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## MEGALOBLASTIC ANEMIAS

### MACROCYTOSIS

An increased MCV can be due to a number of 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").
5. Reticulocytosis
6. Smoking
7. Monoclonal gammopathy

Oval macrocytosis (macroovalocytes) is a sign of problem 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 division are skipped leading to a larger than normal cell. 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 often have **hy**persegmented neutrophils and abnormal platelet morphology.

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 (several weeks) and maintenance of folate stores is dependent on adequate dietary intake. Folate is found in green leafy vegetables, and liver. Folate is absorbed in the small bowel and circulates in a free form or loosely bond to albumin.

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 meal by stomach acid 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 which is required for thymidine synthase. Vitamin B<sub>12</sub> other role is a co-factor 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 synthesize 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-elevated 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. This also leads to the classic sign of tongue smoothing.

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

### **ETIOLOGIES OF FOLATE DEFICIENCY**

Decreased intake- The average intake of folate in the diet has increased dramatically in the past few years due to widespread supplementation of food so this etiology is increasingly rare.

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

#### Malabsorption

Drugs - Patient 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 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.

Abnormal gastric events include being unable to dissociate vitamin B<sub>12</sub> from food due to lack of stomach acid or enzymes. This is a recently recognized group of patients which 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.

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*.

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

Drugs - Metformin, PPIs

### **APPROACH TO THE PATIENT WITH A 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

It turns out that simply measuring serum levels of B<sub>12</sub> or folate is simply inadequate to diagnosis deficiency. Up to 30% of people with low normal B<sub>12</sub> levels will be deficient and many people with low B<sub>12</sub> stores have normal tissue stores. A more reliable method is to assay for the metabolic products that accumulate in B<sub>12</sub> deficiency. Since B<sub>12</sub> is involved in conversion of homocysteine to methionine, lack of B<sub>12</sub> will lead to elevated homocysteine level. Also B<sub>12</sub> is involved in conversion of methylmalonic acid to succinyl so in B<sub>12</sub> deficiency, methylmalonic acid accumulates. Both homocysteine and methylmalonic acid assays are widely available and should be the first line tests for B<sub>12</sub> deficiency.

Serum folate levels are also very unreliable. Since folate is needed for conversion of homocysteine to methionine, serum homocysteine will also accumulate in folate deficiency and is a more sensitive marker of tissue folate stores.

| DEFICIENCY      | HOMOCYSTEINE | METHYLMALONIC ACID |
|-----------------|--------------|--------------------|
| FOLATE          | ELEVATED     | NORMAL             |
| B <sub>12</sub> | ELEVATED     | ELEVATED           |

### Increased Homocysteine level:

B<sub>12</sub> or folate deficiency, renal failure (kidney significant organ for homocysteine metabolism).

#### Implications:

- 1) Marker for possible nutritional deficiency
- 2) Elevated levels of homocysteine are associated with an increased risk of atherosclerosis or venous thrombosis - but multiples trials have shown lowering homocystine with folic acid does not reduce risk of thrombosis.
- 3) Increased levels of homocysteine (reflecting lack of folic acid) in pregnant women is a risk factor for neural tubes defects.

### Increased Methylmalonic Acid

#### Causes:

B<sub>12</sub> deficiency, renal failure (MMA renal excreted), methylmalonic aciduria (rare)

#### Implications:

B<sub>12</sub> deficiency.

## DETERMINING THE UNDERLYING CAUSE

In the majority of patients with folate deficiency, one can determine the underlying cause by history – such as history of bariatric surgery. For patients with B<sub>12</sub> deficiency, again history can provide a strong clue. Specific cause include pernicious anemia and medications. Patients with pernicious anemia can be detected by assaying for autoantibodies but these tests can lack diagnostic specificity. Antibodies to IF are specific but not sensitive and antibodies to parietal cells are sensitive but not specific for pernicious anemia. Common medications such as metformin and proton pump inhibitors can inhibitor B<sub>12</sub> absorption. Many patents are B<sub>12</sub> deficient due to achlorhydria but this is not easy to screen for. Other causes are pancreatic insufficiency, blind loops, and infection with *diphyllobothrium latum* (tapeworm).

## THERAPY

Patients with severe megaloblastic anemia need immediate therapy. One should quickly obtain serum vitamin B<sub>12</sub> and red cell folate levels and then give 1-5 mg of folate and 1000 ug IM of vitamin B<sub>12</sub>. Patients should be treated daily with folate. Schedules for vitamin B<sub>12</sub> replacement vary but a common approach to all is daily therapy for one week to rapidly build up stores and supply vitamin B<sub>12</sub> to tissues, then weekly for a month, then monthly life-long. Patients with severe anemia should have increased reticulocyte by day three and increased hematocrit by day 5. Patients with alcoholism and folate deficiency can take up to three weeks to respond to folate therapy. It used routine to use IM injection to replace vitamin B<sub>12</sub>. Oral therapy with 0.5-2mg/day has been tested and has been found to be just as reliable as IM therapy and has become the standard for long term therapy

Although patients with megaloblastic anemia often present with severe anemia, transfusion therapy is rarely indicated. Since the anemia is rapidly reversible with therapy there is little justification for exposing the patient to the risk of transfusion except if the patient is having life-threatening symptoms such as severe ischemia. A further hazard of transfusion is since some of these patients have high-output heart failure, overzealous transfusion may lead to pulmonary edema.

### **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.

### **CLINICAL DIFFERENCES BETWEEN VITAMIN B<sub>12</sub> AND FOLATE DEFICIENCIES**

|  | <b>Folate Deficiency</b> | <b>Vitamin B<sub>12</sub> Deficiency</b> |
|--|--------------------------|--|
| Most common cause                                  | Dietary deficiency       | Malabsorption                            |
| Time to development                                | Months                   | Years                                    |
| Neurologic abnormalities                           | No                       | Yes                                      |
| Response to treatment with vitamin B <sub>12</sub> | No                       | Yes                                      |
| Response to treatment with folate                  | Yes                      | Hematologic - Yes<br>Neurologic - No     |

### **REFERENCES**

Green R, Allen LH, Bjørke-Monsen AL, Brito A, Guéant JL, Miller JW, Molloy AM, Nexo E, Stabler S, Toh BH, Ueland PM, Yajnik C. Vitamin B(12) deficiency. Nat Rev Dis Primers. 2017 Jun 29;3:17040

Green R, Datta Mitra A. Megaloblastic Anemias: Nutritional and Other Causes. Med Clin North Am. 2017 Mar;101(2):297-317.

Kaferle J, Strzoda CE. Evaluation of macrocytosis. Am Fam Physician. 2009 Feb 1;79(3):203-8.

Stover PJ . Vitamin B12 and older adults. Curr Opin Clin Nutr Metab Care. 2010

Theisen-Toupal J, Horowitz G, Breu A. Low yield of outpatient serum folate testing: eleven years of experience. JAMA Intern Med. 2014 Oct;174(10):1696-7v