dacrocyte)	165	100.0	5323	99.6	Educational

The arrowed cell is a teardrop cell (dacrocyte), as correctly identified by 100.0% of referees and 99.6% of participants. Teardrop cells are usually easily recognizable as such, and often found in scenarios of marrow fibrosis or replacement, although they may also be seen in a variety of other conditions. Teardrop cells may also be seen as an artifact of slide preparation; such teardrops are usually easily recognized from the fact that their "tails" all point in the same direction.

Blood Cell Identification – Ungraded

BCP-10



Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Neutrophil necrobiosis (degenerated neutrophil)	144	87.3	4687	87.7	Educational
Neutrophil, segmented or band	9	5.5	311	5.8	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	4	2.4	56	1.1	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	3	1.8	91	1.7	Educational
Neutrophil with hypersegmented nucleus	2	1.2	48	0.9	Educational
Nucleated red blood cell, normal or abnormal morphology	1	0.6	31	0.6	Educational

The arrowed cell is an example of neutrophil necrobiosis (a degenerated neutrophil), as correctly identified by 87.3% of referees and 87.7% of participants. Neutrophil necrobiosis is a common phenomenon that can be seen both in normal individuals and in patients with a variety of medical conditions but is non-diagnostic and non-specific. Degenerated neutrophils are generally easily identified because they resemble normal segmented neutrophils; the major distinguishing feature is that the nucleus shows karyorrhexis and/or pyknosis. The chromatin pattern is characteristically dense and homogenous, with nuclear fragmentation evident. Necrobiotic neutrophils may also demonstrate cytoplasmic hypogranularity/agranularity, vacuolation and potentially ingested microorganisms (in the correct clinical context).

A minority of both referees (5.5%) and participants (5.8%) identified the arrowed cell as a neutrophil, segmented or band. The cytoplasmic features suggest neutrophil lineage and would not be consistent with erythroid lineage

BCP-10, con't.

or the response of nucleated red blood cell by 0.6% of both referees and participants; moreover, the lack of hair-like filaments connecting the nuclear bodies, and the presence of glassy chromatin, would be inconsistent with a segmented neutrophil. Likewise, the absence of a clearly sausage-shaped nucleus would exclude a band neutrophil. 2.4 % of referees and 1.1% of participants selected toxic neutrophil. The absence of vacuolation and Döhle bodies excludes a toxic neutrophil. Finally, 1.8 % of referees and 1.7% of participants selected dysplastic neutrophil, while 1.2% of referees and 0.9% of participants selected hypersegmented neutrophil; however, features of dysplastic neutrophils or hypersegmented neutrophils are not evident. The glassy pyknotic chromatin helps identify this cell as necrobiotic.

CLINICAL PRESENTATION:

This peripheral blood smear is from a 44-year-old woman who was recently diagnosed with acute myeloid leukemia with t(9;11), *KMT2A-MLLT3* (*MLL-MLLT3*) and persistent/refractory disease. Laboratory data include: WBC = 30.8 x 10E9/L; RBC = 3.22 x 10E12/L; HGB = 9.4 g/dL; HCT = 29.0%; MCV = 90 fL; MCHC = 32.4 g/dL; PLT = 134 x 10E9/L; and RDW = 23%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Residual AML with t(9;11), *KMT2A-MLLT3* (*MLL-MLLT3*)

The provided CBC data are notable for anemia and mild thrombocytopenia, but with prominent leukocytosis. The provided photomicrographs also demonstrate blasts (BCP-08 & BCP-09) and teardrop cells (BCP-07 & BCP-09). The presence of circulating blasts, given the recent diagnosis of AML with t(9;11), *KMT2A-MLLT3* (*MLL-MLLT3*), is suggestive of persistent disease.

AML with t(9;11), *KMT2A-MLLT3* is an acute leukemia that can occur at any age, but is enriched in the pediatric age range (1). The presence of monocytic/monoblastic features (for example, increased amount of gray-blue cytoplasm and/or cytoplasmic vacuolization) can often be observed in this subtype of AML, as also visible in the provided photomicrographs (BCP-08 & BCP-09); entities such as these also have a peculiar predilection for involvement of extramedullary sites such as the gingiva (1).

Blast cells in AML with t(9;11), *KMT2A-MLLT3* demonstrate evidence of myeloid lineage; however, the typical markers of myeloid differentiation such as MPO, CD13 and CD33 may be negative, and the typical markers of immaturity (such as CD34 and CD117) may also be absent (1,2). One or more markers of monocytic differentiation such as CD4, CD14, CD11b/CD11c or lysozyme are typically expressed, however these cannot distinguish monocytic blasts from monocytes (1,2). As such, immunoprofiling of suspect cases in the absence of morphologic assessment runs the risk of under-recognition (2).

Rearrangements involving the *KMT2A* gene are present in 10% of acute leukemias, with a peculiar bimodal incidence: in acute lymphoblastic leukemia (ALL) in the pediatric age range less than 12 months; and in AML in adults (3). The specific *MLLT3* translocation partner accounts for the majority of cases in adult AML, but is relatively infrequent in pediatric ALL (3). The KMT2A protein is essential to proper chromatin remodelling, functioning as a histone methyltransferase (3). *KMT2A* rearrangements lead to abnormal protein function, resulting in abnormal gene expression conferring stem-cell like properties (3).

While most acute leukemias with *KMT2A* rearrangements are generally considered high risk, the specific t(9;11), *KMT2A-MLLT3* subtype is typically classified as intermediate risk in the context of adult AML (1,3). This has the impact of potentially altering the trajectory of care, sometimes in favor of less aggressive consolidation therapy, in patients with otherwise favorable risk profiles (4).

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