



PARTICIPANT STATISTICS

CELL IDENTIFICATION

SECOND QUADRIMESTER 2011

Folate Deficiency Q2-2011

The patient is homeless, 26 years old and 5 months pregnant. She presents with profound fatigue and nausea. Her initial CBC reveals a macrocytic anemia, WBC = 8.2, RBC=2.04, Hgb=7.3, Hct=24.6, MVC=120 and platelets=181,000. Examination of her blood smear adds additional information regarding her anemia. Moderate-marked poikilocytosis, teardrop shaped RBCs, macro-ovalocytes and large, hypersegmented PMNs as well as an occasional 3-lobed eosinophil are seen. This patient, because of her living situation and her pregnancy and her presenting blood picture of a macrocytic anemia, was presumptively diagnosed as suffering from megaloblastic anemia presumably due to inadequate dietary intake of folate.

The normal serum folate is between 6 and 20 ng/ml. When a person stops ingesting folate, their serum folate level can fall below 3 ng/ml, within 3 weeks. However, tissue stores are not exhausted until many weeks later. Conversely when folate deficient patients begin to eat normally, folate levels rise before the tissue stores are replaced. The rapidity with which serum folate levels change with diet diminishes the value of serum folate measurements as a test for clinically significant folate deficiency.

The amount of folic acid in the average diet is not much in excess of the nutritional requirements. Because body folate reserves are relatively meager, folic acid deficiency develops frequently in persons with inadequate diets. It takes only 3 to 4 months for a person to become folate deficient after intake is interrupted. Foliates are present in fresh or lightly cooked green leafy vegetables, fresh fruits, beans, eggs, meats, especially liver and kidney. The normal daily requirement is 50 to 100 ug, increasing to 400 ug in pregnancy, where adequate folate is critical to the normal development of the fetus and insufficient folate will increase the risk that the fetus will develop spina bifida or other neural tube defects. To avoid this risk, folate supplements are an important part of a standard prenatal vitamin regime.

As just mentioned, folates are found in fresh green vegetables and fresh fruits and fruit juices. The word fresh cannot be over emphasized. Folate is lost rapidly during cooking. Heating, during canning, may also destroy folate. Dietary folate intake varies widely in different socioeconomic groups, reflecting both the content of the diet and manner of preparation.

Although folate deficiency occurs primarily as a result of dietary deficiency, other, less common causes include, increased folate requirements, for example in the pregnant or lactating patient and patients undergoing hemodialysis. Folate absorption occurs preferentially in the upper small intestine. Plasma folate circulates only loosely bound to albumin and is taken up by all tissues but with particular avidity by the liver. The liver releases the stored folate for use by other tissues but only by a circuitous route that involves its excretion into the bile and reabsorption from the small intestine into the plasma. Knowing this, it is not surprising that, in addition to dietary deficiency and increased requirements, another cause of folate deficiency are intestinal disorders. These disorders, especially those which affect extensive areas of jejunal absorptive surface, frequently result in the patient becoming folate deficient. Such disorders include gluten enteropathy (nontropical sprue), tropical sprue, lymphoma, scleroderma, amyloidosis, Crohn's disease, Whipple's disease and extensive surgical resection of the small intestine. In addition, certain antiseizure drugs and oral contraceptives have also been reported to impair normal folate absorption. People who have trouble absorbing folic acid take supplements for life.

Folate deficiency and the resulting megaloblastic anemia are frequently seen in patients with chronic liver disease. In these patients the cause of low folate is a combination of poor diet and the fact that when the body is given alcohol, release of folate from liver cells into the bile is impaired, the enterohepatic circulation of folate is interrupted and plasma folate levels begin to fall.

Folate is an inclusive term for folic acid and its derivatives. Folic acid is a molecule made up of a pteridine double ring attached to para-aminobenzoic acid (PABA), which, in turn is attached to from one to nine molecules of glutamic acid. Foliates participate in carbon transfers and are required for several biochemical reactions that occur in normal metabolism including two that are critical to normal DNA synthesis. Vitamin B12 and folic acid are coenzymes that both have essential roles in DNA synthesis. Deficiencies of either or both of these coenzymes effectively blocks or delays cell division, abnormally affecting bone marrow cells and other proliferating cells, such as those lining the GI tract.

Due to abnormal DNA synthesis, the cell nucleus takes longer to mature than does the cytoplasm. However, since RNA synthesis and metabolism are unaffected, the cells have normal cytoplasmic maturation. The resulting abnormal cells are described as having asynchronous nuclear-cytoplasmic maturation meaning that they have mature appearing cytoplasm and less mature nuclei. Additionally, the delayed cell division results in the cells being much larger in size than are normal cells. These abnormal, immature RBCs are termed megaloblasts (megalos meaning large). Megaloblasts are considered to be functionally and morphologically abnormal normoblasts. Megaloblastic anemia is characterized functionally by a striking degree of destruction of developing RBCs within the bone marrow. This destruction is termed *ineffective erythropoiesis*. Iron is ineffectively utilized by the abnormally developing RBCs resulting in abnormal iron accumulation in storage cells in the bone marrow.

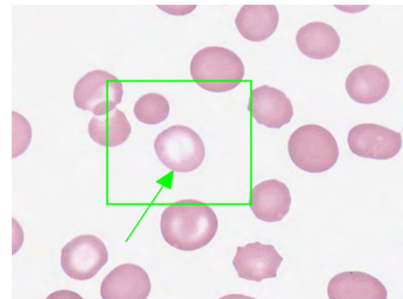
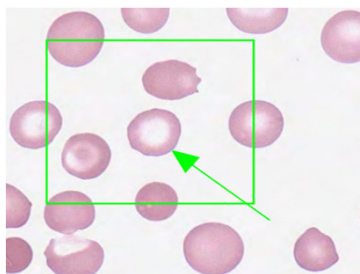
Defective DNA synthesis also can cause ineffective formation and maturation of leukocytes resulting in the large, hyper-segmented PMNs typically seen in the blood of these patients. These abnormal RBCs and WBCs occur with deficiencies of both Vitamin B12 and folic acid. The megaloblastic anemias which results from either cobalamin deficiency (Vit B12) or of folate deficiency look morphologically identical in the blood of the untreated patient

| Specimen 1 | | Specimen 2 | | Specimen 3 | | Specimen 4 | | Specimen 5 | |
|---------------------------------|----------|--|----------|-------------------------------|----------|------------------------|----------|------------------------------------|----------|
| Code - Result | No. Flag | Code - Result | No. Flag | Code - Result | No. Flag | Code - Result | No. Flag | Code - Result | No. Flag |
| 615-Hypersseg. Neutrophil | 645 | 742-Monocyte, any stage | 307 | 194-Teardrop Cell (dacrocyte) | 661 | Eosinophil, any stage | 672 | 170-Macrocytic | 451 |
| 430-Seg. Neutrophil (PMN, poly) | 27 *** | 200-Band Neutrophil (stab) | 186 | 170-Macrocytic | 4 *** | PMN or Band with Toxic | 1 *** | 166-Elliptocyte/Ovalocyte | 140 |
| 875-Abnormal Gran, would refer | 1 | 430-Segmented Neutrophil (PMN, | 48 | 192-Target Cell (codocyte) | 2 *** | Spherocyte | 1 *** | 168-Hypochromic | 50 |
| 100-Abnormal, would refer | 1 | 640-PMN with Pelger-Huet Nucleus | 41 | 742-Monocyte, any stage | 2 *** | Total Population: | 674 | 190-Stomatocyte | 5 *** |
| Total Population: | 674 | 100-Abnormal, would refer | 35 | 870-Abnormal RBC, would refer | 2 | | | 192-Target Cell (codocyte) | 2 *** |
| | | 630-PMN with Toxic Gran | 13 | 100-Abnormal, would refer | 1 | Intended result was | | 870-Abnormal RBC, would refer | 2 |
| Intended result was | | 875-Abnormal Gran, would refer | 13 | 166-Elliptocyte/Ovalocyte | 1 *** | Eosinophil | | 186-Spherocyte | 1 *** |
| Hypersegmented Neutrophil | | 334-Metamyelocyte | 11 | 620-PMN with Dohle Bodies | 1 *** | | | 210-Basophil, any stage | 1 *** |
| | | 713-Lymph, (atypical, Downey) | 3 | Total Population: | 674 | | | 742-Monocyte, any stage | 1 *** |
| | | 712-Lymphocyte, normal | 2 | Intended result was | | | | Total Population: | 674 |
| | | Total Population: | 674 | Teardrop Cell (dacrocyte) | | | | | |
| | | Due to a lack of participant consensus | | | | | | Intended result was | |
| | | Specimen 2 was not graded. | | | | | | Elliptocyte/Ovalocyte or Macrocyte | |
| | | Intended result was | | | | | | | |
| | | Monocyte | | | | | | | |

EDUCATIONAL CHALLENGES

Specimen 1
 Hypochromic
 Erythrocyte, normal RBC
 Elliptocyte/Ovalocyte
 Spherocyte
 Abnormal, would refer
 Basophilic Stippling
 Parasites
 Total Population:
 Intended result was
 Hypochromic RBC

No.
 292
 9
 2
 2
 1
 1
 1
308



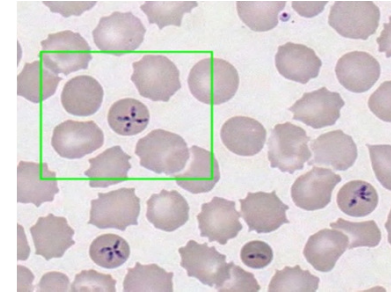
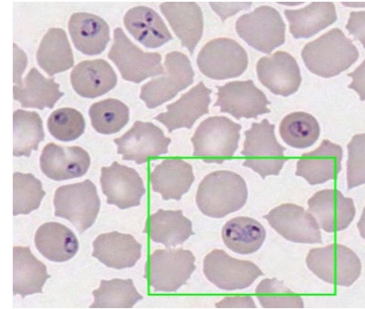
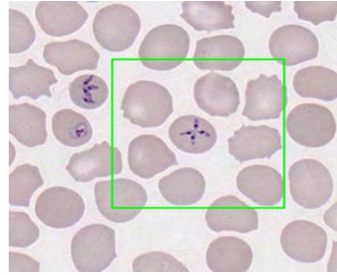
*To see the original full-sized images, please refer to the original CD or sign on to your data entry sheet at <http://www.aab-pts.org/>

Specimen #1: History: A 64-year-old man is seen in the Emergency Department complaining of fatigue, heart palpitations, nosebleeds, and a fever. He recently completed three courses of chemotherapy for renal cancer. On physical examination, he is tachycardic and febrile (temperature 101.4F). Numerous petechiae are present on the oral mucosal membranes as well as on his lower legs. CBC results: WBC 0.8, Hgb 6.1 g/dL, Hct 18.1%, Plts 12,000/ μ L. Identify the indicated cells.

The automated CBC results show a marked decrease in the WBC count, accompanied by anemia and thrombocytopenia. These findings are confirmed upon review of the peripheral blood smear; only a rare WBC and platelet are seen. The red blood cells to be identified are all **hypochromic**. In addition, there is moderate aniso- and poikilocytosis with the presence of spherocytes, target cells, red cell fragments, and polychromasia.

This patient with pancytopenia was diagnosed with **aplastic anemia** secondary to chemotherapy. Although myelosuppression is predictable with some types of chemotherapy agents, the damage to bone marrow stem cells can be severe enough to result in transient marked depletion of all cell lines. This effect is dose-dependent. The reduction in numbers of platelets can lead to bleeding of the gums, easy bruising, nosebleeds, and petechiae. Low numbers of WBCs predisposes the patient to frequent infections, and the anemia results in fatigue, weakness, heart palpitations and tachycardia, and shortness of breath with exertion. Care of the patient is supportive and involves transfusion of RBCs and platelets; granulocyte transfusions are controversial. Prevention and treatment of infections is vital until the bone marrow recovers.

| Specimen 2 | No. |
|-------------------------------------|-----|
| Parasites | 232 |
| Abnormal, would refer | 8 |
| Basophilic Stippling | 3 |
| Hypochromic | 3 |
| Spherocyte | 2 |
| Abnormal RBC, would refer | 2 |
| Pappenheimer Bodies | 1 |
| Schistocyte (bite, blister, helmet) | 1 |
| Blast, undifferentiated | 1 |
| Total Population: | 253 |
| Intended result was | |
| Parasites | |



*To see the original full-sized images, please refer to the original CD or sign on to your data entry sheet at <http://www.aab-pts.org/>

Specimen #2: History: A 62-year-old man is brought to the Emergency Department via ambulance after "passing out" at home. His wife accompanies him and says he was relatively well until 2 weeks ago when he complained of muscle and joint pains and being tired; he has lost 8 lbs since then. His past medical history is remarkable for a splenectomy at age 52 following a motor vehicle accident, and a kidney transplant 2 years ago for which he takes immunosuppressive therapy. Approximately 3 weeks ago, he spent a week camping with his son in the woods of New Jersey. On physical examination, the patient appears acutely ill, pale, febrile, hypotensive and tachycardic. CBC results: WBC 8,000/ μ L, Hgb 5.6 g/dL, Hct 15.8%, Plts 102,000/ μ L. Identify the indicated cells

The automated CBC results show marked anemia, mild thrombocytopenia, and a relatively normal WBC count. Review of the peripheral smear confirms the thrombocytopenia and neutrophils show prominent toxic granulation. The most striking findings are seen in the red blood cells, a large percentage of which contain inclusions. These inclusions are intracellular parasites, specifically *Babesia microti*. This parasite is most often transmitted through the bite of the deer tick, *Ixodes scapularis*, although it can also be transmitted by blood transfusion and transplacental passage to a fetus. This is the same tick that transmits the organism responsible for Lyme disease.

The two most common intracellular RBC parasites are *Babesia* and the *Plasmodium* species that cause malaria. Key features that point to infection with *Babesia* are:

- Multiple ring forms (trophozoites) with one cell;
- Ring forms are typically delicate, but range in size from small to large;
- Trophozoites can have a clear or white vacuole within the ring (see Cell #2);
- Extracellular forms can be present, typically as small groups or sheets of parasites (see area in the vicinity of Cell #3, in the upper left-hand corner of the slide);
- Merozoites form a tetrad (Maltese-cross form) within the cell – this is virtually pathognomonic of *Babesia* (see Cells #1 and #3);
- No pigment is produced.

Babesiosis is endemic in the northeastern United States, parts of the Midwest, and California. Individuals most at risk for infection are the elderly, immunocompromised, and those without a spleen (recall that this patient was taking immunosuppressive medication as well as being asplenic). Patients older than 50 years are most at risk of developing a severe infection and dying from the disease. Many infected individuals do not remember being bitten by a tick, but do give a recent travel history to an endemic area. The incubation period ranges from one to four weeks.

Once the tick bites, parasites are injected into the bloodstream and subsequently infect the red blood cells where they multiply. They then cause rupture of the red cell membrane, leading to a hemolytic anemia, and go on to infect other RBCs. Thrombocytopenia is not uncommon. Signs and symptoms include chills, muscle aches, fever, weight loss, fatigue, night sweats, cough, and nausea and vomiting. The degree of parasitemia does not necessarily correlate with the severity of illness; however, individuals without a spleen are at risk for high levels of parasitemia and are considered a medical emergency.

Treatment for severely infected patients includes clindamycin and quinine. More recently, atovaquone and azithromycin have been shown to be an acceptable alternative. In some patients, red blood cell exchange transfusion may be necessary to decrease the parasite load and resultant hemolysis.