Blood Cell Identification – Graded

Case History

This peripheral blood smear is from a 16-year-old girl with abdominal discomfort and jaundice. Laboratory data include: WBC = 2.9 x 10E9/L; RBC = 3.19 x 10E12/L; HGB = 6.5 g/dL; HCT = 22.5%; MCV = 71 fL; RDW = 22%; and PLT = 69 x 10E9/L. Identify the arrowed object(s) on each page.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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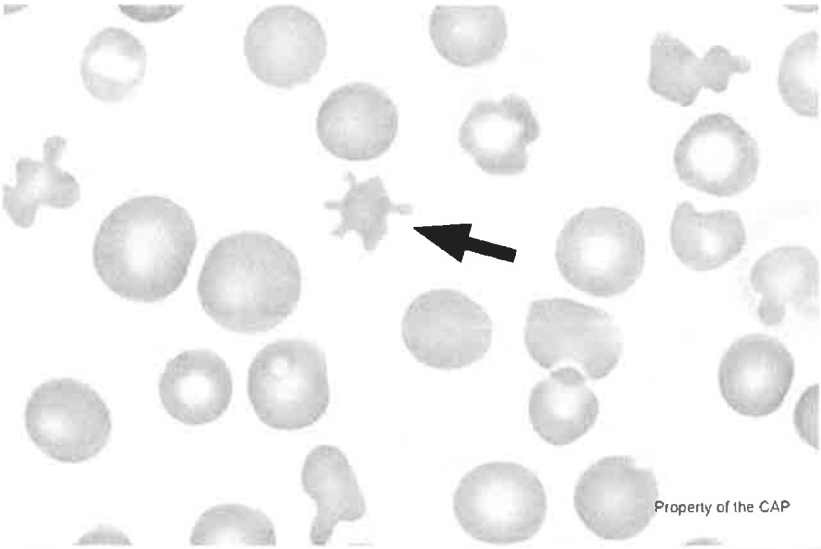
BCP-11

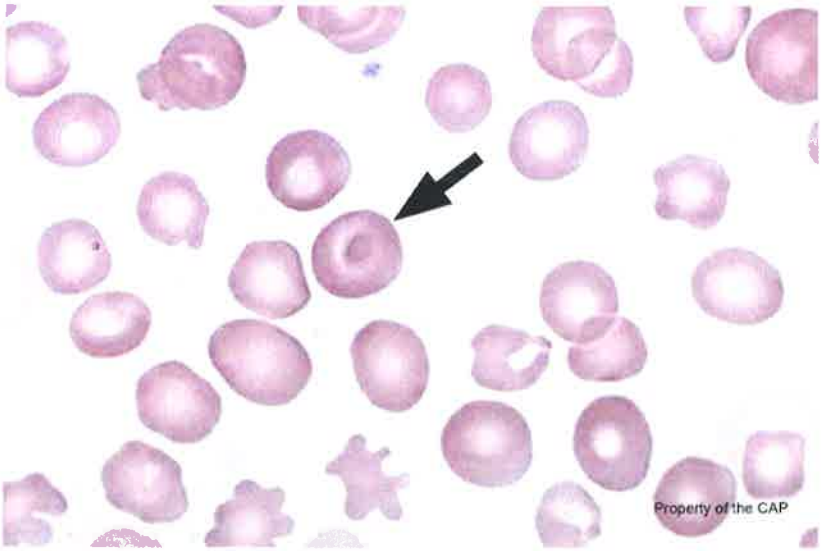


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Acanthocyte (spur cell)	111	96.5	5304	92.5	Good
Echinocyte (burr cell, crenated cell)	2	1.7	313	5.5	Unacceptable
Fragmented red blood cell (schistocyte, helmet cell, keratocyte, triangular cell)	2	1.7	111	1.9	Unacceptable

The arrowed cell is an acanthocyte (spur cell), as correctly identified by 96.5% of the referees and 92.5% of the participants. Acanthocytes are densely stained red blood cells that lack central pallor and characteristically demonstrate multiple, irregularly distributed, thorn-like spicules of variable size, often with blunted or 'drumstick-like' ends. Acanthocytes are classically seen in association with hereditary abetalipoproteinemia but are much more commonly encountered in significant numbers in other settings such as severe liver disease, post splenectomy, and chronic starvation.

Blood Cell Identification – Graded

BCP-12

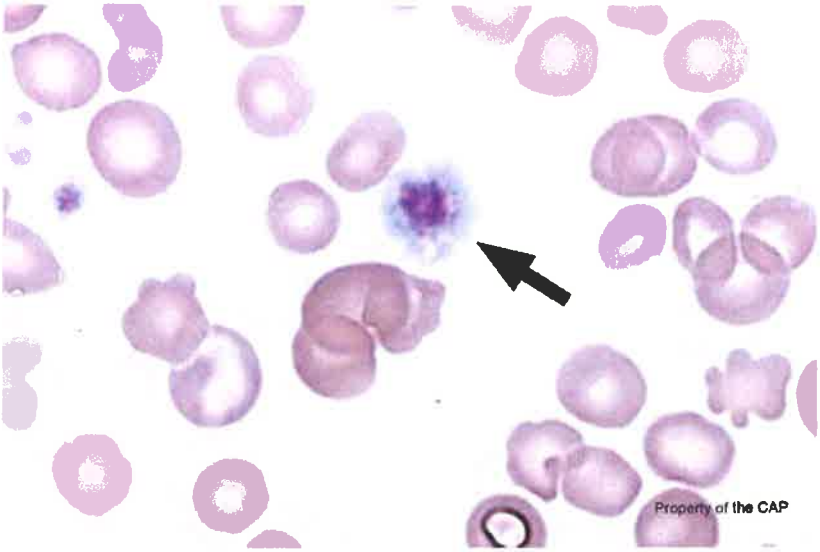


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Target cell (codocyte)	114	99.1	5685	99.1	Good
Acanthocyte (spur cell)	1	0.9	4	0.1	Unacceptable

The arrowed cell is a target cell (codocyte), as correctly identified by 99.1% of the referees and 99.1% of the participants. Target cells are red blood cells with a 'targetoid' appearance due to a ring of pallor caused by redundant cytoplasm with central hemoglobin accounting for the bull's-eye in the center. Target cells are commonly observed in patients with hemoglobinopathy, liver disease, iron deficiency, and hyposplenism. Rare target cells (< 5%) may be seen in normal individuals.

Blood Cell Identification – Graded

BCP-13

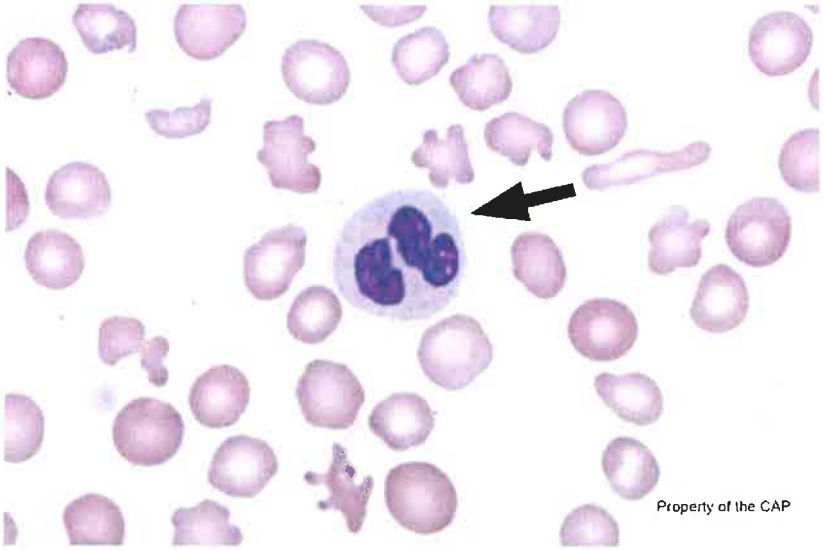


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Giant platelet (macrothrombocyte)	114	99.1	5626	98.1	Good
Monocyte	1	0.9	2	0.0	Unacceptable

The arrowed cell is a giant platelet (macrothrombocyte), as correctly identified by 99.1% of the referees and 98.1% of the participants. Platelets (also known as a 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. Giant platelets are usually 10 - 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 arrowed thrombocyte is much larger than the red cells in the background and is therefore best classified as a giant platelet.

Blood Cell Identification – Graded

BCP-14



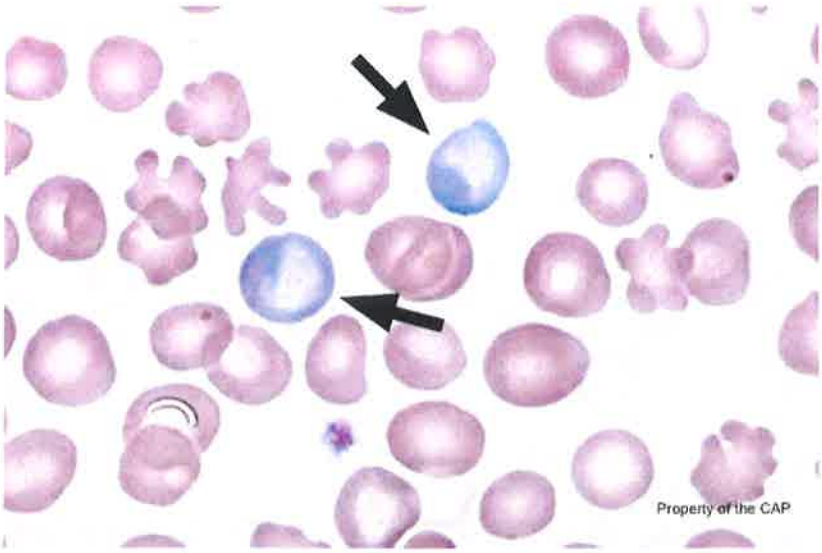
Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Neutrophil (segmented or band)	106	92.2	5153	89.8	Good
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	5	4.3	492	8.6	Unacceptable
Neutrophil containing Pelger-Huët nucleus (acquired or congenital)	4	3.5	61	1.1	Unacceptable

The arrowed cell is a neutrophil (segmented or band), as correctly identified by 92.2% of the referees and 89.8% of the participants. Neutrophils are 10 - 15 µm in size and contain moderate pale pink cytoplasm containing fine, eosinophilic granules. The mature neutrophil has a segmented nucleus usually comprised of two to five lobes with condensed nuclear chromatin. Segmented neutrophils are mature granulocytes and usually represent the most predominant while cells in adult blood. A band neutrophil is nearly mature, with a curved, band-like nucleus that has not yet fully segmented.

4.3 % of referees and 8.6% of the participants incorrectly identified the cell as a neutrophil with dysplastic nucleus and/or hypogranular cytoplasm. The indicated neutrophil has normal segmentation (three irregularly sized lobes) and lacks the characteristic bilobed or "pince-nez" appearance due to hyposegmentation seen in dysplastic neutrophils. The cytoplasm also has a somewhat "dirty" blue appearance indicative of normal granulation rather than the washed out, pale blue appearance that is characteristic of thehypogranular cytoplasm of dysplastic neutrophils. Often dysplastic neutrophils the cytoplasm is so pale that cytoplasmic borders cannot be easily distinguished from the slide background.

Blood Cell Identification – Graded

BCP-15



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Polychromatophilic (non-nucleated) red blood cell	110	95.7	5333	93.0	Good
Hypochromasia	2	1.7	83	1.4	Unacceptable
Blister cell/Prekeratocyte	1	0.9	142	2.5	Unacceptable
Hemoglobin C crystal	1	0.9	75	1.3	Unacceptable

The arrowed cell is a polychromatophilic (non-nucleated) red blood cell, as correctly identified by 95.7% of the referees and 93.0% of the participants. These are immature erythrocytes and constitute a minor subset of reticulocytes which are identifiable on routine smears. Polychromatophilic red blood cells are typically larger than mature erythrocytes and their cytoplasm appears pinkish-gray or pale purple in Wright-Giemsa stained smears due to relatively increased ribosomal content. Reticulocytosis with polychromasia is typically observed in the setting of hemolytic anemia but can also be seen in a variety of other conditions associated with a hematologic stress response. Decreased reticulocytes, particularly in an anemic patient, suggest a hypoplastic or hypoproductive bone marrow state.

Case Presentation:

This peripheral blood smear is from a 16-year-old girl with abdominal discomfort and jaundice. Laboratory data include: WBC = 2.9 x 10E9/L; RBC = 3.19 x 10E12/L; HGB = 6.5 g/dL; HCT = 22.5%; MCV = 71 fL; RDW = 22%; and PLT = 69 x 10E9/L. Identify the arrowed object(s) on each page.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case Discussion: Acanthocytosis

Acanthocytes are densely stained, spheroidal red blood cells that lack central pallor and have multiple (a few up to 20), irregularly distributed, thorn-like spicules of variable length, often with a blunted or knob-like tip. Spicules may occasionally have branches. Acanthocytes should be differentiated from other spiculated red blood cells. Echinocytes (burr cells), for example, have numerous (10 - 30) short evenly spaced spicules and central pallor is preserved. Teardrop cells are characterized by a single spicule forming a tear-drop shape. Morphologic variants of schistocytes such as helmet cells, triangulocytes or horn cells may sometimes mimic acanthocytes. In this context, visualization of two or more pointed ends may be helpful in identification of fragmented cells.

Rare acanthocytes may be encountered in peripheral blood smears from normal individuals. In such cases they represent older, senescent red blood cells approaching their end of life in circulation (120 days). However, when acanthocytes are moderately increased or many in number (ie, 5 - 20% or greater than 20% of red cells, respectively), the finding should prompt consideration of possible causes. Although the finding of acanthocytes is a nonspecific morphologic finding, a relatively narrow differential diagnosis can be generated upon their identification, particularly when adequate clinical information is available.

The classic association of acanthocytes is with hereditary abetalipoproteinemia (hereditary acanthocytosis). In addition, acanthocytes are often seen in significant numbers in severe liver disease or hepatorenal failure of various etiologies. The underlying cause in these cases is most likely related to altered plasma lipid composition due to accumulation of abnormal lipoprotein(s). Acanthocytes form because of the resulting abnormal red cell membrane lipid composition. Acanthocytes seen in combination with spherocytes, target cells, and Howell-Jolly bodies should raise suspicion of a post-splenectomy state or possibly functional hypoplenism, as may occur in patients with sickle cell disease. Acanthocytes are readily found in blood smears in the post-splenectomy state because of diminished splenic activity in removal of these poikilocytes.

Other disorders in which acanthocytes may be prominent include myeloproliferative neoplasms, fat malabsorption, vitamin E deficiency, McLeod red blood cell phenotype, anorexia nervosa, and chronic starvation. Notably, in the latter two disorders, the acanthocytes may appear as irregularly shaped erythrocytes with multiple blunt projections imparting an 'animal cracker-like' appearance. Rare neuromuscular disorders may demonstrate acanthocytosis and this may be an indication for peripheral smear examination in some settings. Ultimately, close correlation of patient clinical and family history with physical exam, CBC findings and other laboratory studies is required in order to assess exact significance of acanthocytes when they are encountered in significant numbers on peripheral blood smears.

Jay Patel, 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*. Northfield, IL: College of American Pathologists; 2018.
2. Kjeldsberg CR, Perkins SL, eds. *Practical Diagnosis of Hematologic Disorders*. 5th ed. Singapore:American Society for Clinical Pathology; 2010.
3. Palmer L, et al. ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. *Int J Lab Hematol.* 2015 Jun;37(3):287-303.

Blood Cell Identification – Ungraded

Case History

This peripheral blood smear is from an 18-year-old woman presenting with diarrhea and renal failure after eating at a fast food restaurant where several other people became ill. Laboratory data include: WBC = 36.9 x 10E9/L; RBC = 2.20 x 10E12/L; HGB = 6.8 g/dL; HCT = 18.9%; MCV = 86 fL; MCH = 30.7 pg; MCHC = 35.9 g/dL; and PLT = 10 x 10E9/L. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

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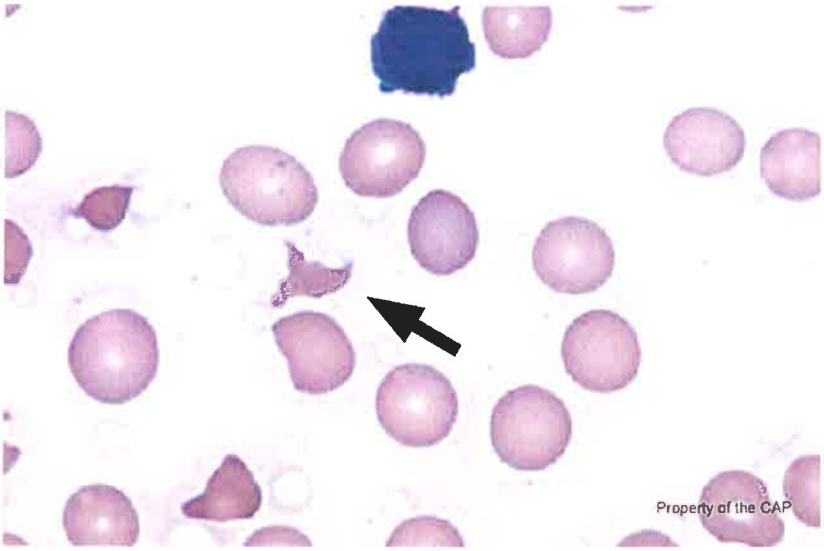
BCP-16



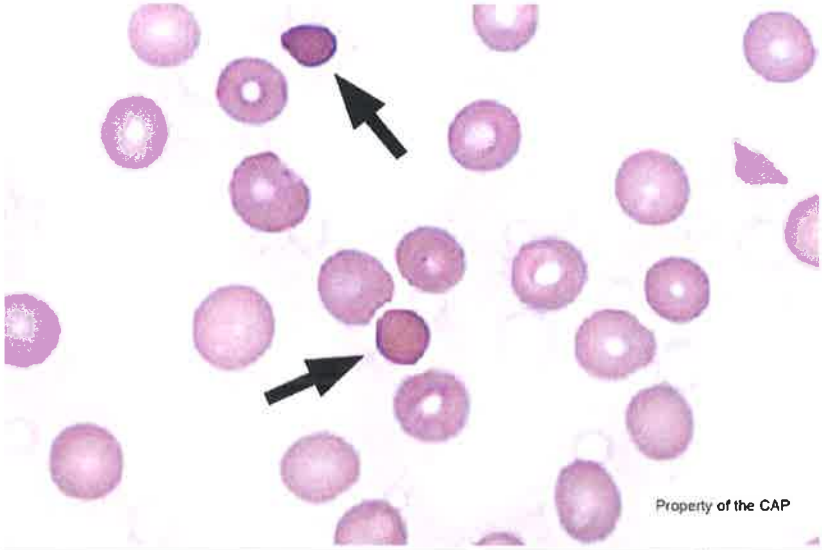
Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Fragmented red blood cell (schistocyte)	108	97.3	5477	96.6	Educational
Acanthocyte (spur cell)	3	2.7	154	2.7	Educational

The arrowed cell is a fragmented red blood cell (schistocyte), as correctly identified by 97.3% of referees and 96.6% of participants. Fragmented red blood cells are red blood cells that have undergone rips and tears, and have two or more points resulting in helmet cells, keratocytes (horn cells), triangulocytes and a more indecisive term, schistocyte. A zone of central pallor is rarely present in fragmented cells.

Fragmented cells are seen in severe burns, disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), and other microangiopathic hemolytic anemias, in patients with prosthetic cardiac valves or severe valvular stenosis, malignant hypertension, or other mechanical trauma to the cell (eg, march hemoglobinuria, marathon running). When present in large numbers, they may cause the MCV to fall into the microcytic range or interfere with platelet enumeration.

Blood Cell Identification – Ungraded

BCP-17

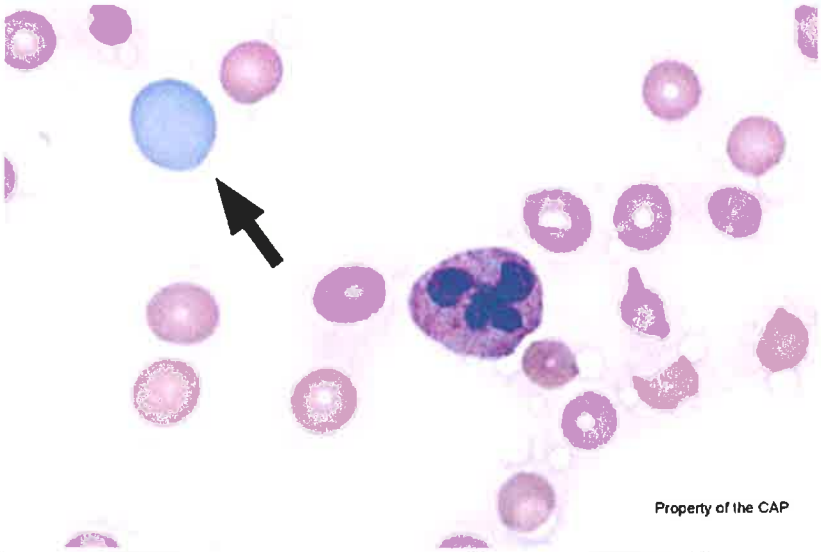


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Spherocyte	105	94.6	5335	95.6	Educational
Microcyte (with increased central pallor)	6	5.4	214	3.8	Educational

The arrowed cells are spherocytes, as correctly identified by 94.6% of referees and 95.6% of participants. Spherocytes are densely staining, spherically shaped red blood cells that lack central pallor and have a decreased diameter compared to normal red blood cells. Spherocytes may be found in patients with the red blood cell membrane disorder, hereditary spherocytosis, or in patients with immune hemolytic anemia. Occasional spherocytes are almost invariably present in association with fragmented cells, as a result of re-sealed rounded-up red blood cell fragments. Increased spherocytes may also be seen as an artifact in very thin areas of a blood film.

Blood Cell Identification – Ungraded

BCP-18

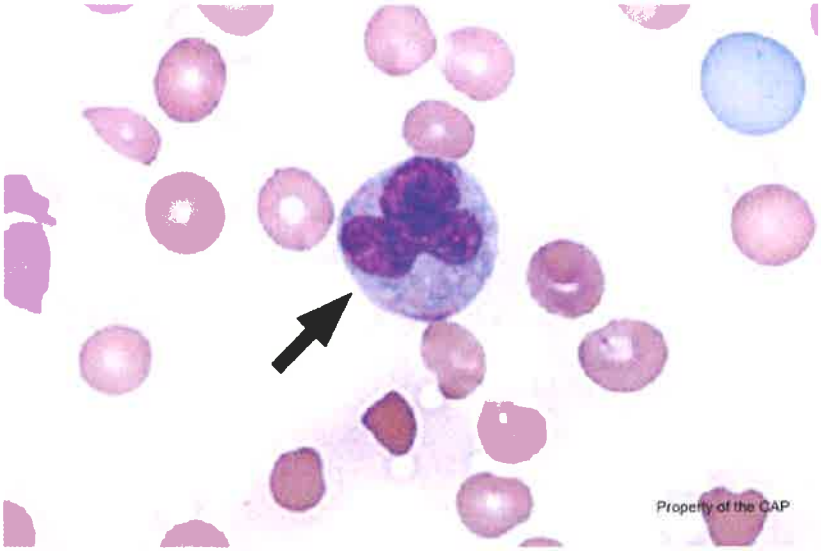


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Polychromatophilic (non-nucleated) red blood cell	111	100.0	5535	99.2	Educational

The arrowed cell is a polychromatophilic (non-nucleated) red blood cell (RBC), as correctly identified by 100.0% of referees and 99.2% of participants. Mature red blood cells (erythrocytes) are round to oval disc shaped cells measuring ~7 µm in diameter, and contain central pallor occupying less than one third (2 - 3 µm) of the cell diameter. In contrast, reticulocytes appear polychromatophilic on Wright stains and are slightly larger in size compared to mature RBCs, have a blue-grey cytoplasm and occasionally contain fine basophilic granules.

Blood Cell Identification – Ungraded

BCP-19



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Monocyte	92	82.9	4321	77.4	Educational
Neutrophil, segmented or band	8	7.2	534	9.6	Educational
Neutrophil, metamyelocyte	4	3.6	95	1.7	Educational
Monocyte, immature (promonocyte, monoblast)	2	1.8	207	3.7	Educational
Neutrophil, giant band	2	1.8	79	1.4	Educational
Neutrophil, toxic	2	1.8	102	1.8	Educational

The arrowed cell is a monocyte, as correctly identified by 82.9% of referees and 77.4% of participants. In a normal peripheral blood smear, the monocyte is relatively infrequent, representing up to 10% of peripheral white blood cells. Monocytes are approximately 12 - 20 µm in size with convoluted or folded nucleus and abundant blue-grey cytoplasm. Cytoplasmic vacuolization is common, and eosinophilic granules can occasionally be appreciated. In contrast to a lymphocyte, the monocyte has a fine and lacy chromatin pattern.

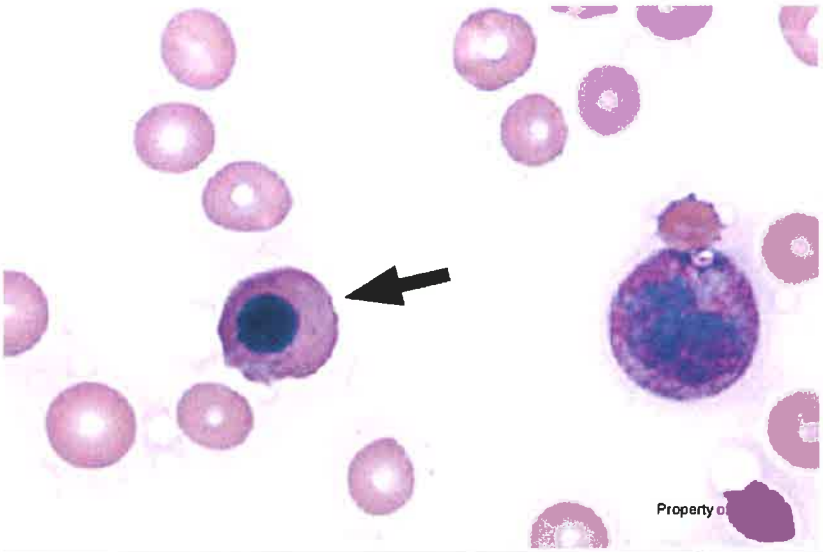
BCP-19 (cont)

Segmented neutrophils and their immediate precursors, bands, (as selected by 7.2% of referees and 9.6% of participants) constitute 12% to 25% of the nucleated cells in the bone marrow. Band neutrophils, also known as stabs, are 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: S-, C-, or U-shaped. The cytoplasm is similar to that of other post-mitotic neutrophils, with specific granules predominating in an otherwise pale cytoplasm. The segmented neutrophil 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 highly 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. The cell in question is not a neutrophil, segmented or band, in that the chromatin is too fine and lacy. Moreover, the abundant cytoplasm is mostly blue-grey with few eosinophilic granules, which is typical of a monocyte. A neutrophil, on the other hand, will have many specific granules in pale cytoplasm. Finally, the nuclear shape of this cell is more in keeping with a monocyte.

Metamyelocytes (as selected by 3.6% of referees and 1.7% of participants) 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. 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 is not a metamyelocyte in that the chromatin is too fine and lacy. Moreover, the abundant cytoplasm is mostly blue-grey with few eosinophilic granules, which is typical of a monocyte. A metamyelocyte would have many specific granules with possible rare primary granules in pale cytoplasm. Finally, the nuclear shape of this cell is more in keeping with a monocyte, (ie, more convoluted). A metamyelocyte usually has a single nuclear indention.

Blood Cell Identification – Ungraded

BCP-20



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Nucleated red blood cell (nRBC), normal or abnormal morphology	110	99.1	5448	97.6	Educational
Neutrophil necrobiosis (degenerated neutrophil)	1	0.9	102	1.8	Educational

The arrowed object is a nucleated red blood cell (nRBC), as correctly identified by 99.1% of referees and 97.6% of participants. As is generally the case, the marked circulating nucleated red blood cell is at the orthochromic stage of differentiation with a small pyknotic nucleus and hemoglobinized cytoplasm. For the purposes of proficiency testing, it is adequate to identify a cell as a nucleated red blood cell when it is present in the peripheral blood, be it normal or abnormal (ie, exhibits megaloblastic or dysplastic changes).

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FH(1-4, 6, 9-10, 13)-B 2018: Microangiopathic Hemolytic Anemias

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The following authors/planners have no financial relationships to disclose:

Parul Bhargava, MD
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 diagnostic criteria for microangiopathic hemolytic anemia (MAHA) and thrombotic microangiopathy (TMA).
2. Identify the clinical, morphologic, and common laboratory features of TMA.
3. Understand the clinical conditions and differential diagnosis of patients presenting with MAHA and thrombocytopenia.
4. Understand the clinical course and prognosis of patients with TMA.

Case Presentation:

This peripheral blood smear is from an 18-year-old woman presenting with diarrhea and renal failure after eating at a fast food restaurant where several other people became ill. Laboratory data include: WBC = 36.9 × 10E9/L; RBC = 2.2 × 10E12/L; HGB = 6.8 g/dL; HCT = 18.9%; MCV = 86 fL; MCH = 30.7 pg; MCHC = 35.9 g/dL; and PLT = 10 × 10E9/L.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

INTRODUCTION

Microangiopathic hemolytic anemia (MAHA) is a descriptive term for non-immune mediated (ie, Coombs negative) intravascular hemolysis. It is characterized by the presence of red blood cell (RBC) fragments (schistocytes) that are produced as a result of intravascular mechanical damage. RBCs may fragment from shearing by fibrin strands in the microvasculature (eg, in disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), and other thrombotic microangiopathies), in patients with prosthetic cardiac valves or severe valvular stenosis, malignant hypertension, other mechanical trauma to the cell (eg, march hemoglobinuria, marathon running), or in severe burns.

Thrombotic microangiopathy (TMA) is a pathologic condition in which intravascular thrombi are formed in capillaries and microvasculature. While not all MAHAs are due to TMA, nearly all TMAs lead to development of MAHA and thrombocytopenia. Thus, the presence of (1) schistocytes and (2) thrombocytopenia on a blood film examination, in the appropriate clinical context, is strongly suggestive of an underlying thrombotic microangiopathy. There are several underlying causes of TMA summarized in Table 1 below. For the purposes of this activity, there will be focus on TTP and hemolytic uremic syndrome (HUS).

Table 1. Causes of TMA

Thrombotic thrombocytopenic purpura (TTP) Hereditary TTP or Upshaw-Shulman syndrome (occurs in patients with hereditary deficiency of ADAMTS13 protein) Acquired TTP (occurs in patients with auto-antibodies to ADAMTS13 protein resulting in an acquired deficiency of ADAMTS13)
Hemolytic Uremic Syndrome (HUS) Shiga-like toxin mediated HUS (typical HUS) Complement-mediated TMA (atypical HUS) Hereditary Acquired
Other uncommon inherited defects associated with TMA Vitamin B12/Cobalamin metabolism defects, eg, hereditary mutation in MMACHC gene (MethylMalonic Aciduria and Homocystinuria type C), with cobalamin C deficiency Coagulation-mediated TMA, eg, mutations in genes encoding thrombomodulin (TM), plasminogen, and diacylglycerol kinase epsilon (DGKE)
Drug-induced TMA Immune mediated Dose-dependent, toxicity mediated (chemotherapy, immune-suppressives, vascular endothelial growth factor inhibitors, narcotics, illicit drugs)
Systemic disorders associated with MAHA and thrombocytopenia Pregnancy complications, severe hypertension, sepsis, malignancies, post-bone marrow or organ transplant, rheumatic disorders, disseminated intravascular coagulation, severe B12 deficiency

CLINICAL PRESENTATION

The clinical presentation of patients with TMA varies according to the underlying cause. Symptoms vary in intensity and onset and may include systemic, gastrointestinal, and neurologic manifestations.

HUS, as the name implies, is the diagnostic term used to describe the constellation of intravascular *hemolysis* (MAHA) and *uremia* ie, acute renal failure. Pathogenetically, this may occur due to (a) Shiga-toxin exposure (called "typical" HUS), (b) complement deficiency (atypical HUS), or in (c) systemic illness.

In Shiga-toxin induced TMA, patients have a history of exposure to contaminated food/water, and acute-onset gastrointestinal symptoms such as severe abdominal pain, nausea, vomiting, and diarrhea, which may be bloody. Elevation in creatinine, MAHA, and thrombocytopenia develop after a few days.

In the case of TTP, most patients have a few days of nonspecific symptoms such as malaise, gastrointestinal symptoms (eg, nausea, diarrhea), and purpura. Some may have relatively minimal symptoms and the diagnosis may be suspected based on peripheral smear findings of schistocytes and thrombocytopenia. Neurologic symptoms may be present including nonspecific findings such as headache, which occurs in one-third of the patients, and more specific neurologic defects (eg, diplopia, unstable gait) in another third of the patients. Fever is less commonly present. The historically described "pentad" of (a) schistocytes, (b) thrombocytopenia, (c) renal insufficiency, (d) neurologic symptoms, and (e) fever is obsolete as all 5 symptoms are present in less than 10% of patients with acute TTP.

PATHOGENESIS

A. HUS

In **typical HUS,** patients are infected by Shiga toxin producing *Escherichia coli* via ingestion of contaminated food or water. There have been several well-publicized outbreaks of HUS due to contamination of water sources in farming (scallions, spinach, lettuce) or contamination of meat during processing (especially hamburger or other mixed meat products). The toxin produced by the bacteria binds to cell membrane glycolipid Gb3, and eventually leads to apoptosis of affected cells. Such damage may occur in endothelial cells, red blood cells, and platelets. Additionally the toxin enhances expression of tissue factor by endothelial cells, which may lead to thrombosis in the microvasculature. There is high expression of glycoprotein Gb3 in the endothelium of glomerulus, leading to kidney damage and the characteristic renal failure associated with this disease. Shiga-toxin associated HUS accounts for 90% of all pediatric cases of HUS.

The complement pathway proteins are part of our innate immune system that can help in killing microorganisms through the formation of "membrane attack complexes." Rarely patients have a genetic or acquired defect in the regulation of complement proteins, such that they may be over-activated or proceed uninhibited. This may lead to damage of "self" cells such as renal endothelium, with activation of coagulation cascade and development of thrombotic microantiopathy. This form of HUS, which is not

associated with Shiga-toxin, is called **atypical HUS.** Complement-mediated HUS is a rare disorder with a prevalence of 7 per 1 million children, according to one European study.

In rare situations, **secondary HUS** can develop after systemic conditions (eg, infections, transplantation, autoimmunity, cancer, pregnancy, cytotoxic drugs) which lead to direct cell damage, activate complement, or enhance destruction of self/host cells after complement activation.

B. TTP

TTP is another important, and potentially life-threatening cause of TMA. There is a protein called ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13) that is responsible for breaking down high molecular weight multimers of von Willebrand Factor (vWF). Some patients have a congenital deficiency or acquired inhibitors of ADAMTS-13. They are unable to break down high molecular weight vWF multimers, which leads to formation of microthrombi, with development of MAHA and thrombocytopenia.

DIAGNOSIS

Laboratory workup is critical for (a) establishing the diagnosis of TMA and (b) identifying the specific cause of TMA, which is necessary for instituting appropriate therapy.

A diagnosis of HUS is made in patients with MAHA, thrombocytopenia, and acute renal failure. A search for Shiga-toxin producing bacteria by stool cultures may also be performed. TTP, on the other hand, has MAHA and thrombocytopenia, but renal involvement is a late finding. Confirmation of a diagnosis of TTP generally requires measurements of levels of ADAMTS-13 protein and evaluation of its inhibitors. In all causes of TMA, the anemia is due to hemolysis with elevation of lactate dehydrogenase (LDH), indirect bilirubin (generally greater than 2 mg/dL), with absent haptoglobin, and negative Coombs test.

Complete blood count

Complete blood count (CBC) evaluation shows anemia with hemoglobin levels generally below 8 mg/dL. Thrombocytopenia is seen, with platelet counts averaging 40 × 10E9/L in HUS, and may be below 10 × 10E9/L in TTP. White blood cell count may be elevated if patient has an infection or other systemic illness. Reticulocytes are generally elevated (> 2.5%).

Peripheral blood evaluation

Review of peripheral smear is a key step in making a diagnosis of TMA. Identification of 1% of greater schistocytes, together with thrombocytopenia, point to a diagnosis of TMA. Schistocytes are fragmented RBCs with 2 or more points and generally lacking true central pallor (See Figure 1). Some authors allow for a minor degree of central pallor to be present. Schistocytes may be distinguished from bite cells as the latter generally have preserved central pallor, and the arc-like defect is generally smaller. Presence of schistocytes is generally accompanied by a small number of microspherocytes, as fragmented RBCs re-seal and become more rounded. Since the anemia is from an intravascular cause, the marrow tries to compensate by putting out increased reticulocytes, visible as polychromatophils on a Wright stained blood film. Depending on the severity of anemia, occasional

nucleated RBCs may also be seen. Platelet counts are moderately to severely reduced in number; occasional larger forms may be seen. Platelet granulation is normal. White blood cell count and differential are often not affected, unless the patient additionally has an underlying systemic illness, in which case toxic changes (toxic granulation, Döhle bodies, toxic vacuolation), and possible left-shift with circulating immature granulocytes may be encountered.

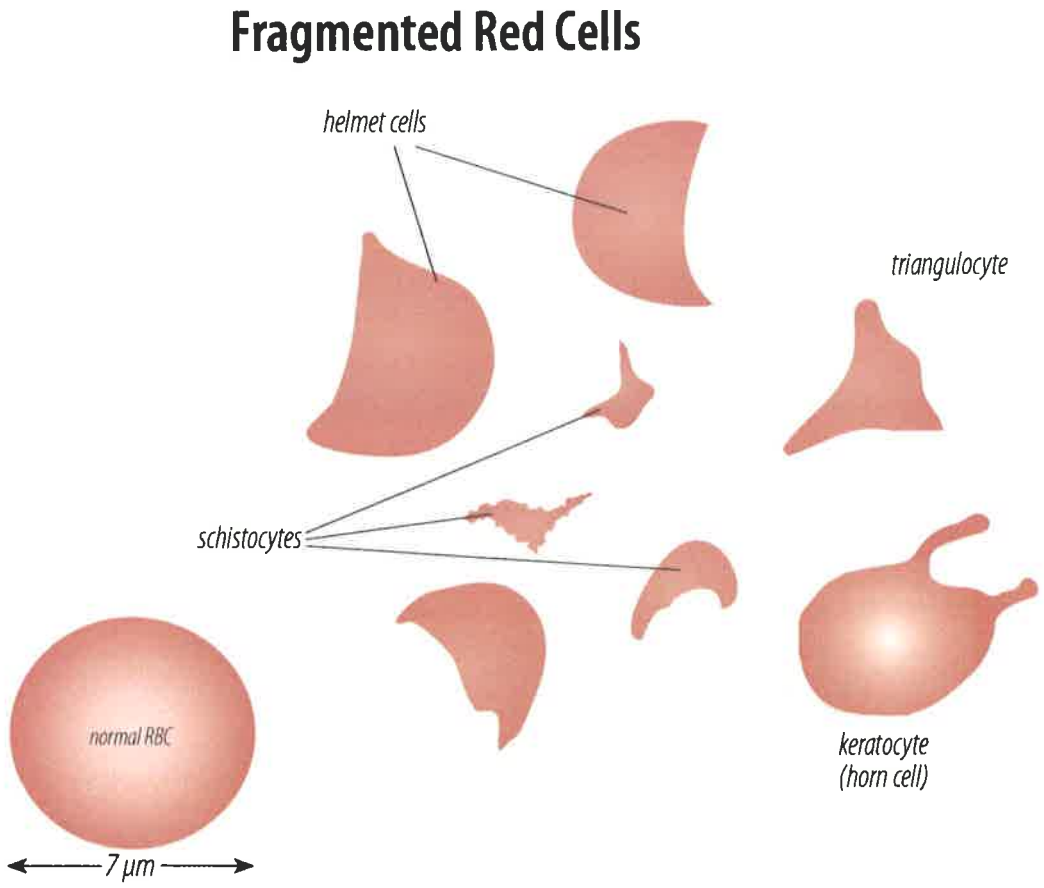

Figure 1. Schistocytes. From Glassy EF, ed. *Color Atlas of Hematology: An Illustrated Field Guide Based on Proficiency Testing*, 2nd ed. Peripheral Blood. Northfield, IL: College of American Pathologists; 2018:68. Reproduced with permission.

Other laboratory studies

Coagulation parameters (prothrombin time, activated partial thromboplastin time) are generally normal. Coombs testing is negative, with elevated LDH, elevated indirect bilirubin, and undetectable serum haptoglobin. Reticulocytes are often elevated. Urinalysis may show proteinuria and hematuria. Serum creatinine and plasma urea may be increased, especially in HUS. Cardiac tropoinin may also be increased.

Stool culture
All patients with TMA without a systemic illness, who have abdominal symptoms and/or known exposure to possible contaminated food/water should have a stool culture for enterohemorrhagic *Escherichia coli* (United States, Europe) and/or *Shigella* (Asia). Testing for Shiga toxin is also recommended.

ADAMT-13 evaluation
ADAMTS-13 measurement with inhibitor assessment should be done in all patients where TTP is suspected. TTP is a life-threatening disorder which requires specific treatment (see therapy section), and demonstrating low levels of ADAMTS-13 are required to definitively establish a diagnosis of TTP.

A. ADAMTS-13 levels may be obtained by functional assays, or via immunochemical assays such as enzyme-linked immunosorbent assay (ELISA)-based methods.

   Less than 10% ADAMTS-13 activity supports the diagnosis of TTP.

B. ADAMTS-13 inhibitors are assessed when ADAMTS-13 levels are low.

DIFFERENTIAL DIAGNOSIS

All causes of TMA (as listed in Table 1) are in the differential diagnosis when there is evidence of MAHA and thrombocytopenia. Evaluation of systemic causes such as pregnancy, severe hypertension, sepsis, etc, that may secondarily lead to TMA is generally undertaken first. In the absence of systemic illnesses/conditions, clinical factors such as patient's age, rapidity of onset of symptoms, degree of renal dysfunction, exposure to farm animals/contaminated food/water, and medications are taken into consideration.

THERAPY

Therapy for TMA is based on providing adequate supportive care (eg, fluid/electrolyte balance, transfusions), treatment of underlying diseases, if any, and assessment for specific therapies such as anti-complement therapy (for complement mediated HUS) or plasma exchange therapy (for TTP).

Treatment of HUS is primarily supportive. RBC transfusions are given when hemoglobin drops below a critical threshhold (generally below 6 or 7 mg/dL), and platelets may be transfused in actively bleeding patients. Fluid and electrolyte balance has to be maintained, and dialysis may be necessary in some patients.

TTP is a life-threatening disease with a historic mortality rate of 80% - 90%. With the understanding of the pathogenesis of TTP, including presence of inhibitors to ADAMTS-13 in plasma and/or deficiency of ADAMTS-13, therapeutic plasma exchange (PEX) has become the mainstay of treatment. PEX serves to remove ADAMTS-13 antibodies when present, and replace ADAMTS-13. In any patient with suspected TTP, PEX is instituted early without necessarily waiting for laboratory confirmation. Immunomodulatory drugs (eg, steroids, rituximab) may be used in certain cases with ADAMTS-13 inhibitors.

Eculizumab, a monoclonal antibody to complement factor 5, is a drug that inhibits complement and is used in the treatment of complement mediated HUS. Generally, this is tried in children presenting with HUS (ie, possible atypical HUS), and in postpartum women presenting with HUS.

For drug-induced TMA, the implicated drug needs to be stopped. High-dose hydroxycobalamin along with betaine and folinic acid have been used to treat cobalamin C deficiency-mediated TMA.

Prognosis

A majority of patients (60% - 70%) recover completely after Shiga-toxin induced HUS. However, based on a large retrospective study, approximately 2.9% patients died due to end-organ damage, sepsis, coagulopathy, or hyperkalemia. In the remaining patients, there may be residual irreversible renal dysfunction.

PEX has dramatically reduced mortality in TTP; however mortality remains 10% - 20% even when appropriate therapeutic measures are taken.

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