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PARTICIPANT STATISTICS | **CELL IDENTIFICATION** | **THIRD QUADRIMESTER 2018**

Q3-2018

HELLP Syndrome and Microangiopathic Hemolytic Anemia (MAHA)

This 28 year-old woman is 32 weeks pregnant. She presents with mild confusion, headaches, nausea, easy bruising and petechiae. Laboratory values are as follows: WBC - 9.7, RBC - 2.97, Hgb -7.7, Hct -23.0, Platelets-23,000, abnormal RBC indices, including elevated RDW. Peripheral blood smear examination reveals many schistocytes, occasional nRBC and polychromasia. She was diagnosed with HELLP syndrome at which point she was hospitalized.

HELLP (H for hemolysis, EL for elevated liver enzymes, and LP for low platelets) syndrome is a known obstetric complication with an occurrence rate on the order of 0.2 to 0.6 per cent of allpregnancies versus an occurrence rate of 5 to 7 per cent for preeclampsia. Initially HELLP was believed to be a variant of preelampsia. However, it is now thought it may be a separate entity since the syndrome has also been reported on its own as well as superimposed on preeclampsia. Complicating the picture is the fact that the following three obstetric disorders, pregnancy induced hypertension, preelampsia and HELLP syndrome, overlap in their presentations. HELLP syndrome is characterized by multiorgan dysfunction but the pathogenesis of HELLP still remains unclear. It has not yet been determined whether the syndrome manifests as a result of some singular anomaly or whether is precipitated by the simultaneous presence of several mitigating elements.

Although the syndrome most commonly presents in the third trimester of pregnancy, nearly a third of cases present postpartum. For those obstetric patients ultimately diagnosed with HELLP syndrome most initially presented with malaise, over half complained of epigastric pain and nearly a third complained of nausea and vomiting or headache. It is primarily due to the vague nature of the presenting symptoms that HELP syndrome is frequently initially misdiagnosed. The syndrome has been reported to have morbidity and mortality rates as high as 25 per cent making early diagnosis critical. Pregnant woman in the third trimester who present with malaise or a viral type illness are now routinely evaluated with a CBC and LFTs.
In HELLP syndrome the platelet count is < 150,000/mm^3, LFTs are elevated, with the AST greater than 70 U/L and the LDH greater than 600 U/L. The anemia associated with the hemolysis is the last of the three markers to appear so the hematocrit may be or low or normal. If the first two markers, low platelets and elevated LFTs, are present but the hematocrit is normal then it is necessary to measure the serum haptoglobin level. Below normal serum haptoglobin levels occur when there is on-going hemolysis. Regardless of the hematocrit, peripheral blood smear examination will reveal abnormal RBC morphology

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As mentioned earlier, the pathogenesis of HELLP still remains unclear. But what is understood is that in early in the development of the syndrome, there is intravascular platelet activation and endothelial cell damage to the capillaries and arterioles. Endothelium refers to cells that line the interior surface of blood vessels and lymphatic vessels. It forms an interface between circulating blood or lymph in the lumen and the rest of the vessel wall. Vascular endothelial cells line the entire circulatory system, from the heart to the smallest capillaries. These cells have unique functions in vascular biology. These functions include fluid filtration, such as in the glomerulus of the kidney, blood vessel tone, hemostasis, neutrophil recruitment, and hormone movement.

After endothelial cells are damaged, they become susceptible to deposition of fibrin, also referred to as microthrombi. The presence of microthrombi severely narrows the internal diameter of the affected blood vessels. During passage through the compromised microcirculation, many RBCS are damaged or fragmented. The morphologic evidence of a microangiopathic hemolytic anemia (MAHA) is seen on the peripheral blood smear in the form of schistocytes, spherocytes, triangle cells and burr cells. The appearance of RBC polychromasia and nRBCs on the peripheral blood smear provides evidence of bone marrow response to the anemia.
The thrombocytopenia that develops in HELLP syndrome has been attributed to increased consumption and/or destruction of platelets. Based on the platelet count, the HELLP syndrome is further divided into three classes. Class I patients have a platelet count of less than 50,000/mm^3, class 2 is between 50,000 and 100,000/mm^3, and class 3 is more than 100,000/mm^3.

Obstruction of the hepatic blood flow by fibrin deposits in the hepatic sinusoids can cause widespread endothelial dysfunction and hepatocellular damage. The presence of specific, hepatotoxic, placental -derived proteins damage hepatocytes resulting in the release of liver enzymes. The obstruction of the hepatic blood flow also increases intrahepatic pressure and the resulting hepatic dysfunction can then, rarely, progress to liver necrosis, hemorrhages, and in the worst, most life threatening cases, subcapsular hematoma and hepatic rupture.
A diagnosis of full HELLP syndrome is made only if all three criteria are present. A diagnosis of partial HELLP syndrome is made if only one or two criteria are present, and a diagnosis of severe pre-eclampsia is made if none is present. Patients with full HELLP syndrome are likely to have a higher incidence of stroke, cardiac arrest, DIC, placental abruption, need for blood transfusion, pleural effusion, renal failure, and wound infections.

When first described in the early 80's, it was believed that prompt delivery was the best treatment option available. Since then, treatment protocols have continued to evolve and current treatment approaches are more conservative and primarily based on the gestational age combined with the condition of the mother and baby. Whenever possible, patients with gestational age less than 32 weeks are treated with bed rest, fluids, close monitoring of the BP and urine output and administration of corticosteroids. This conservative treatment has successfully allowed many of these HELLP syndrome patients to prolong their pregnancy for up to two weeks. Severe cases of HELLP syndrome or those who develop DIC are not eligible for conservative treatment regimes. In these cases, prompt delivery, by C- section, is usually considered the best treatment option.
The laboratory abnormalities in HELLP syndrome typically worsen after delivery. Usually by four days postpartum, they begin to normalize. But, if at 72 hours postpartum, the platelet count remains below 30,000/mm^3, there is continued elevation of LFTs and/or repeat transfusions are needed order to maintain their hematocrit, treatment with plasmapheresis will be considered.

Our case study patient remained hospitalized for 2 weeks, during which time she received extensive supportive treatment. At 34 weeks gestation she was successfully delivered of a 3lb. 2oz. baby girl. Her lab results normalized over the succeeding 6 weeks.

Cell Identification

Specimen 1

Result	No.	Flag
Polychromatophilic RBC	231	
Macrocytic	10	***
Abnormal, would refer	4	
Dimorphic RBC	3	***
Immature RBC, would refer	2	***
Schistocyte (bite, blister, helmet)	1	***
Reticulocyte (supravital stain)	1	***
Basophilic Stippling	1	***

Total Population **253**

Intended result: Polychromatophilic RBC

Specimen 2

Result	No.	Flag
Promyelocyte	79	
Myelocyte	78	
Abnormal, would refer	20	
Immature WBC, would refer	18	
Basophil, any stage	18	***
Metamyelocyte	13	***
Abnormal Granulocyte, would refer	8	
PMN with Toxic Granulation/Vacuolization	6	***
Lymphocyte, reactive	4	***
Basophil, any stage	3	***
Eosinophil, any stage	2	***
Lymphocyte, normal	1	***
Abnormal Lymphocyte, would refer	1	***
PMN with Dohle Bodies	1	***
Lymphocyte, abnormal/atypical	1	***
Lymphocyte; atypical, Downey, variant	1	***

Total Population **253**

Intended result: Myelocyte

19 of 21 Referees corectly identified
the intended result of Myelocyte.

Specimen 3

Result	No.	Flag
Monocyte, any stage	182	
Monocyte, normal/any stage	20	
Abnormal, would refer	12	***
PMN with Toxic Granulation/Vacuolization	12	***
Segmented Neutrophil (PMN, poly)	11	***
Hypersegmentated Neutrophil	4	***
Lymphocyte; atypical, Downey, variant	3	***
Megakaryocyte	3	***
Segmented Neutrophil (PMN, poly)	2	***
Abnormal Granulocyte, would refer	2	***
Abnormal Lymphocyte, would refer	1	***
PMN with Degenerated Nucleus (pyknotic PM	1	***

Total Population **253**

Intended result: Monocyte, any stage

20 of 21 Referees corectly identified
the intended result of Monocyte

Specimen 4

Result	No.	Flag
Lymphocyte, normal	128	
Blast, undifferentiated	38	
Lymphocyte; atypical, Downey, variant	20	
Myeloblast	17	
Lymphocyte, reactive	13	
Myelocyte	10	
Abnormal, would refer	8	
Abnormal Lymphocyte, would refer	6	
Immature WBC, would refer	5	
Lymphocyte, abnormal/atypical	4	
Abnormal Granulocyte, would refer	1	
Promyelocyte	1	
Auer Rods (myeloblast)	1	
Monocyte, any stage	1	

Total Population **253**

Intended result: Lymphocyte, normal

Specimen 4 was not evaluated due to lack of peer consensus.
16 of 21 Referees corectly identified
the intended result of Lymphocyte, normal

Specimen 5

Result	No.	Flag
Nucleated RBC, any stage	234	
Lymphocyte, normal	8	***
Abnormal, would refer	4	
Immature RBC, would refer	3	
Lymphocyte, normal	2	***
Abnormal Lymphocyte, would refer	1	***
Lymphocyte; atypical, Downey, variant	1	***

Total Population **253**

Intended result: Nucleated RBC

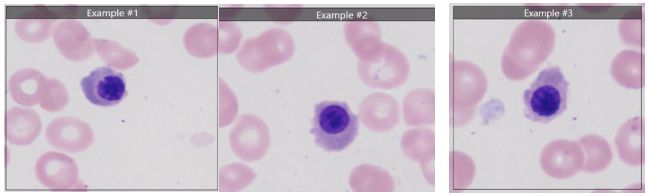
Correct responses are defined as those reflecting agreement among 80% or more of all participants or referees. Unacceptable responses are indicated by "*****" on the Flagging line of each specimen.

Cell Identification - Educational Challenge

Specimen 1

	No.
Nucleated RBC, any stage	169
Immature RBC, would refer	2
Plasma Cell, any stage	2
Lymphocyte, normal	1
Abnormal Lymphocyte, would refer	1
Reticulocyte (supravital stain)	1
Total Population:	**176**

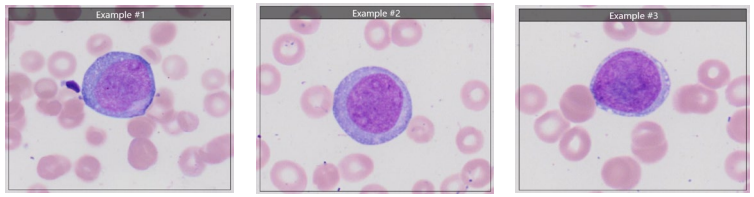
Intended result: Nucleated RBC



Specimen 2

	No.
Blast, undifferentiated	78
Myeloblast	38
Immature WBC, would refer	17
Abnormal, would refer	11
Promyelocyte	5
Monocyte, any stage	4
Immature RBC, would refer	3
Abnormal Lymphocyte, would	3
Myelocyte	3
Plasma Cell, any stage	3
Lymphocyte, abnormal/atypical	2
Monocyte, normal	1
Abnormal Granulocyte, would	1
Metamyelocyte	1
Lymphocyte, normal	1
Lymphocyte; atypical, Downey,	1
Lymphocyte, reactive	1
Megakaryoblast	1
Total Population:	**174**

Intended result: Blast



*To see the original fullsized images, please sign on to your data entry sheet at http://www.aab-pts.org/

Sample 18Q3 - Clinical Discussion

In this educational challenge, a 52-year-old man is brought to the emergency department by his wife following a fall secondary to an episode of dizziness this morning. There was no loss of consciousness. Over the last month, the patient noted increasing fatigue, shortness of breath after performing routine tasks, and bleeding of his gums with tooth brushing. He further states that he has lost approximately 10 lbs. during the past 2-3 weeks. Being a stubborn individual (confirmed by his wife), he refused to seek medical help until the episode this morning.

On physical examination, he is found to have a temperature of 99.8F, accompanied by tachycardia. His gingival mucosa is pale and numerous petechiae are noted on this back and chest, as well as unexplained ecchymoses on his lower extremities. e an elevated blood pressure (168/88 mmHg). Initial CBC results showed: WBC 78.6 x 10^3/µL, Hgb 6.2g/dL, Hct 18.3%, Plts 15,000/µL. Review of the peripheral smear shows a normochromic, normocytic red cell morphology. The subsequent red cell indices showed a normocytic hypochromic anemia; however, examination of the peripheral smear shows marked anisocytosis with a mixture of microspherocytes, normocytic RBCs, and polychromatophilic RBCs, all leading to a "normal" MCV. Basophilic stippling is present as well as the occasional giant platelet.

The cells to be identified are nucleated RBCs and blasts. **Nucleated red blood cells** (NRBC), or orthochromic normoblasts, represent the last stage of RBC differentiation prior to extrusion of the nucleus and are normally confined to the bone marrow. The NRBC has a round nucleus with tightly condensed chromatin without nucleoli; this pyknotic nucleus is undergoing degeneration prior to being expelled with resulting formation of a reticulocyte. The thin rim of cytoplasm is gray-pink to pink, consistent with the cytoplasmic coloration of a mature RBC. NRBCs may be confused with small mature lymphocytes. Both have round nuclei with tightly condensed chromatin; however, the cytoplasm of a lymphocyte is typically blue to blue-gray and may contain azurophilic granules. The nucleus of an NRBC may be eccentrically placed if nuclear extrusion will occur soon. In this instance, the NRBC may be confused with a plasma cell, which also has an eccentrically placed nucleus. However, much like the lymphocyte, the cytoplasm of a plasma cell is blue and the nuclear chromatin is not as condensed as is seen with the NRBC. Most hematology analyzers are able to differentiate WBCs from NRBCs and will "flag" the results for NRBCs. Older analyzers, however, may not consistently flag NRBC levels of <5%. The number of NRBCs should be assessed based on review of the peripheral smear and are reported as the number of NRBCs per 100 WBCs.

Except during the neonatal period, the presence of NRBCs in the peripheral blood is generally considered abnormal. Although a rare NRBC may be seen during pregnancy, the numbers of NRBCs present on this peripheral smear signify bone marrow damage or stress. With severe anemia, such as seen in this male patient, the reduced hemoglobin concentration leads to reduction in the oxygen-carrying capacity of the blood. This results in increased production of erythropoietin by the kidneys, which is turn stimulates the bone marrow to increase its erythropoietic activity. This accelerated compensatory activity leads to the release of NRBCs into the peripheral blood.

Blasts are also present on the peripheral smear and outnumber the normal component of WBCs. These cells vary in size; however all are much larger than adjacent neutrophils, lymphocytes, or monocytes. The blasts have blue, agranular cytoplasm and an increased nuclear-to-cytoplasmic ratio. The nucleus is composed of fine immature chromatin and contains one or more nucleoli. It can be very difficult, if not impossible, to determine whether these are myeloblasts or lymphoblasts. With the exception of finding Auer rods in the cytoplasm – diagnostic of myeloid differentiation – immunophenotyping, special stains, and/or cytogenetic studies are typically necessary to make a final diagnosis. In this patient, the circulating blast count is high enough to perform many of these studies; nonetheless, most physicians prefer to obtain a bone marrow biopsy to assess the degree of marrow involvement.

The presence of a large number of blasts (>20%) in the peripheral blood, along with elevated NRBCs is consistent with acute leukemia. The differential diagnosis depends on the primary stem cell defect in the bone marrow and includes acute myelocytic leukemia (AML) and acute lymphoblastic leukemia (ALL). In AML, the stem cell defect occurs in the myeloid progenitor cell, while in ALL the defect occurs in the lymphoid progenitor cell. The neoplastic cells slowly replace the normal cells in the bone marrow, leading to pancytopenia (decreased platelets, RBCs, and WBCs). Bone marrow aspirate and biopsy with flow cytometric analysis would be the next step for this patient in determining the final diagnosis.

The patient was ultimately diagnosed with **acute myelocytic leukemia (AML)**. However, given the lack of a prior history of a myeloid disease, it was not clear whether this was a de novo AML or secondary AML arising from myelodysplastic syndrome (MDS). Myelodysplastic syndromes and AML exist along a continuum, with the disease spectrum beginning with MDS and ultimately progressing to AML, which is often aggressive and ultimately fatal. Approximately one-third of all MDS cases evolve to become AML. The majority of patients with MDS-AML are not cured with conventional therapies.

AML is one of the most common acute leukemia affecting adults and is more common in men than in women. It is also diagnosed in the very young. The signs and symptoms of AML are the result of the cytopenias, and include fever, easy bruising, bleeding from the nose and gums, petechiae, frequent infections, lethargy, fatigue, pallor, bone pain, shortness of breath, and weight loss. The etiology of most cases of AML is unknown; however, it has been shown to be associated with exposure to radiation, certain chemotherapy agents, and repeated exposure to specific chemicals, such as benzene and fluorocarbons (found in petroleum products and cigarette smoke). A genetic predisposition has also been identified. An individual with Down Syndrome has a 10- to 18-fold increase in risk for developing AML.

This is in contrast to MDS, which is considered a disease of aging; it rarely occurs in individuals younger than 60 years of age. In early-stage MDS, no symptoms may be present, and when symptoms do occur, they vary from individual to individual. The more common symptoms mimic those seen with AML – fatigue, fever, easy bruising, weight loss, weakness, shortness of breath, and frequent infections. Risk factors for MDS include smoking, prior chemotherapy or radiation, exposure to high levels of radiation (e.g., nuclear reactor accident), and long-term exposure to certain chemicals such as benzene. Like AML, specific inherited disorders are associated with development of MDS. These include Fanconi anemia, severe congenital neutropenia, and Shwachman-Diamond syndrome. There are several types of MDS and they are classified based upon the cytology of cells in the blood and bone marrow, immunophenotyping, cytochemistry, and cytogenetics.

As the bone marrow is replaced with dysfunctional cells, the patient becomes pancytopenic and is often dependent on transfusions to survive. The standard of care for patients with MDS is the use of hypomethylating agents (drugs that inhibit DNA methylation), such as azacitidine and decitabine. Although patients may initially respond to these agents, the defective stem cells in the bone marrow are not eliminated and eventually become resistant to treatment and relapse. The eventual outcome is death or progression to AML. The risk of developing AML depends on the type of MDS present at the time of diagnosis.

There are several subtypes of AML and treatment and prognosis varies depending on the subtype. Since AML is an acute process, death occurs within weeks to months if left untreated. Initial treatment is chemotherapy with agents such as cytarabine, daunorubicin, or idarubicin, the goal being to induce remission. If these therapies fail, bone marrow and peripheral blood stem cell transplants have been used and there are several clinical trials available using various drug combinations or new drugs. For older patients (>70-75 yrs) or those with cardiac or renal morbidities, alternate chemotherapeutic agents must be considered with a lower toxicity potential.