

## **ANEMIA: AN APPROACH TO DIAGNOSIS**

"I want to say in a single sentence what it takes books for other philosophers to say"- Frederich Nietzsche

### **General Principles**

1. Anemia is a sign, not a disease.
2. Anemias are a dynamic process.
3. Although the elderly are more prone to anemia, being elderly is not a cause of anemia.
4. The diagnosis of iron deficiency anemia mandates further work-up.

### **Initial Work-up**

1. Good H & P-Ask about blood loss, duration of anemia, family history of anemia, medication use etc. The exam should include a careful search for splenomegaly, blood in the stool, etc...
2. A careful review of the peripheral smear will often reveal many diagnostic clues, especially in the complex patient.
3. The reticulocyte count provides insight into whether a marrow problem is involved or if the anemia is due to blood loss or destruction.
4. Armed with the above knowledge one can then order specific tests further to explain the etiology of the anemia.
5. Given the frequency of iron and B<sub>12</sub> deficiency and the non-specificity of RBC indices, serum ferritin and methylmalonic acid should be checked in all anemic patients.
6. A word should be mentioned about the RDW. Once touted at the diagnostic panacea for anemia, more recent studies have shown it is of little help in the diagnosis of thalassemia, iron, folate or B<sub>12</sub> deficiency, myelodysplasia, or the anemia of chronic disease (ACD).

### **Reticulocyte Count**

The reticulocyte count is a measure of the new cells the marrow is producing. Since you turn over about 1% of your red cells daily, to maintain a steady hematocrit your reticulocyte count should be 1%. The reticulocyte count has to be adjusted for the hematocrit since a retic 1% at a hematocrit of 45% is the same as one of 4.5% at a hematocrit of 10%. Several ways exist to do this:

**Absolute retic count:** % retic x red cell count (normal is 50 - 85,000/mm<sup>3</sup>)

**Corrected:** retic % x (patient's hct/45)

Increased reticulocytes (greater than 2-3% corrected reticulocyte count or 100,000/mm<sup>3</sup> total) are seen in blood loss and hemolytic processes, although up to 25% of hemolytic anemias will present with a normal reticulocyte count due to immune destruction of red cell precursors and/or folate deficiency. The key idea is that if a patient has a hematocrit of 10% and a "normal" absolute reticulocytes count this is abnormal given the situation. Retic counts are most helpful if extremely low (<0.1%) or greater than 3% (100,000/mm<sup>3</sup> total).

### **Anemia: Etiologies**

1. Production defects:
  - A. Nutritional deficiencies-Vitamin B<sub>12</sub>, folate, copper, or iron deficiency.
  - B. Inflammation/chronic disease.
  - C. Primary marrow disorders-pure red cell aplasia, myelodysplasia.
2. Sequestration (hypersplenism)-usually associated with mild pancytopenia.
3. Dilutional-common in hospitalized patients. A patient's plasma volume increases with lying down and when they quit smoking. Possibly responsible for as much as a 3-6% drop in the hematocrit in the first two days of hospitalization.

4. Blood loss.
5. Blood destruction.

## **IRON DEFICIENCY**

In adults the most common cause of iron deficiency is blood loss. In men and in post-menopausal women the source of the blood loss is most often the gastrointestinal track with cancer being found in 10-15%. Patients who have had bariatric surgery can have impaired iron absorption. Iron deficiency, especially refractory to iron therapy, can be a clue to celiac disease that can be seen in up to 1:250 Caucasians. Finally, infection with *helicobacter pylori* is being recognized as a cause of iron deficiency.

### **Iron Deficiency: Diagnosis**

1. RBC indices are of little diagnostic value unless the MCV is below 70fl which is only seen in iron deficiency and thalassemia.
2. Serum iron can be decreased in a variety of states including iron deficiency, inflammation, and stress. The serum iron level varies tremendously from morning to evening and from day to day. The minuscule amount of iron in a multivitamin can falsely elevate the serum iron for up to 24 hours.
3. The total iron binding capacity is very specific for iron deficiency (near 100%) but has poor sensitivity (less than 30%).
4. The iron saturation (Fe/TIBC x 100) can be decreased below 16 percent in *both* anemia of chronic disease and iron deficiency and is of little help in distinguishing between the two.
5. In the normal patient the serum ferritin is directly correlated with iron stores. This relationship holds true even in inflammatory states although the curve is "shifted to the left." That is, for a given level of storage iron in a patient with an inflammatory state the serum ferritin is higher. A ferritin level of greater than 100ng/ml rules out iron deficiency anemia in most patients. One good rule of thumb is that in patients more than 65 years of age a ferritin below 50 ng/ml is associated with iron deficiency. The measurement of the serum ferritin is the most useful and cost-effective test of iron stores.

The most efficient approach to detecting iron deficiency is to perform a serum ferritin. If it is more than 100 ng/dl, this eliminates iron deficiency. Very low values are diagnostic of iron deficiency. Although laboratories will often state that ferritins of over 12-36 ng/dl are in the "normal range" it is important to remember that many older patients may be iron deficiency with ferritin in the 50-80 ng/dl range.

Oral iron is the best treatment option. The gut can only absorb so much iron so there is no utility in taking more than one pill per day. Taking iron with some food can help with GI tolerance. Meals that contain meat will double iron absorption. Vitamin C 500 units can also help absorption. Tea within one hour of oral iron should be avoided as this will decreased absorption.

Some patients cannot replete their iron stores with oral iron and will benefit from intravenous iron. The most expedient is 1000mg of iron dextran over several hours but multiple other preparations are on the market.

With effective iron therapy, the reticulocyte count should rise in one week and the hematocrit should increase by 3% in two weeks. Unless the cause of the blood loss is obvious, all patients with iron deficiency should undergo a gastrointestinal evaluation. In older patients studies show that 50% will have an identifiable source of blood loss and that 10-15% of iron deficiency patients will have colon cancer. In patients with iron deficiency that is resistant to iron therapy one should check anti-glidian and anti-endomyoseil antibodies to check for celiac disease and for *h. Pylori*. Recently achlorhydria due to anti-parietal cell antibodies has been implicated in refractory iron deficiency.

### **Microcytic Anemia: Differential Diagnosis**

1. Iron Deficiency. The lack of iron results in decreased hemoglobin available to the developing red cell. Thus, the erythrocytes produced are underhemoglobinized which result in smaller cells. The earliest sign of iron deficiency is decreased iron stores. This stage has a normal CBC and indices, although one can see microcytic/hypochromic cells on the smear. The anemia gradually evolves into the classic microcytic- hypochromic anemia. Diagnosis is made by showing decreased iron stores on bone marrow examination. Biochemically the diagnosis is established by a high TIBC or a low ferritin. The major diagnostic difficulty is distinguishing iron deficiency from anemia of chronic disease.

**2. Anemia of Chronic Disease.** (anemia of defective iron utilization). In patients with inflammatory states iron is sequestered in the RE system and is unavailable for use by the developing red cell (defective iron utilization). Thus at the erythrocyte level the defect is identical to iron deficiency and therefore results in production of underhemoglobinized red cells. This can result in a microcytic/hypochromic anemia. Increase production of the protein hepcidin is response for the multiple changes in iron metabolism seen in inflammation. Additional factors including shorten red cell survival and decreased levels of erythropoietin add to the hypoproliferative state. The inflammatory state also leads to a decreased serum iron and decreased TIBC.

Recently the spectrum of diseases associated with anemia of chronic disease has expanded. Besides the classic association of temporal arteritis (may be a presenting sign), rheumatoid arthritis, cancer etc., anemia of chronic disease has been found in patients with non-inflammatory medical conditions such as congestive heart failure, COPD and diabetes. Patients with anemia of chronic disease can have hemoglobins decreased into the lower 20% range and many (20-30%) will have red cell indices in the microcytic range.

Diagnosis is made by proving ample bone marrow iron stores with decrease sideroblasts (iron containing red cell precursors). Biochemically anemia of chronic disease remains a clinical diagnosis of exclusion with the key test is to rule out iron deficiency. The serum erythropoietin level is inappropriately low vales when compared with the hematocrit. The serum iron is decreased in both conditions and the TIBC is low in states where iron deficiency and chronic disease co-exists thus rendering these tests useless. The finding of an elevated ferritin over 100ng/ml is an adequate demonstration of good iron stores. In the older patient or one with back pain, one should also rule-out the presence of multiple myeloma by performing a serum protein electrophoresis. In difficult cases one can resort to assessing bone marrow stores of iron. In the future assays of hepcidin levels will be helpful as this protein is the chemical mediator of the anemia of chronic disease.

There is no specific therapy for the anemia of chronic disease except treating the underlying disorder. Patients with low erythropoietin levels who are symptomatic may response to erythropoietin injections with either erythropoietin 40,000/wk or darbopoeitin 300 ug/every 2-3 weeks but this has become controversial in recent years. One should insure there are adequate iron stores before starting growth factors.

**3. Thalassemia.** In this disorder it is the defective production of hemoglobin that leads to microcytosis. The main types are the beta-thalassemia, alpha-thalassemia and Hemoglobin E.

Patients who are heterozygotes for beta-thalassemia have microcytic indices with mild (30ish) anemias. Homozygotes have very severe anemia. Peripheral smears in heterozygotes reveal microcytes and target cells. Diagnosis is established in by hemoglobin electrophoresis that shows an increased HbA<sub>2</sub>. Beta-thalassemia occurs in a belt ranging from Mediterranean countries, the Middle East, India, and Pakistan to Southeast Asia. Patients with beta-thalassemia traits who are of child bearing age need to have their spouse screened for beta-thalassemia and Hemoglobin E.

Alpha-thalassemia also presents with microcytosis. Patients with alpha-thalassemia will have normal hemoglobin electrophoresis. The diagnosis of alpha-thalassemia is made by excluding other causes of microcytosis, a positive family history of microcytic anemia, and a life-long history of a microcytic anemia. Exact diagnosis requires DNA analysis. Alpha-thalassemia is distributed is a similar pattern to beta-thalassemia except it very high frequency in Africa (up to 40%). In patients of African descent the finding of alpha-thalassemia requires no further evaluation. In patients from Asia of child bearing age the spouse should be screened (if need be with DNA analysis) to assess the risk of bearing a child with severe thalassemia.

Hemoglobin E is a beta-hemoglobin chain defect that presents in a similar fashion to the thalassemia. . Hemoglobin E occurs in Southeast Asia, especially in Cambodia, Laos and Thailand. Patients who are heterozygotes are not anemic but are microcytic. Patients who are homozygotes are mildly anemic with microcytosis and target cells. The importance of Hemoglobin E lies in the fact that patients with both genes for Hemoglobin E and beta-thalassemia have severe anemia and behave in a similar fashion to patients with homozygote beta-thalassemia.

Thalassemia	MCV	Hgb	Electrophoresis	Other Features
<b>Beta-Thalassemia</b>				
Major	50-75	< 7	Raised HbA2	Severe anemia,
Intermedia	50-75	< 9	Raised HbA2	Target cells on smear
Trait	65-75	9-10	Raised HbA2	Target cells on smear
<b>Alpha Thalassemia</b>				
Trait-1 ( $\alpha\alpha/\alpha-$ )	80-85	NI	Normal	
Trait-2 ( $\alpha-\alpha-$ ) or ( $\alpha\alpha/--$ )	65-75	12-13	Normal	
Hemoglobin H ( $\alpha-\alpha-$ )	60's	9-8	HgbH	Hemolysis, splenomegaly
Hemoglobin Barts( $--/--$ )	-	-	HgbH, Hbg Barts	Hydrops fetalis
<b>Hemoglobin E</b>				
Heterozygous	80-85	12	HgbE present	Rare target cells on smear
Homozygous	70's	11-12	HgbE predominant	Target cells on smear

**4. Sideroblastic Anemia.** Defective production of the heme molecule is the basis of this disorder. The deficit of heme leads to the underhemoglobinization of the erythroid precursors and microcytosis. Sideroblastic anemia can be congenital, can be due to toxins such as alcohol, lead, INH, or can be an acquired bone marrow disorder. The peripheral smear may show basophilic stippling in lead poisoned patients, a dimorphic (macrocytic and intensely microcytic red cells) in patient with acquired sideroblastic anemia, or stigmata of a myelodysplastic syndrome. Diagnosis is made by the finding of ringed sideroblasts on the bone marrow iron stain. Iron studies in patients with sideroblastic anemia usually show signs of iron-overload.

#### HEMOLYTIC ANEMIAS

The laboratory signs of hemolytic anemias include:

1. Increased LDH (LDH1) -sensitive but not specific.
2. Increased indirect bilirubin-sensitive but not specific.
3. Increased reticulocyte count-specific but not sensitive
4. Decreased haptoglobin-specific but not sensitive.
5. Urine hemosiderin-presence of any is specific but not sensitive.

The indirect bilirubin is proportional to the hematocrit, so with a hematocrit of 45% the upper limit of normal is 1.00 mg/dl and with a hematocrit of 22.5% the upper limit of normal for the indirect bilirubin is 0.5mg/dl. Since tests for hemolysis suffer from a lack of sensitivity and specificity, one needs a high index of suspicion for this type of anemia.

Autoimmune hemolytic anemias (AIHA) are due to red cell destruction by autoantibodies. AIHA may be idiopathic or associated with malignancies, drugs or other autoimmune disorders. The major subgroups are Warm antibody hemolytic anemia (IgG), Cold antibody hemolytic anemia (IgM) and Drug induced. (See Table 1) In patients with AIHA one usually sees microspherocytes on the peripheral smear and splenomegaly may be present on exam. The diagnosis is established by the finding of a positive direct antibody test (direct Coombs). Not all patients with a positive direct antibody test will have AIHA. The direct antibody test will detect IgG and occasionally complement in patients with warm antibody disease. Cold antibody disease will only demonstrate complement and not IgG.

Patients with warm antibody disease should be started on prednisone 60 mg/day and rituximab 1000mg x 2 14 days apart. Rituximab has been shown to improve the durability of steroid remissions. In those who do not respond or require high doses of prednisone splenectomy may induce remission in 50%.

Many patients will require further immunosuppression with agents such as mycophenolate. Treatment of cold antibody disease is difficult as these patients will not respond to steroid or splenectomy. Rituximab has been reported to be effective and should be the initial therapy for symptomatic patients. Drug induced hemolytic anemia requires stopping the implicated drug. All patients with hemolysis can become folate

deficiency so folate replacement should be given to all.

Microangiopathic hemolytic anemias are due to mechanical destruction of red cells. The most common associated diseases are disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, hemolytic-uremic syndrome, valvular disease, or the presence of an artificial heart valve. One sees schistocytes in the peripheral smear and an elevated LDH. The exact cause of the microangiopathic hemolytic anemia is determined by the history and laboratory testing.

Paroxysmal nocturnal hemoglobinuria is an acquired hemolytic anemia that is due to a clonal proliferation of erythrocytes abnormally sensitive to the action of complement. Hemolysis may be more conspicuous at night leading to the characteristic hemoglobinuria. Patients with paroxysmal nocturnal hemoglobinuria demonstrate the routine lab abnormalities of hemolysis such as a very high LDH. The diagnosis is made by performing flow cytometry to demonstrate the lack of glycosyl phosphatidylinositol-anchored proteins. Patients are often pancytopenic and can present with aplastic anemia. Patients with PNH also have a high incidence of thrombosis including visceral vein thrombosis. Treatment consists of giving the C5 complement inhibitor eculizumab or the longer acting ravulizumab.

### **CONGENITAL HEMOLYTIC ANEMIA**

Three fundamental processes lead to congenital hemolytic anemia: 1) membrane defects, 2) hemoglobin defects, or 3) enzyme defects.

One of the most common congenital causes of hemolysis is hereditary spherocytosis. In this disease the red cell membrane is abnormal leading to increased splenic destruction. Patients often have a family history of gallstones. Another rare cause of hereditary hemolysis due to membrane defects includes hereditary elliptocytosis. The diagnosis of hereditary spherocytosis is made by finding that spherocytes are present on the peripheral smear and splenomegaly is present on exam. The laboratory values are consistent with hemolysis and the MCHC is elevated. The diagnosis is established by the finding of increased osmotic fragility or demonstrating Band 3 defects.

The most common hemoglobin defect is sickle cell anemia. In this disease the abnormal hemoglobin leads to destruction of the red cell. Diagnosis is established by hemoglobin electrophoresis. Patients may also have chronic hemolysis due to unstable hemoglobins. These patients will often have "Heinz" bodies present on a specially stain blood smear and may have an abnormal hemoglobin electrophoresis.

Enzyme deficiencies such as glucose-6-phosphate dehydrogenase deficiency are also important causes of hereditary hemolytic syndromes. The same population at risk for thalassemia is also at risk for G-6-PD deficiency. It is sex linked and thus only affects males. This defect is in the hexose monophosphate shunt and renders the RBC to be unable to withstand oxidative stress. Most people with this disease have hemolysis only with such stressors as infections and intake of oxidative drugs. There are two main subtypes-African (A-) and Mediterranean that tends to be more severe. Such drugs as dapson, pyrimethoprine, pyridium, and sulfamethoxazole may provoke severe hemolysis in these patients.

### **MACROCYTOSIS**

An increased MCV can be due to many reasons but careful review of the patient's history and blood smear can narrow the diagnostic possibilities. The differential can be divided into two broad categories based on RBC morphology.

Round macrocytosis-due to abnormal lipid composition of the erythrocyte membrane. Common etiologies include:

1. Alcoholism.
2. Liver Disease.
3. Renal Disease.
4. Hypothyroidism ("myxedema of the red cell").

Oval macrocytosis (macroovalocytes) is a sign of problems with cell DNA replication. The

developing red cell has difficulty in undergoing cell division but RNA continues to be translated and transcribed into protein leading to growth of the cytoplasm while the nucleus lags behind. Often one or more cell divisions are skipped leading to a larger than normal cells. Common causes are:

1. Drug effect including cytotoxic chemotherapy
2. Megaloblastic Anemias-Folate Deficiency or Vitamin B<sub>12</sub> deficiency - Patients will have hypersegmented neutrophils on review of the peripheral smear.
3. Myelodysplasia - Patients have often **hypos**segmented neutrophils and abnormal platelet morphology.

Patients with RBC autoantibodies or cold agglutinins can have a spurious increase in the MCV due to red cell clumping in the automatic counters. Patients with increased reticulocyte counts can also have an increase MCV due to the large size of the reticulocyte (MCV = 160).

### **ABSORPTION AND METABOLISM OF VITAMIN B<sub>12</sub> AND FOLATE**

Folate-The body stores very little folate (four weeks) and maintenance of folate stores is dependent on an adequate dietary intake. Folate is found in green leafy vegetables, fruits and liver. Folate is absorbed in the small bowel and circulates in a free form or loosely bond to albumin. Due to mass supplementation of food, folate deficiency now very rare.

Vitamin B<sub>12</sub>- In contrast to folate the body stores copious amounts of vitamin B<sub>12</sub> (2-6 years). This is fortunate as the absorption of vitamin B<sub>12</sub> is complex and can be interrupted by a variety of mechanisms. Vitamin B<sub>12</sub> is synthesized by microbes and the major dietary source is animal protein. When animal protein is ingested, vitamin B<sub>12</sub> is freed from the protein and binds to "R proteins". The R protein-vitamin B<sub>12</sub> complex travels to the duodenum where pancreatic enzymes destroy the R protein. This allows intrinsic factor (IF) to bind to vitamin B<sub>12</sub>. This IF-vitamin B<sub>12</sub> complex is absorbed only in the last 1-2 feet of terminal ileum. Vitamin B<sub>12</sub> binds to transcobalamin II and is delivered to tissues.

### **VITAMIN B<sub>12</sub> AND FOLATE- METABOLIC PATHWAYS**

Both vitamin B<sub>12</sub> and folate are key components in the synthesis of DNA due to their role in conversion of uridine to thymidine. When methyltetrahydrofolate loses a methyl group to form tetrahydrofolate, vitamin B<sub>12</sub> "shuttles" the methyl group to homocysteine converting it to methionine. Tetrahydrofolate is eventually converted to methylenetetrahydrofolate required for thymidine synthase. Vitamin B<sub>12</sub> other role is a cofactor in the conversion of methymalonyl-CoA to succinyl-CoA.

### **CONSEQUENCES OF VITAMIN B<sub>12</sub> OR FOLATE DEFICIENCY**

When vitamin B<sub>12</sub> or folate is deficient, thymidine synthase function is impaired and DNA synthesis is interrupted. As described above this leads to megaloblastic changes in all rapidly dividing cells. The inability to synthesized DNA leads to ineffectual erythropoiesis. There is often erythroid hyperplasia in the marrow but most of these immature cells die before reaching maturity. This process, intramedullary hemolysis, leads to the classic biochemical picture of hemolysis-raided LDH and indirect bilirubinemia. The LDH level is often in the 1,000's in patients with megaloblastic anemia. The lack of DNA synthesis affects the neutrophils leading to nuclear hypersegmentation. The anemia is of gradual onset and is often very well tolerated despite low hematocrits. Often a mild pancytopenia is seen but thrombocytopenia can be severe.

Other rapidly dividing tissues are influenced by the megaloblastic process. In the GI tract this can lead to atrophy of the luminal lining and further malabsorption.

As discussed further below, only vitamin B<sub>12</sub> deficiency leads to neurological damage. The mechanism is unknown.

### **ETIOLOGIES OF FOLATE DEFICIENCY**

Decreased dietary intake - uncommon in this era

Increased requirements-Patients who are pregnant have hemolytic anemia, or psoriasis have increased needs for folate that can cause them rapidly to develop folate deficiency if intake is not kept up.

Malabsorption

Drugs - Patients with underlying mild folate deficiency are more susceptible to trimethoprim/sulfa,

pyrimethamine and methotrexate toxicity. Oral contraceptive and anticonvulsants lead to increase consumption of folate.

Alcohol- Alcohol affects several aspects of folate metabolism. Alcoholics have a poor intake of folate. In addition, folate metabolism is interfered with leading to a functional folate deficiency. Alcoholics have an inability to mobilize folate stores and can have depleted tissue stores with normal serum levels of folate.

**ETIOLOGIES OF VITAMIN B<sub>12</sub> DEFICIENCY**

Inadequate intake is rare but seen in very strict vegans (no eggs or milk).

Abnormal gastric events include being unable to dissociated vitamin B<sub>12</sub> from food due to lack of stomach acid or enzymes. This is a recently recognized group of patients that may compose a very large subset of patients with vitamin B<sub>12</sub> deficiency. 10-30% percent of patients with partial gastrectomy will develop vitamin B<sub>12</sub> deficiency. The recent promiscuous use of H<sub>2</sub> and proton pump blockers is leading to an increased incidence of patient's not absorbing vitamin B<sub>12</sub>. Finally, infection with H. pylori is also associated with decreased B12 absorption.

Deficient intrinsic factor most commonly occurs due to destruction of parietal cells by autoantibodies (pernicious anemia).

Abnormal small bowel events include pancreatic insufficiency, blind loops syndromes (bacterial absorbing vitamin B<sub>12</sub>-IF complexes) and patients infested with *Diphyllobothrium latum*. Metformin blocks B<sub>12</sub> absorption at this step.

Abnormal mucosal events including malabsorption syndromes and surgical removal of the terminal ileum.

**APPROACH TO THE PATIENT WITH AN MEGALOBLASTIC ANEMIA**

1. Recognizing that a megaloblastic anemia is present.
2. Diagnosing vitamin B<sub>12</sub> and/or folate deficiency
3. Determining the underlying cause.
4. Therapy

**DIAGNOSING VITAMIN B<sub>12</sub> AND/OR FOLATE DEFICIENCY**

When a patient is believed to have a megaloblastic anemia or a process consistent with vitamin B<sub>12</sub> deficiency, one should draw a serum methylmalonic acid, a serum homocysteine level (a more sensitive indicator of tissue stores than serum or red cell folate) and since up to 30% of patients with megaloblastic anemia have concurrent iron deficiency, a serum ferritin.

Recent data suggests that B<sub>12</sub> levels may not be accurate especially in older patients. Many patients will have low B<sub>12</sub> levels but not tissue deficiency. Up to 15% of patients with normal B<sub>12</sub> levels will have tissue deficiencies. Measuring serum levels of methylmalonic acid, a metabolic precursor that is increased in B<sub>12</sub> deficiency, is now the preferred method of diagnosis especially in older patients. Many patients who are B<sub>12</sub> deficiency will also have elevated serum homocysteine levels. Patients with high homocysteine levels should have a methylmalonic acid drawn to insure that they do not have B<sub>12</sub> deficiency.

**Folate deficiency**

Like B<sub>12</sub> levels, serum folate levels do not reflect body stores of folate. Serum homocysteine levels are more accurate.

	B <sub>12</sub> Deficiency	Folate Deficiency
Methylmalanoic acid <0.4 umol/L	Elevated	Normal
Homocysteine 4-12 umol/L	Elevated	Elevated

## **VITAMIN B<sub>12</sub>- NEUROLOGICAL CONSEQUENCES**

Recently it has become clear the patients can have neurological damage due to vitamin B<sub>12</sub> deficiency without anemia. In fact as many as 30% of patients with neurological disease due to vitamin B<sub>12</sub> deficiency will have no or only subtle hematological symptoms. Patients with the most severe neurological manifestation often have mild hematological disease. Thus it is appearing that vitamin B<sub>12</sub> deficiency may exhibit two different types of disease states in humans - hematological or neurological. Neurological symptoms are reversible if found early but those present for over a year slowly, if ever, improve.

The neurological symptoms include:

- < Paresthesias-most often in fingers and toes. The most common symptom of vitamin B<sub>12</sub> deficiency.
- < Diminished vibratory sense
- < Gait ataxia
- < Increases deep tendon reflexes
- < Memory loss
- < Personality change
- < Orthostatic hypotension

## **VITAMIN B<sub>12</sub> AND THE ELDERLY**

On routine screening as many as 10-23% of elderly patients will have low vitamin B<sub>12</sub> levels. One study found that 14.5% had levels below 300 pg/ml with 56% of these patients having increased levels of homocysteine and methylmalonic acid indicative of tissue vitamin B<sub>12</sub> deficiency. The most common mechanism is inability to absorb vitamin B<sub>12</sub> from food. It is speculated the rapid rise in the use of H<sub>2</sub> blockers will increase this problem in this patient population. Patients with dementia have lower levels of vitamin B<sub>12</sub> than those without but treatment with vitamin B<sub>12</sub> is often not effective, perhaps due to the long duration of the neurological damage. Studies are underway to examine the relationship of vitamin B<sub>12</sub> deficiency to neurological disease in the elderly and the effects of early intervention.

## **B12 DEFICIENCY- THERAPY**

For severe deficiency B<sub>12</sub> subcutaneous injection of 1000 ug should be given followed by long term management can consist of either monthly B<sub>12</sub> shots or oral therapy with 1-2 mg/day. Patients who have had their bowel or stomach resected should receive routine B<sub>12</sub> supplementation. There is overwhelming evidence that oral B<sub>12</sub> replacement at a dose of 0.5-2 mg/day is just as effective as injections.

## **MYELODYSPLASIA**

The myelodysplastic syndromes are a group of bone marrow diseases marked by various cytopenias, morphologically abnormal blood cells, dysplastic bone marrow changes and a propensity to evolve into acute leukemia. The changes on the peripheral smear can range from very abnormal looking blood smears to subtle changes. Hallmarks on the peripheral smear are pseudo-Pelger Huet cells (two-lobed neutrophils), macroovalocytes and hyposegmented neutrophils. This is commonly a disease of older patients and manifests itself as an anemia with normal iron, vitamin B12, and folate studies. Often these patients are misdiagnosed and treated ineffectually with iron or vitamin shots. Another group of patients in whom the myelodysplastic syndromes are common is patients who have undergone therapy for malignancy. Diagnosis is by bone marrow examination and genetics studies. Often cytogenetic abnormalities will be present and aid in diagnosis and assessing prognosis. Increasingly searching for specific mutations via next generation sequencing helps with both diagnosis and therapy. Therapy is very dependent on age and underlying disease. Only stem cell transplant is curative but newer therapies can reduce transfusion requirements. One specific type of myelodysplasia - 5q minus syndromes - responds robustly to lenalidomide.

## **APLASTIC ANEMIA/PURE RED CELL APLASIA**



Destruction of the hemopoietic cells of the marrow by whatever means leads to the clinical condition of aplastic anemia. The patient with this condition presents with pancytopenia. A thorough history should be taken from the patient to try to identify any possible drug or toxin exposure, although in most patients the cause is unknown. Splenomegaly is absent. The peripheral smear shows a decreased number of normal red cells. Diagnosis is established by bone marrow biopsy that reveals a hypocellular marrow. Since paroxysmal nocturnal hemoglobinuria can initially present as aplastic anemia, when marrow function recovers, a Ham's test should be performed. In young patients with aplastic anemia, bone marrow transplantation is the treatment of choice. Since the prognosis is worse in these patients if they had received transfusion, one should not transfuse these patients unless absolutely necessary.

Pure red cell aplasia is the condition where the red cell precursors are selectively destroyed. Since this condition can be associated with thymoma (and responsive to its removal), this should be sought with radiographic studies. Parvovirus B19, which is selectively toxic to the developing red cell, can cause a chronic infection that leads to clinical picture resembling pure red cell aplasia in susceptible patients. Parvovirus infection is also hazardous in patients dependent on increased RBC production such as those with congenital hemolytic anemia or sickle cell anemia. In those patients parvovirus infection may lead to a dramatic "aplastic crisis."

### **ANEMIA AND ALCOHOLISM**

Anemia of complex etiologies is often present in the alcoholic patient. Alcohol has a direct toxic effect on the bone marrow that leads to decreased red cell production. Folate metabolism is also interfered with leading to functional folate deficiency. This is aggravated by the poor dietary intake of folate by alcoholics and impaired absorption of folate in these patients. The alcohol abusing patient may also have increased blood losses due to gastrointestinal bleeding and trauma. Hypersplenism due to liver disease can be a factor to the anemia. This group of patients will often have coexistent inflammatory states that will lead to defective iron use. Heavy alcohol use can even lead to a sideroblastic anemia.

Diagnosis of specific defects in the alcoholic can be difficult due to the myriad of problems. The serum ferritin is a dependable gauge of marrow iron stores. Although the MCV may be normal, most alcoholics with folate deficiency will have either macroovalocytes or hypersegmented neutrophils present on the peripheral smears. Sources for blood lost should be aggressively sought. Often a bone marrow examination is required fully to explain the etiology(s) of the anemia.

### **A BRIEF BUT IMPORTANT PARAGRAPH ABOUT COPPER**

Copper plays a key role in hematopoiesis. Patients can become copper deficiency most commonly by:

1. Lack of intact (anorexia, bariatric surgery)
2. Excessive zinc intake (Cu and Zn share the same transporter)
  - a. Eating too many coins (especially pennies)
  - b. Too many Zn supplements

The classic signs of copper deficiency are the following 4 findings

1. Anemia – can be severe
2. Neutropenia – can be severe
3. Thrombocytopenia – very RARE
4. Neurological findings – peripheral neuropathy

Treatment is by replacing copper and convincing the patient to stop eating coins.

### **INDICATIONS FOR A BONE MARROW ASPIRATION AND BIOPSY**

1. Pancytopenia.
2. Leukoerythroblastic blood smear (presence of immature white cells and nucleated red cells).
3. Staging of the lymphomas and of small cell lung cancer (not in the diagnosis of these diseases!).
4. Unexplained anemia.
5. Blood smear suggestive of myelodysplasia or of leukemia.
6. Monoclonal gammopathy.

7. Anemia with a very low (less than 0.1%) reticulocyte count.

Table 1: Drugs implicated in hemolytic anemia, (DeLoughery 1998)

<b>Hapten</b> (Drug binding to RBC membrane)	<b>Ternary Complex</b> (drug-antibody complex binds to RBC)	<b>Autoantibody</b> (induction of AIHA)	<b>Unknown</b>
Penicillin Cephalosporin Tetracycline Tolbutamide Ampicillin Methicillin Carbenicillin	Amphotericin B Cefotaxime Ceftriaxone Cephalosporins Chlorpropamide Chlorpromazine Diclofenac Doxepin Hydrochlorothiazide Fenoprofen Isoniazid Melphalan Nomifensine Probenecid Quinidine Quinine Probenecid Rifampin Tetracycline Thiopental Tolmetin	Cephasporins Tolmetin Nomifensine Methyldopa Levodopa Mefenamic acid Teniposide Procainamide Diclofenac	Chlorpromazine Melphalan Isoniazid Acetaminophen Thiazides Ibuprofen Erythromycin Sulindac Omeprazole Sulfa drugs Rifampin Tricyclic antidepressants