Active $B_{12}$
The Next Level of B12 Testing

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At the end of the program, participants will be able to describe:

- The clinical presentation of patients with vitamin B12 deficiency.

- The physiology of B12 and the B12-binding proteins.

- The clinical utility of tests to diagnose B12 deficiency with special reference to active B12.
Vitamin B12 Deficiency

Scope:
- History of B12 deficiency
- Prevalence of B12 deficiency
- Review of normal physiology
- Causes of B12 deficiency
- Clinical Manifestations of B12 deficiency
- Diagnosing B12 deficiency
- Holotranscobilamin (Active B12) and its usefulness
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Pernicious Anemia: Description (Cabot, 1908)

Anemia associated with a diagnostic triad of:

- Sore tongue (glossitis)
- Jaundice
- Spinal cord damage

Pernicious Anemia was an inevitably fatal disease prior to the Nobel Prize-winning discoveries of Minot, Murphy and Whipple.

Kaplan-Meyer survival curve for 320 patients with pernicious anemia pre-1926 (Cabot 1908)
The Conquest of Pernicious Anemia &
The Characterization of Vitamin B12

Minot, Whipple & Murphy Nobel Prize for
Physiology & Medicine 1934 – “Cure of PA”

Karl Folkers and Lester Smith 1948 – Anti-pernicious
anemia principle crystallized from liver; B12 named

Dorothy Hodgkin – Nobel Prize for Chemistry
for studies on X-Ray crystallographic structure
of B12 and proteins
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Prevalence of B12 Deficiency in the United States

- Adults age >65 years
  - 2-3% have pernicious anemia
  - 30-40% have food B12 malabsorption

- Sacramento Area Latino Study on Aging (SALSA)
  - Elderly Latinos, age >60 years
  - 6.5% with total serum B12 <200 pg/ml
  - 18% with total serum B12 200-300 pg/ml

World Population by Age

Source: United Nations Data
Vitamin B12 Deficiency

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Vitamin B12 Absorption

Intestinal Lumen

Intestinal Cell (ileum)

Blood

IF-B12

IF-B12

IF

IF

B12

B12

B12

B12

B12

B12

B12

TC (transcobalamin)

TC

TC

TC

TC

TC

CH₃-B12

CH₃-B12

CH₃-B12

CH₃-B12

CH₃-B12

CH₃-B12

B12-TC

B12-TC

B12-TC

B12-TC

B12-TC

B12-TC

Adenosyl-B12

Adenosyl-B12

Adenosyl-B12

Adenosyl-B12

Adenosyl-B12

Adenosyl-B12

Vitamin B12
Vitamin B12 Cellular Uptake

Blood → B12-TC → B12-TC → B12

TC → CH₃-B12 → Adenosyl-B12

Tissue Cell
Vitamin B12-Dependent Reactions

Homocysteine* + MethylTHF → Methionine + THF

Methylmalonyl-CoA → Succinyl-CoA

Methylmalonic Acid*

*Levels rise in B12 deficiency
# Plasma B12 Transport Proteins

<table>
<thead>
<tr>
<th></th>
<th>Haptocorrin (TC I + III)</th>
<th>Transcobalamin (TC II)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Source</strong></td>
<td>granulocytes</td>
<td>endothelial cells, gut</td>
</tr>
<tr>
<td><strong>Transport functions</strong></td>
<td>storage, excretion of B12 analogues, antimicrobial</td>
<td>cellular B12 uptake</td>
</tr>
<tr>
<td><strong>Binding specificity</strong></td>
<td>low specificity, binds B12 analogues</td>
<td>binds B12 with higher specificity</td>
</tr>
<tr>
<td><strong>Membrane receptors</strong></td>
<td>non-specific asialoglycoprotein receptors on hepatocytes</td>
<td>specific receptors on most cells</td>
</tr>
<tr>
<td><strong>Saturation</strong></td>
<td>high (mainly &quot;holo&quot;)</td>
<td>low (mainly &quot;apo&quot;)</td>
</tr>
<tr>
<td><strong>Fraction of total B12</strong></td>
<td>70-90%</td>
<td>10-30%</td>
</tr>
<tr>
<td><strong>Plasma clearance</strong></td>
<td>slow (t$_{1/2}$ ≈ 10 days)</td>
<td>rapid (t$_{1/2}$ ≈ 6 min)</td>
</tr>
<tr>
<td><strong>Molecular Weight</strong></td>
<td>60,000</td>
<td>38,000-45,000</td>
</tr>
</tbody>
</table>
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Vitamin B12 Deficiency

**Causes:**
- Dietary deficiency (rare, primarily occurs in strict vegans and their offspring)
- **Malabsorption**
  - Nitrous oxide (irreversibly oxidizes B12)
  - Transcobalamin deficiency
  - Genetic enzyme defects
Causes of Vitamin B12 Malabsorption

- Atrophic Gastritis (achlorhydria or loss of stomach acid)
- Autoimmune production of IF or parietal cell antibodies (pernicious anemia)
- Gastrectomy
- Pancreatic insufficiency
- Bacterial overgrowth (H. pylori)
- Diphyllobothrium latum (fish tapeworm)
- HIV Infection
- Ileal disease or resection
- Selective Vitamin B12 Malabsorption –Immerslund-Gräsbeck Syndrome (Autosomal Recessive Megaloblastic Anemia (MGA1) –defects in cub, amn.
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Symptoms and Signs of Vitamin B12 Deficiency

Clinical Manifestations:

- Megaloblastic anemia
- Subacute combined degeneration (SACD) (demyelination with central and peripheral neuropathy, most notably in spinal cord)
- Gait ataxia
- Cognitive deficits (can be Alzheimer-like)
- Glossitis
- Increased risk of vascular disease, cancer, neural tube defects
- Osteopenia/osteoporosis
Patients in Whom to Suspect Possible B12 Deficiency

- Symptoms and signs of B12 deficiency
- Anemia with or without macrocytosis
- Neurological disturbances with or without anemia
Vitamin B12 Deficiency

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- **Diagnosing B12 deficiency**
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Diagnosis of Vitamin $B_{12}$ Deficiency

- Macrocytic megaloblastic anemia with or without neurological involvement.
- Atypical presentations (neurological syndrome without anemia or macrocytosis
- Low plasma B12 as an isolated lab finding
- Raised plasma metabolites (methylmalonic acid and homocysteine
- Low transcobalamin B12 (HoloTC) = “Active B12”
Approach to the diagnosis of Pernicious Anemia

Assessing B12 Status

Total Serum B12

Holohaptocorrin

- Fraction of total B12 bound to haptocorrin.

~70-80%

Holotranscobalamin (Active B12)

- Fraction of total B12 bound to transcobalamin.
- Delivers B12 to all tissues.

~20-30%

Wide-Range
<10% to >70%
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Theoretical Advantages of HoloTC (Active B12) in the Diagnosis of B12 Deficiency

- TC delivers B12 to all tissues; haptocorrin does not. Except on the liver no cellular receptors exist for the B_{12} carried by haptocorrin (HC)
- Genetic TC deficiency leads to life-threatening functional B12 deficiency; genetic haptocorrin deficiency is relatively benign.
- HoloTC has a short half-life (~6 min) and is therefore expected to fall early during states of B12 malabsorption.
- It can take months, even years, for a significant fall in HoloHC levels and so the more rapid decline in HoloTC (Active-B12) may be masked when measuring total serum B12
Not all vitamin $\text{B}_12$ in serum is active

Around 20% of circulating $\text{B}_12$ is carried on transcobalamin.

- Holohaptocorrin (holoHC)
  - Biologically inert

- Active-B12 (holotranscobalamin)
  - Biologically active

- $70\text{-}90\%$

- $10\text{-}30\%$

- MMA → Hcy → Methyl-$\text{B}_{12}$ → Adenosyl-$\text{B}_{12}$ → TCII

- Adenosyl-$\text{B}_{12}$ → Methyl-$\text{B}_{12}$
Sequence of Changes in Developing B12 Deficiency*

1. Early: low holoTC (Active B12)

2. Cellular: low serum B12, depletion of body stores

3. Metabolic: increased Hcy and MMA

4. Clinical: macrocytic anemia, neurological impairment

*Victor Herbert 1987
# How vitamin B$_{12}$ deficiency develops (hypothesis)

<table>
<thead>
<tr>
<th>Normal B$_{12}$ status</th>
<th>Early serum depletion</th>
<th>Cell depletion</th>
<th>Damaged metabolism</th>
<th>Clinical damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Active-B12 ↓</td>
<td>Active-B12 ↓</td>
<td>Active-B12 ↓</td>
<td>Active-B12 ↓</td>
</tr>
<tr>
<td>Active-B12 &gt; 35 pmol/L</td>
<td>MMA &lt; 271 nmol/L</td>
<td>MMA &amp; tHcy ↑</td>
<td>MMA &amp; tHcy ↑</td>
<td>MMA &amp; tHcy ↑</td>
</tr>
<tr>
<td>MMA &lt; 271 nmol/L</td>
<td>tHcy &lt; 12 µmol/L</td>
<td>tHcy ↓</td>
<td>tHcy ↓</td>
<td>tHcy ↓</td>
</tr>
<tr>
<td>B$_{12}$ &gt; 300 pmol/L</td>
<td>Normal erythropoiesis</td>
<td>MRI &amp; tHcy</td>
<td>MRI &amp; tHcy</td>
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**Active-B12 levels react early in the process.**

1. [Modified from V. Herbert, Am J Clin Nutr 1994](#)
Assessing B12 Status

Total Serum B12

- ~70-80% Holohaptocorrin
  - Fraction of total B12 bound to haptocorrin.
- ~20-30% Holotranscobalamin
  - Fraction of total B12 bound to transcobalamin.
  - Delivers B12 to all tissues.

Wide-Range <10% to >70%
TC-B12 or Active B12 in B12 Absorption and Transport

Dietary B12

Stomach

Intestinal Lumen

IF

B12

IF-B12

Ileal Cell

Portal Circulation

TC-B12

Stool

Liver

Bile

HC-B12

Extrahepatic Tissues

General Circulation

TC-B12

TC-B12

HC-B12

Kidney

Urine
Active-B12 reaction schematics
2-Step sandwich MEIA

Active-B12 specific Mab (mouse, monocl.) immobilised on latex microparticle.

Sample B12 bound to transcobalamin (red) and haptocorrin (magenta).

Only B_{12}-TC (Active-B12) will bind to solid phase.

Suspension moved to glass fiber matrix and washed to remove unbound sample.

Anti-TC:ALP conjugate (mouse, monocl.) is added.

Conjugate binds to TC bound to solid phase.

Unbound conjugate is removed.

Rate of fluorescent MU formation is directly proportional to [Active-B12] in sample.
Active-B12 levels are low in patients with biochemical signs of vitamin B$_{12}$ deficiency

B$_{12}$ deficiency defined by $^9$:
- MMA >400 nmol/l and
- Normal renal function

Data suggests improved identification of B$_{12}$ deficient patients with Active-B12 compared to total serum B$_{12}$.

Recently Proposed Algorithm for $\text{B}_{12}$ Deficiency Subjects

Subjects at risk of $\text{B}_{12}$ deficiency

- $\text{B}_{12} < 150 \text{ pmol/L}$: Likely deficient
- $\text{B}_{12} 150-250 \text{ pmol/L}$: Additional testing, like Active-B12
- $\text{B}_{12} > 250 \text{ pmol/L}$: Unlikely deficient

Resolve $\text{B}_{12}$ indeterminate samples

Note: Due to many false negative total $\text{B}_{12}$ results the negative population could also be confirmed with Active-B12 TC assay.

Adapted from Schneede J., Scan J Clin Lab Invest 2003; 63: 369 - 376
Approach to the diagnosis of Pernicious Anemia