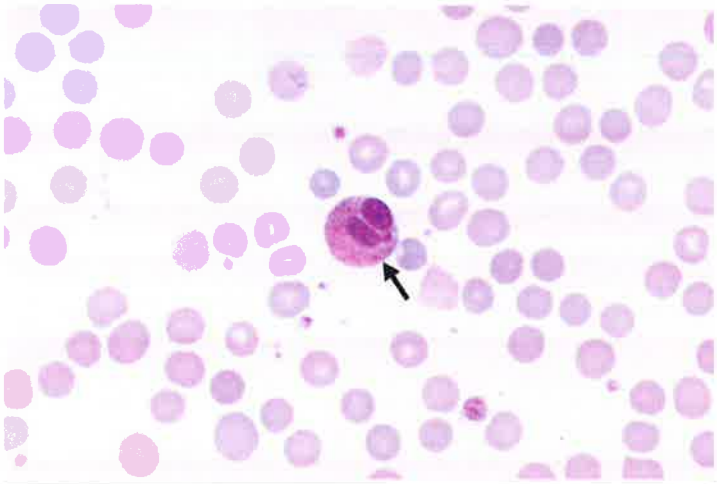
Cell Identification

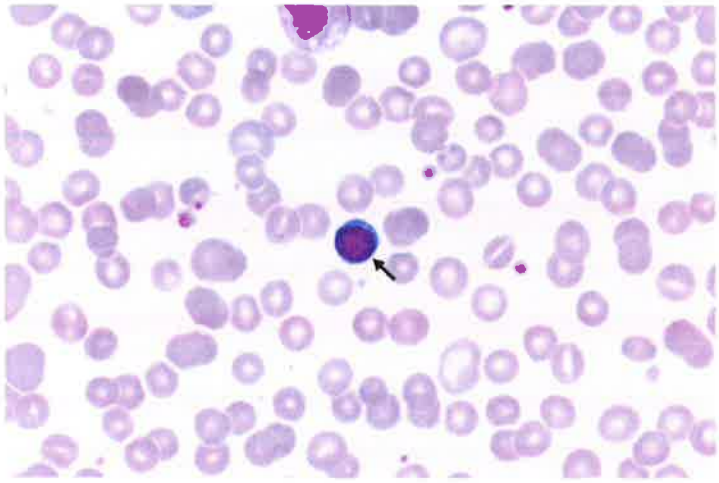
VPBS-20



Identification	Participants N	Participants %	Evaluation
Eosinophil, any stage	1330	99.7	Educational
Basophil, any stage	2	0.1	Educational
Lymphocyte, large granular	1	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 99.7% of participants. Eosinophils are round-to-oval leukocytes that are recognizable by their characteristic coarse, orange-red granulation. They are comparable in size to neutrophils. The cytoplasm is generally evenly filled with numerous coarse, orange-red, refractile granules of uniform size. The majority of segmented eosinophils will have the classic bilobed nucleus.

VPBS-21



Identification	Participants N	Participants %	Evaluation
Lymphocyte	1302	97.6	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	25	1.9	Educational
Lymphocyte, large granular	3	0.2	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	1	0.1	Educational
Monocyte	1	0.1	Educational
Nucleated red blood cell, normal or abnormal morphology	1	0.1	Educational
Platelet, hypogranular	1	0.1	Educational

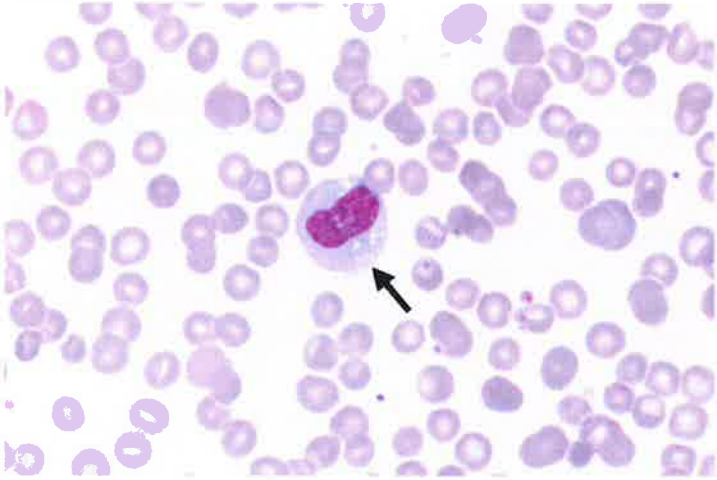
The arrowed cell is a normal lymphocyte, as correctly identified by 97.6% of participants. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 µm with an N:C ratio ranging from 5:1 to 2:1. Most have round to oval nuclei that may be slightly indented or notched. The chromatin is diffusely dense or coarse and clumped. Nucleoli are not visible, although some cells may exhibit a small, pale chromocenter that may be mistaken for a nucleolus. Most lymphocytes have a scant amount of pale blue to moderately basophilic, agranular cytoplasm.

The arrowed cell was incorrectly identified as a reactive lymphocyte by 1.9% of participants. The most common types of reactive lymphocytes are larger lymphocytes (so-called Downey type II and Downey type III cells) with sizes ranging from 10 to 25 µm, and plasmacytoid lymphocytes. Downey type II cells have round to oval nuclei, moderately condensed chromatin (giving a smeared appearance), and absent or indistinct nucleoli. In sharp contrast to the arrowed cell here, Downey type II cells contain abundant pale gray-blue cytoplasm with their amoeboid cytoplasm displaying a darker-staining, furled margin as they partially surround adjacent red cells. Downey type III cells are immunoblasts and immunoblast-like large reactive lymphocytes (15 to 20 µm) with round to oval nuclei. They have finely to moderately dispersed chromatin with abundant parachromatin and one or more prominent nucleoli, and may resemble lymphoma cells or blasts. Their cytoplasm is moderately abundant and stains deeply basophilic. Their large size and chromatin pattern readily distinguish them for normal lymphocytes. Plasmacytoid lymphocytes are intermediate in size (10 to 20 µm). As their name implies, they can mimic plasma cells with their round to oblong shape, centrally placed or slightly eccentric nuclei, and slightly to moderately coarse chromatin resembling that of plasma cells. The cytoplasm is moderately abundant, homogeneous, and light blue to deep slate-blue, and it may show a perinuclear clear zone, or hof. Lastly, a type of rare reactive lymphocyte is a Downey type I cell.

VPBS-21, cont'd.

The amount of basophilic cytoplasm can be scant to moderate in a Downey type I cells; however, their nuclei often appear indented, folded, or lobulated. Downey type I reactive lymphocyte chromatin is condensed, and few small vacuoles and granules may be apparent. This is in contrast to the features in the arrowed cell of this normal lymphocyte, which exhibits relatively smooth nuclear contours and is devoid of cytoplasmic granules and vacuoles.

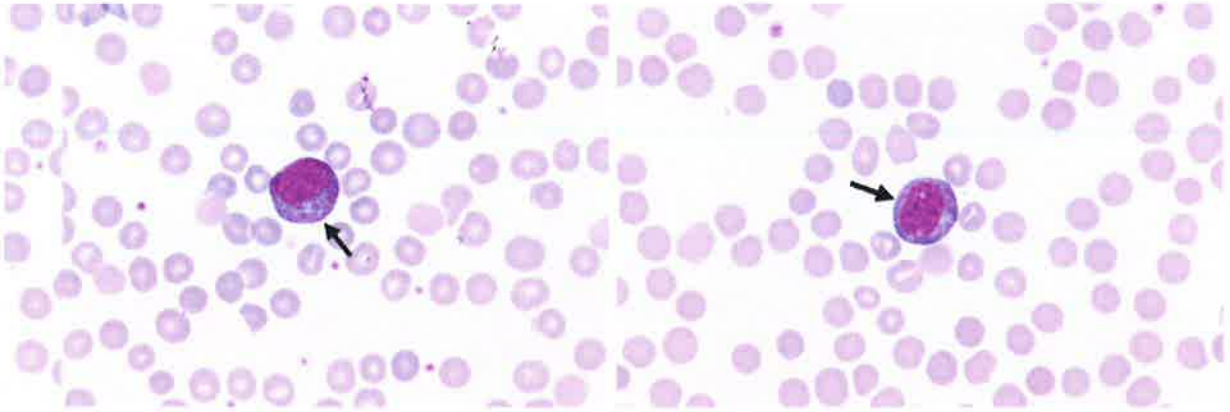
VPBS-22



Identification	Participants N	Participants %	Evaluation
Monocyte	1317	98.7	Educational
Monocyte, immature (promonocyte, monoblast)	5	0.4	Educational
Neutrophil, giant band or giant metamyelocyte	3	0.2	Educational
Neutrophil, segmented or band	3	0.2	Educational
Neutrophil, metamyelocyte	2	0.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.1	Educational
Neutrophil, promyelocyte, abnormal with/without Auer rod(s)	1	0.1	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	1	0.1	Educational
Platelet, giant (macrothrombocyte)	1	0.1	Educational

The arrowed cell is a monocyte, as correctly identified by 98.7% 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. Their 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 usually less dense than that of a neutrophil or lymphocyte. Nucleoli are generally absent, but occasional monocytes may contain a small, inconspicuous nucleolus.

VPBS-23



Identification	Participants N	Participants %	Evaluation
Neutrophil, myelocyte	920	69.0	Educational
Neutrophil, promyelocyte	332	24.9	Educational
Immature or abnormal cell, would refer for identification	19	1.4	Educational
Neutrophil, metamyelocyte	15	1.1	Educational
Lymphocyte, large granular	13	1.0	Educational
Monocyte, immature (promonocyte, monoblast)	9	0.7	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	5	0.4	Educational
Blast cell	4	0.3	Educational
Myeloblast with Auer rod	4	0.3	Educational
Neutrophil, promyelocyte, abnormal with/without Auer rod(s)	4	0.3	Educational
Basophil, any stage	3	0.2	Educational
Basophilic stippling (coarse)	1	0.1	Educational
Eosinophil, any stage	1	0.1	Educational
Monocyte	1	0.1	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	1	0.1	Educational
Platelet, normal	1	0.1	Educational

The arrowed cells are neutrophil, myelocytes, as correctly identified by 69.0% of participants. Myelocytes are usually confined to the bone marrow where they constitute approximately 10% of the nucleated cells. In pathologic states, such as this case of a leukemoid reaction, myelocytes are seen in blood. Myelocytes are smaller than a promyelocytes, usually 10 to 18 µm. They are round to oval in shape and have a nuclear-to-cytoplasmic ratio of 2:1 to 1:1. Their nucleus is slightly eccentric, lacks a nucleolus, and begins to demonstrate chromatin clumping; one side often shows slight flattening. Sometimes a clear space or hof is seen adjacent to the nucleus, indicating the location of the Golgi apparatus. The cytoplasm is relatively more abundant than in earlier precursors and is amphophilic. Both azurophilic and specific granules are present in the cytoplasm, with specific granules coming to predominate as maturation progresses. The response of immature or abnormal cell, would refer for identification, classified by 1.4% of participants, is an acceptable response.

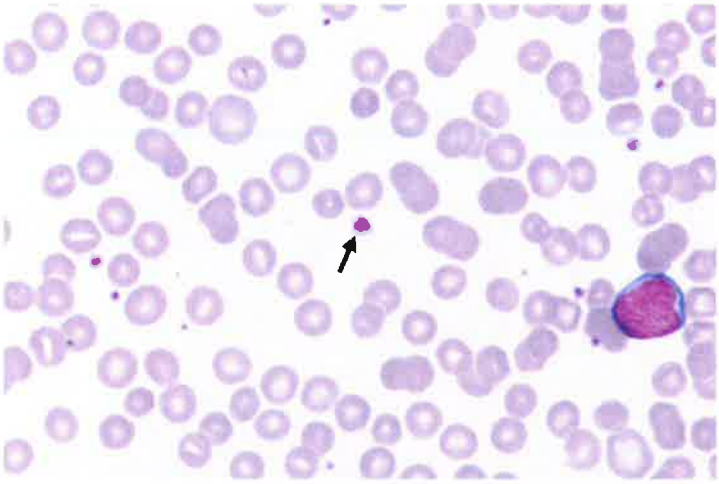
Many participants (24.9% of participants) identified these cells as promyelocytes. Promyelocytes are round to oval cells that are larger than myelocytes, with a diameter of 12 to 24 µm and N:C ratio usually ranging from 5:1 to 3:1. Their nuclei are round-to-oval with fine chromatin and distinct nucleoli, while their cytoplasm is basophilic and contain multiple distinct azurophilic (primary) granules. A paranuclear hof or cleared space is

typically present. The intended response for the arrowed cells are neutrophil, myelocytes given the presence of both azurophilic (primary) granules, as well as the presence of fine lilac or pale orange/pink specific granules. Admittedly, these cells show a spectrum of maturation, with the last VPBS-23 cell displaying distinct nucleoli and prominent primary granules, making this cell one that is transitioning from promyelocyte to an early myelocyte. However, the other 2 cells in this VPBS-23 selection are further along in their neutrophilic maturational sequence and have less distinct nucleoli, fewer primary granules, and (importantly) more specific granules that allow identification as a neutrophilic precursors. Thus, the correct identification for these cells are neutrophil, myelocytes.

A small percentage of participants (1.1%) incorrectly identified these arrowed cells as neutrophil, metamyelocytes. Metamyelocytes are the first of the postmitotic myeloid precursors. They have similar overall size, shape, and N:C ratios to myelocytes, are approximately 10 to 18 µm in diameter, round to oval, and have a N:C ratio of 1.5:1 to 1:1. Similar to myelocytes, their cytoplasm may contain fewer azurophilic or purple (primary) granules and many fine lilac or pale orange/pink specific granules. However, in contrast to myelocytes, metamyelocytes are further along in maturation. Thus, their nuclear chromatin is more condensed than myelocytes, and their 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). Myelocyte nuclear contours can be flattened on one side, but are not indented, as metamyelocyte contours are.

Finally, 1.0% of participants classified these arrowed neutrophilic cells incorrectly as lymphocyte, large granular. Large granular lymphocytes are medium to large cells with round nuclei, dense chromatin, and no visible nucleoli. The cytoplasm is moderate to abundant, clear and lightly basophilic, and contains several variable coarse, unevenly distributed, small azurophilic granules. These cells are found in small numbers in blood smears from normal individuals, but they may be increased in association with other reactive lymphocytes. Cell surface marker studies show that these cells are natural killer cells or suppressor/cytotoxic T-lymphocytes. The abundant number of granules and the quality of primary and neutrophil-specific granules in these arrowed cells should allow confident recognition as a neutrophilic precursor, and exclude these from inappropriate classification as lymphoid cells.

VPBS-24



Identification	Participants N	Participants %	Evaluation
Platelet, normal	1184	88.8	Educational
Platelet, giant (macrothrombocyte)	140	10.5	Educational
Platelet, hypogranular	3	0.2	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	2	0.1	Educational
Blast cell	1	0.1	Educational
Lymphocyte	1	0.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.1	Educational
Neutrophil, promyelocyte	1	0.1	Educational
Platelet satellitism	1	0.1	Educational

The arrowed object is a platelet, as correctly identified by 88.8% of participants. Platelets are small, blue-gray fragments of megakaryocytic cytoplasm. Most measure 1.5 to 3 µm in diameter. A few small platelets, less than 1.5 µm in diameter, and a few large platelets, 4 to 7 µm in diameter, may also be seen in normal blood films. Fine, purple-red granules are dispersed throughout the cytoplasm or are sometimes aggregated at the center. These granules are platelet alpha granules. Platelet delta granules (or dense granules) are not visible on light microscopy. Platelets may be variable in shape, but most normal platelets are round or very slightly elliptical. Some have short cytoplasmic projections or ruffled margins.

The arrowed object was incorrectly identified as a giant platelet (macrothrombocyte) by 10.5% of participants. While the cytomorphologic quality is essentially identical for normal platelets and giant platelets, the size of giant platelets are much larger, usually measuring 10 to 20 µm in diameter. For proficiency testing purposes, the term "giant platelet" is used when the platelet is larger than the size of the average red blood cell in the field (assuming a normal MCV, as in this case). In this field, the average red blood cell is at least three times larger than the platelet, excluding it from the definition of giant platelet.

Case Presentation:
This peripheral blood smear is from a 23-year-old man with a history of rhabdomyolysis (compartment syndrome). Laboratory data include: WBC = 17.1 x 10E9/L; RBC = 3.72 x 10E12/L; HGB = 11.6 g/dL; HCT = 33.9 %; MCV = 89 fL; PLT = 218 x 10E9/L; and RDW = 15%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Rhabdomyolysis due to compartment syndrome

Rhabdomyolysis is characterized by muscle damage with potential for significant morbidity and mortality, if not appropriately managed in a timely fashion. Severity can vary from asymptomatic elevations in serum muscle markers to life-threatening disease with acute renal failure, liver dysfunction, and disseminated intravascular coagulation.

The classic presentation is that of a patient who complains of muscle pain or weakness and reports dark-colored urine, with extremely high serum creatine kinase (CK) levels and evidence of urine myoglobin. Interestingly, this constellation of features is present in fewer than 10% of patients with rhabdomyolysis. While many patients endorse muscle weakness or pain, they also report other nonspecific symptoms, like swelling, nausea, vomiting, palpitations, confusion, or decreased urine output.

Question 1. True or False statement: The clinical diagnosis of rhabdomyolysis requires the combined history of muscle pain and dark-colored urine.

A. False
B. True

The diagnostic laboratory finding in a patient with new acute-onset rhabdomyolysis is a serum creatine kinase level that is greater than five times the upper limit of the normal reference range, with high levels in the skeletal muscle component (CK-MM). Dark urine is not due to hemoglobin (ie, hemoglobinuria) nor red cells (ie, hematuria), but rather myoglobin, a muscle breakdown product excreted in the urine. Though myoglobinuria would also be characteristic for rhabdomyolysis, the short half-life of myoglobin (3 hours, as opposed to 36 hours for CK) makes it a poorly sensitive marker for disease. Severe cases can develop a number of metabolic derangements leading to kidney and liver failure, as well as activation of the coagulation pathway resulting in disseminated intravascular coagulation. In such stressful settings, acute phase reactants and other inflammatory markers are expected to be elevated. An example of this would be an increased white blood cell count with neutrophilic left-shift, as was observed in this patient's case.

Question 2. Which laboratory abnormality is essential for the diagnosis of rhabdomyolysis?

A. High serum creatinine resulting in low glomerular filtration rate
B. Increased numbers of red blood cells on microscopic urinalysis
C. Leukocytosis with neutrophilic predominance and left shift
D. Markedly elevated serum creatine kinase (CK-MM)

There are many etiologies for rhabdomyolysis; they can be categorized into three broad mechanisms: traumatic with muscle compression, non-traumatic exertional (including intense exercise, hyperthermia, metabolic myopathies), and non-traumatic non-exertional (caused by exposure to certain drugs, toxins, and infections). The first broad mechanism of rhabdomyolysis - traumatic muscle compression - is the most common mechanism and

the one experienced by the patient in this vignette. Traumatic muscle compression can be seen in motor vehicle accidents, prolonged immobilization, crush injury, severe extremity injury complicated by compartment syndrome, and electrical injury.

Acute compartment syndrome occurs commonly with significant injury or trauma to limbs with long bones (like the leg or forearm). In this setting, swelling within the extremity exceeds the confines of the inflexible fascia in that region, increasing pressure and compromising blood circulation to, and function of, tissues in that space. The widespread necrosis of muscle cells liberates intracellular components into the interstitial tissue and into the circulation, including myoglobin, calcium, phosphate, potassium, uric acid, and serum creatine kinase. As myocytes in the extremity break down, resultant increased water content within cells and the interstitium further contribute to increased pressures within the compartment. This eventually will cause blood vessels to collapse, resulting in ischemia and oxygen deprivation to tissues.

Acute limb compartment syndrome must be managed emergently by fasciotomy, which is a surgical procedure to decompress the symptomatic compartment. In addition, careful administration of intravenous fluids in the proper settings may be warranted to protect organ functions, particularly renal output.

Maria Vergara-Lluri, MD
Hematology and Clinical Microscopy Committee

REFERENCES:

1. Torres PA, Helmstetter JA, Kaye AM. Rhabdomyolysis: pathogenesis, diagnosis, and treatment. *Ochsner* 2015;15:58-69.
2. Long B, Koyfman A, Gottlieb M. An evidence-based narrative review of the emergency department evaluation and management of rhabdomyolysis. *Am J Emerg Med.* 2019;37:518-523.
3. Duckworth AD, McQueen MM. The diagnosis of acute compartment syndrome: a critical analysis review. *JBJS. Rev* 2017;5:e1.

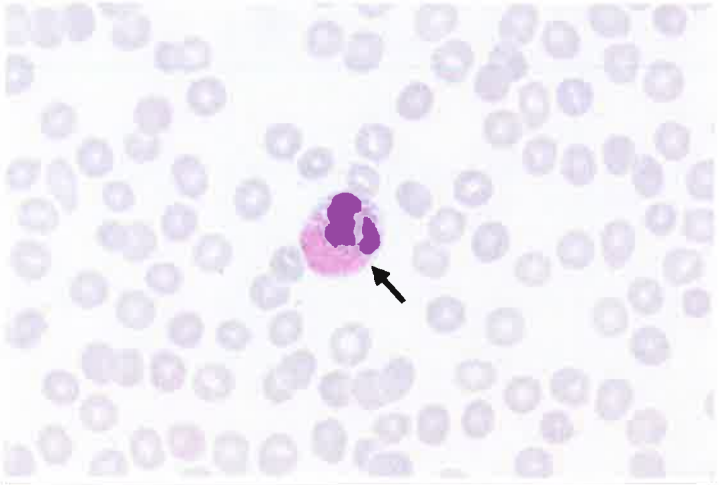
ANSWERS TO QUESTIONS:

Question 1. Answer: A. False
While myalgias and dark-colored urine are part of the classic clinical presentation of rhabdomyolysis, along with markedly elevated serum creatine kinase levels, only a small minority of patients (fewer than 10%) will present with all three of these findings. In particular, fewer than 5% of patients with rhabdomyolysis report the finding of dark-colored urine – which makes this sign a particularly unreliable marker of disease.

Question 2. Answer: D. Markedly elevated creatine kinase with markedly elevated CK-MM
The diagnostic laboratory test in a patient with a new-onset acute neuromuscular disorder is a serum creatine kinase level that exceeds five times the upper limit of the reference range. A leukemoid reaction with neutrophilia and left-shift may be seen, but is a non-specific finding. markedly elevated levels of serum creatinine would certainly be observed in cases of acute renal failure; however, demonstration of these levels is not required for the diagnosis of rhabdomyolysis. While high levels of myoglobinuria would be supportive of the diagnosis, the finding of hematuria (ie, increased numbers of RBCs on urine microscopy) is not.

Cell Identification

VPBS-26

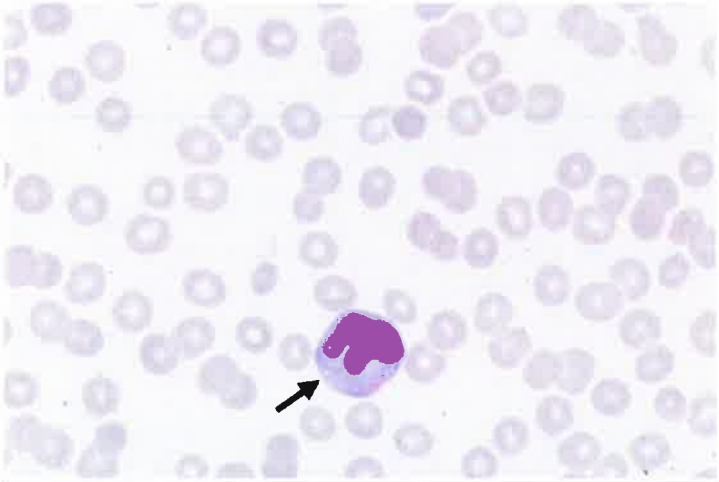


Identification	Participants N	Participants %	Evaluation
Eosinophil, any stage	1296	97.3	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	25	1.9	Educational
Neutrophil, segmented or band	4	0.3	Educational
Basophil, any stage	3	0.2	Educational
Leukocyte containing Chediak-Higashi anomaly inclusion(s)	2	0.1	Educational
Erythrocyte with overlying platelet	1	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 97.3% of the participants. Eosinophils are round-to-oval leukocytes that are recognizable by their characteristic coarse, orange-red granulation. They are comparable in size to neutrophils, ie, 10 to 15 µm in diameter in their mature forms. 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. The nucleus segments into two or more lobes connected by a thin filament. About 80% of segmented eosinophils will have the classic bilobed nuclei. 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 three lobes, and an occasional cell will exhibit four to five lobes.

1.9% of participants incorrectly identified the cell as a neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization). The cytoplasmic granules of toxic neutrophils, while coarse, are smaller and less eosinophilic that those of eosinophils. In addition, neutrophils typically have three or more nuclear segments, while eosinophils typically have two nuclear lobes.

VPBS-27



Identification	Participants N	Participants %	Evaluation
Monocyte	1233	92.6	Educational
Neutrophil, segmented or band	33	2.5	Educational
Monocyte, immature (promonocyte, monoblast)	30	2.3	Educational
Neutrophil, metamyelocyte	14	1.1	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	6	0.5	Educational
Neutrophil, giant band or giant metamyelocyte	5	0.4	Educational
Lymphocyte	3	0.2	Educational
Immature or abnormal cell, would refer for identification	2	0.1	Educational
Neutrophil, myelocyte	2	0.1	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	2	0.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.1	Educational
Mitotic figure	1	0.1	Educational

The arrowed cell is a monocyte, as correctly identified by 92.6% 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 and/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 usually less dense than that of a neutrophil or lymphocyte. Nucleoli are generally absent, but occasional monocytes may contain a small, inconspicuous nucleolus.

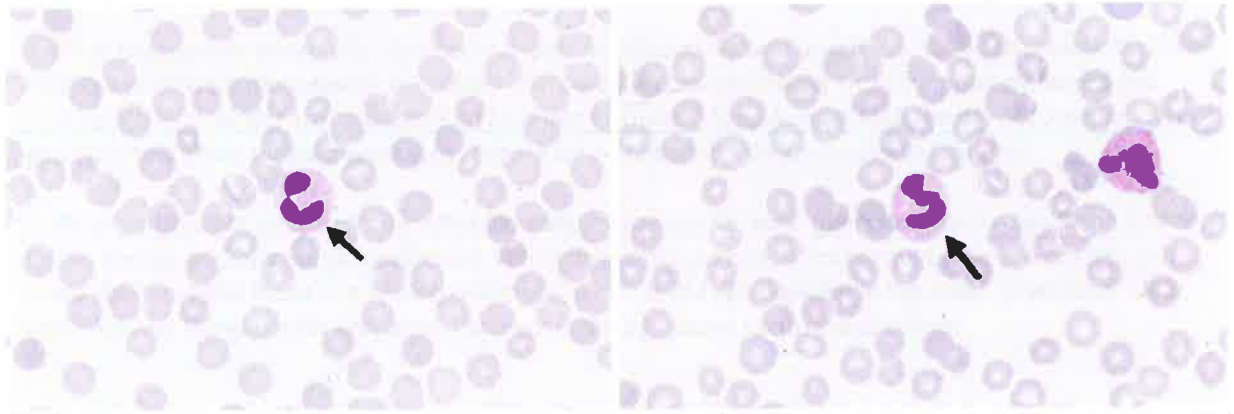
2.3% of participants incorrectly identified the cell as a monocyte, immature (promonocyte/monoblast). Immature monocytes, promonocytes, and monoblasts show progressively higher nuclear:cytoplasmic ratios, less to no nuclear indentation, finely dispersed nuclear chromatin, and occasional distinct nucleoli than mature monocytes show.

VPBS-27, cont'd.

2.5% of participants incorrectly identified the cell as a neutrophil, segmented or band. Neutrophils have relatively thin nuclear segments, whereas monocyte nuclei are mononuclear as their name implies and show squat, kidney-shaped morphology. While monocytes may have occasional fine cytoplasmic granules, especially in activated states, monocyte granules are significantly less abundant than those in the cytoplasm of neutrophils. Monocytes also may show prominent cytoplasmic vacuoles, which are not present in non-toxic neutrophils.

1.1% of participants incorrectly identified the cell as a neutrophil, metamyelocyte. The metamyelocyte is a neutrophil precursor normally found only in the bone marrow but that may circulate in the peripheral blood under stress conditions. While the nuclei of metamyelocytes and monocytes both show indentation and show similar nuclear chromatin features of variable/subtle condensation, the cytoplasm differs considerably: as above, cytoplasmic granules are abundant in metamyelocytes, as in neutrophils, and are fine and few at most in monocytes.

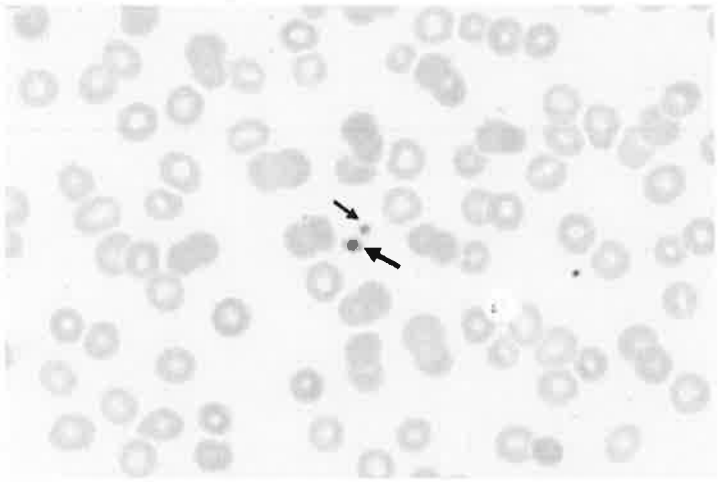
VPBS-28



Identification	Participants N	Participants %	Evaluation
Neutrophil, segmented or band	1260	94.6	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	35	2.6	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	18	1.4	Educational
Neutrophil with Pelger-Huët nucleus (acquired or congenital)	11	0.8	Educational
Neutrophil necrobiosis (degenerated neutrophil)	2	0.1	Educational
Echinocyte (burr cell, crenated cell)	1	0.1	Educational
Metastatic tumor cell or tumor cell clump	1	0.1	Educational
Mitotic figure	1	0.1	Educational
Monocyte	1	0.1	Educational
Neutrophil with hypersegmented nucleus	1	0.1	Educational
Platelet, hypogranular	1	0.1	Educational

The arrowed cells are neutrophils, as correctly identified by 94.6% of the participants. Segmented neutrophils and their immediate precursors, bands, constitute 12% to 25% of the nucleated cells in the bone marrow. Band neutrophils, also known as stabs, constitute 5% to 10% of the nucleated cells in the blood under normal conditions. The band is round-to-oval and 10 to 18 µm in diameter. The N:C ratio is 1:1.5 to 1:2, and the nuclear chromatin is condensed. The nucleus is indented to more than half the distance to the farthest nuclear margin, but the chromatin is not condensed to a single filament (as is the defining feature of the fully mature neutrophil). The nucleus can assume many shapes: it can be band- or sausage-like; S-, C-, or U-shaped; and twisted or folded on itself. The cytoplasm is similar to that of other post-mitotic neutrophils, with specific granules predominating in an otherwise pale cytoplasm. 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 N:C ratio is 1:3 and the nuclear chromatin is variably condensed. The nucleus is segmented or lobated (with a normal range of three to five lobes). The lobes are connected by a thin filament that contains no internal chromatin, giving it the appearance of a solid, dark, thread-like line. The presence of these thread-like filaments is the basis for distinguishing the segmented neutrophil from the band neutrophil. However, in repeated proficiency testing studies, it has not been possible to achieve consistent differentiation between bands and segmented neutrophils. Therefore, for the purposes of proficiency testing, it is not required that they be differentiated.

VPBS-29



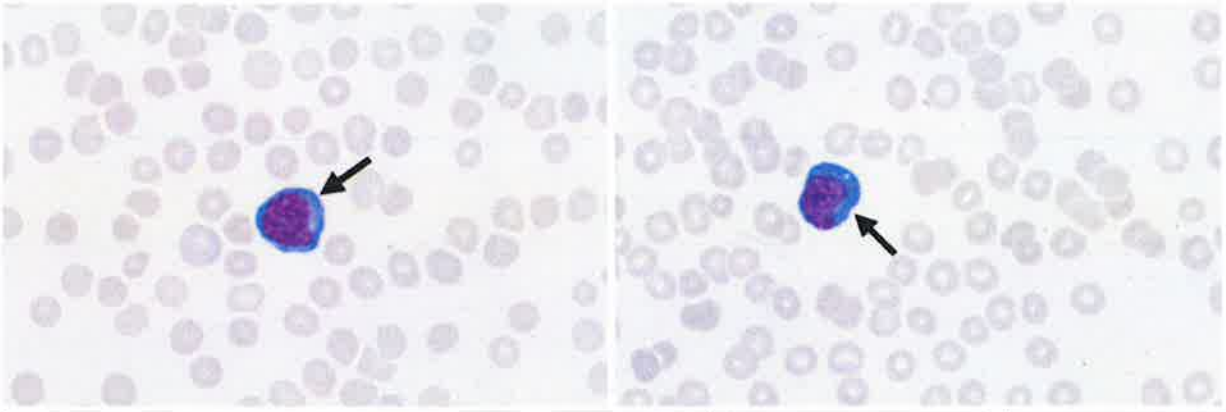
Identification	Participants N	Participants %	Evaluation
Platelet, normal	1258	94.4	Educational
Platelet, hypogranular	35	2.6	Educational
Platelet, giant (macrothrombocyte)	26	2.0	Educational
Platelet satellitism	4	0.3	Educational
Megakaryocyte (normal, abnormal, or nuclear fragment)	3	0.2	Educational
Neutrophil, myelocyte	2	0.1	Educational
Fungi, extracellular	1	0.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.1	Educational
Monocyte	1	0.1	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	1	0.1	Educational

The arrowed objects are normal platelets, as correctly identified by 94.4% of the participants. Platelets, also known as thrombocytes, are small, blue-gray fragments of megakaryocytic cytoplasm. Most measure 1.5 to 3 µm in diameter. A few small platelets, less than 1.5 µm in diameter, and a few large platelets, 4 to 7 µm in diameter, may also be seen in normal blood films. Fine, purple-red granules are dispersed throughout the cytoplasm or are sometimes aggregated at the center. These granules are platelet alpha granules. Platelet delta granules (or dense granules) are not visible on light microscopy. Platelets may be variable in shape, but most normal platelets are round or very slightly elliptical. Some have short cytoplasmic projections or ruffled margins. They are typically single but may form aggregates, particularly in fresh (fingerstick) preparations, in the presence of EDTA anticoagulant (some patients), and in disease states as in this case.

2.6% of participants incorrectly identified the objects as platelets, hypogranular. Hypogranular platelets either lack granules entirely or have a substantially reduced number of the granules found in normal platelets. The cytoplasm stains pale blue or blue gray. The platelets in the furnished images have abundant granules, consistent with normal platelets.

2.0% of participants incorrectly identified the objects as platelets, giant (macrothrombocyte). Giant platelets are larger than 7 µm, usually measuring 10 to 20 µm in diameter. For proficiency testing purposes, the term "giant platelet" is used when the platelet is larger than the size of the average red blood cell in the field, assuming a normal MCV. The depicted platelets are smaller than adjacent red blood cells.

VPBS-30



Identification	Participants N	Participants %	Evaluation
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	996	74.8	Educational
Lymphocyte	115	8.6	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	83	6.2	Educational
Blast cell	58	4.3	Educational
Immature or abnormal cell, would refer for identification	19	1.4	Educational
Malignant lymphoid cell (other than blast)	13	1.0	Educational
Lymphocyte, large granular	8	0.6	Educational
Nucleated red blood cell, normal or abnormal morphology	8	0.6	Educational
Neutrophil, metamyelocyte	5	0.4	Educational
Neutrophil, myelocyte	5	0.4	Educational
Neutrophil, promyelocyte	5	0.4	Educational
Basophil, any stage	4	0.3	Educational
Monocyte	4	0.3	Educational
Monocyte, immature (promonocyte, monoblast)	3	0.2	Educational
Platelet, normal	3	0.2	Educational
Basophilic stippling (coarse)	1	0.1	Educational
Myeloblast with Auer rod	1	0.1	Educational
Protozoa (non-malarial)	1	0.1	Educational

The arrowed cells are reactive lymphocytes, as correctly identified by 74.8% of the participants. Compared to resting lymphocytes, reactive lymphocytes tend to be larger, with increases in volume of both nucleus and cytoplasm and with occasionally prominent cytoplasmic granules. Most reactive lymphocytes in viral illnesses are T lymphocytes. However, plasmacytoid lymphocytes (B lymphocytes) are also frequently observed. Plasmacytoid lymphocytes are medium-sized cells with irregular, densely clumped chromatin, absent to indistinct nucleoli, abundant basophilic cytoplasm, often with a paranuclear clear zone (hof). Immunoblasts are large reactive lymphocytes with round to oval nuclei, fine, delicate chromatin, prominent nucleoli, and moderate amounts of deeply basophilic cytoplasm.

The distinction between normal and reactive lymphocytes is often difficult and subjective; however, it is more important to distinguish reactive lymphocytes from lymphoma cells. The reactive lymphocyte usually has a distinct, smooth nuclear membrane in contrast to the often irregular nuclear membrane of lymphoma cells. Also, in contrast to malignant lymphoproliferative disorders which demonstrate a monotonous population of neoplastic cells, there is usually a spectrum of lymphocyte morphology present in reactive conditions. In some situations, differentiation of reactive from malignant lymphocytes may require the use of ancillary techniques, including flow cytometry and molecular analysis.

VPBS-30, cont'd.

8.6% of participants incorrectly identified the cells as lymphocytes. Resting lymphocytes are smaller than reactive lymphocytes, with nuclei of similar size to normocytic red blood cells and scant cytoplasm.

6.2% of participants incorrectly identified the cells as plasma cells, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body). Morphologically mature plasma cells have round nuclei with coarse (so-called "clock face") nuclear chromatin, eccentrically oriented cytoplasm, and perinuclear clearing (known as a "hof"). While reactive lymphocytes may frequently show plasmacytoid features, true mature plasma cells in peripheral blood are uncommon and abnormal, and may signify progression of plasma cell myeloma, a cancer of plasma cells. Dutcher bodies, intranuclear pseudo-inclusions of immunoglobulin in plasma cells, are abnormal and highly suggest malignancy.

4.3% participants incorrectly identified the cells as blast cells. The nuclear chromatin of blast cells is finely dispersed, more so than that of reactive lymphocytes. The cytoplasm of blasts also tends to be scant, but when it is relatively abundant, the cytoplasm of blasts does not show the amoeba-like molding around adjacent red blood cells that the cytoplasm of reactive lymphocytes does. Features of the overall peripheral blood smear may help to confirm blasts versus reactive lymphocytes as well: blasts have a more uniform, monomorphous appearance with one another, whereas reactive lymphocytes almost always show a spectrum of changes.

1.4% of participants identified the cells as immature/abnormal cells, would refer for identification. This is a reasonable approach in morphologically difficult cases. The nuclear chromatin of reactive lymphocytes can be relatively dispersed, mimicking an immature cell. A spectrum of changes in the population of lymphocytes is often helpful to reassure that they are reactive.

1.0% participants identified the cells as malignant cells. Similarly, this is a reasonable approach in challenging cases, as morphologic distinction between malignant lymphoma cells and reactive lymphocytes is not always possible as above. Malignant lymphoma cells tend to show more monomorphous features as a population than reactive lymphocytes do, but in many cases immunophenotypic (flow cytometry) work-up is necessary to establish a diagnosis of circulating lymphoma.

Case Presentation:

This peripheral blood smear is from a 19-year-old woman presenting with a fever. Laboratory data include: WBC = 3.7 x 10E9/L; RBC = 2.28 x 10E12/L; HGB = 8.9 g/dL; HCT = 25.9 %; MCV% = 112 fL; PLT = clumped; and RDW = 15%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Platelet clumping

Platelet clumping, a potential and common cause of pseudothrombocytopenia, is present in approximately 1% of peripheral blood smears and is important to identify. Recognition of platelet clumping by clinical laboratory technologists and scientists will lead to appropriate collection of a new peripheral blood sample for an accurate platelet count. It can also prevent patients from undergoing unnecessary medical tests to look for cause(s) of truly low platelets (thrombocytopenia) and/or experiencing delays in necessary medical procedures. This author has seen an example of heart surgery being postponed in a patient with chest pain and clumped platelets on peripheral blood smear over concern about bleeding risk from "low platelets"!

The causes of platelet clumping are well described. Peripheral blood specimens for the complete blood count (CBC) are collected in lavender (purple)-top phlebotomy tubes, which are coated with ethylene-diamine-tetra-acetic acid (EDTA) to prevent the blood from clotting before analysis. The glycoprotein IIb/IIIa protein complex (GPIIb/IIIa) on platelets becomes exposed on their surface *ex vivo* (outside the body) and in the presence of EDTA *in vitro* (in a test tube). Some people have naturally occurring antibodies against GPIIb/IIIa that then clump the platelets together. As an aside, a number of drugs designed to block GPIIb/IIIa activation are used to help prevent heart attack and stroke, eg, clopidogrel.

Question 1. Which pre-analytical laboratory factor is most associated with platelet clumping?

E. Anticoagulant in phlebotomy tube
F. Hemolyzed specimen
G. Volume of blood draw
H. Specimen transport time

Some people make transient anti-GPIIb/IIIa or similar antibodies, so their platelets only clump under certain conditions. Acute illnesses can cause an inflammatory state with increased autoantibody production. Infections with a wide variety of viruses have been implicated in pseudothrombocytopenia in an anti-GPIIb/IIIa-dependent or other manner. Examples include acute hepatitis A, Epstein Barr (EBV), and human immunodeficiency virus (HIV) infections. The patient in this case study had undergone a bone marrow transplant for aplastic anemia, had many peripheral blood draws in the past, and had never had platelet clumping before. Her fever raises consideration for a transient infection. Other conditions in which pseudothrombocytopenia can occur include antiphospholipid antibody syndrome and other autoimmune and rheumatologic conditions.

Question 2. Which disease state is most associated with platelet clumping?

A. Disseminated intravascular coagulation
B. Iron deficiency anemia
C. Leukemia
D. Viral illness

To help prevent platelet clumping, and correct the automated platelet count, peripheral blood may be redrawn into a phlebotomy tube containing a different anticoagulant. Sodium citrate-containing tubes (light blue) are the most commonly used in this situation. The patient's medical record should also be flagged so that future platelet counts may be performed on samples collected without EDTA and that all caregivers interpret future thrombocytopenia with caution.

Alexandra E. Kovach, MD
Hematology and Clinical Microscopy Committee

REFERENCES:

1. Manthorpe R, Kofod B, Wiik A, Saxtrup O, Svehag SE. Pseudothrombocytopenia. In vitro studies on the underlying mechanism. *Scand J Haematol.* 1981;26:385-392.
2. Berkman N, Michaeli Y, Or R, Eldor A. EDTA-dependent pseudothrombocytopenia: a clinical study of 18 patients and a review of the literature. *Am J Hematol.* 1991;36:195-201.
3. Casonato A, Bertomoro A, Pontara E, Dannhauser D, Lazzaro AR, Girolami A. EDTA-dependent pseudothrombocytopenia caused by antibodies against the cytoadhesive receptor of platelet gpIIB-IIIA. *J Clin Pathol.* 1994;47:625-630.
4. Bizzaro N, Brandalise M. EDTA-dependent pseudothrombocytopenia: association with antiplatelet and antiphospholipid antibodies. *Am J Clin Pathol.* 1995;103:103-107.
5. Bizzaro N. EDTA-dependent pseudothrombocytopenia: a clinical and epidemiological study of 112 cases, with 10-year follow-up. *Am J Hematol.* 1995;50:103-109.
6. Fiorin F, Steffan A, Pradella P, Bizzaro N, Potenza R, De Angelis V. IgG platelet antibodies in EDTA-dependent pseudothrombocytopenia bind to platelet membrane glycoprotein IIb. *Am J Clin Pathol.* 1998;110:178-183.
7. Chia J, Hsia CC. Pseudothrombocytopenia. *Blood.* 2011;117:4168.
8. Froom P, Barak M. Prevalence and course of pseudothrombocytopenia in outpatients. *Clin Chem Lab Med.* 2011;49:111-114.
9. Choe W-H, Cho Y-U, Chae J-D, Kim S-H. Pseudothrombocytopenia or platelet clumping as a possible cause of low platelet count in patients with viral infection: a case series from single institution focusing on hepatitis A infection. *Int J Lab Hematol.* 2013;35(1):70-76.
10. Schuff-Werner P, Steiner M, Fenger S, et al. Effective estimation of correct platelet counts in pseudothrombocytopenia using an alternative anticoagulant based on magnesium salt. *Br J Haematol.* 2013;162:684-692.

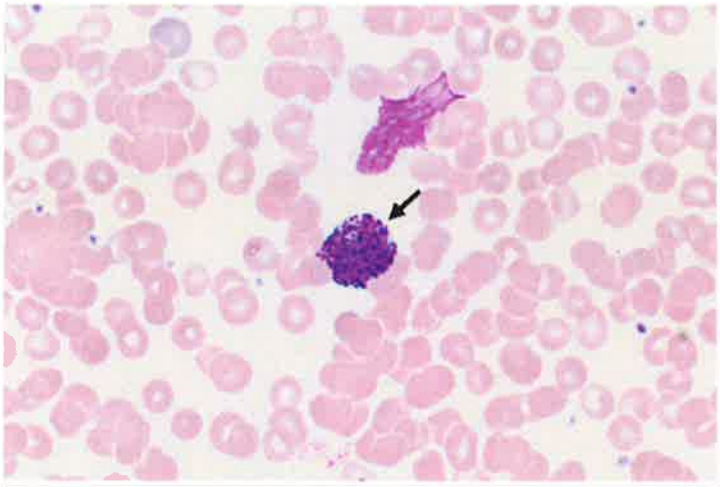
ANSWERS TO QUESTIONS:

Question 1. Answer: A. Anticoagulant in phlebotomy tube.
The ethylene-diamine-tetra-acetic acid (EDTA) anticoagulant in lavender (purple)-top phlebotomy tubes for the complete blood count (CBC) exposes the glycoprotein IIb/IIIa protein complex (GPIIb/IIIa) on the surface of platelets. In people whose blood contains antibodies to GPIIb/IIIa, platelets will aggregate (clump), leading to pseudothrombocytopenia on the automated CBC.

Question 2. Answer: D. Viral illness.
Many viruses including hepatitis A, Epstein Barr (EBV), and HIV have been implicated in transient pseudothrombocytopenia.

Cell Identification

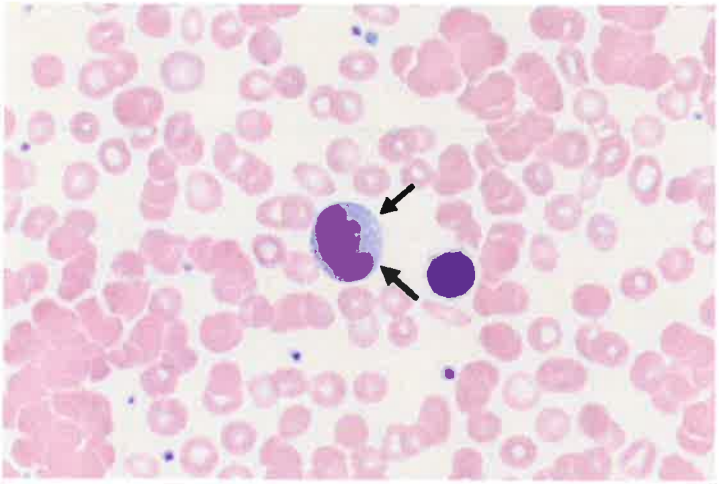
VPBS-32



Identification	Participants N	Participants %	Evaluation
Basophil, any stage	1316	98.9	Educational
Basophilic stippling (coarse)	5	0.4	Educational
Mast cell	3	0.2	Educational
Eosinophil, any stage	2	0.1	Educational
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	1	0.1	Educational
Leukocyte containing Alder (Alder-Reilly) anomaly inclusion(s)	1	0.1	Educational
Mitotic figure	1	0.1	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	1	0.1	Educational
Platelet, giant (macrothrombocyte)	1	0.1	Educational

The arrowed cell is a basophil, as correctly identified by 98.9% of participants. Basophils are similar in size to neutrophils (typically 10 - 15 µm in diameter) but contain characteristically dark blue-black coarse granules. These granules typically obscure the basophil nucleus. The basophil nucleus demonstrates segmentation similar to other granulocytes.

VPBS-33

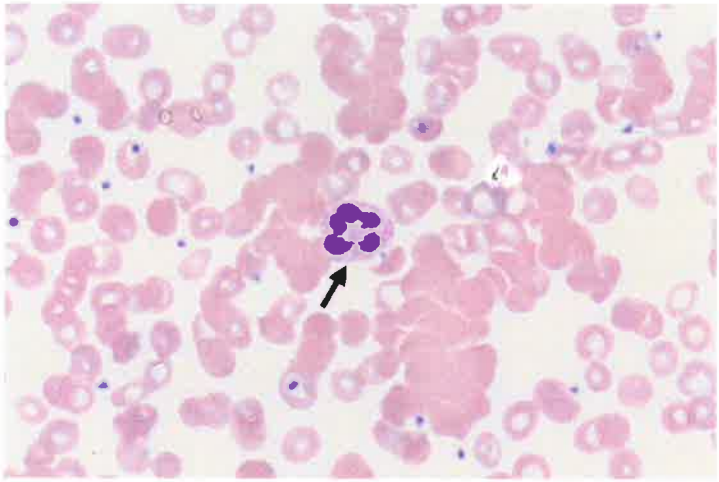


Identification	Participants N	Participants %	Evaluation
Monocyte	1303	97.9	Educational
Monocyte, immature (promonocyte, monoblast)	17	1.3	Educational
Neutrophil, metamyelocyte	4	0.3	Educational
Immature or abnormal cell, would refer for identification	1	0.1	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.1	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	1	0.1	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	1	0.1	Educational
Neutrophil, segmented or band	1	0.1	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	1	0.1	Educational
Ovalocyte (elliptocyte)	1	0.1	Educational

The arrowed cell is a monocyte, as correctly identified by 97.9% of participants. Monocytes range in size from 12 to 20 µm in diameter and the N:C ratio ranges from 4:1 to 2:1. The majority of monocytes are round with smooth edges, but some may demonstrate pseudopod-like cytoplasmic extensions. The cytoplasm is typically abundant, with a gray or gray-blue ground-glass appearance, and a variable number of vacuoles or fine, evenly distributed azurophilic granules may be present. 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.

This cell was incorrectly identified as an immature monocyte by 1.3% of participants. While immature monocytes can share many characteristics with mature forms, they demonstrate features of immaturity including finely dispersed chromatin and increased N:C ratio. Immature monocytes include monoblasts and promonocytes. The former characteristically demonstrates blast-like nuclear features in combination with abundant monocytic cytoplasm. Promonocytes are characterized by nuclei with much more open lacey chromatin, often with variable delicate nuclear folds.

VPBS-34



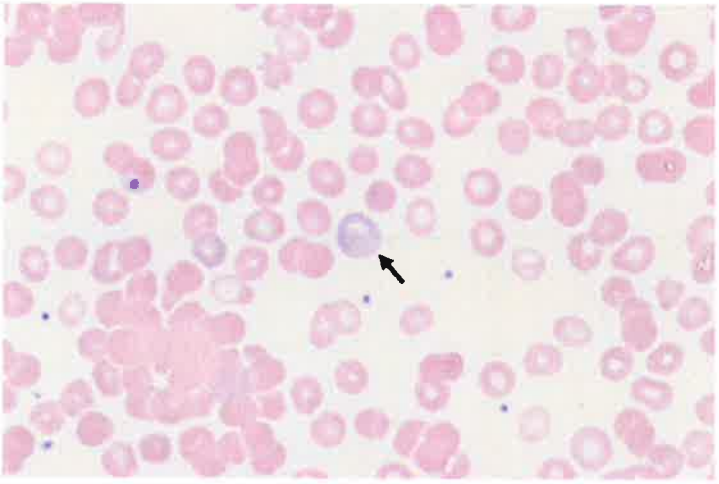
Identification	Participants N	Participants %	Evaluation
Neutrophil, segmented or band	1211	91.0	Educational
Neutrophil with hypersegmented nucleus	86	6.5	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	25	1.9	Educational
Eosinophil, any stage	2	0.1	Educational
Neutrophil, polyploid	2	0.1	Educational
Echinocyte (burr cell, crenated cell)	1	0.1	Educational
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	1	0.1	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	1	0.1	Educational
Neutrophil, myelocyte	1	0.1	Educational
Neutrophil necrobiosis (degenerated neutrophil)	1	0.1	Educational

The arrowed cell is a segmented neutrophil, as correctly identified by 91.0% of participants. Neutrophils are usually 10 to 15 µm in diameter, round to oval, with segmented to lobated nuclei (typically, three to five lobes), with pale pink granular cytoplasm. The chromatin is highly condensed, and the lobes are connected by dark, thread-like filaments with no internal chromatin. For the purposes of proficiency testing, band and segmented neutrophils are identified together.

This cell was incorrectly identified as a neutrophil with a hypersegmented nucleus by 6.5% of participants. For the purposes of proficiency testing, hypersegmented neutrophils should demonstrate six or more lobes, each separated from others by a thin filament of chromatin. The depicted cell contains five nuclear lobes.

This cell was incorrectly identified as a toxic neutrophil by 1.9% of participants. For the purposes of proficiency testing, toxic neutrophils should contain all the cardinal features of toxicity, that is toxic granulation, toxic vacuolation and Döhle bodies.

VPBS-35

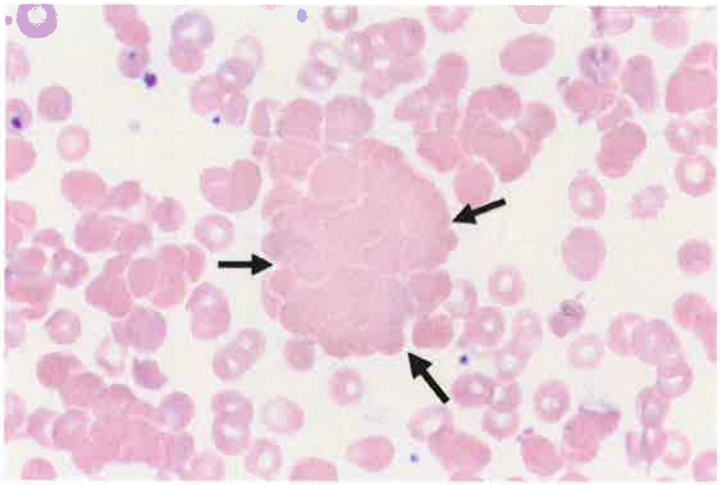


Identification	Participants N	Participants %	Evaluation
Polychromatophilic (non-nucleated) red blood cell	1301	97.8	Educational
Macrocyte, oval or round (excluding polychromatophilic red blood cell)	14	1.1	Educational
Basophilic stippling (coarse)	11	0.8	Educational
Erythrocyte, normal	2	0.1	Educational
Immature or abnormal cell, would refer for identification	1	0.1	Educational
Neutrophil, segmented or band	1	0.1	Educational
Platelet, normal	1	0.1	Educational

The arrow points to a polychromatophilic (non-nucleated) red blood cell, as correctly identified by 97.8% of participants. The polychromatophilic red cell is larger than typical red blood cells and represents a less mature form. The polychromatophilic nature of these cells is evident in the gray-pink or purple hue of the cytoplasm. This coloration is attributable to the relatively higher cytoplasmic content of RNA in the less mature red cell, which can also be highlighted using supravital stains (used to enumerate reticulocytes). Polychromatophilic red blood cells are normally relatively few, but will increase with increased marrow output of red cells (eg, hemolysis or in response to bleeding).

This cell was incorrectly identified as an oval or round macrocyte by 1.1% of participants. While polychromatophilic red blood cells are often large and macrocytic, they are distinguished from oval/round macrocytes by their much grayer hue, as is evident in the case when the cytoplasm is compared to that of surrounding red blood cells.

VPBS-36



Identification	Participants N	Participants %	Evaluation
Red blood cell agglutinates	1290	96.9	Educational
Rouleaux	35	2.6	Educational
Cryoglobulin	3	0.2	Educational
Basket cell/smudge cell	1	0.1	Educational
Lymphocyte, large granular	1	0.1	Educational
Neutrophil, segmented or band	1	0.1	Educational

The arrows point to red blood cell agglutinates, as correctly identified by 96.9% of participants. Red blood cell agglutinates refer to clumped red blood cells, usually under the influence of an antibody. Red blood cell agglutinates can vary in size and may involve clumps of numerous red blood cells.

2.6% of participants incorrectly identified the arrowed objects as rouleaux. Agglutination should be distinguished from rouleaux, the latter is characterized by a much more orderly linear stack of red blood cells.

Case Presentation:

This peripheral blood smear is from an 84-year-old woman presenting with unexplained weight loss and lymphadenopathy. Laboratory data include: WBC = 7.6 x 10E9/L; RBC = 3.37 x 10E12/L; HGB = 11.3 g/dL; HCT = 33.4 %; MCV% = 93 fL; PLT = 160 x 10E9/L; and RDW = 26%.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

CASE DISCUSSION: Agglutination without correction on warming

Agglutination refers to the process of clumping of red blood cells under the influence of one of more antibodies. Agglutinins effect red cell clumping by binding to red cell antigens shared by groups of red cells. Red cell agglutination is exploited in the lab for the purposes of antigen-based red cell typing. Agglutination also occurs "in vivo" in autoimmune hemolytic anemia (AIHA), in which the two main classes of disease are warm antibody mediated (autoimmune) hemolytic anemia (wAIHA) and cold agglutinin disease (CAD).

wAIHA is the most common form of autoimmune hemolysis and is characterized by agglutination at temperatures of at least 37°C. wAIHA can in turn be subclassified into primary/idiopathic subtypes or secondary subtypes. In the latter, wAIHA occurs owing to antibodies arising secondarily to other conditions such as lymphoma and autoimmune diseases (eg, systemic lupus erythematosus). wAIHA may also be seen in the context of drug exposures (eg, antibiotics such as penicillins). wAIHA is usually mediated by IgG autoantibodies, and hemolysis is typically mediated by splenic degradation rather than intravascular lysis.

CAD, in contrast, is characterized by antibody activity limited to cooler temperatures (less than 37°C). Like wAIHA, CAD may be idiopathic or secondary to lymphoproliferative diseases; CAD may also be seen in association with certain infections (eg, mycoplasma pneumonia and infectious mononucleosis). CAD typically results from IgM antiantibodies, which are red cell bound at the relatively low temperatures of the peripheral circulation and fix complement. The latter then results in splenic degradation.

Question 1. Which of the following statements relating to autoimmune hemolysis is CORRECT?

A. Cold autoimmune hemolysis typically involves IgM antibodies, with complement fixation, resulting in extravascular hemolysis
B. Cold autoimmune hemolysis typically involves IgG antibodies, with complement fixation, resulting in intravascular hemolysis
C. Warm autoimmune hemolysis typically involves IgM antibody binding to red cells, resulting in agglutination
D. Warm autoimmune hemolysis typically occurs due to intravascular lysis resulting from complement pathway activation

---

AIHA is typified by anemia with reticulocytosis, often with additional biochemical features suggestive of hemolysis (eg, elevated LDH or bilirubin and low haptoglobin). CAD also demonstrates peripheral blood smear agglutination at ambient temperatures, which may resolve with specimen warming. wAIHA, by contrast, is characterized by agglutination at ambient temperatures that persists with warming. Peripheral blood polychromatophilic red cells are typical as well, often with evident spherocytosis.

Additional laboratory testing for AIHA should include direct antiglobulin (DAT or Coombs) testing. By this test, red cell coating antibodies are detected using anti-IgG and anti-C3 sera. DAT test positivity for IgG is typical of wAIHA (with or without accompanying C3 positivity). By contrast, CAD is typically C3 positive, but lacks IgG. Antibody-antigen specificity can occasionally be delineated using an indirect antiglobulin test; in this test, patient serum is

mixed with antigen-specific red cells. Cold agglutinin titers are generally recommended, to establish a sense of the severity of agglutination. Clinically relevant agglutination typically demonstrates high titers (eg, in excess of 1:64 dilutions).

Question 2. Which statement relating to laboratory testing in the context of autoimmune hemolysis is CORRECT?

A. Cold agglutinin titers are not associated with severe agglutination
B. Direct antiglobulin testing will demonstrate C3 positivity in warm autoimmune hemolytic anemia
C. Peripheral smears are generally not required for the work-up of autoimmune hemolytic anemia
D. Peripheral smears are invaluable in the work-up of autoimmune hemolytic anemia

---

Therapeutic strategies for AIHA patients vary greatly and are targeted to the underlying etiology and severity of hemolysis. Urgent intervention with blood transfusion may be required in patients with profound anemia. In contrast, patients with well-compensated hemolysis, without cardiovascular or thrombotic concerns may not require intervention. Patients are typically counseled to avoid cold exposure in CAD and avoid the use of or exposure to other identified offending agents. Unlike many other autoimmune conditions, steroids may not be effective, especially in CAD. However, intravenous immunoglobulin or immunomodulatory interventions, such as rituximab, may be effective. In rare instances of protracted hemolysis, splenectomy may be required.

Question 3. Which statement relating to the treatment of autoimmune hemolysis is CORRECT?

A. Cold exposure/avoidance is required for patients with warm autoimmune hemolysis
B. Corticosteroids are typically effective, and generally recommended in all cases of hemolysis
C. Some patients may not require clinical intervention, provided they are clinically stable and that their anemia is well compensated
D. Upfront splenectomy is often required in patients with both warm and cold autoimmune hemolysis

---

Etienne Mahé, MD, MSc, FRCPC, FCAP
Hematology and Clinical Microscopy Committee

REFERENCES:

1. Berentsen, Sigbjørn. 2021. "How I Treat Cold Agglutinin Disease." *Blood.* 137(10):1295–1303.
2. Berentsen, Sigbjørn, and Tatjana Sundic. 2015. "Red Blood Cell Destruction in Autoimmune Hemolytic Anemia: Role of Complement and Potential New Targets for Therapy." *BioMed Research International.* 2015:1–11.

ANSWERS TO QUESTIONS:

Question 1: A. Cold autoimmune hemolysis typically involves IgM antibodies, with complement fixation, resulting in extravascular hemolysis. Both warm and cold autoimmune hemolysis are characterized by extravascular hemolysis, mainly in the spleen. Cold autoimmune hemolysis typically involves IgM autoantibodies, whereas IgG autoantibodies are implicated in warm autoimmune hemolysis.

Question 2: D. Peripheral smears are invaluable in the work-up of autoimmune hemolytic anemia. The work-up of autoimmune hemolytic anemia requires peripheral blood smear evaluation. This work-up is supplemented by evaluation of both ambient temperature and warmed blood specimens. The direct antiglobulin (Coombs) test demonstrates C3 positivity typically in cold autoimmune hemolysis (less so in warm cases). The indirect antiglobulin test can occasionally be used to identify specific red cell antigens.

Question 3: C. Some patients may not require clinical intervention, provided they are clinically stable and that their anemia is well compensated. Corticosteroid use in autoimmune hemolysis is often ineffective, and splenectomy is typically reserved for recalcitrant cases or prolonged disease. Cold exposure avoidance is recommended for patients with cold autoimmune hemolysis.