Definition: Macrocytic Anemia

- MCV > 100 fL
- Impaired DNA formation due to lack of:
  - B12 or folate in ultimately active form
  - Use of antimetabolite drugs
- Macrocytosis also caused by:
  - Liver disease with inadequate cholesterol esterification
  - Alcohol abuse independent of folate (MCV 100-105)
  - Myelodysplasia
  - Post-splenectomy
  - HIV drugs
  - Dilantin
Vitamin B12: Cobalamin

- Meat and dairy products only
- Typical American diet contains 5-7mcg/d
- Minimum daily requirement 6-9 mcg/d
- Total body store 2-5 mg (half in liver)

- Helps to synthesize thiamine, thus deficiency leads to problems with DNA replication
B12: Cobalamin absorption

- Initially bound to protein in diet, liberated by acid and pepsin, then binds to R factors in saliva and gastric acids
- Freed from R factors by pancreatic proteases then binds to Intrinsic Factor secreted by gastric parietal cells
- Absorbed together (Cbl + IF) in ileum
- Released from IF in ileal cell then exocytosed bound to trans-Cbl II
- Cbl bound to transcobalamin II binds to cell surface receptors and is endocytosed
Actions of Cobalamin & Folate

A

(Homocysteine)
\[ \text{CH}_2 - \text{CH}_2 - \text{CH} - \text{COOH} \]
| \( \text{SH} \) | \( \text{NH}_2 \) |

\[ \text{Methionine synthase} \]
\[ \text{CH}_2 - \text{CH}_2 - \text{CH} - \text{COOH} \]
| \( \text{S} \) | \( \text{NH}_2 \) |
| \( \text{CH}_3 \) |

\[ \text{Methylcobalamin} \]

\[ \text{Cobalamin (enzyme bound)} \]

\[ \text{CH}_3\text{-THF} \]

\[ \text{THF} \]

B

(Propionyl-CoA)
\[ \text{CH}_3 - \text{CH}_2 \]
| \( \text{O} = \text{C} - \text{SCoA} \) |

\[ \text{Methylmalonyl-CoA} \]
\[ \text{CH}_3 - \text{C} - \text{COOH} \]
| \( \text{O} = \text{C} - \text{SCoA} \) |

\[ \text{Methylmalonyl-CoA} \] mutase

\[ \text{Succinyl-CoA} \]
\[ \text{CH}_2 - \text{CH}_2 - \text{COOH} \]
| \( \text{O} = \text{C} - \text{SCoA} \) |

Cofactor = Adenosylcobalamin
Causes of B12 Deficiency: Pernicious Anemia

- Autoantibody to Intrinsic Factor detectable in <70%
  - Highly specific, but insensitive
  - 2 types of anti-IF antibody
    - Blocks attachment of Cbl to IF
    - Blocks attachment of Cbl-IF complex to ileal receptors

- Chronic atrophic gastritis
  - Autoantibody against parietal cells (H-K-ATPase) though pathology indicates destruction by CD4+ T cells
  - Increased risk of gastric cancer (carcinoid and intestinal-type)
Causes of B12 Deficiency: Helicobacter pylori?

- In 1 study from Turkey
  - 138 total pts, HP infection documented by endoscopy in 77
    - B12 level returned to normal in all 31 pts with successful eradication of bacteria without additional supplementation
Causes of B12 Deficiency: Growing Older

- Usually mild and subclinical
- Age >65 approx 5%
- Age >75 approx 10%+, up to 40% in institutionalized patients
- Unclear mechanism
  - gastric atrophy
  - inadequate intake
  - Achlorhydria
Causes of B12 Deficiency: Surgery, Medication, Worms, Etc.

- Gastrectomy/Bariatric surgery
- Ileal resection or bypass
- Ileal disease (TB, lymphoma, amyloid, post-radiation, Crohn’s)
- Enteropathies (protein losing, chronic diarrhea, celiac sprue)
- Fish tapeworm (Diphyllobothrium latum) infection
- Bacterial overgrowth
- HIV infection
- Chronic alcoholism
- Sjogren’s syndrome
- Pancreatic Exocrine Insufficiency
- Strict vegan diet
- Inherited
  - Trans-Cbl II or IF deficiency
  - decreased uptake of IF-Cbl (Imerslun-Grasbeck’s or juvenile megaloblastic anemia) - also presents with proteinuria
  - Homocysteinuria, severe abnormalities of methionone synthesis, abnormal lysosomal exporter
- Decreased absorption from medication
  - Neomycin
  - Metformin (biguanides) up to 10-25%
  - PPI
  - Nitric oxide (inhibits methionine synthase)
B12 Deficiency Symptoms

- Atrophic glossitis (shiny tongue)
- Shuffling broad gait
- Anemia and related sx
- Vaginal atrophy
- Malabsorption
- Jaundice
- Personality changes
- Hyperhomocysteinemia
- Neurologic symptoms (next slide)
- Copper deficiency can cause similar neurologic symptoms
B12 Symptoms: Neurologic

- Paresthesias
- Memory loss
- Numbness
- Weakness
- Loss of dexterity due to loss of vibration and position sense
- Symmetric neuropathy legs>arms
- Severe weakness, spasticity, clonus, paraplegia and incontinence

- Subacute combined degeneration of the dorsal (posterior) and lateral spinal columns
- Due to a defect in myelination

- NOT ALL PATIENTS WITH B12 DEFICIENCY RELATED NEUROLOGIC ABNORMALITIES ARE ANEMIA OR MACROCYTOSIS
Subacute Combined Degeneration

Degeneration and demyelination of the dorsal (posterior) and lateral spinal columns
B12 Lab findings

- Macroovalocytic anemia with elevated serum bili and LDH
  - Increased red cell breakdown due to ineffective hematopoiesis
- Retic, WBC & platelets normal to low
- Hypersegmented neurophils
  - Also occur in renal failure, iron deficiency, inherited
Bone Marrow

- Hypercellular marrow
  - Megaloblastic erythroid hyperplasia
  - Giant metamyelocytes

Due to slowing of DNA synthesis and delayed nuclear maturation
Methionine deficiency may play a central role
Folate

- Animal products (liver), yeast and leafy vegetables
- Normal requirement 400mcg/day
- Pregnancy/Lactation: 500-800mcg/day
- Increased requirement in hemolytic anemia and exfoliative skin disease
- Body stores: 5-10mg
Folate Metabolism

- Binds to folate receptor, becomes polyglutamated intracellularly
- Many drugs (trimethoprim, methotrexate, pyrimethamine) inhibit dihydrofolate reductase
Causes of Folate Deficiency

- **Malnutrition:** Destroyed by heat during cooking
- **Alcoholism** (decreased in 2-4 days): impairs enterohepatic cycle and inhibits absorption
- **Increased requirement** in hemolytic anemia, pregnancy, exfoliative skin disease
- **IBD, celiac sprue**
- **Drugs**
  - Trimethoprim, Methotrexate, Primethamine (inhibit DHFR)
  - Phenytoin: blocks FA absorption, increases utilization (mech unknown)
Folate deficiency symptoms

- Similar symptoms as B12 save for neurologic symptoms
- Presentation is different classically:
  - Alcoholic
  - Very poor dietary intake
  - Older
  - Depressed
  - Living alone
Whom should you test for B12 or Folate deficiency?

- MCV >100 with or without anemia
- Hypersegmented neutrophils
- Pancytopenia of uncertain cause
- Unexplained neurologic s/sx
- Alcoholics
- Malnourished, particularly the elderly
- Vegans if no hx of supplementation
- Diabetics on metformin with new onset neuropathy
### Lab testing for diagnosis

<table>
<thead>
<tr>
<th></th>
<th>Serum B12</th>
<th>Serum Folate</th>
<th>MMA</th>
<th>Homocysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&gt;300</td>
<td>&gt;4</td>
<td>70-270</td>
<td>5-14</td>
</tr>
<tr>
<td>Deficiency</td>
<td>&lt;200</td>
<td>&lt;2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confirm B12</td>
<td>200-300</td>
<td></td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Confirm folate</td>
<td>2-4</td>
<td></td>
<td>Normal</td>
<td>High</td>
</tr>
</tbody>
</table>

High amount of seaweed in the diet can interfere with the B12 assay as can a single meal. It is best to add-on tests to blood already in the lab, particularly for inpatients due to the variability of the test.

Intrinsic factor antibody assay can be falsely positive if pt has recently received a B12 shot with B12 >800, thus important to add-on.
1. **PART 1**: Oral labeled B12 and IM unlabeled B12 at the same time to saturate tissue stores
2. 24h urine to assess absorption
   - >5% normal
   - <5% impaired
3. **PART 2**: Repeat w/oral IF
   - if now normal = PA
   - if abnormal = malabsorption
4. Can continue with antibiotics to look for bacterial overgrowth, pancreatic enzymes for exocrine insufficiency

<table>
<thead>
<tr>
<th>Part 1 test result</th>
<th>Part 2 test result</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>-</td>
<td>Normal or vitamin B12 deficiency</td>
</tr>
<tr>
<td>Low</td>
<td>Normal</td>
<td>Pernicious anemia</td>
</tr>
<tr>
<td>Low</td>
<td>Low</td>
<td>Malabsorption</td>
</tr>
</tbody>
</table>
B12 Deficiency: Treatment

- IM B12 1000mcg daily x 1 wk
  - then 1000mcg weekly x 1 month
  - Then 1000mcg monthly for life for PA

- Oral high dose 1-2 mg daily
  - As effective but less reliable than IM
  - Currently only recommended after full parenteral repletion

- Sublingual, nasal spray and gel formulations available
Vegan B12 Recommendations

- Daily multivitamin with 10 mcg/d
- Available in a few specific commercial nutritional yeasts most of which contain B12 from Pseudomonas sp., Propionibacterium sp. or Streptomyces sp.
  - Red Star Vegetarian Support Formula
  - Twinlab Natural Nutritional Yeast
- Probiotics are NOT sufficient to provide adequate B12
- Keep supplements in the fridge and out of light
- Encourage supplement for prenatal counseling of vegan or ovo-lacto vegetarian women (prenatal vitamin is sufficient unless deficient)

- B12: Are You Getting It? By Jack Norris, RD
- VeganHealth.org
Folate Deficiency Treatment

- Oral folate 1mg daily for 4 months or until hematologic recovery
- Rule out B12 deficiency prior to treatment as folic acid will not prevent progression of neurologic manifestations of B12 deficiency
- Repeat testing for B12 deficiency may be reasonable for those on long-term folic acid therapy if hematologic (macrocytosis or ↑LDH) or neurologic sx persist