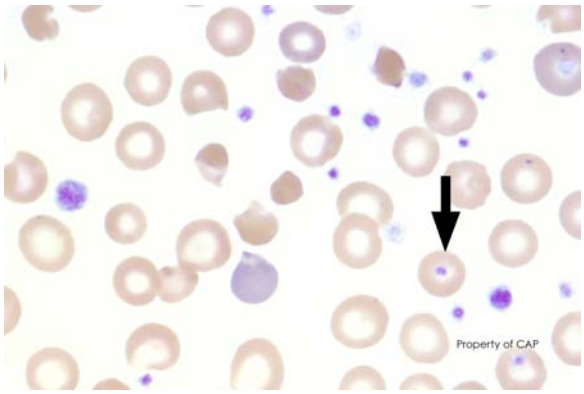
Blood Cell Identification – Graded

Case History

This peripheral blood smear is from a 2-week-old female found to be jaundiced with an elevated bilirubin. Laboratory data include: WBC = 17.6 × 10^9/L; RBC = 2.04 × 10^{12}/L; HGB = 7.1 g/dL; HCT = 21.3%; MCV = 100 fL; MCHC = 34.7 g/dL; RDW = 16.2; and PLT = 957 × 10^9/L. Identify the arrowed object(s) on each image.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

BCP-21

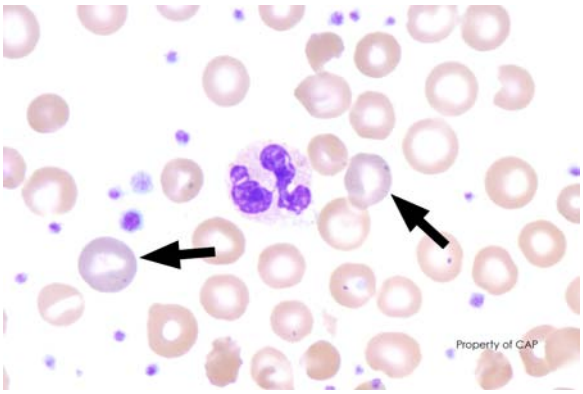


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Erythrocyte with overlying platelet	89	98.9	4919	98.9	Good
Platelet, normal	1	1.1	23	0.5	Unacceptable

The arrowed cell is an RBC with overlying platelet as correctly identified by 98.9% of the referees and 98.9% of the participants. Platelets overlying red cells must be distinguished from other red cell inclusion or parasites. Correct interpretation depends on carefully examining the morphology of the platelet and comparing its characteristics to other known platelets in the field, as well as determining if the platelet is in the same field of focus as the red cell. In this image, the platelet overlying the red cell shows similar size and staining characteristics to other platelets in the field, and is surrounded by a clear halo which is not a feature of most genuine red cell inclusions. A blister cell is also present in this image (left upper portion of image). This blister cell is characterized by concentration of hemoglobin on one side of the cell with just a thin membrane on the other side. This finding is seen with oxidative hemolysis, as well as in sickle cell disease.

Blood Cell Identification – Graded

BCP-22

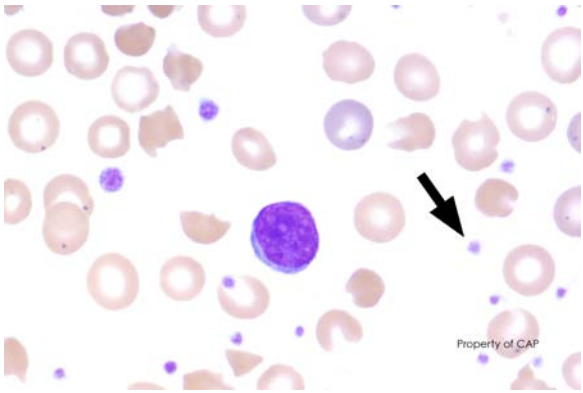


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Polychromatophilic (non-nucleated) red cell	90	100.0	4855	97.7	Good

The arrowed cells are polychromatophilic red cells as correctly identified by 100.0% of the referees and 97.7% of the participants. The polychromatophilic red cell is a non-nucleated, round or ovoid cell, which represents the final stage of red cell maturation after exiting the bone marrow. It is larger than a mature erythrocyte; stains pink-gray or pale purple on Wright-Giemsa stained slides, and often lacks central palor. These cells can be stained as reticulocytes using supravital stains, such as new methylene blue, or counted on automated analyzers by supravital staining or fluorescent methods. An increased number of polychromatophilic cells is seen as the bone marrow response to anemia due to bleeding or red cell destruction. In this case, hemolytic anemia due to a red cell oxidative insult is present. Also of note in this image, are several erythrocytes that contain small, angular dark inclusions representing Pappenheimer bodies.

Blood Cell Identification – Graded

BCP-23

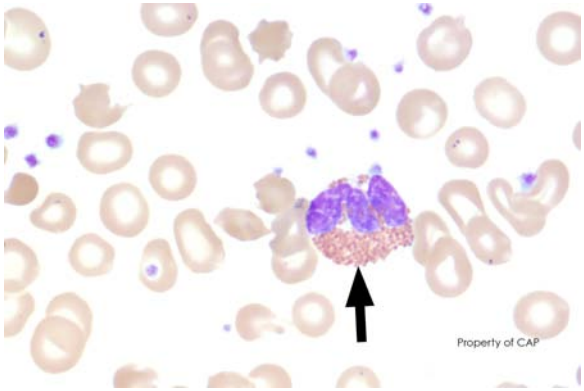


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Platelet, normal	90	100.0	4957	99.7	Good

The arrowed cell is a platelet, as correctly identified by 100.0% of the referees and 99.7% of the participants. Platelets (also known as thrombocytes) are small, blue-gray fragments of megakaryocytic cytoplasm, typically measuring 1.5 to 3 µm in diameter. Fine, purple-red granules are aggregated at the center or dispersed throughout the cytoplasm. They are typically single but may form aggregates. This image also contains bite cells, a finding seen due to hemoglobin precipitation associated with oxidative red cell injury.

Blood Cell Identification – Graded

BCP-24

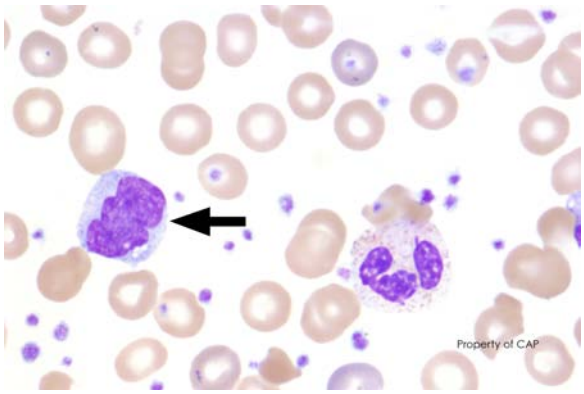


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Eosinophil, any stage	90	100.0	4960	99.7	Good

The arrowed cell is an eosinophil as correctly identified by 100.0% of the referees and 99.7% of the participants. Eosinophils are round to ovoid leukocytes, ranging in size from 10 to 15 µm and having an N:C ratio of approximately 1:3. They contain abundant cytoplasm that is usually evenly filled by numerous coarse, orange-red granules of uniform size. These granules exhibit a refractile appearance by light microscopy; although this finding is not apparent in photomicrographs. Mature eosinophils most often have two or three nuclear lobes; occasional eosinophils will exhibit four or five nuclear lobes.

Blood Cell Identification – Graded

BCP-25



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Monocyte	86	95.6	4850	97.7	Good
Monocyte, immature (promonocyte, monoblast)	4	4.4	79	1.6	Unacceptable

The arrowed cell is a monocyte as correctly identified by 95.6% of the referees and 97.7% of the participants. Monocytes are slightly larger than neutrophils, 12 to 20 µm in diameter. The majority of monocytes are round with smooth edges, but some have pseudopod-like cytoplasmic extensions. The cytoplasm is abundant and gray to gray-blue (ground-glass appearance) and may contain fine, evenly distributed, azurophilic granules, or vacuoles. The nuclear-to-cytoplasmic ratio is 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 less dense than that of a neutrophil or lymphocyte. Nucleoli are generally absent, but occasional monocytes may contain a small, inconspicuous nucleolus.

Discussion:

Oxidative Hemolytic Anemia: BCP 21-25

This case presentation illustrates oxidative hemolytic anemia presenting in a neonate. Oxidative hemolytic anemia, sometimes also referred to as Heinz body hemolytic anemia, is the result of hemoglobin precipitation within the red cell due to an oxidative insult.

Review of the peripheral blood smear is critical for diagnosis of and classification of hemolytic anemia. When hemolytic anemia presents in a newborn, a variety of etiologies should be considered, including:

- Production of abnormal hemoglobins (hemoglobinopathies), such as sickle cell disease
- Decreased production of normal globin chain (thalassemia)
- Red cell cytoskeletal defects, including hereditary spherocytosis and hereditary elliptocytosis
- Abnormalities of glycolytic enzymes, such as G6PD deficiency and pyruvate kinase deficiency
- Immune mediated hemolytic anemias, often resulting from transplacental transfer of antibodies
- Disseminated intravascular coagulation (DIC)

In our case presentation, the patient has a normochromic, normocytic anemia with increased polychromatophilia, bite cells, blister cells (also referred to as eccentrocytes), schistocytes, and a minor population of spherocytes. In particular, the presence of bite and blister cells suggests an oxidative hemolytic anemia with G6PD deficiency being the most common underlying inherited abnormality.

G6PD Deficiency

G6PD deficiency is an X-linked disorder and the most common enzymatic disorder of red blood cells, affecting approximately 400 million people worldwide. G6PD is an enzyme necessary in red cells for production of NADPH. NADPH is required to produce reduced glutathione, a protein that functions within red cells as a reducing agent to prevent oxidative injury. When G6PD is deficient, insufficient reduced glutathione is available to neutralize oxidants and oxidative damage to the cell. These oxidants produce oxidized hemoglobin that precipitates and results in hemolysis. The disease severity of G6PD deficiency is variable, depending on the type of abnormality (mutation) and has been classified by the World Health Organization (WHO) in to five classes:

Class I variants: severe deficiency (<10% of normal) and have chronic hemolytic anemia.
Class II variants: severe deficiency but usually only with intermittent acute hemolytic episodes associated with drugs, infections, or chemicals. Includes G6PD Mediterranean mutation.
Class III variants: moderate enzyme deficiency (10-60% of normal) with intermittent acute hemolytic episodes associated with drugs, infection, or chemicals. Includes G6PD A- mutation.
Class IV variants: no enzyme deficiency or hemolysis.
Class V variants: increased enzyme activity.

The gene for G6PD is located on the X chromosome. Males carrying a variant gene generally express deficiency of the enzyme while heterozygous females (due to the presence of two X chromosomes) may be normal or in some cases, have reduced G6PD depending on the degree of X-chromosome inactivation (lyonization).

Common variants of G6PD include:

G6PD B: the normal wild type enzyme with normal enzymatic activity.
G6PD A: an unstable enzyme that has nearly normal activity in bone marrow cells and reticulocytes with activity falling dramatically as the red cell ages; found in 10-15% of African-Americans.
G6PD Mediterranean: an enzyme synthesized at a reduced rate with markedly reduced catalytic activity that may result in severe hemolysis; most common variant in Caucasians and also found throughout the Mid-East in Arabic populations.

Most individuals with G6PD deficiency are asymptomatic and lack anemia or red cell morphologic abnormalities in the steady state. If exposed to an oxidative insult, acute hemolysis can occur. Potential oxidative insults include various medications (eg, dapsone, sulfamethoxazone, primaquine, quinine), foods (eg, fava beans, other legumes, menthol, ascorbic acid, vitamin K, some Chinese herbs), infections, and other metabolic abnormalities (eg, diabetic ketoacidosis). The oxidative insult results in hemoglobin oxidation and precipitation with Heinz body formation. Heinz bodies are not visualized on Wright-Giemsa stained blood smears but may be seen with special staining, such as crystal violet or Brilliant cresyl blue, which can be used to visualize Heinz bodies by light microscopy. As these red cells transverse the spleen, the Heinz body is removed along with a portion of the red cell membrane and cytoplasm, resulting in the formation of bite cells and blister cells, findings that can be detected on the blood smear. Red cells may contain multiple "bites" or even may have morphology resembling an "apple core." As the bone marrow responds to the resulting red cell destruction, increased polychromaphilia, a finding corresponding to reticulocytosis, ensues and is usually apparent within 5 days of the onset of hemolysis. In some patients, including those with G6PD A-, the acute hemolysis will resolve after a week or so as the older red cells are replaced with younger red cells containing sufficient G6PD. In other patients, such as those with G6PD Mediterranean, hemolysis will continue for a more extended period of time, even following removal of the oxidative insult, because the G6PD variant is more unstable and has a short half life.

Laboratory testing is useful to establish a diagnosis of G6PD deficiency and should be performed when the patient is in steady state (not actively hemolyzing or responding to a hemolytic event), since the red cells with the lowest G6PD (the oldest red cells) are destroyed during an acute hemolytic event. If G6PD testing is done during an acute hemolytic event, a false negative test result can occur as younger red cells may have higher levels of the enzyme present. Testing for G6PD deficiency is based on the function of the normal enzyme. In these assays, red cell hemolysate is added to a mixture of substrate (glucose-6-phosphate) and cofactor (NADP). Spectrophotometry is used to measure the rate of production of NADPH which is directly related to the functional activity of G6PD. Once a diagnosis of G6PD is established, the patient should avoid oxidative insults whenever possible.

References:

1. Beutler E. Glucose-6-phosphate dehydrogenase deficiency: a historical perspective. *Blood.* 2008;(111):16-24.
2. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. *Lancet* 2008;(371):64-74.
3. Greer JP, Foerster J, Rodgers GM, et al. Glader B. Hereditary hemolytic anemias due to red blood cell enzyme disorders. *Wintrobe's Clinical Hematology*. 2009;933-940.
4. Mason PJ, Bautista JM, Gilsanz F. G6PD deficiency: the genotype-phenotype association. *Blood Rev.* 2007;(21):267-283.

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Hematology and Clinical Microscopy Resource Committee

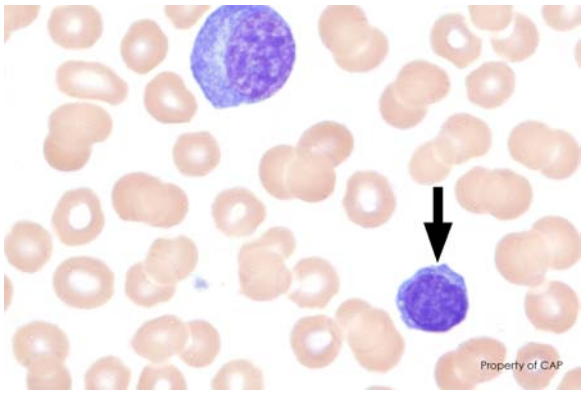
Blood Cell Identification – Ungraded

Case History

This peripheral blood smear is from a 52-year-old male with recent travel to the Caribbean and Ghana who presents with fever.
Laboratory data included: WBC = 14.0 × 10^9/L; RBC = 4.66 × 10^{12}/L; HGB = 13.1 g/dL; HCT = 38.8%; MCV = 83.2 fL; RDW = 15.1 and PLT = 127 × 10^9/L. Identify the arrowed object(s) on each image.

(BLOOD, WRIGHT-GIEMSA)

BCP-26

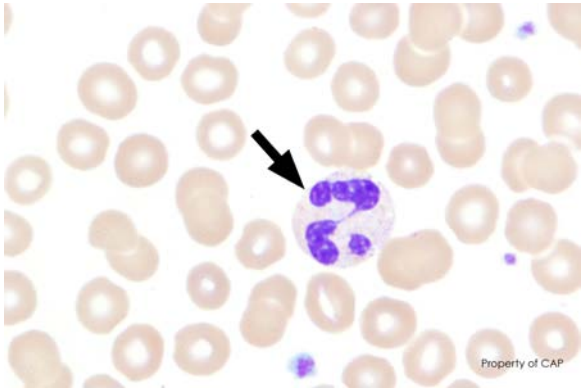


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Lymphocyte	86	98.9	4695	95.9	Educational
Lymphocyte, reactive	1	1.2	163	3.3	Educational

The arrowed cell is that of a lymphocyte as correctly identified by 98.9% of the referees and 95.9% of the participants. Lymphocytes are leukocytes that are equal to, or slightly larger than, a red blood cell. Lymphocytes have scanty to medium amounts of blue cytoplasm, round to oval nuclear contours, and dense coarse chromatin. At the top of the image is a plasmacytoid lymphocyte which has a round nucleus, with moderately coarse chromatin, that is eccentrically placed. A perinuclear clear zone, or hof, is present.

Blood Cell Identification – Ungraded

BCP-27

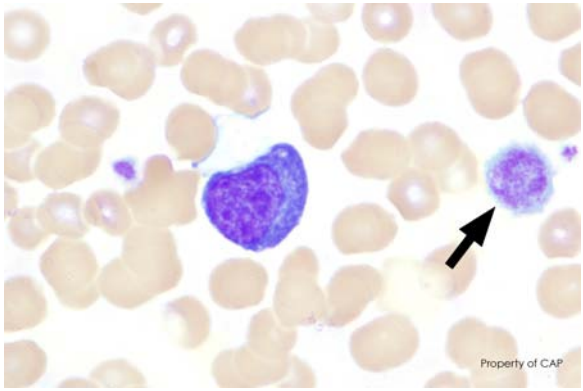


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Neutrophil, segmented or band	84	96.6	4705	96.1	Educational
Neutrophil, toxic	2	2.3	76	1.6	Educational
Megakaryocytic cell	1	1.2	1	0.0	Educational

The arrowed cell is that of a neutrophil as correctly identified by 96.6% of the referees and 96.1% of the participants. A neutrophil is a large leukocyte that represents the predominant white cell population in the blood. Neutrophils have abundant pale pink cytoplasm with many lilac granules. They are multiple lobated (2-5 lobes) that are joined together by fine filament. The chromatin is condensed and lacks nucleolus. Under reactive states, neutrophils can undergo toxic changes, including coarse cytoplasmic granulation and cytoplasmic vacuolization.

Blood Cell Identification – Ungraded

BCP-28

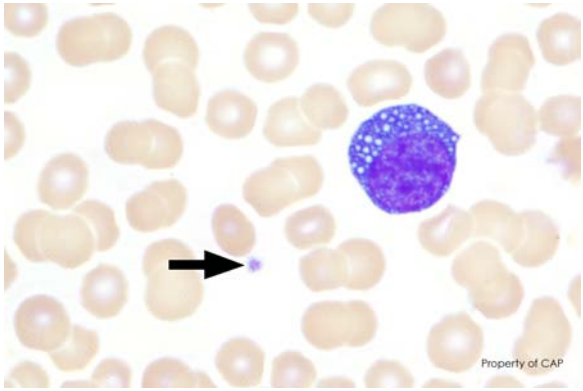


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Platelet, giant	86	98.9	4819	98.5	Educational
Megakaryocytic cell	1	1.2	48	1.0	Educational

The arrowed cell is that of a giant platelet (macrothrombocyte) as correctly identified by 98.9% of the referees and 98.5% of the participants. Giant platelets are larger than a normal red blood cell. Unlike leukocytes, giant platelets do not have a nucleus. A giant platelet is composed of exclusively blue-gray cytoplasm with variable amounts of fine purple to red granules. Giant platelets have variable cytoplasmic contours from smooth and round to scalloped. The nucleated cell present in the center of the image is a plasmacytoid lymphocyte, which is intermediate in size, with moderately abundant basophilic cytoplasm; the nucleus contains moderately coarse cytoplasm with two small nucleoli.

Blood Cell Identification – Ungraded

BCP-29

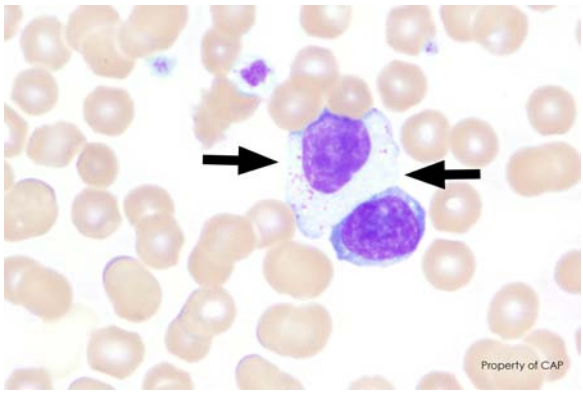


Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Platelet, normal	87	100.0	4862	99.3	Educational

The arrowed cell is that of a platelet as correctly identified by 100.0% of the referees and 99.3% of the participants. A platelet is a small anucleated component of blood that represents fragments of megakaryocyte cytoplasm. They typically have round to elliptical contours, and blue-gray cytoplasm containing scattered fine purple to red granules. Platelets are different from giant platelet because they are smaller than a red blood cell. The leukocyte present in the image is that of a plasma cell. The moderately abundant cytoplasm contains multiple small globules which represent accumulations of immunoglobulin; these cells are also called “Mott cells.” Plasma cells may be seen in association with reactive lymphocytoses of various etiologies, as in this case.

Blood Cell Identification – Ungraded

BCP-30



Identification	Referees No.	Referees %	Participants No.	Participants %	Evaluation
Lymphocyte, large granular	46	52.9	2486	50.8	Educational
Lymphocyte, reactive	40	46.0	2261	46.2	Educational
Leukocyte with phagocytized bacteria	1	1.2	23	0.5	Educational

The arrowed cell is that of a large granular lymphocyte as correctly identified by 52.9% of the referees and 50.8% of the participants. Large granular lymphocytes are large leukocytes with distinctively abundant amount of light blue cytoplasm and scattered azurophilic granules. The cytoplasmic contours of large granular lymphocytes are variable and may become indented by neighboring cells. The nuclear contour and chromatin can have variable appearance. Nucleoli may also be present in variable number. The large granular lymphocyte in this field is immediately adjacent to a lymphocyte, which is small in size, with a small to moderate amount of basophilic cytoplasm. Large granular lymphocytes are typically seen as part of the reactive lymphocyte spectrum in patients with viral infections

Discussion:

C Dengue Fever Discussion: BCP 26-30

This case is an excellent example of reactive lymphocytes, including plasmacytoid lymphocytes and circulating plasma cells, that may be seen with viral infections. In this case, images were selected to show a variety of plasmacytoid lymphocytes and plasma cells in the peripheral blood of this patient. A reactive lymphocytosis will show a wide range of cellular sizes and shapes. While the classic example of a reactive lymphocytosis in most laboratories is infectious mononucleosis (Epstein-Barr viral infection), other viral infections will also show a reactive lymphocytosis in the peripheral blood. In such a reaction, a variety of lymphocytes are seen, ranging from small bland lymphocytes with round nuclei and condensed chromatin to larger reactive lymphocytes with abundant pale-blue cytoplasm, round to oval nuclei, and moderately condensed chromatin. The cytoplasm of these reactive lymphocytes may hug adjacent red cells and show a basophilic rim at their margins. Immunoblasts are frequently present, being large lymphocytes with round to oval nuclei, containing one or more prominent nucleoli. The cytoplasm of an immunoblasts is abundant and deeply basophilic. Plasmacytoid lymphocytes and even frank plasma cells may also be seen in viral infections, as demonstrated in this case.

Dengue fever is most commonly seen in Southeast Asia and in the Caribbean and is caused by any of four related viruses (of the Flavivirus family) that are spread by mosquitoes. According to the Centers for Disease Control (www.cdc.gov), more than 100 million people are infected annually. In the United States, most cases are 'imported' via travelers, with indigenous transmission occasionally seen (typically in Texas); it is also endemic in Puerto Rico. Three clinical presentations are seen with Dengue infection. Dengue fever, also known as 'breakbone fever,' presents in patients with a sudden high fever followed by a measles-like rash. Other symptoms include headache, eye pain, joint/muscle/bone pain, and mild bleeding. A low white blood cell count is common. Dengue hemorrhagic fever is characterized by fever, hemorrhage, thrombocytopenia less than 100 x 10^9/L, and evidence of plasma leakage (ie, hemoconcentration, pleural effusion, ascites, and hypoproteinemia). Dengue shock syndrome presents with features of Dengue hemorrhagic fever and superimposed circulatory failure.

The diagnosis of Dengue infection can be made by detection of the virus in tissue, blood, cerebrospinal fluid, or body fluid by polymerase chain reaction (PCR), immunofluorescence, or immunohistochemistry. A real-time RT-PCR test is now available for detection of all four Dengue serotypes. Serologic detection of antibodies can also be used. There is no specific treatment for Dengue infection and there is no vaccine. Rest, fluids, and pain relief with acetaminophen is recommended for Dengue fever, with hospitalization and fluid replacement recommended for Dengue hemorrhagic fever and shock syndrome.

References:

1. Chunhakan S, Butthep P, Yoksan S, et al. Early diagnosis of dengue virus infection by detection of dengue viral antigen in peripheral blood mononuclear cell. *Pediatr Infect Dis J*. 2009;28(12):1085-1088.
2. Gerome P, Foucher B, Otto MP, et al. [Plasmacytosis and dengue fever: an underestimated abnormality?]. *Rev Med Interne*. 2012;33(6):343-345.
3. Wrammert J, Onlamoon N, Akondy RS, et al. Rapid and massive virus-specific plasmablast responses during acute dengue virus infection in humans. *J Virol.* 2012;86(6):2911-2918.

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